AN ABSTRACT OF THE THESIS OF

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Title: ISOLATION AND PARTIAL CHARACTERIZATION OF BOVINE RUMEN MUSCLE PROTEINS

Abstract approved: _____
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Investigations were conducted to determine the nature, type, functional properties and the amounts of different proteins found in bovine rumen tissue.

Data concerning the effect of pH and salt concentration on the extractability of proteins from rumen tissue indicated that 92% of the proteins could be extracted in aqueous solutions at either pH 3.0 or 10.0. Rumen tissue proteins showed the least solubility in the pH range of 5 to 6. Two protein isolates having distinct compositional differences were obtained from the pH 10.0 aqueous extract by successively lowering the pH to 7.0 and 5.4. The supernatant remaining from the isoelectric precipitation at pH 5.4 contained one major sodium dodecyl sulfate (SDS) gel electrophoretic band corresponding to a subunit molecular weight of 67,000. However, bands corresponding to the four major contractile proteins were not detected.

SDS-gel electrophoresis was used to identify the proteins in fresh rumen tissue, in the various extracts and in the protein isolates. The gels were subjected to densitometric analysis to obtain an estimate of the contractile proteins contained in the various samples. The protein isolate obtained at an isoelectric precipitation of pH 7.0 contained 28% myosin, 65% actin and 7% unidentified proteins but no troponin or tropomyosin. The isolate obtained at pH 5.4 contained 20% myosin, 40% actin, 19% troponin, 11% tropomyosin and 10% unidentified proteins. The protein extract at pH 10.0 contained 12% myosin, 35% actin, 9.5% troponin, 13% tropomyosin and 30.5% unidentified proteins. Based on 92% protein extractability at pH 10.0 and assuming only the stroma proteins to be insoluble at this pH, the protein composition of the fresh bovine rumen tissue was calculated to be 11% myosin, 32% actin, 9% troponin, 12% tropomyosin, 8% stroma, and 28% unidentified proteins. When the rumen tissue was homogenized in distilled water at neutral pH, 5% of the total soluble protein was myosin, 45% actin, 22% troponin and 20% tropomyosin. However, once the contractile proteins of the rumen tissue were purified, their solubility properties were similar to those of skeletal muscle contractile proteins.

Contractile proteins purified from rumen and skeletal muscle yielded identical R_m values on SDS gel electrophoresis, and their estimated molecular weights were similar. Myosin from both rumen

and skeletal muscle gave similar elution patterns on DEAE Sephadex A-50 columns. In each case, the major myosin peak emerged at a KCl concentration of about 0.1 M.

The ATPase activity of rumen myosin was lower at low ionic strengths but higher at high ionic strengths than that of skeletal myosin. The activity of both types of myosin was stimulated by Ca^{++} and EDTA but inhibited by Mg^{++}.

Amino acid analysis of rumen and skeletal myosins were similar, although rumen myosin had lower levels of lysine, aspartic acid, isoleucine, leucine and phenylalanine, and higher levels of glycine, valine, methionine and tyrosine, than skeletal myosin. Rumen and skeletal actin differed somewhat in amino acid composition. The most notable differences were the higher contents of glutamic acid and lysine in the rumen actin.

The emulsifying capacity of the two rumen protein isolates were about 30% lower than that of the skeletal muscle proteins. However, the rumen protein isolates showed excellent stability and consistency which were judged to be comparable to those of skeletal muscle proteins.

Rumen protein extracts and isolates showed excellent whippability and foam stability characteristics that were found to be equivalent to dried, reconstituted egg albumen.

Isolation and Partial Characterization of Bovine Rumen Muscle Proteins

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INTRODUCTION

Many compelling forces have been developing in recent years that demand a more efficient world-wide distribution, utilization and conservation of food. The world's food demands have been increasing at an alarming rate. Providing adequate amounts of protein poses a much greater problem than providing adequate supplies of general calories (fats or carbohydrates). Proteins not only are more costly to produce than fats or carbohydrates but the daily requirement per kg of bodyweight remains constant throughout adult life. Conversely, requirements for general calories usually decrease with age.

While there is a severe shortage of food proteins of high biological value in the developing countries, the cost of proteins of animal origin has been accelerating in the developed countries. These circumstances have led scientists to look for new sources of proteins adaptable for food use. The tremendous world wide interest in this direction is amply demonstrated by the vast amount of literature published on new or unconventional sources of food proteins (single-cell proteins, leaf proteins, etc.) during the past 15 years.

In view of the probable food protein deficits, it seems relevant and timely to reassess some of the slaughter plant waste materials or

by-products for their potential value as human food. Since the by-products of the meat packing industry are not directly edible or have very limited appeal in the fresh or frozen markets, many create pollution problems or are under-utilized by being processed into animal feeds (Levin, 1970). Some of these waste materials are relatively rich in good quality proteins (Olson, 1970).

Bovine stomach tissue, more commonly known as "tripe", is one of the more intriguing by-products of slaughter plants that appears to have potential value as a source of food protein. Recent research findings have indicated that about 70% of the total tripe proteins can be readily extracted in the native state at ambient temperatures with dilute salt solutions adjusted to pH 10. Once solubilized, fairly pure protein isolates can be obtained from the protein extracts by isoelectric precipitation. It is visualized that these isolates could be used to help fabricate palatable texturized foods or used as binders or emulsifiers in existing foods.

With a trend towards increasing centralization of the meat packing industry (Anonymous, 1969), collection of slaughter plant by-products for specialized processing appears to be both practical and economically feasible.

Although a limited amount of applied research has been directed towards better utilization of the meat packing industry by-products in the past, the results have not been particularly encouraging.

Much of the fault lies with the fact that the proteins were extracted or concentrated with organic solvents. Proteins subjected to such treatments usually undergo considerable denaturation resulting in an excessive loss of functionality and decreased nutritive value. Thus, their utility is very limited in the manufacture of food.

In order to maximize their potential value, a clear understanding of these proteins would be invaluable. The present work was undertaken to gain basic knowledge about the type and nature of the proteins of the bovine rumen tissue in relation to those of skeletal muscle and to evaluate some of the more critical functional properties that are so essential in the food processing industry.

LITERATURE REVIEW

There is a growing awareness among responsible individuals for the need to optimize available food resources at both national and international levels. It is well known that nearly one-third of a beef carcass is not used directly for human consumption (Levin, 1970). Table 1 shows the material balance sheet of the typical processing of a 1200 lb steer.

Table 1. Average dressing weight of a 1200 lb steer.

Item	Weight (lbs)	Percent
Live weight	1200	
Carcass	700	58.3
Hide	75	6.2
Head, feet and knees	45	3.7
Oleo fat	80	6.6
Liver	12	1.0
Heart	3	0.03
Lungs	20	1.6
Tongue	5	0.41
Cheek meat	5	0.41
Rough tallow	84	7.0
Liquid blood	46	3.8
Stomach and contents	106	8.8
Lips and weasand meat	4	0.3
Tail, bung and casings	15	1.2

One of the by-products that has a potential for use as food that currently is more or less "wasted" is stomach tissue (tripe). Ruminant stomach consists of four compartments. The protein contents of each of the tissues may vary slightly (Young and Lawrie, 1974). However, Table 2 summarizes the weight of the various stomach components and their relative protein contents.

Table 2. Percentage, weight and protein content of the bovine stomach compartments.

Stomach compartments	Total stomach tissue (%)	Wet weight (lbs)	Protein (%)	Protein (lbs)
Rumen	55	27	11.0	3.0
Omasum	25	12	9.0	1.1
Abomasum	13	7	8.0	0.6
Reticulum	7	4	9.5	0.4

Recent research findings have shown that about 70% of the stomach tissue protein can be extracted by homogenization-solubilization in water at high pH, subsequently followed by iso-electric precipitation (Young and Lawrie, 1974).

The number of cattle slaughtered in the United States during 1974 was in excess of 35 million (U.S. Bureau of Census, 1975). This number is likely to increase during the coming years. These

data, together with the fact that the meat packing industry is fast becoming centralized, aptly display the feasibility of producing a protein isolate from stomach tissue. Table 3 gives an estimation of the amount of pure protein that could be isolated from tripe annually.

Table 3. Estimation of total protein that could be isolated from rumen tissue.

35 million beef animals slaughtered per year	
@ 50 lbs tripe/animal	= 1.75 billion lbs
@ 10% protein content	= 175 million lbs protein
@ 70% protein recovery	= 122.5 million lbs protein
or 61,250 tons of pure protein per year	

Despite the possibility of producing a tremendous amount of protein, there is a serious lack of information about these proteins, their functional properties, and amounts found in the stomach tissue. In subsequent pages attempts will be made to elucidate the nature of smooth muscle proteins which appear to be similar to those of rumen tissue.

Smooth and Striated Muscles

A wealth of experimental data are available concerning the properties of the various myofibrillar or contractile proteins and

their specific functions in the contractile mechanism of vertebrate striated muscle. On the other hand, in spite of the importance of smooth muscle for visceral and vascular functions, relatively little is known about their proteins (Pollard and Weihing, 1974). Even the contractile mechanisms by which smooth muscles undergo contraction and relaxation is still only vaguely understood (Bulbring et al., 1970). Thus, striated muscle proteins are considered "typical" and it is not unusual to compare the contractile proteins from other muscle systems such as smooth muscle with those of striated muscle.

Vertebrate smooth muscle differs from vertebrate striated muscle in many respects (Needham and Shoenberg, 1967; Hamoir, 1973; Shoenberg and Needham, 1976). In order to understand its peculiar properties, a clear appreciation of the diversity of the two types of muscle is necessary. Hamoir (1973) summarized some of the differences between striated, cardiac, uterus and vascular muscles. The extracellular space in the myometrium (uterus muscle) and vascular muscle is about two to three times larger than that of skeletal muscle. Smooth muscles also are characterized by their extremely small cell size, whereas skeletal muscle cells are several hundred times larger. Smooth muscles have a high serum albumin content. In addition to collagen and elastin, they also contain large amounts of insoluble components such as glycoproteins and acid

mucopolysaccharides. These components are not extracted by the salt solutions used to extract striated muscle proteins.

Extractability of Contractile Proteins of Smooth Muscle

The extractability of smooth muscle proteins is hindered by the presence of large amounts of insoluble components (Bulbring et al., 1970; Hamoir, 1973). Hence, drastic extraction conditions such as homogenizing for long periods of time or grinding with sand must be employed. This contrasts to the mild and gentle conditions used for the extraction of skeletal muscle proteins. One of the most ambiguous and least understood characteristics of smooth muscle contractile proteins is their high solubility at low ionic strengths. This solubility can be further enhanced by incorporating 1 mM ATP in the extraction medium (Csapo, 1948, 1950 a, b). Hamoir and Laszt (1962) found that about one-third of smooth muscle tropomyosin is extracted at low ionic strengths (≤ 0.05 μ) as compared to only a small percentage in the case of striated muscle.

When a buffered 0.5 M KCl extract of skeletal muscle was diluted to 0.04 M KCl, the dilution precipitate formed consisted mainly of actomyosin and myosin. However, when uterus muscle was subjected to the same treatment, about 45% of the dilution precipitate consisted mostly of actomyosin with little or no myosin being

present (Needham and Shornberg, 1967). Thus, in order to isolate pure myosin from actomyosin a dissociating agent such as ATP is used to split away actin which is then polymerized in the presence of Mg^{++} and separated from myosin by ultracentrifugation as a gelatinous aggregate.

The unusually high solubility of the contractile proteins of smooth muscle at low ionic strengths suggests the labile nature of the ultrastructure of these muscles. Also, the high solubility of smooth muscle myosin at low ionic strengths may lead to an insight about its in situ occurrence. Small and Sobieszek (1972), using electron microscopy, have shown that smooth muscle myosin exists in the form of ribbons, whose core consists of tropomyosin associations. However, according to a personal communication by these authors to Shoenberg and Needham (1976), they no longer hold this view. Biochemical and electron microscopic data collected by Schoenberg et al. (1966) suggested that in the relaxed smooth muscle, myosin existed mostly in a colloidally dispersed phase, which also could explain its ready solubility.

Even though there is some doubt about the presence of myosin thick filaments, F-actin thin filaments have been clearly identified in smooth muscle (Hanson and Lowy, 1963; Shoenberg et al., 1966; Elliot, 1964, 1967; Panner and Honig, 1967). The high solubility of actin, as mentioned earlier, cannot be explained on the basis of

the drastic extraction conditions employed, because F-actin filaments are known to be preserved under such conditions (Bulbring et al., 1970).

Smooth muscle actomyosin can be completely precipitated from an extract by adjusting the pH to 5.5 (Hamoir, 1973). However, some tropomyosin remains in solution which can be precipitated between pH 4.2 and 5.2. These isoelectric precipitates obtained at various pH values are contaminated with numerous ill-defined proteins typical of smooth muscle. They may also contain large amounts of nucleic acids and some salt-soluble collagen (Needham and Williams, 1963a; Gaspar-Godfroid et al., 1968).

Smooth Muscle Proteins

In the following pages a more detailed treatment of the biochemical characteristics of the individual contractile proteins found in smooth muscle will be undertaken.

Actin

Actin seems to be the most constantly occurring contractile protein of muscles, in general, and in other motile cells. It is probably ubiquitous, in that it is also known to occur in numerous non-muscle cells ranging from amoeba and cellular slime molds to mammalian fibroblasts, nerve cells, and platelets (Anonymous,

1970, 1971; Mark, 1975). Present evidence indicates that actin, together with myosin may play an important part, not only in muscle contraction, but also in cell motility and mitosis. According to Mark (1975), more is known about actin of both muscle and non-muscle cells than about myosin. In smooth muscle and non-muscle cells, actin is known to occur as close-packing microfilaments (Rice et al., 1966, 1970, 1971; Somlyo and Somlyo, 1968; Heumann, 1970; Lowy and Small, 1970; Devine and Somlyo, 1971; Devine et al., 1972; Cooke and Fay, 1972; Cooke, 1976).

Actin obtained from skeletal, cardiac, and smooth muscles, as well as from cytoplasm of non-muscle cells, greatly resemble each other. The monomeric globular form of actins from various sources are known to have a molecular weight of the order of 45,000. They also have similar gel electrophoretic migration patterns, sedimentation constants, peptide maps, and amino acid compositions (Gosselin-Ray et al., 1969; Needham and Shoenberg, 1967). This close resemblance of actin from a variety of cells and species is probably why it is difficult to prepare antibodies against actin (Shoenberg and Needham, 1976). Actin prepared from smooth muscle of the arterial wall was shown to undergo depolymerization on homogenization with 0.1 mM ATP, and repolymerized on addition of salt, a behavior characteristic of skeletal muscle actin. However, the slow rates of polymerization and depolymerization of smooth

muscle actin appear to be due to a minor structural difference (Hamoir, 1973).

Needham and Williams (1963) found that purified uterine actin reacted normally with skeletal myosin to give a viscous actomyosin. A similar reaction occurred between purified uterine myosin and skeletal actin.

Smooth muscle actin is known to stimulate the ATPase activity of chicken gizzard myosin in the presence of Mg^{++}, but this activity was very low compared with that of skeletal muscle (Barany et al., 1966). This low actin activated ATPase activity of smooth muscle myosin is related to the low contractability of smooth muscles (Needham and Williams, 1963). As in the case of skeletal muscle, actin is believed to be intimately associated with tropomyosin, which was shown by the amount of tropomyosin contamination of actin preparations from smooth muscle (Needham and Shoenberg, 1967).

Actomyosin and Myosin

The complex formed when actin and myosin are mixed in vitro is known as actomyosin. This complex together with troponin and tropomyosin was first used as a model system by Ebashi to explain the muscle contraction in vivo (Cohen, 1975). It is well known that purified actin and myosin isolated from smooth and skeletal muscles as well as from non-muscle cells, complex with each other to form

actomyosin (Yamaguchi et al., 1970; Pollard and Korn, 1972; Pollard and Weihing, 1974). These actomyosin complexes are known to have high viscosity and birefringence. Even though actin by itself has no enzymatic activity, the presence of it in the actomyosin complex is known to modify the ATPase activity of myosin. In the case of both skeletal and smooth muscle myosin, magnesium ions tend to inhibit the ATPase activity. However, in the case of actomyosin, magnesium ions seem to activate the ATPase activity (Needham and Shoenberg, 1964; Barany et al., 1966; Yamaguchi et al., 1970). This magnesium activation of the ATPase activity was found to be several times lower in smooth muscle actomyosin than in the skeletal actomyosin. Needham and Williams (1959) found that trypsin treatment of uterus actomyosin increased the ATPase activity to about the same order of magnitude as skeletal actomyosin. This trypsin effect was not observed with skeletal actomyosin.

Smooth muscle actomyosin can be extracted at low ionic strengths (0.05 M KCl) in the presence of small amounts of ATP (Needham and Shoenberg, 1964). They found that the sensitivity to ATP as measured by fall in viscosity in such extracts was very small compared to that of skeletal actomyosin and higher concentrations of ATP were required for maximum effect. This was thought to be due to the heterogeneity of the proteins and the presence of lateral complexes not readily dissociated by ATP.

Actomyosin content of smooth muscle is strikingly lower than that of skeletal muscle (Needham and Williams, 1963). They also found that only about 45% of the dilution precipitate of a smooth muscle extract contained actomyosin and there was little or no myosin present in this precipitate.

Myosin is equally widespread as actin in muscle and non-muscle cells (Pollard, 1972, 1973, 1974; Clarke and Spudich, 1974). However, it is not found in high concentrations in all of these cells as is actin. Cytoplasmic myosins may differ from one another and from skeletal myosin quite significantly. However, they appear to resemble vertebrate smooth muscle myosin in structure fairly closely. In spite of the differences that are found in myosins of different species, two functions essential for the development of contractile forces are found in all myosins (Clarke and Spudich, 1974). These are the ability to bind actin filaments reversibly and to hydrolyze ATP.

The ATPase activity of smooth muscle myosin, as well as platelet myosin, is lower than that of skeletal myosin, which suggests a structural difference at the active site of the molecule (Cohen et al., 1976). Gaspar-Godfroid (1968) showed that the ATPase activity of myosin preparations from cow carotid depended on the degree of SH protection. Smooth muscle myosins also are known to have a higher Ca^{++}-ATPase activity at higher ionic strengths than

skeletal myosin, which is a distinguishing characteristic. However, their actin activated ATPase activity in the presence of Mg^{++} is lower than those of their skeletal counterpart. Smooth muscle myosin obtained from rabbit intestine had two pH optima of increasing magnitude, occurring at pH 6 and 7.5, and a third of negligible value around pH 9.5, for Ca^{++}-ATPase activity. On the other hand, rabbit skeletal myosin was characterized by three well defined pH optima of increasing magnitude at pH 6, 7.8, and 9.1 (Bogach et al., 1976). Bogach and co-workers also found that by increasing the temperature, both skeletal and smooth myosin ATPase activity increased until a temperature of 37^{o} was reached. Above this temperature, skeletal myosin ATPase activity rapidly decreased and at 50^{o} the enzyme activity was completely lost. In the case of smooth muscle myosin, the maximum activity was attained at 45^{o} and inactivation occurred at 55^{o}. They also found that Ca^{++}-ATPase activity of skeletal myosin was about thrice that of smooth myosin at their respective temperature optima. They further concluded that the ATPase activity of myosin of skeletal and smooth muscles was affected differently by the environmental changes such as pH, temperature, ionic strenght, etc., and that these variations may be related to the differences in the protein structure. Cohen and co-workers (1976) recently found that the lysyl residues at the active site of platelet and gizzard myosin had a similar location which was different from that found

in skeletal myosin.

Choi (1962) and Needham and Shoenberg (1964), working with homogenates of chicken gizzard, guinea-pig taenia coli and rabbit uterus, observed sparsely and irregularly distributed thick filaments of uneven lengths in their electron micrographs, which were believed to be superimposed thin filaments. Hanson and Lowy (1964) later showed that these thick filaments were indeed myosin. The long time of homogenization necessary to disrupt the connective tissue in smooth muscle was believed to cause disintegration of the myosin thick filaments. More recently, Kaminer et al. (1976) observed that reconstituted myosin filaments from smooth muscle were shorter than those from striated muscle. The hybrid filaments showed a progressive decrease in length as the proportion of smooth muscle myosin increased. They concluded from their experiments that smooth muscle myosin may have less specific bonds available for assembly.

The amino acid compositions of smooth and skeletal muscle myosins show considerable differences (Hamoir, 1973; Shoenberg and Needham, 1976). Histidine content was found to be lower in smooth muscle myosin than in any other of the myosin preparations studied. In general, an increase in the acidic amino acids and a decrease in the basic amino acids of smooth muscle myosin were noteworthy. Hence, the net negative charge of smooth muscle

myosin at neutral pH is probably higher than that of the skeletal myosin. The proline and glycine contents also are generally lower in smooth muscle myosin except in the case of chicken gizzard myosin.

One good way of differentiating the myosin isozyme, or determining its location in the cell, is by immunochemical techniques (Shoenberg and Needham, 1976; Hamoir, 1973). Several authors (Becker and Murphy, 1969; Groschel-Stewart and Doniach, 1969; Groschel-Stewart, 1971) have used this technique to show that the antibodies against red and white skeletal muscle myosins and against smooth muscle myosins were not species specific, even though they were specific for each of the three types of muscle. Antisera prepared against the myosins of red and white skeletal muscles and those against cardiac myosins were found to cross-react, indicating the immunological similarities of these myosins. Conversely, antisera against smooth muscle myosin failed to cross-react with skeletal or cardiac myosin, but reacted with myosins of non-muscle cells such as blood platelets and endothelial cells. From these observations the conclusion was drawn that a structural dissimilarity exists between myosin of smooth muscle and that of skeletal muscle. Since the antibody is known to combine with heavy meromyosin and not with light meromyosin (Groschel-Stewart, 1971), it is probable

that the structural dissimilarity of the two types of myosins resides with the globular part of the molecule.

Smooth muscle myosins have been purified by various investigators (Barany et al., 1966; Yamaguchi, 1970; Groschel-Stewart, 1971; Ebashi, 1976). The most common procedure for purification was to dissociate the actomyosin complex with ATP and collect the actin by ultracentrifugation. The supernatent was subjected to ammonium sulfate fractionation. Pure myosin was obtained in the fraction between 45-55% ammonium sulfate saturation. Recently, Mergerman and Murphy (1975) obtained pure myosin from arterial smooth muscle by selectively precipitating it from an actomyosin extract using polyethylene glycol-6000 to depolymerize the actin.

The myosin molecule is cleaved by trypsin into two fragments called light meromyosin (LMM) and heavy meromyosin (HMM). The latter is further sub-divided into two fragments designated by HMMS-1 and HMMS-2. A schematic representation of the myosin molecule (Figure 1) shows the location of the various fragments and the light chains of myosin.

Huriaux and co-workers (1967) and Huriaux (1972) isolated and studied the various fragments of bovine carotid artery myosin, while Bailin and Barany (1971) studied the subunits of chicken gizzard myosin. The overall substructure of smooth muscle myosin does appear to differ significantly from that of the skeletal or cardiac

muscle. The light meromyosin (LMM) from bovine carotid myosin showed greater solubility at low and high ionic strenghts than the corresponding skeletal muscle protein. The amino acid composition of these two proteins also differed, particularly the low values of cysteine and high levels of phenylalanine observed in carotid LMM. As judged by the higher electrophoretic mobility, carotid LMM probably had a higher net negative charge at neutral pH. Since myosin molecules form thick filaments, it is the fibrous LMM that is responsible for the association, and the aforementioned properties of LMM would probably lower the stability of myosin filaments of vertebrate smooth muscle. This property in turn could reflect on the high solubility of these myosins at low ionic strengths.

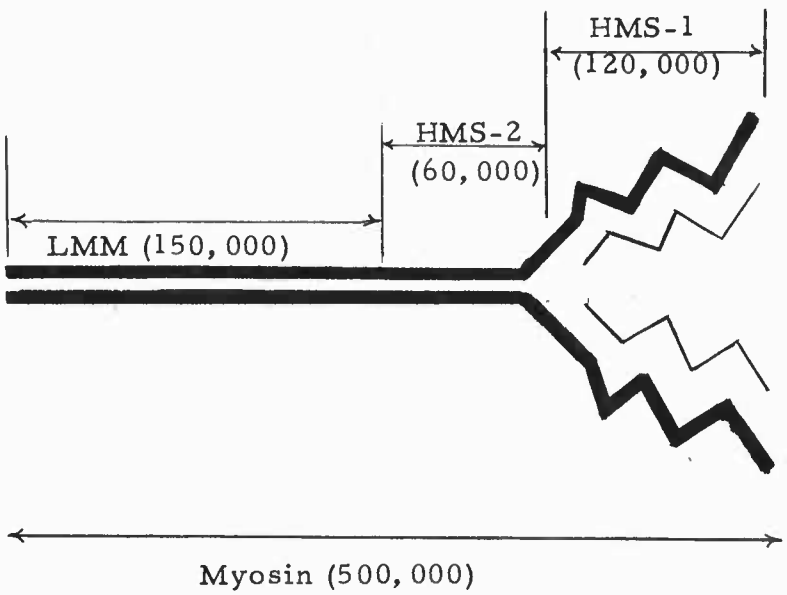


Figure 1. Schematic representation of the myosin molecule. Myosin heavy chains are represented by thick lines and the light chains by thin lines. Molecular weights are given in brackets.

In studying the sub-fragments of different myosins it was observed that cow carotid myosin, like chicken gizzard and cardiac myosin, was more resistant to tryptic digestion than rabbit skeletal myosin (Barany et al., 1966; Tada et al., 1969).

Heavy Meromyosin Sub-fragment 1 (HMMS-1)

Huriaux (1970) prepared HMMS-1 from cow carotid myosin by tryptic digestion. Most of its properties such as the sedimentation coefficient, concentration dependence of S, molecular weight (110,000) and the diffusion coefficient were quantitatively similar to the corresponding skeletal muscle protein. This sub-fragment retained the ATPase activity of the original myosin molecule as was the case for rabbit skeletal and bovine cardiac myosin sub-fragment 1. It combined with actin to yield a viscous complex which was dissociated by the addition of ATP. Recently, this sub-fragment has found use as a convenient label for actin filaments (Mark, 1975).

Light Chains of Myosin

Light chains of skeletal myosin were detected by Tsao in 1953. There are three known light chains in skeletal myosin having molecular weights of 17,000, 19,000, and 21,000. The 19,000 molecular weight component can be removed without loss of ATPase activity.

These light chains are located in the globular head of the myosin molecule as shown in Figure 1.

In contrast to the skeletal myosin, only two light chains having molecular weights of 20,000 and 17,000 were observed in smooth muscle myosin (Kendrick-Jones, 1973). In chicken gizzard myosin, these two proteins make up about 16% of the total molecule. The 17,000 component could be removed from the myosin molecule by incubation in 4 M urea, which resulted in an irreversible loss of the ATPase activity. These two light chains also had extensive differences in their amino acid compositions. Preliminary studies indicate that they were different from the light chains of skeletal and cardiac myosin.

Leger and Focant (1973) characterized the light chains of cow carotid, uterine and gastric muscle myosins and found only two light chains of molecular weights 19,800 and 17,200. They were found to be different from each other in their amino acid composition and also from that of skeletal muscle light chains.

Burridge (1974) found identical electrophoretic patterns for heavy and light chains of chicken gizzard and fibroblast myosins. Both myosins had light chains of identical molecular weights, namely 17,000 and 20,000, as observed by other workers.

Tropomyosin

Tropomyosin was first described by Bailey in 1946. It is soluble in water, giving highly viscous solutions. The viscosity decreases rapidly upon addition of salt up to 0.1 M concentration. It is highly resistant to denaturing agents and its amino acid composition resembles that of myosin.

It is well documented that much higher amounts of tropomyosin are found in smooth muscle than in striated ones (Needham and Shoenberg, 1967). Hamoir (1973) reported that about one-third of the cow carotid tropomyosin was extracted at low ionic strengths, compared to only a small percentage in the case of skeletal muscle. When extraction was repeated several times, tropomyosin seemed to be liberated progressively. This higher solubility may be due to the disruption of the actin filaments during extraction, since these two proteins are known to exist in close association.

Carsten (1968) obtained a highly pure tropomyosin from smooth muscle of human and sheep uterii which showed similarities in the sedimentation constant and intrinsic viscosities to those of rabbit skeletal muscle tropomyosin. The amino acid analysis and electrophoretic mobility suggested a higher negative charge at neutral pH than the corresponding skeletal muscle protein. This was further confirmed by peptide map analysis which showed six to eight less

peptides in the case of uterus tropomyosin than in the skeletal tropomyosin.

Small and Sobieszak (1972) studied the in vitro ribbons formed from actomyosin extracts of smooth muscle. They concluded that the core material of these ribbons consisted of tropomyosin, which provided a surface suitable for interaction with myosin. However, such a hypothesis would make it difficult to explain the regulatory nature of tropomyosin in vertebrate smooth muscle.

Ebashi et al. (1966) were the first to prepare tropomyosin from smooth muscle. Both skeletal and smooth muscle tropomyosins were found to be highly sensitive to trypsin. Subjecting gizzard actomyosin to a trypsin treatment resulted in the complete loss of its calcium sensitivity. However, the calcium activity could be restored upon the addition of native tropomyosin. Recently, Sands (1975) isolated actomyosin from bovine tracheal smooth muscle which did not show any calcium sensitivity. He found no troponin, tropomyosin activity in these muscle fractions.

Troponin

There is considerable doubt about the presence of troponin in smooth muscle. However, Carsten (1971) claims to have prepared a tropomyosin-free troponin from cow uterus. She purified the protein by isoelectric precipitation and ammonium sulfate

fractionation. SDS gel electrophoresis of this preparation gave at least six components. The four major components had molecular weights of approximately 14,500, 26,000, 43,000 and 56,000. These components in the presence of tropomyosin would restore the calcium sensitivity of both desensitized skeletal and uterine actomyosin. She thus concluded that the components of the regulatory system in skeletal muscle also are found in smooth muscle.

It is now well established that skeletal muscle troponin is a complex of three components (Hartshorne and Dreizen, 1972; Greaser et al., 1972; Drabikowski et al., 1972; Perry et al., 1972). These components are known as the calcium sensitizing factor of troponin-C (TN-C), the inhibitory factor or troponin-I (TN-I) and the tropomyosin-binding factor or troponin-T (TN-T). These have molecular weights of 18,000, 24,000 and 39,000 respectively.

Some workers have reported the absence of a band corresponding to troponin-T in smooth muscle (Shoenberg and Needham, 1976). Driska and Hartshorne (1974) studied the regulatory proteins of chicken gizzard and found that actomyosin could be desensitized by a mild trypsin digestion. However, the desensitized actomyosin would recombine with the material released in digestion to restore the calcium activity. More recent studies (Driska and Hartshorne, 1975) indicate that in the gizzard muscle, the regulatory system is located in the thin filaments. However, sub-units similar to troponin

sub-units were not detected, and they concluded that troponin was absent in chicken gizzard muscle. Sobieszek and Bremel (1975), and Sobieszek and Small (1976), working with gizzard also found no troponin. Calcium sensitivity was retained in actomyosin preparations from which all tropomyosin had been removed. They found that this calcium sensitivity was lost when a 20,000 mass light chain was cleaved from the molecule by trypsin. These studies support the view that in smooth muscle or at least in the gizzard tissue, the regulation is brought about by the myosin light chains. More work is underway in various laboratories for evidence of such a myosin-linked regulatory system in vertebrate smooth muscle (Shoenberg and Needham, 1976).

EXPERIMENTAL PROCEDURE

The research carried out in this study was conducted in three phases. The first phase dealt with an assessment of the extractability of rumen tissue proteins in aqueous extracting solutions at differing pH levels and varying salt concentrations. The second phase was concerned with the isolation and partial characterization of the rumen tissue proteins. The functionality of the various extracts and protein isolates derived from the rumen tissue was evaluated in the third phase.

Sample Preparation

Fresh rumen tissue and skeletal muscle (M. sternomandibularis) from the neck area were obtained from a beef animal immediately after slaughter. These materials were chilled in crushed ice and then thoroughly washed with ice-water to remove blood and extraneous matter. All subsequent preparatory work was carried out at 4°. After the mucous linings and membraneous-type materials were scraped from the rumen tissue, this material was flash-frozen and ground for the immediate extraction of myosin. All external fat and connective tissue surrounding the skeletal muscle were trimmed away after which, the muscle was diced and passed through a previously cooled (4°) meat grinder. Appropriate samples were

taken immediately for isolation of the various skeletal muscle proteins with the remainder being stored at -40° for further analysis.

Extractability of Rumen Tissue Proteins

The experimental procedure used was similar to that described by Young and Lowrie (1974). The effect of pH on protein extractability was determined by grinding 2 g of diced rumen tissue and sand with a mortar and pestle for 5 min. The macerated tissue was dispersed in 50 ml of distilled water. The dispersion was adjusted to the desired pH by the addition of either 0.2 M NaOH or 0.2 M HCl to encompass a pH range of 3 to 10. Distilled water was added to make a final volume of 60 ml. Dispersions were agitated continuously for 2 hours at ambient temperature.

The effect of the metal chelator, ethylene diamine tetraacetic acid (EDTA), upon the extraction of rumen tissue proteins over a range of pH values of 3 to 10 was conducted exactly in the manner described above using a solution of 0.01 M EDTA in place of water.

The effect of salt concentration upon the extractability of rumen tissue proteins was determined in a similar manner. Differing ionic strengths were achieved by adding 0.05 M, 0.01 M, 0.1 M and 0.5 M KCl or NaCl into the extracting solution, the pH of which was adjusted to 7.2 as before.

The dispersions were centrifuged at 34,000 x G for 15 min. In each case, the supernatants were collected and filtered through glass wool. Total protein (N x 6.25) was determined by the microkjeldahl procedure (A. O. A. C., 1970) on 2 ml aliquots of the clarified supernatants. The total protein of the rumen tissue was also determined by the microkjeldahl method. Extractability was expressed as a percentage of the amount of protein in the supernatant in relation to the total amount of protein in the rumen tissue.

Preparation of Isolates

Two isolates were prepared from pH 10.0 extracts by adjusting the pH to 7.0 using 0.5 M HCl. The precipitate was collected by centrifugation at 6,000 x G for 10 min at 4°. The pH of the supernatant was then adjusted to 5.4 and the precipitate resulting from this acidification was collected by centrifugation as before.

Isolation of Contractile Proteins

Rumen Myosin

The method of Yamaguchi et al. (1970) was modified for the extraction of rumen myosin. To macerate the sample initially, 250 g of ground rumen tissue was blended intermittantly in an Osterizer blender at high speed for 2 min. An equal volume of 5 mM

histidine buffer (wash solution), pH 7.0, containing 0.1 mM dithiothreitol (DTT) and 0.7% glycerol was added. Homogenization was conducted at 5 sec intervals for 1 min. Cold wash solution was added to provide a final volume of 1000 ml and homogenization was continued as before for another 5 min. Care was taken to avoid foaming as much as possible. The homogenate was centrifuged at 13,000 x G for 15 min. The residue was rinsed with the wash solution and suspended in 750 ml of 0.6 M KCl buffer, pH 7.0, containing 1 mM ATP and 20 mM histidine. The contents were stirred continuously for 12 hr at 4°. The suspension was centrifuged at 13,000 x G for 15 min. The supernatant was filtered through nylon parachute cloth and glass wool to remove suspended particles. The filtrate was diluted 10-fold with cold distilled water and gentle stirring. The precipitate was allowed to settle for 1 hr. The clear liquid was siphoned away and the gelatinous white residue was collected by centrifugation at 1,000 x G for 10 min. The precipitate was placed in a graduated cylinder with a minimum of water and solid KCl was added to form a final concentration of 0.6 M KCl. The contents were stirred gently until all of the precipitate was dissolved and a clear solution was formed. The protein content was adjusted, when necessary, with 0.6 M KCl solution. This protein solution was centrifuged at 34,000 x G for 20 min to remove suspended particles and the supernatant was diluted 10-fold as before.

This process of resolubilization-reprecipitation was carried out for a total of 4 cycles. The final precipitate was dissolved in 0.6 M KCl so that the final protein concentration was in the range of 12-18 mg/ml. A stock solution at ATP, $MgSO_4$ and KCl in Tris-HCl buffer, pH 8.0, was prepared and gently stirred into the protein solution so that the final concentrations of the mixture components were: 10 mM ATP, 10 mM $MgSO_4$, 0.6 M KCl, 20 mM Tris, and 6 to 10 mg/ml of protein.

The suspensions were centrifuged at 105,000 x G for 3 hr at 2°. The upper third of the supernatant from each of the tubes was carefully removed, pooled and dialyzed overnight against a solution of 0.6 M KCl. A saturated $(NH_4)_2SO_4$ solution containing 10 mM EDTA and adjusted to pH 7.0 with ammonia was added until a saturation of 45% was achieved. The contents were allowed to stand for 30 min and the precipitate formed was removed by centrifugation at 6,000 x G for 10 min at 4°. The volume of supernatant was measured and saturated $(NH_4)_2SO_4$ was added to 55% saturation. After standing for 30 min, the precipitate was collected by centrifugation at 6,000 x G for 10 min at 4° and dialyzed against several changes of buffer solutions that were to be used in subsequent analysis. The $(NH_4)_2SO_4$ fractionation was repeated as needed.

Skeletal Myosin

Skeletal myosin was prepared from bovine neck muscle (M. sternomandibularis) by the procedure of Tsao (1953). Two hundred and fifty grams of ground muscle were homogenized intermittantly for 30 sec with 3 volumes of 0.3 M KCl in 0.1 M potassium phosphate buffer, pH 6.8. The homogenate was stirred for 30 min and the residue was separated by centrifugation at 13,200 x G for 10 min at 4^{o}. The supernatant was filtered through nylon parachute cloth and glass wool. The filtrate was diluted 10-fold with distilled water. The precipitate formed was collected and redissolved in 0.5 M CKl and the pH was adjusted to between 6.7 and 6.8. The solution was diluted to 0.28 M KCl by the addition of distilled water. Actomyosin that precipitated was removed by centrifugation. Myosin was precipitated from the supernatant by diluting to 0.04 M KCl. The precipitate was dissolved in 0.5 M KCl and the above procedure was repeated twice more. The final precipitate was dissolved in a minimum of water and saturated $(NH_4)_2SO_4$ was added to 40% saturation. Myosin that precipitated between 40% and 50% saturation of $(NH_4)_2SO_4$ was collected and dialyzed against several changes of the appropriate buffer solutions used in subsequent tests.

Rumen Actin

Ground frozen rumen tissue was homogenized in an Osterizer blender for 2 min and then suspended in 5 volumes of 2 mM Tris-HCl buffer containing 0.2 mM ATP and 0.5 mM β-mercaptoethanol (β-ME) adjusted to pH 8.0. The contents were agitated for 3 hr and the residue was collected by centrifugation at 13,200 x G for 15 min at 4°. The supernatant was filtered through glass wool and the pH was adjusted to 6.8. The precipitate was collected by centrifugation at 6,000 x G for 10 min at 4°. Three volumes of acetone were slowly added to the precipitate and the contents were stirred for 10 min. After the liquid was decanted, the residue was mixed with additional acetone and stirred for another 10 min. The residue was collected and air-dried for 10 hr. Actin was extracted from this acetone powder by adding 20 volumes of 0.2 mM ATP solution adjusted to pH 7.5 and allowing the mixture to stand for 2 hr at 0°.

Actin was precipitated from this solution by the addition of 10 volumes of acetone plus a few drops of acetate buffer, pH 4.6. The actin that precipitated in the form of a gelatinous mass was collected by centrifugation at 5,000 x G for 10 min at 4°. The precipitated actin was dissolved in water to yield a solution of globular actin (G-actin).

To prepare fibrous actin (F-actin), G-actin was allowed to polymerize overnight in a 0.05 M KCl-0.002 M $MgCl_2$ solution. This solution was centrifuged at 80,000 x G for 3 hr at 2° after which the pellet was dissolved in 0.05 M KCl-2 mM ATP, pH 7.0, to yield F-actin.

Skeletal Actin

Skeletal actin was isolated by the procedure of Feuer et al. (1948). The residue remaining from the skeletal myosin extraction was suspended in 5 volumes of 0.4% $NaHCO_3$ and stirred for 30 min at 25°. The mixture was gently squeezed through nylon parachute cloth.

The residue was suspended in 1 volume of 0.01 M $NaHCO_3$-0.01 M Na_2CO_3 solution at 4°. The suspension was stirred for 10 min and then diluted with 10 volumes of glass distilled water at 25°. The suspension was quickly squeezed through nylon parachute cloth. The residue was weighed and each gram of material was suspended in 3 ml of acetone at 25°. After stirring for 10 min, the residue was collected and resuspended in one-third of the former amount of acetone. After 10 min of stirring, the residue was once again squeezed through nylon parachute cloth and air-dried at ambient temperature.

Skeletal actin was extracted from the air-dried material using 20 volumes of CO_2-free glass distilled water for 30 min at 0°. The solution was filtered through glass wool and then diluted by the addition of 10 volumes of acetone plus a few drops of acetate buffer, pH 4.6. The resulting precipitate was handled in the same manner as that described for rumen actin to obtain the skeletal G- or F-actin.

Rumen and Skeletal Troponin

Both rumen and skeletal troponin were prepared by the procedure of Greaser and Gergely (1971, 1973). The ground tissues were stirred with 2 volumes of a solution containing 50 mM Tris-0.1 mM $CaCl_2$, pH 7.5, for 5 min. The suspensions were filtered through nylon parachute cloth. For skeletal muscle, the above washing procedure was repeated 4 times. The residues were stirred with 2 volumes of 95% ethanol for 10 min. The ethanol washing was done twice. The suspensions were pressed through nylon parachute cloth and washed in diethyl ether for 10 min. This step was repeated twice after which the residues were air-dried at ambient temperature.

The dried material was extracted overnight with 2 volumes of 1 M KCl-25 mM Tris-HCl-0.1 mM $CaCl_2$-1 mM DTT, pH 8.0, at ambient temperature. After centrifugation at 13,000 x G for 15 min at 4°, residues were once more extracted with 1 volume of the above

solution. The extracts were pooled per tissue type and filtered several times through nylon parachute cloth and glass wool.

The filtrates were adjusted to pH 4.6 with 0.5 M HCl to precipitate tropomyosin. The latter was collected by centrifugation at 6,000 x G for 10 min at 4° and retained for further analysis. The supernatants were neutralized, cooled to 0° and subjected to $(NH_4)_2SO_4$ fractionation. Fractions precipitating between 40% and 60% saturation were collected by centrifugation at 6,000 x G for 10 min at 4° and dissolved in 5 mM Tris buffer, pH 7.5. They were dialyzed against several changes of 5 mM Tris buffer, pH 7.5, containing 0.1 mM DTT and 0.1 mM $CaCl_2$.

Solid KCl was added to 1 M concentration and the protein content was adjusted to 2 to 3 mg/ml with 1 M KCl. The pH was lowered to 4.6 by addition of 0.5 M HCl to precipitate residual tropomyosin. The supernatant was neutralized and solid $(NH_4)_2SO_4$ was added to 70% saturation. The precipitate was collected by centrifugation at 6,000 x G for 10 min at 4° and dissolved in 5 mM Tris buffer, pH 7.5, as before and dialyzed overnight. Tropomyosin was once more precipitated by lowering the pH to 4.6. The neutralized supernatant was dialyzed against 2 mM Tris, pH 7.5, containing 0.1 mM DTT and was then lyophilized.

Rumen and Skeletal Tropomyosin

The procedure of Greaser and Gergely (1973) was followed to prepare both rumen and skeletal tropomyosin. Precipitates obtained by the lowering of the pH to 4.6 in the troponin preparation were dissolved in 0.25 M KCl-10 mM EDTA solution and brought to neutral pH. the protein content was adjusted to 1 to 2 mg/ml and $(NH_4)_2SO_4$ fractionation between 53 and 60% saturation was repeated several times as needed to obtain a pure preparation.

Analytical Procedures

Viscosity Measurements

The ATPase sensitivity of both rumen and skeletal myosin was determined by viscosity measurements carried out at 10^{o}. An Ostwald viscometer having a water flow-through time of 116 sec at 10^{o} was used to measure viscosity of the myosin preparations obtained by $(NH_4)_2SO_4$ fractionation. Myosin concentrations were adjusted to 3 mg/ml in 0.5 M KCl-4 mM Tris-HCl, pH 7.2, for a total volume of 4 ml/sample. To the latter, 0.05 ml of an 80 mM ATP solution was added and mixed well prior to the viscosity measurement. The final ATP concentration in the solution was 1 mM.

ATPase sensitivity was calculated from the appropriate data using the equation of Weber and Portzehl (1952):

$$\text{ATPase sensitivity} = \frac{\eta_{rel} - \eta_{rel\ ATP}}{n_{rel\ ATP}} \times 100$$

ATPase Activity

The hydrolysis of ATP was determined by measuring the amount of inorganic phosphate liberated at 37° by the method of Fiske and Subbrow (1925) as outlined in detail by Quass and Briskey (1968).

DEAE - Sephadex Chromatography

The chromatography of myosin was carried out by the method of Richards et al. (1967). Myosin preparations obtained by $(NH_4)_2SO_4$ fractionation were dissolved in 0.04 M $Na_4P_2O_7$ buffer, pH 7.5. These myosin solutions were dialyzed for 24 hr against several changes of the 0.04 M $Na_4P_2O_7$ buffer. A column (2.5 x 50 cm) packed with diethylaminoethyl (DEAE) Sephadex A-50 (Pharmacia, particle size 40-120 μ) was equilibrated at 4° with 0.04 M $Na_4P_2O_7$ buffer, pH 7.5. Twenty ml of the dialyzed myosin solutions containing 8-10 mg/ml of protein were applied to the column. Myosin was eluted with a linear gradient using 250 ml of 0.04 M $Na_4P_2O_7$

buffer, pH 7.5, and 250 ml of 0.04 M $Na_4P_2O_7$-0.5 M KCl buffer, pH 7.5. The absorbance of effluent from the column was continuously recorded at 280 nm by a Gilson UV absorption meter. Ten ml fractions were collected. The absorbance of each of the fractions was measured at 260 and 280 nm in a Beckman DB recording spectrophotometer.

Polyacrylamide Gel Electrophoresis

Quantitative estimation of contractile proteins in the various extracts and in fresh rumen tissue were determined by incorporating sodium dodecyl sulfate treatment with polyacrylamide gel electrophoresis (SDS PAGE). The procedure used to prepare the samples for electrophoresis was similar to that described by Weber and Osborn (1969) with certain modifications.

If the protein solutions contained potassium ions, they were dialyzed extensively against a solution of 0.01 M sodium phosphate buffer, pH 7.2, before SDS was added. The protein contents were adjusted to 1 mg/ml and β-ME and SDS were added to a concentration of 1% each in that respective order. The contents were mixed well and 5 ml were placed in small beakers and heated in an oven at 100° for 5 min. The maximum temperature attained by the protein-SDS solutions during this time was about 60°. The samples were cooled at ambient temperature and dialyzed against 0.01 M sodium phosphate

buffer, pH 7.2, containing 0.1% each of SDS and β-ME. Two hundred and fifty µl of dialyzed SDS-protein solutions were carefully mixed with 10 µl of β-ME, 10 µl of 1% bromophenol blue (marker dye) and 25 mg of powdered sucrose in small test tubes. The final protein content of sample solutions was 0.85 µg/ml. These samples were retained for electrophoresis.

An electrophoresis assembly similar to that described by Davis (1964) was operated according to the continuous buffer procedure of Weber and Osborn (1969). Stock solutions needed were prepared as follows:

A gel buffer consisting of 0.1 M sodium phosphate buffer, pH 7.2, and 0.1% SDS.

An electrode buffer consisting of 0.05 M sodium phosphate buffer, pH 7.2, and 0.1% each of SDS and β-ME. An acrylamide stock solution containing 22.2 g of recrystallized acrylamide plus 0.8 gm of methylbisacrylamide in 100 ml of distilled water.

To prepare 10% gels, 15 ml of gel buffer and 31.5 ml of acrylamide stock solution were deareated and twice filtered through Whatman No. 1 filter paper. To the filtrate, 20 µl of N, N, N', N'-tetramethyl ethylenediamine (TEMED) and 1.5 ml of freshly prepared ammonium pensulfate at a concentration of 7.5 mg/ml were added and mixed well. The contents were quickly poured into precision

bore glass columns (0.5 x 13 cm) held vertically in a leveling rack. The bottom end of each column was sealed with parafilm. Columns were filled within 2 cm of the top. After air bubbles were removed by gently tapping the sides of the columns, a few drops of water were carefully layered over each gel column. The columns were allowed to stand undisturbed until polymerization had occurred. One hr after gel polymerization the parafilm and water layer were removed and the top of each gel was washed with electrode buffer. The gel tubes were placed in the electrophoresis assembly and both the upper and lower buffer compartments were filled with electrode buffer.

For the quantitative estimation of the various proteins in the different extracts, each sample was run in duplicate at six different volumes ranging from 3 μl to 25 μl. Electrophoresis was carried out at a 5 mA per tube for 5-6 hr at ambient temperature. Upon completion of the electrophoretic run, gels were very carefully removed from the tubes and placed in appropriate sized test tubes containing the protein stain, coomassie Blue, and stained for 10 hr. Excess stain was removed with an electrophoretic destainer by Petropakis (1968) for 45 minutes. The gels were then allowed to destain by diffusion for ten days before they were taken for densitometric scanning.

Molecular Weight Determination

An inverse linear relationship exists between the relative electrophoretic migration distances and the logarithm of the molecular weights of protein subunits liberated by SDS treatment (Shapiro et al., 1967; Weber and Osborn, 1969). Thus, the SDS PAGE procedure provides a rapid and simple means of estimating the size of proteins and polypeptides.

The electrophoretic system previously described was employed to prepare a calibration curve of mobility versus log-molecular weight using the following enzymes and proteins as standards; myoglobin (17,200), carboxypeptidase (34,600), skeletal tropomyosin (36,000), skeletal troponin (39,000), skeletal actin (42,000), fumerase (49,000), bovine serum albumin (68,000), phosphorylase B (92,000), and skeletal myosin heavy chain (210,000).

These standard proteins were run under exactly the same electrophoretic conditions as were the unknown rumen proteins. Total volume per sample applied to each of the gels was of the order of 10-15 μl. Three microliters of skeletal actin was incorporated into the standards and unknowns. Relative mobility was calculated as a ratio of the distance of migration of a given protein from the origin to that of skeletal actin from the same origin.

Quantitative Densitometry

The destained gels were scanned with a Beckman DU spectophotometer equipped with a Gilford scanning accessory at 550 nm and optical density setting of 1.0. The gels were placed in 10 cm cuvettes with 7% acetic acid to fill the interspaces between the gels and the sides of the curvettes. The speed of the recorder and that of the gel were adjusted so that the tracing length to gel length ratio was 4:1. The areas of the peaks were computed by multiplying the peak height by the width at half-height for each peak.

Protein Determination

Protein concentration in the various extracts and preparations was estimated by the biuret method (Torten and Whitaker, 1964) using bovine serum albumin as the standard. The standard curve was prepared using the protein values obtained by the microkjeldahl method (A.O.A.C., 1970). When protein determinations could not be done accurately by the biuret method, the microkjeldahl method was used.

Amino Acid Analysis

Amino acid analysis was carried out by Dr. R. R. Becker, Department of Biochemistry and Biophysics, Oregon State University.

Lyophilized samples of purified rumen and skeletal muscle protein were hydrolyzed with 6 N HCl at 110° for 22 hr. The hydrolyzates were analyzed with a Beckman Model 120C Amino acid analyzer based on the principle of Spackman et al. (1958).

Emulsifying Capacity

A model system similar to that described by Carpenter and Saffle (1964) was used to determine the emulsifying capacity (EC) of the various proteins under study. The system consisted of an Osterizer blender base connected in series with a rheostat. A 4-bladed cutter assembly was screwed onto a 250 ml jar that was inverted onto the blender base. The opposite end of the jar had two holes, one at the center through which oil was added and another near the side for the insertion of a copper electrode. Rotational frequency of the cutter assembly was calibrated at 10,000 rpm with a stroboscope.

All solutions of protein extracts and isolates to be tested were adjusted to meet the following standard conditions: pH 7.0, a protein concentration of 6 mg/ml and 3% NaCl content.

Twenty ml of protein solutions was placed in the jar assembly and weighed. Twenty-five ml of cottonseed oil was added and the contents homogenized for 30 sec at low speed after which, the cutter assembly was activated to the calibrated rpm. At this point, oil

was added at a rate of 0.9 ml/sec for 1 liter separatory funnel.

The break point or emulsion collapse was determined by the electrical resistance method of Cummins (1975). The direct current resistance of the emulsion was measured by inserting a copper electrode into the emulsion through the side hole of the jar. The other electrode was attached to the cutter assembly base. The electrodes were connected to an ohm meter to monitor the electrical resistance of the emulsion. During emulsification, the electrical resistance ranged between 30-40 Kohm but abruptly increased to infinity as the emulsion collapsed. At this point, addition of oil was stopped. The jar assembly was weighed and the difference in weight was recorded as the amount of oil emulsified. The emulsifying capacity was expressed as gm of oil that was emulsified by 100 mg of protein.

Emulsion Stability

Emulsion stability was measured by the method of Acton and Saffle (1970) modified in the following way. Emulsions were prepared in the manner as described for the EC tests although the addition of oil was stopped just prior to the emulsion break point. Ten ml of each emulsion was placed in the test tubes in duplicate. Five ml of emulsion was immediately weighed in aluminum drying pans in duplicate and the moisture was determined *in vacuo* at a temperature

of 105° for eight hours. The tubes containing the emulsions were incubated in a water bath at 37° for two hours. At the end of the incubation period five ml of the emulsion was removed from the bottom of each tube and the moisture content determined as described above.

The stability rating was calculated for each sample on the basis of the percentage change in moisture by the following equation:

$$\text{Stability rating} = \frac{100-M_{test}}{100-M_{original}} \times 100$$

where M_{test} is the moisture of the bottom 5 ml incubation at 37° and $M_{original}$ is the moisture of the emulsion before incubation.

Whippability and Foam Stability

These tests are empirical. Whippability is the ability of an aqueous protein solution to foam when whipped. Foam stability refers to the ability of the foam to resist "weeping" or release of moisture with time.

A series of aqueous protein solutions ranging in protein concentrations from 1-6 mg/ml were prepared with each of the tissue extracts and isolates used in the previous experiments. All solutions were adjusted to pH 7.0. One hundred ml of each protein

concentration was whipped with a Hamilton Beach food mixer at a speed setting of whipping for 4 min. The foam was transferred to a 100 ml beaker having a known volume when filled to the brim. The foam was levelled at the brim and immediately weighed. The beaker was then inverted and placed in an appropriate-sized glass funnel atop of a graduated cylinder. The volume of liquid released was recorded at 30 min intervals over a 90 min test period. Foam stability was expressed as the ml of liquid released at any given time.

Whippability, according to Lawhon et al. (1972), is usually expressed as foam over-run per mg of protein. Foam over-run was calculated from the expression:

$$\text{Foam over-run} = \frac{\text{volume of foam X 100}}{\text{weight of foam (g)}}$$

RESULTS AND DISCUSSION

Extractability of Rumen Tissue Proteins

The average proximate composition of the bovine rumen tissue utilized in this study is given in Table 4. All values are expressed on a wet weight basis.

Table 4. Proximate composition of bovine rumen tissue.

Component	Percent[a]
Moisture	82.33
Protein (N x 6.25)	16.50
Lipid	1.05

[a] Average of 3 determinations.

The effect of pH on the extractability of proteins from bovine rumen tissue is illustrated in Figure 2. The curves show a minimum protein solubility in the pH range 5.0 to 6.0. Only 11 to 14% of the total protein was extracted in this pH range. These data are in close agreement with the results of Young and Lawrie (1974) who obtained similar curves for the extractability of proteins from various bovine offal tissues at room temperature. However, one major difference between the results of this study and those of Young and Lawrie is the greater protein extractability on the acidic side of the

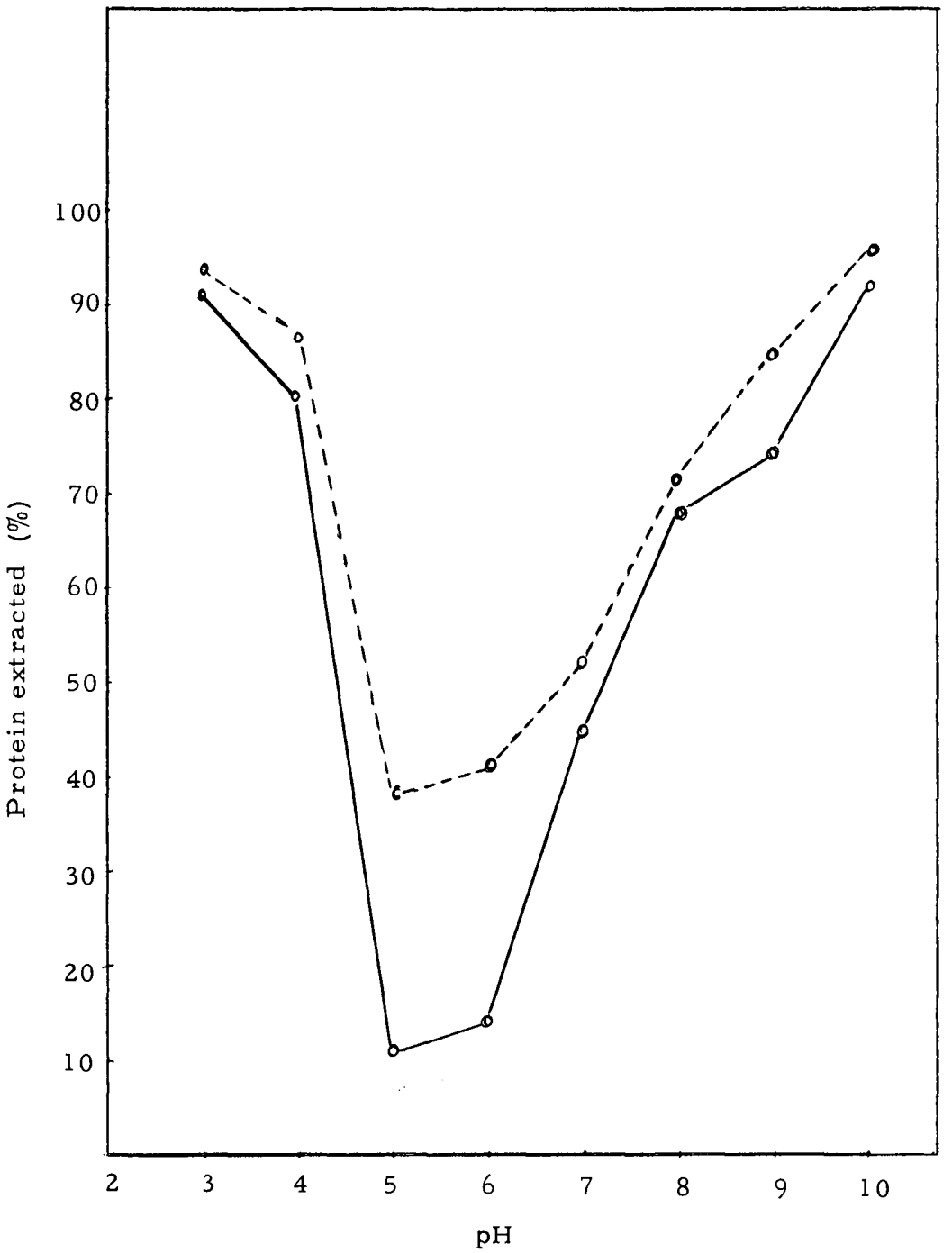


Figure 2. The effect of pH and EDTA on the water extractability of protein from bovine rumen tissue. (——) Without EDTA; (- - -) with EDTA.

pH range in the present study. About 80% of the rumen tissue proteins were extracted at pH 4.0 in the present study as contrasted to 60% extractability reported by Young and Lawrie at the same pH.

The effect of the addition of 0.01 M EDTA to the extracting medium on subsequent protein extractability is noteworthy. Figure 2 shows that the addition of EDTA increased protein solubility over the entire pH range studied, a pH range of 3 to 10. The effect is more pronounced in the range between pH 4.5 to 6.5 which is normally the isoelectric region for muscle proteins. It is most likely that EDTA chelates some of the bivalent metallic ions such as calcium and magnesium, both of which cause solubilized muscle proteins to aggregate in solution (Briskey and Fukazawa, 1971). In preliminary studies it was noted that the extractability of rumen tissue proteins decreased when $MgSO_4$ was added to the extracting medium. Young and Lawrie (1974) reported that $CaCl_2$ caused a marked repression of protein extractability. Thus, removal of such inhibitory factors should result in the improvement of protein solubility and/or extractability.

Figure 3 shows the effect of various concentrations of KCl and NaCl on the extractability of rumen tissue proteins at neutral pH. There was no difference in the solubility data of the proteins extracted at salt concentrations of 0.05 M and 0.5 M irrespective of whether NaCl or KCl was used. However, NaCl was more effective in

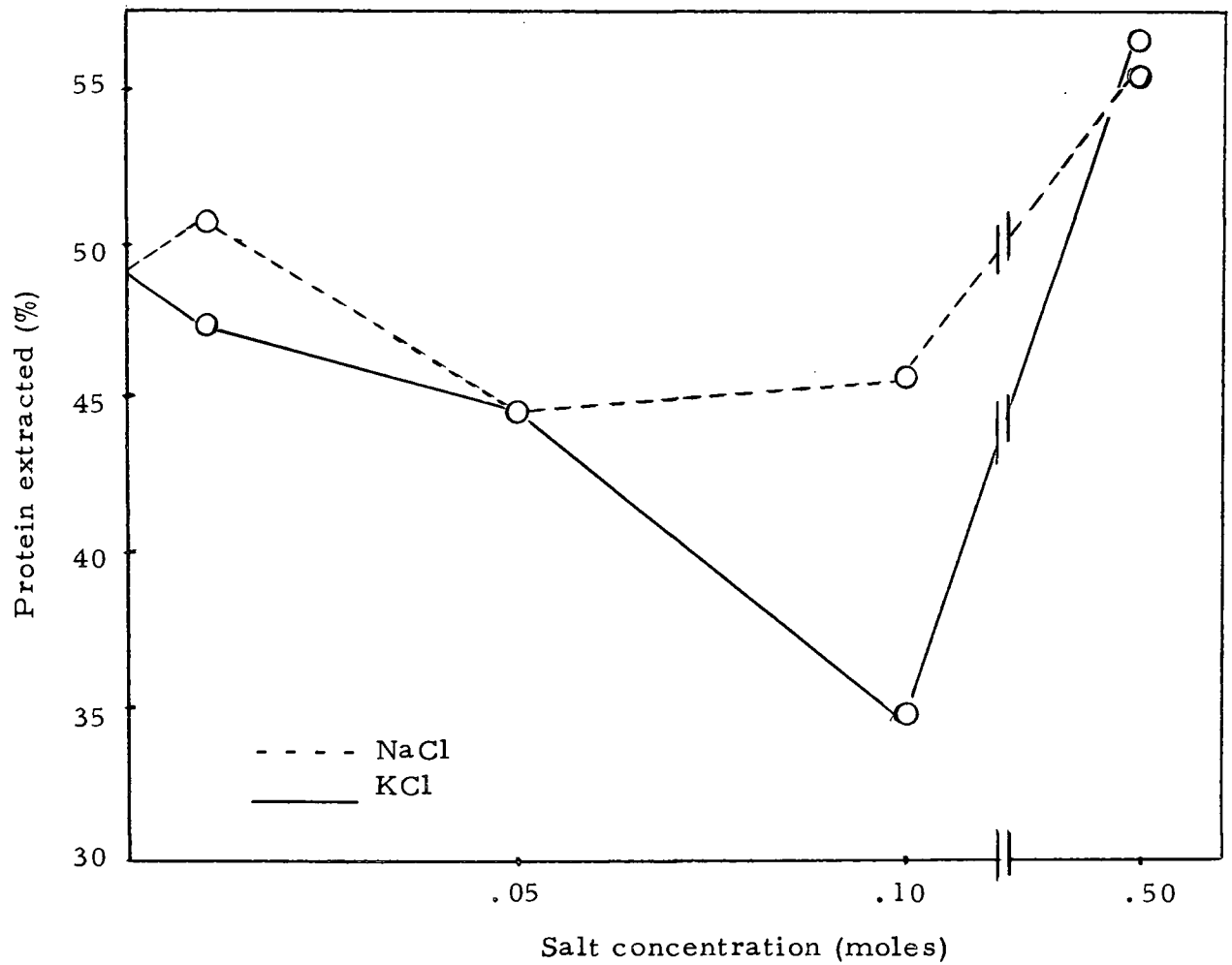


Figure 3. The effect of various salt concentrations on the extractability of rumen tissue proteins. (- - -) With NaCl; (——) with KCl.

solubilizing the rumen tissue proteins at both 0.01 M and 0.1 M concentrations than was KCl. This finding was not expected since KCl is more effective than NaCl in solubilizing skeletal muscle proteins.

It has been documented many times that salt enhances the extractability of contractile proteins of skeletal muscle. It also will be shown later in this study (Table 9) that the same holds true for extracting this type of protein from rumen tissue. However, the addition of salt to the extracting medium does not markedly improve the total extractability of proteins from rumen tissue. About 45% of the total rumen proteins are water extractable at pH 7.0 (Figure 2) as compared to slightly over 50% extractability in 0.5 M NaCl at the same pH (Figure 3). Thus, differential extraction might be useful for preparing protein extracts possessing varying degrees of functionality. For example, the contractile muscle proteins have greater emulsifying capacities and emulsifying stabilization properties than do the sarcoplasmic or connective tissue proteins (Saffle, 1968).

Rumen muscle proteins seem to denature rather readily by heat. Washing the rumen muscle in 70^{o} water for a few minutes decreased the extractability to almost 5 to 10%. It was also interesting to note that a well homogenized rumen muscle mince, readily polymerized into a network of long strands on heating, which

possessed considerable tensile strength.

In developing a new protein source for human consumption, the solubility characteristics determined in a variety of ionic environments provide much useful knowledge. This information not only helps to assess the functional properties of the protein but may also provide an indication about its potential use or non-use in specific food systems. For example, from the data presented in Figure 2 concerning the water extractability of the rumen tissue proteins at pH 10, it can be assumed that about 85% of the protein would be isolated by isoelectric precipitation at pH 5.0. On the other hand, if the same isoelectric precipitation was applied to the extract containing EDTA, about 40% of the protein would remain in solution. Thus, in a commercial isolation process, it would be reasonable to anticipate higher losses of the protein, whenever the extractant contained EDTA. However, if the rumen tissue proteins are to be used in a food system whose pH is below 7.0 (which is the case with most food systems), then according to the solubility curve, (Figure 2) one might deduce that the EDTA extracted proteins would exhibit more functionality than those of the water extract, since more protein is soluble in the former than in the latter.

The shape of the solubility curve also is a source of other information. The solubility curve for the water extract shown in Figure 2 has a point of inflection around pH 8.5. Thus, one might

expect to obtain two distinct isolates from this extract. This was indeed found to be the case. On lowering the pH of the extract, a precipitate began to form at about pH 8.5 and ceased around pH 7.0. On further lowering the pH, another precipitate began to form at pH 6.2 and ended at pH 5.4. Subsequent analysis of these two isolates which are discussed in greater detail later (see Table 9) revealed a distinct compositional difference of proteins in the two isolates.

In order to make a valid assessment of the rumen tissue proteins as a food ingredient, it was deemed necessary to compare the proteins of the rumen tissue with those of skeletal muscle. Such a comparison is justified because much information is available on the skeletal muscle proteins. Furthermore, if applicable, the rumen tissue proteins logically would be expected to be used initially as a partial replacement or substitute for the skeletal muscle proteins in certain processed meat products. To carry out this assessment it was important to determine what proteins were common to both tissues and in what proportions they were found in each. Also, it was important to gain information about some of their similarities and differences in terms of physical and chemical properties. The answers to these questions could only be gained by working with purified proteins. Preliminary studies employing sodium dodecyl sulfate (SDS) polyacrylamide gel electrophoresis (PAGE) indicated that rumen tissue contained the four major contractile proteins

(myosin, actin, tropomyosin and troponin) found in skeletal muscle in fairly high concentrations. This observation was based on the thickness of the electrophoretic bands. Consequently, efforts were directed to the isolation and purification of the contractile proteins from rumen tissue.

Isolation of Myosin

Isolation of rumen muscle was attempted by the methods outlined by Needham and Williams (1963) and Needham and Shoenberg (1964) in which ATP and ultracentrifugation were employed. Although following these procedures resulted in an increase in the amount of myosin isolated as judged by the thickness of the electrophoretic bands, numerous other proteins were also carried along as contaminants. Actin was observed to be one of the most prominant contaminants. Attempts were made to purify the myosin preparations by chromatography on DEAE Sephadex A-50 columns. These attempts were unsuccessful since most of the contaminants were eluted at the same time as myosin.

Needham and Williams (1963) found that the dilution precipitate of 0.5 M KCl extract of a smooth muscle consisted of only about 45% actomyosin and very little or no myosin. It is known that the myosin content of smooth muscle is lower than that of skeletal muscle (Needham and Shoenberg, 1964) and that myosin in muscle

extracts exists mostly as actomyosin. Thus, in isolating myosin from smooth muscle, it was thought to be appropriate to use the dilution precipitate mentioned above as a starting material. Even though less than half of this precipitate was actomyosin, it is generally referred to as the actomyosin precipitate. Hence, when one uses the term "actomyosin" in the case of smooth muscle extracts, it refers to a complex of proteins which includes actomyosin.

SDS gel electrophoresis of the "actomyosin" used in the preparation of myosin gave over 20 bands, and actin was judged to be the most abundant component as inferred by the intensity of the stained protein bands.

Myosin isolation was also attempted by the procedure of Bailin and Barany (1971). Ion exchange chromatography of these preparations on DEAE Sephadex failed to separate the myosin component from the impurities.

The procedure employed by Driska and Hartshorne (1975) for the extraction of highly purified actomyosin from chicken gizzard also failed to yield a homogenous product from rumen tissue. Notable contaminants were troponin and tropomyosin in addition to numerous other unidentified components.

Myosin was successfully purified by the procedure of Yamaguchi et al. (1970) which was modified as described in the

Experimental Procedure. However, the myosin obtained by the $(NH_4)_2SO_4$ fractionation between 45-55% saturation step of this procedure still contained small amounts of actin, tropomyosin and troponin as shown by the SDS PAGE pattern in Figure 4.

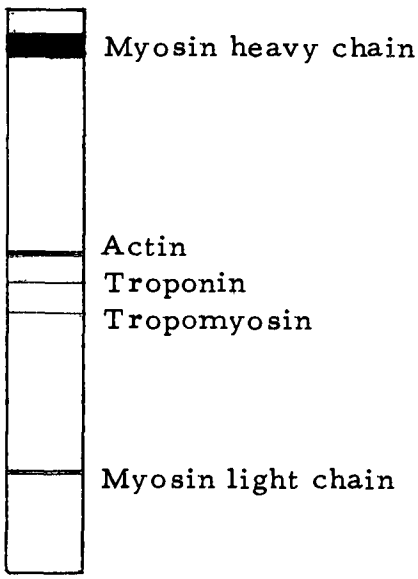


Figure 4. SDS gel electrophoretic pattern of rumen myosin fractionated between 45-55% $(NH_4)_2SO_4$ saturation.

In the presence of ATP, actomyosin is split into actin and myosin accompanied by a simultaneous reduction of viscosity (Weber and Portzehl, 1952; Ebashi, 1961). Thus, the presence of actomyosin in a myosin preparation can be easily detected by viscosity measurements. Such measurements can be carried out rather quickly and are very useful for checking the purity of the myosin preparation.

Viscosity measurements of rumen myosin fractionated at 45-55% $(NH_4)_2SO_4$ saturation showed a lowering of relative viscosity upon the addition of ATP (Table 5).

Table 5. Relative viscosity and ATPase sensitivity of rumen and skeletal myosins.

Type of myosin	Log^{η}_{rel}	Log^{η}_{relATP}	ATPase sensitivity (%)
Rumen myosin	0.253	0.228	9.9
Skeletal myosin	0.257	0.252	2.0

A higher ATP sensitivity value was obtained with rumen myosin than with skeletal myosin. This finding indicated that the rumen myosin obtained by the above mentioned $(NH_4)_2SO_4$ fractionation still contained some actomyosin which was confirmed by electrophoretic analysis.

Purification of Myosin by DEAE Sephadex Chromatography

Ion exchange chromatography of myosin on DEAE Sephadex columns has been shown to remove nucleotides and other minor contaminants from myosin (Richards et al., 1967). In this study, chromatography patterns of rumen and skeletal myosin were similar (Figures 5 and 6). They also showed a close resemblance to those obtained by Richards et al. (1967) for various skeletal

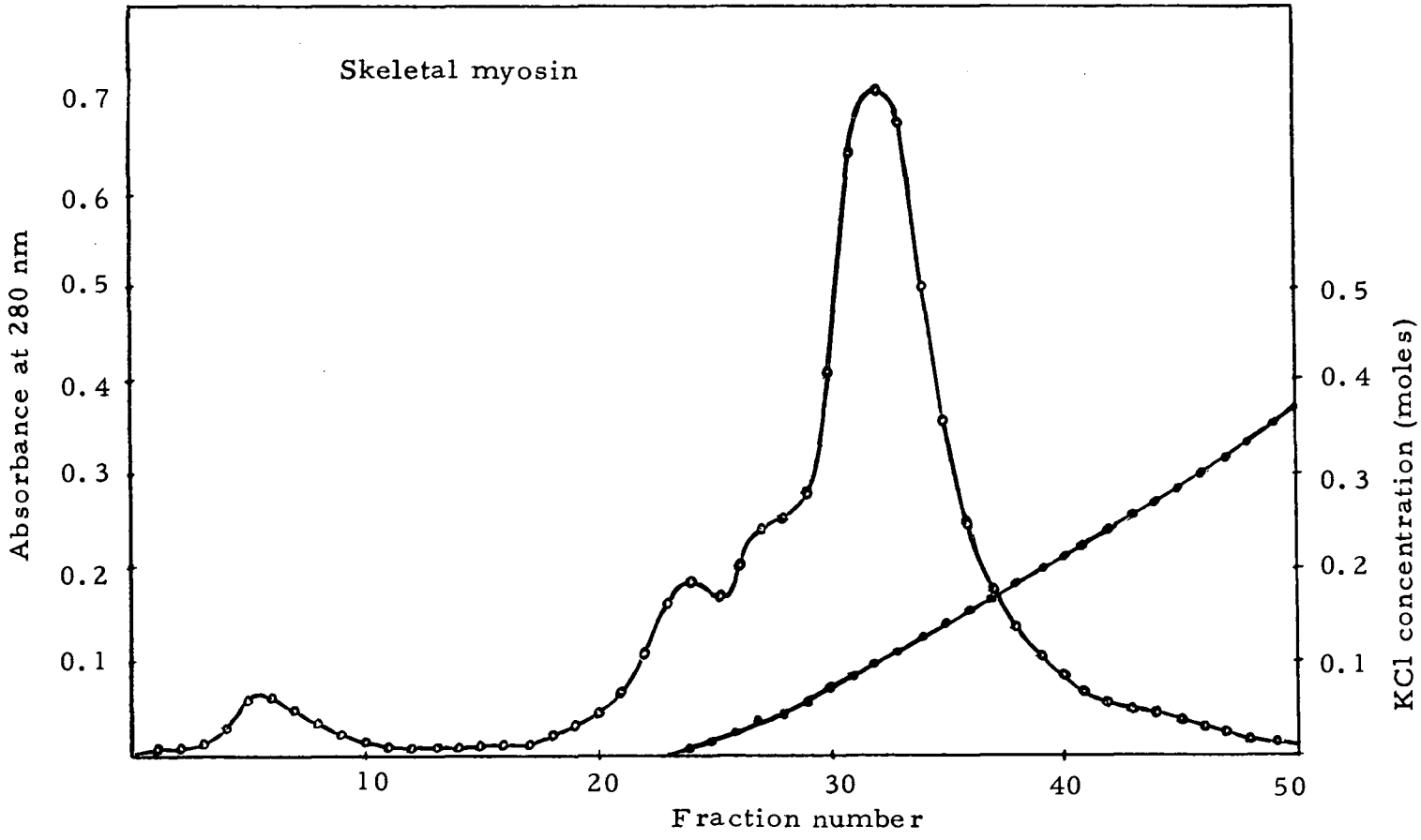


Figure 5. Chromatography of skeletal myosin on DEAE Sephadex A-50. Myosin (20 ml of 10 mg/ml) in 0.04 M $Na_4P_2O_7$, pH 7.5, was applied to a 2.5 x 50 cm column equilibrated with the same buffer. A linear gradient (250 ml 0.04 M $Na_4P_2O_7$, 250 ml 0.5 M KCl in 0.04 M $Na_4P_2O_7$, both at pH 7.5) was applied at 20 ml/hr. Fractions of 10 ml were collected. (o-o) Absorbance at 280 nm; (●-●) KCl concentration in the eluate.

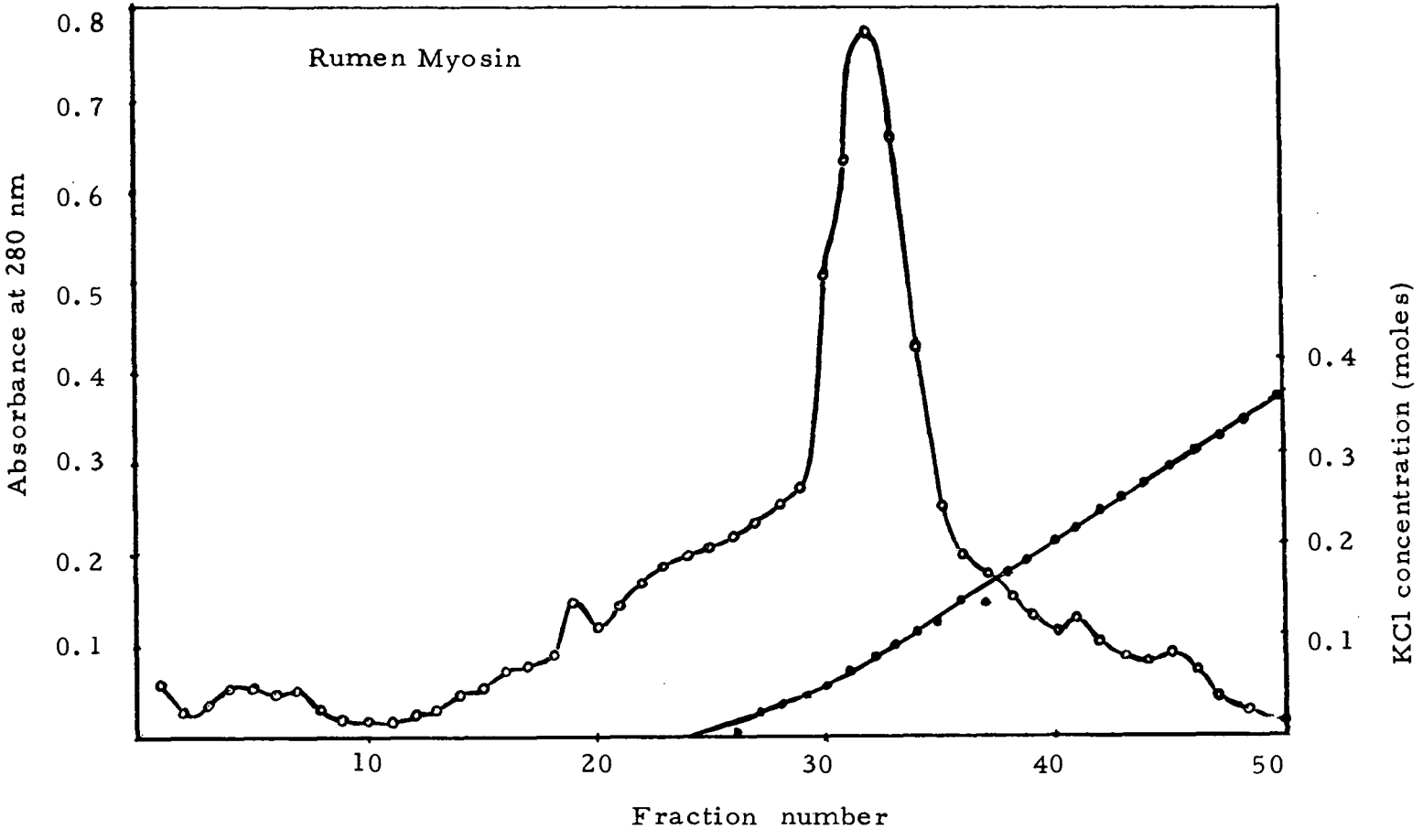


Figure 6. Chromatography of rumen myosin on DEAE Sephadex A-50. Myosin (20 ml of 10 mg/ml) in 0.04 M $Na_4P_2O_7$, pH 7.5, was applied to a 2.5 x 50 cm column equilibrated with the same buffer. A linear gradient (250 ml 0.04 M $Na_4P_2O_7$, 250 ml of 0.5 M KCl in 0.04 M $Na_4P_2O_7$, both at pH 7.5) was applied at 20 ml/hr. Fractions of 10 ml were collected. (o-o) Absorbance at 280 nm; (●-●) KCl concentration in the eluate.

myosin preparations. In the case of rumen myosin a small peak appeared just before the KCl gradient was applied (Figure 6). SDS gel electrophoresis of this peak revealed the presence of small amounts of actin, troponin and tropomyosin. It also probably contained some nucleotides as judged by the lesser values for the ratio A_{280}/A_{260}. This ratio gradually increased as the major peak began to emerge and reached the highest value within the major peak. From Table 6 it was found that the major peak was essentially free of nucleotides and nucleic acids. These impurities began eluting beyond the major peak and the A_{280}/A_{260} ratio continued to decrease.

In both rumen and skeletal myosin preparations, the major peak emerged at a KCl concentration of 0.1 M. SDS gel electrophoresis of this peak showed that it was essentially free of contaminating proteins. In the case of skeletal myosin, the electrophoretograms showed a thick band corresponding to the heavy chains of myosin and two moderately faint bands and a very faint band in the low molecular weight region corresponding to the three light chains of myosin. In the case of rumen myosin one thick band corresponding to the myosin heavy chains and only one faint band corresponding to the light chains were observed (Figure 7). This may indicate that rumen myosin may be structurally different from that of skeletal myosin and also from any other smooth muscle myosin thus far studied (Leger and Focant, 1973). The assumption

Table 6. Estimated nucleic acid contents in the various fractions of rumen and skeletal myosins separated by DEAE Sephadex A-50 chromatography.

Fraction number	Rumen myosin		Skeletal myosin	
	A_{280}/A_{260} (ratio)	Nucleic acid (%)[a]	A_{280}/A_{260} (ratio)	Nucleic acid (%)[a]
5	2.13	0.00	2.00	0.00
10	1.77	0.00	1.92	0.00
15	1.90	0.00	2.00	0.00
20	1.26	1.50	2.00	0.00
24	1.56	0.50	1.80	0.00
28	1.75	0.00	1.54	0.50
29	2.00	0.00	1.56	0.50
30	1.90	0.00	1.75	0.00
31	2.29	0.00	1.76	0.00
32	2.34	0.00	1.77	0.00
33	2.25	0.00	1.76	0.00
34	2.30	0.00	1.70	0.10
36	1.62	0.25	1.66	0.20
40	1.58	0.30	1.54	0.50
45	1.34	1.10	1.23	1.50
50	0.93	4.00	0.85	5.50

[a] Obtained from section IV, Layne (1957).

was that some of these light chains of myosin were not removed during the process of purification. It is unlikely that the purification steps may have removed some of these light chains, because it is well known that their release requires extensive denaturation (Potter, 1974).

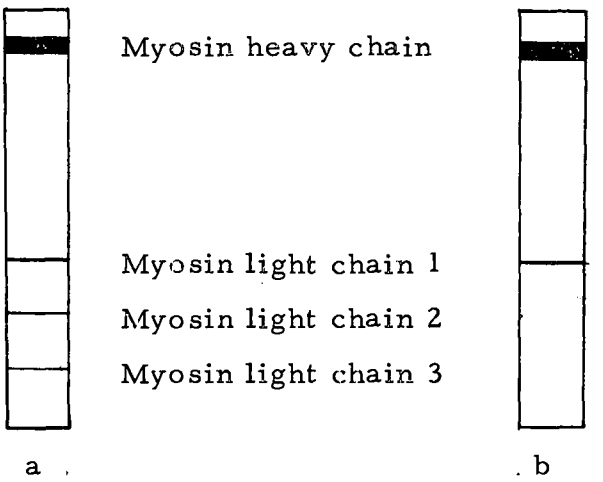


Figure 7. SDS gel electrophoretic patterns of the myosins purified by DEAE Sephadex A-50 chromatography. (a) Skeletal myosin; (b) rumen myosin.

ATPase Activity

The versatility of myosin as a structural protein comes from its ability to function as an enzyme. It derives the energy needed for muscular contraction by the enzymic hydrolysis of ATP. Hence, the ATPase activity of myosin which varies in the presence of various co-factors is an excellent parameter for the partial characterization of myosin. ATPase activity is dependent upon environmental

conditions such as pH, temperature, ionic concentration, etc. (Bogach et al., 1976). For smooth muscle myosin, they found that the maximum ATPase activity was obtained at a pH of 7.6. In the case of skeletal myosin the maximum activity was found to be at a pH of 9.1 (Szent-Gyorgyi, 1951). However, for comparative purposes, the activity of both types of myosin was determined at pH 7.6.

The ATPase assay yielded reproducible values. The specific activity was calculated from the slope of the graph of inorganic phosphate liberated with time. A typical graph is represented in Figure 8. The curves do not pass through the point of origin because the blank did not contain ATP. In the case of the sample, the ATP has undergone a certain amount of hydrolysis other than that due to the enzyme activity.

The ATPase activity of rumen myosin was lower than that of skeletal muscle as would be expected. However, this difference was not as large as has been reported for other smooth muscles (Bogach et al., 1976; Yamaguchi et al., 1970). Table 7 summarizes the ATPase activity of rumen and skeletal myosins.

The specific activities of the ATPase, activated by Mg^{++}, in the presence of 0.05 M KCl were similar, although rumen myosin had a slightly higher activity than skeletal myosin. That activated by Ca^{++} in the presence of 0.05 M KCl resulted in a several fold

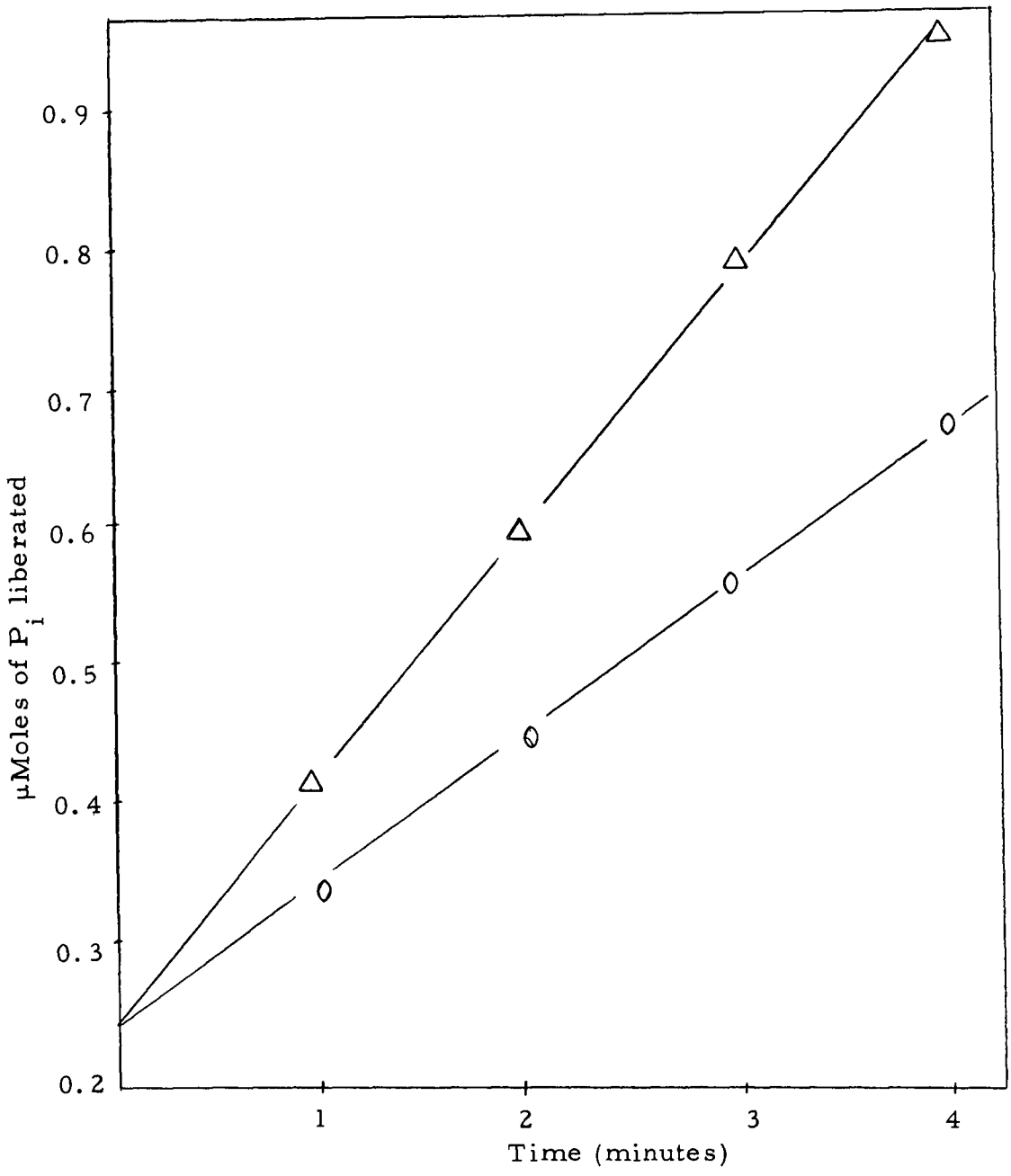


Figure 8. ATPase activity of rumen and skeletal myosins purified by DEAE Sephadex A-50 chromatography. Assay conditions: incubation temperature, 37°; 1.0 ml of myosin in a 10 ml reaction mixture; 2.0 ml aliquots taken at 1 min intervals for determination of inorganic phosphate by Fiske Subbarow method. (Δ -Δ) Skeletal myosin; (o-o) rumen myosin.

increase in the activities, with skeletal myosin having almost twice the activity of rumen myosin. This trend continued for Ca^{++} activation in the presence of 0.5 M KCl and showed the highest activity for either myosin in the case of EDTA activation.

Table 7. ATPase activity of rumen and skeletal myosins.

	ATPase activity as $\mu M\ P_i$/min/mg protein[a]			
	0.5 M KCl		0.05 M KCl	
	EDTA	Ca^{++}	Ca^{++}	Mg^{++}
Rumen myosin	1.570	0.880	0.680	0.051
Skeletal myosin	2.875	1.050	1.310	0.036

[a] ATPase activity was measured in a system containing 5 mM ATP and 30 mM histidine buffer, pH 7.6; Ca^{++}-ATPase in the presence of 5 mM $CaCl_2$ and either 0.5 M or 0.05 M KCl; Mg^{++}-ATPase in the presence of 1 mM $MgCl_2$ and 0.05 M KCl; EDTA-ATPase in the presence of 1 mM EDTA and 0.5 M KCl.

The overall trend in the ATPase activities was similar to that found by Yamaguchi et al. (1970) for horse esophagus myosin. However, the values obtained for rumen myosin ATPase activated by EDTA, Ca^{++} and Mg^{++} were found to be higher than those obtained for horse esophagus. This difference in the ATPase activities may be due to the difference in the physiological activity of these two muscle tissues. Barany (1967) studied the ATPase activities of myosin isolated from 14 different muscles. He concluded that the maximum speed of muscle shortening was proportional to the myosin ATPase activity.

Sodium Dodecyl Sulfate (SDS) Gel Electrophoresis

SDS gel electrophoresis is a valuable technique to evaluate the compositional differences that may exist between extracts of various muscle tissues. Young and Lawrie (1975) used this procedure to compare various protein extracts obtained from the offal tissues of meat industry by-products.

In the present study, actin, troponin and tropomyosin were also isolated and purified, in addition to myosin, from both bovine skeletal and rumen muscles. In each case the SDS gel electrophoretic pattern obtained was used as a criterion of purity of these preparations.

Actin purified from either type of muscle gave a single band. The same was true for the tropomyosin preparations. Troponin prepared according to Greaser and Gergely (1973) gave essentially a single band corresponding to troponin-T. Any other bands corresponding to troponin sub-units as reported by various investigators (Carsten, 1971; Driska and Hartshorne, 1974; Shoenberg and Needham, 1976) were either removed in the purification process or too faint to be detected.

The relative migration (R_m) of these four contractile proteins purified from the rumen muscle were identical to the corresponding

proteins of the skeletal muscle as determined by the SDS gel electrophoretic system employed in the present study. However, R_m values were found to vary considerably if different protein concentrations were applied on the gels. In order to avoid this anomaly, the conditions were standardized so that the samples and standards both contained the same amount of protein in the same volume of buffer.

Molecular Weight Estimation by SDS Gel Electrophoresis

Determining the molecular weight is one way of characterizing a protein. SDS gel electrophoresis affords a convenient and excellent means of estimating molecular weights (Weber and Osborn, 1969; Neville, 1971). This is based on the fact that SDS binds to protein on a gram to gram ratio (Reynolds and Tanford, 1970a, b). Thus, the charge to mass ratio (e/m) of the SDS-protein complexes is the same. Their migration in polyacrylamide gel medium under the influence of an electric field depends on the size and therefore, on the molecular weight of a given protein. Stoklosa and Latz (1975) found that the relative migration of SDS-protein complexes may vary depending on the type of protein and the amount of SDS used. For high molecular weight proteins (i.e., those that have low R_m), the influence of changes in the SDS concentration on R_m was very small or negligible. Conversely, for low molecular weight proteins (i.e.,

those that have high R_m) this effect was more pronounced. They also found that to have consistant and reproducible R_m values, the SDS to protein ratio should be 4.5 or above. The conditions employed for the SDS gel electrophoresis in the present study ensured that the SDS to protein ratios were above the critical value of 4.5.

The time taken for a typical run was normally five to six hours. The bromophenol tracking dye-band at the end of the run was so faint and diffused that it was not possible to calculate reproducible R_m values with respect to this band. Hence, purified skeletal actin was used as a marker protein for each of the standards and the samples. Relative migration was calculated as the ratio of the distance of migration of a given protein from the point of origin to that of the actin marker protein from the same origin. Actin was chosen as a marker, because it was easily identifiable and there were no interfering proteins around it in the gels.

In order to standardize the conditions employed in the gel system, all buffers including the electrode buffer were prepared in bulk. SDS was recrystallized from chloroform and iso-propanol. The acrylamide was also recrystallized from chloroform. They were dried in a vacuum desiccator. Once prepared, the acrylamide stock solution was refrigerated in a dark bottle and was discarded after two weeks. Ammonium persulfate solution which was used as

a catalyst in the polymerization of acrylamide gels was prepared fresh daily.

The standard curve for the estimation of molecular weights is shown in Figure 9. It is noteworthy to discuss the shape of the calibration curve. Deviation from linearity was observed for proteins having molecular weights greater than 70,000. Above this molecular weight the curve tends to bend upward. Similar results were reported by Green and Pastewka (1976) using a continuous buffer system much in the same manner as was reported in this study. In using the discontinuous buffer system with SDS gel electrophoresis, Neville (1971) reported that the standard curve was a shallow sigmoidal type having a linear region in the molecular weight range of 17,000 to 70,000. Hence, it is important to have sufficient standard proteins throughout the whole molecular weight range that one is interested in, to obtain an accurate standard curve. However, one limitation to this is the lack of suitable standard proteins in pure enough form in the higher molecular weight region. The use of purified skeletal myofibrillar proteins in addition to the other proteins in the present study is therefore justifiable.

Table 8 summarizes the molecular weights of various rumen tissue proteins estimated from the caliberation curve with reference to their relative migration (R_m) values.

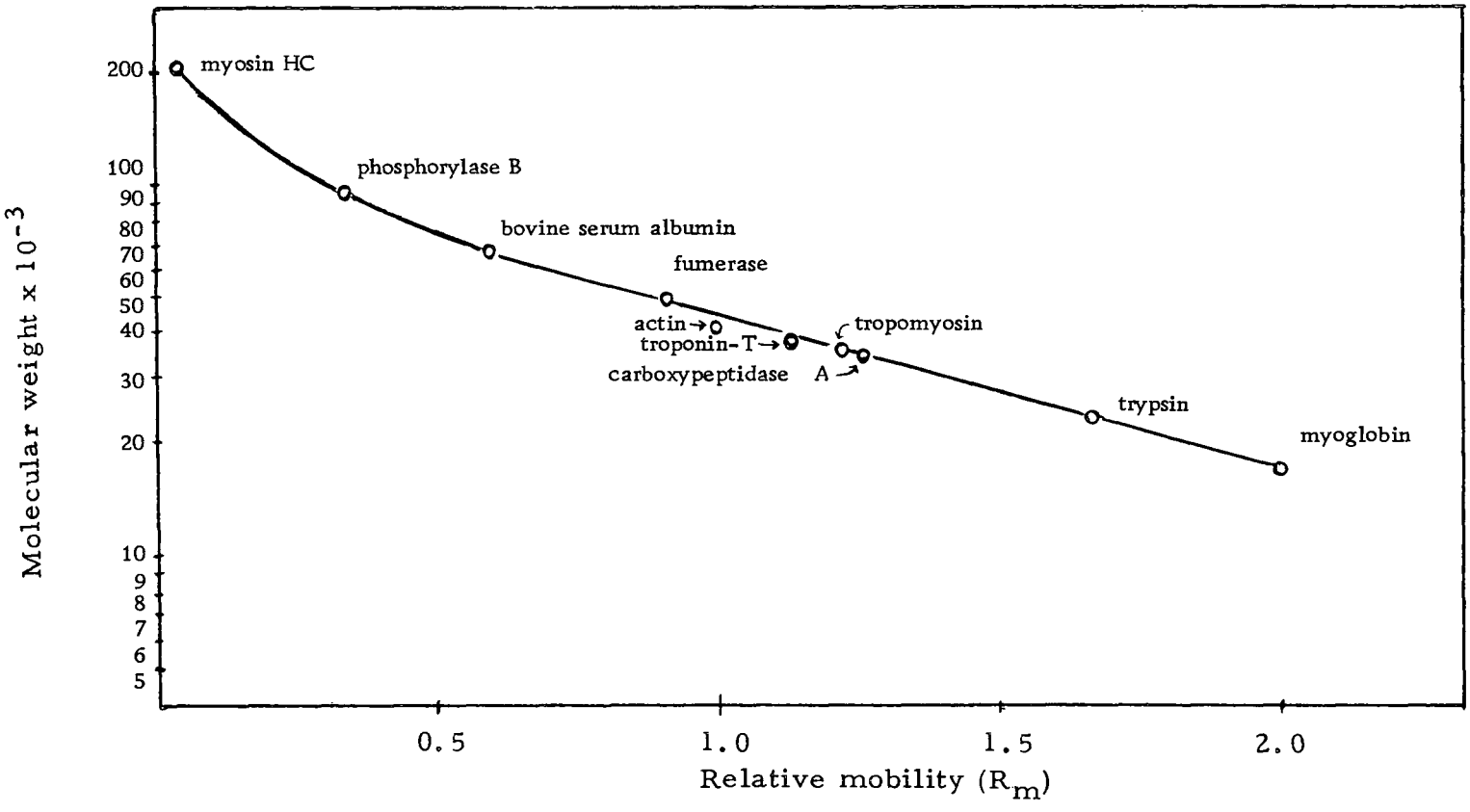


Figure 9. Plot of the logarithm of the molecular weights of standard proteins treated with SDS versus their relative mobilities on 10% polyacrylamide gels. Gel concentration: 10% total, 2.63% cross-linker (10% T, 2.63% C).

Table 8. Relative mobilities (R_m) and estimated molecular weights of some rumen muscle proteins solubilized in the pH 10.0 extract.

Protein	Intensity[a]	R_m	Molecular weight
Myosin heavy chain	++	0.036	210,000
Actin	++++	1.000	45,000
Troponin	++	1.137	39,000
Tropomyosin	++	1.214	36,000
Unidentified protein	++	0.195	130,000
Unidentified protein	++	0.243	115,000
Unidentified protein	+	0.305	102,500
Unidentified protein	++	0.329	96,000
Unidentified protein	+	0.457	80,000
Unidentified protein	++	0.586	67,000
Unidentified protein	++	0.691	60,000
Unidentified protein	++	1.743	21,500

[a] (++++) highest intensity
(++) moderate intensity
(+) low intensity

The extent of the intensity of the dyed protein bands is denoted by (+) signs. However, as will be seen later, each protein has a different dye binding ability and these intensities cannot be taken as an indication of the relative abundance of the respective proteins. The estimated molecular weights in Table 8 were different from those obtained by Young and Lawrie (1975), especially in the higher molecular weight region. The highest molecular weight they obtained for the proteins extracted at pH 10.0 was about 130,000. In the lower molecular weight region the present values were very similar to what they obtained. The reason for this variation may be due to differences in the standard curves. Young and Lawrie (1974, 1975) extrapolated the linear portion of the standard curve to obtain molecular weights in the high molecular weight region. If the standard curve is, indeed, curvilinear as shown in the present study and that of Green and Pastewka (1976), then calculation of molecular weights from a standard curve assumed to be linear over the entire molecular weight region would produce considerably lower values for molecular weights in the higher molecular weight region.

Quantitative Estimation of Proteins by SDS Gel Electrophoresis

The anomalous solubility properties of the contractile proteins of the rumen tissue made it difficult to quantify them by the classical

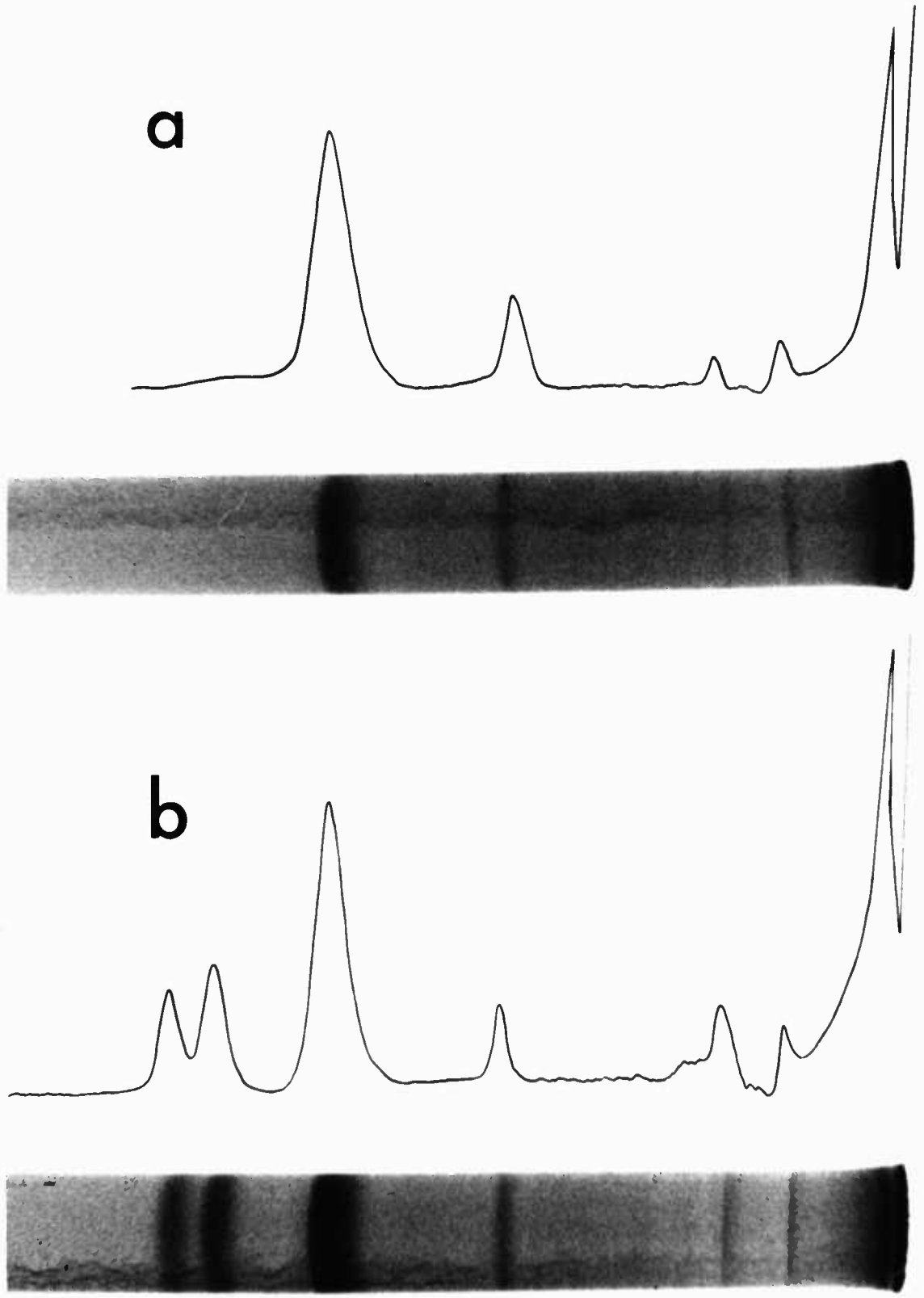
procedures employed for striated muscle. Hence, it was decided to use quantitative electrophoretic and densitometric methods to quantify the various proteins. Quantitative estimation of proteins by electrophoretic separation coupled with densitometric scanning is nothing new. Fazehar de St. Groth in 1963 used a similar method to estimate various proteins on paper electrophoretic strips. More recently, Lowy and Risby (1971), Tregear and Squire (1973) and Potter (1974) have successfully used this method to estimate myofibrillar proteins in various tissues ranging from insect muscle to rabbit muscle.

To determine the weight percentages of the myofibrillar proteins in the various extracts and isolates from rumen muscle, the proteins were solubilized in SDS and subjected to gel electrophoresis. Initial attempts to resolve the proteins by the gel system employed by Laemmli (1970) as modified by Potter (1974) were not successful. Abnormal electrophoretic patterns devoid of any bands in the upper region of the gel often resulted, even though the other bands were sharply focused. Such anomalous electrophoretic patterns were also reported by Richter-Landsberg et al. (1974) in the discontinuous gel electrophoresis of membrane proteins.

In the current study, the modified Weber-Osborn (1969) gel system consistently yielded reproducible results. However, electrophoresis over a period of five to six hours resulted in an

increase in curvature of the protein bands. Thus, electrophoresis was not conducted beyond this critical time. The electrophoretic conditions were similar to those employed for molecular weight estimations. In order to minimize the variability of various parameters from one run to another, all gels were prepared from the same stock solution as was the case for all buffers used. The time of electrophoresis was fixed at 5 hours at a current of 5 mA per gel. Staining was for exactly 10 hours. The most crucial step was believed to be the destaining step. If destaining was not uniform, it would affect the intensity of the stained protein bands which in turn would affect the peak areas in the densitometric scanning. In order to minimize this effect, destaining was done electrophoretically for exactly 45 minutes at a current of 3 amperes. The gels were then placed in test tubes containing destaining solvent and allowed to destain for ten days by diffusion. At the end of this period the gels were taken for densitometric scanning. It was assumed that this procedure would minimize any differential destaining of the protein bands, or at least the effect would be the same from one batch to another and that the peak areas corresponding to stained protein bands could be compared with one another. Figure 10 represents the electrophoretic patterns and densitometric scans of two preparations. The dark band at the origin is an artifact produced during photography. The original gels did not show any such band.

Figure 10. SDS gel electrophoretic patterns and densitometric scans of (a) isoelectric precipitate of the pH 10.0 extract obtained by adjusting the pH to 7.0, and (b) the second isoelectric precipitate obtained by adjusting the pH of the supernatant from the previous precipitation to 5.4.


a
b

The staining intensities of myosin heavy chain, actin, tropin and tropomyosin are shown in Figure 11. The staining intensity is expressed in terms of the peak areas obtained. It can be seen that the peak area for each of the proteins increased linearly with increase in the amount of the purified proteins applied over the protein range studied. The staining intensities of troponin and tropomyosin were about the same. The myosin heavy chain (HC) stained the most, while the actin staining intensity was intermediate between that of myosin and troponin or tropomyosin.

Figure 12 illustrates the plot of the peak areas of myosin-HC, actin, troponin and tropomyosin calculated from the densitometric scans versus the total microgram of proteins of the water extract, pH 7.0, applied on the gels. Each point is the mean of four readings. The linearity of the curves over the range of proteins applied shows that Beer's law holds. Hence, the amount of each of the proteins at a given total protein concentration can be calculated from the standard curve. The adherence of Beer's law for myosin-HC and actin over a wide range of total applied proteins for insect flight muscle, myosin heavy and light chains, and guinea pig taenia coli were confirmed by Bullard and Reedy (1972), Lowy and Risby (1971), and Tregear and Squire (1973), respectively. Similar plots were made for 3% NaCl extract (3% NaCl extract 1) (Figure 13), 3% NaCl extract of the residue from the first extraction (3% NaCl extract 2)

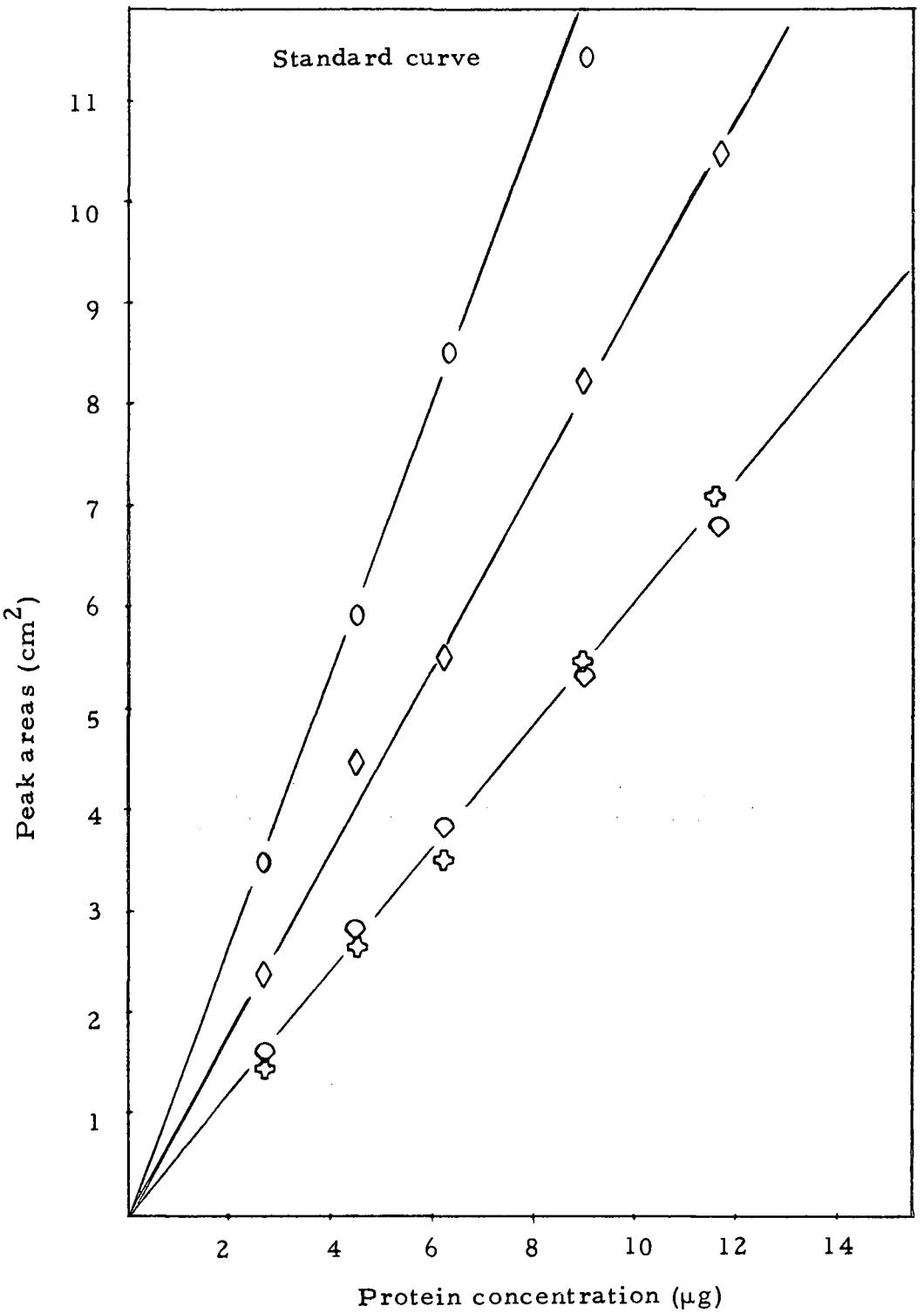


Figure 11. Dependence of densitometric peak areas on the concentration of purified proteins applied. (O-O) Myosin HC; (◊-◊) actin; (○-○) troponin-T; (✧-✧) tropomyosin.

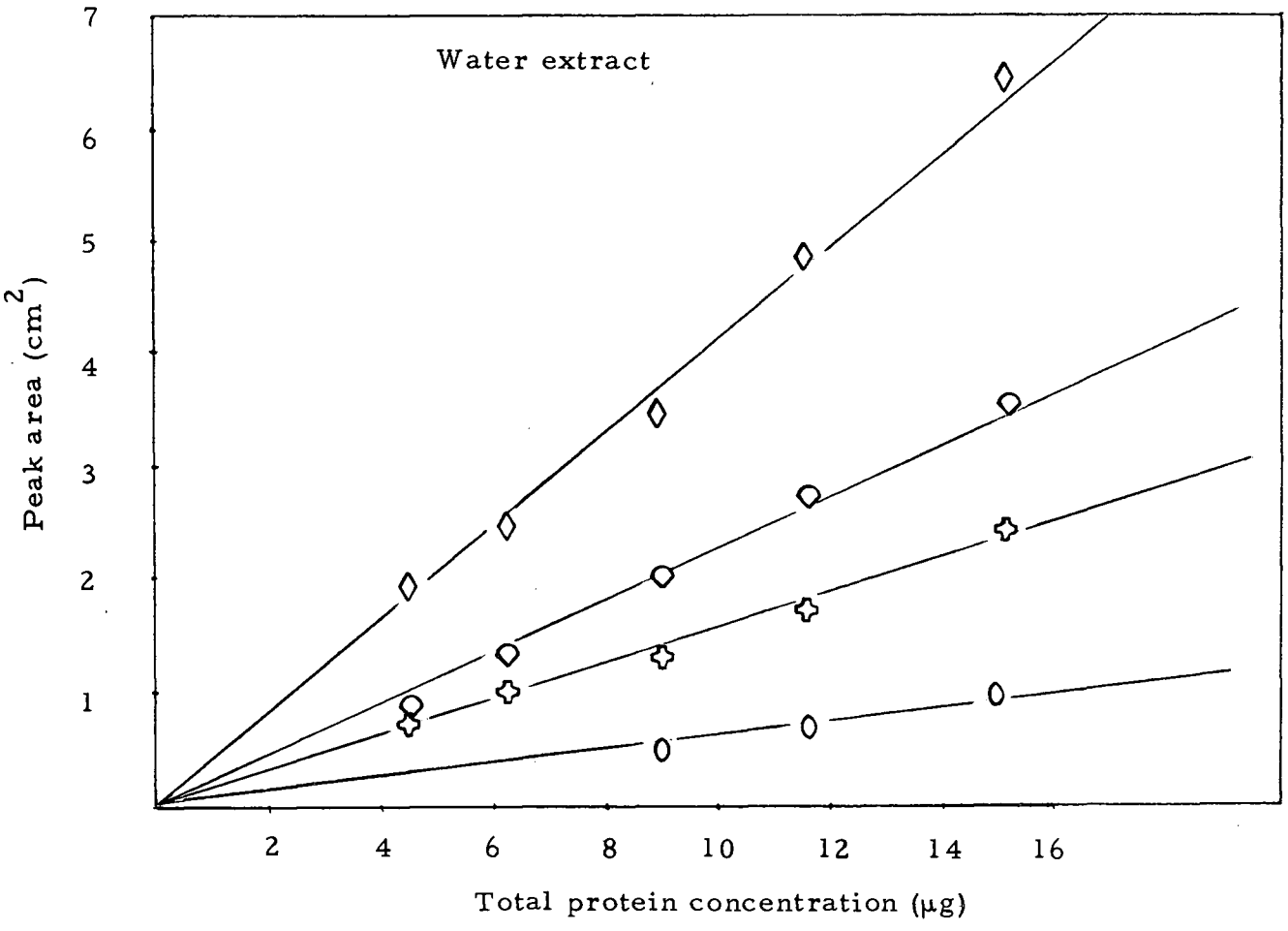


Figure 12. Dependence of densitometric peak areas on total protein concentration of the water extract, pH 7.0. The peak areas for each protein are plotted as a function of the total applied proteins of the water extract. (O-O) Myosin HC; (◇-◇) actin; (○-○) troponin-T; (✩-✩) tropomyosin.

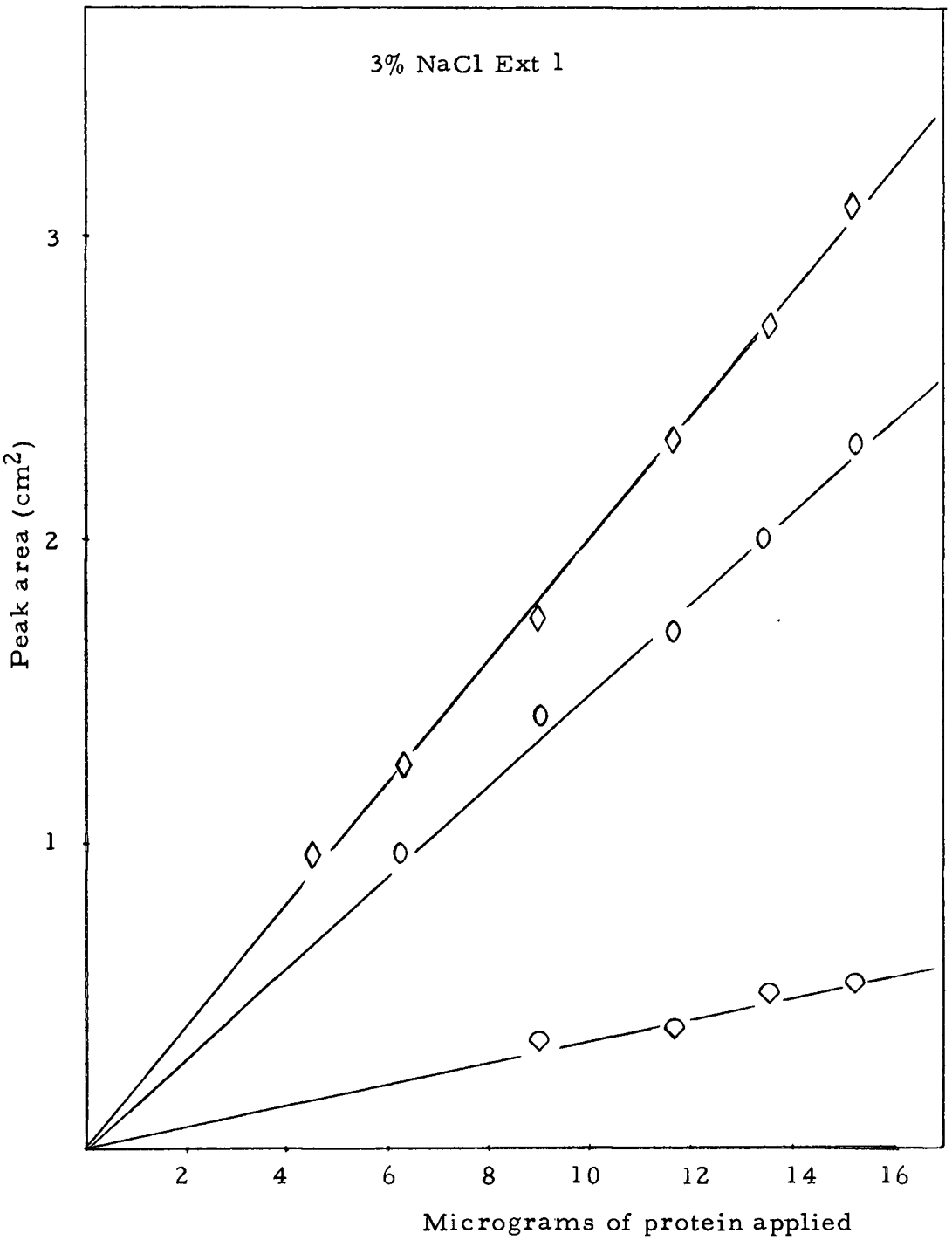


Figure 13. Dependence of densitometric peak areas on total protein concentration of the 3% NaCl extract 1. The peak areas for each protein are plotted as a function of the total applied proteins of the 3% NaCl extract 1. (◊-◊) myosin HC; (◊-◊) actin; (○-○) troponin-T.

(Figure 14), pH 10.0 extract (Figure 15), isoelectric precipitate of the pH 10.0 extract adjusted to pH 7.0 (IEP at pH 7.0) (Figure 16), and second isoelectric precipitate at pH 5.4 (IEP at pH 5.4) (Figure 17).

Table 9 summarizes the peak areas and the weight percentages of myosin, actin, troponin and tropomyosin in each of the extracts and isolates mentioned above.

All the major contractile proteins of the rumen tissue were soluble in water at neutral pH to varying degrees. Actin was the most soluble. This phenomenon is strikingly different in skeletal muscle because the contractile proteins are solubilized only in salt solution at neutral pH (Briskey and Fukazawa, 1971; Hamoir, 1973). This peculiar solubility of the contractile proteins may reflect upon the nature of the ultrastructure of rumen muscle. It might be possible that at least part of the contractile proteins in the rumen muscle cell exist as disaggregated molecular entities which could account for their solubility. Shoenberg et al. (1966) showed that the vertebrate smooth muscle myosin may exist as a disaggregated, colloidally dispersed phase.

It also can be seen from Table 9 that extracting the rumen muscle with 3% NaCl increased the extractability of myosin while decreasing that of actin. It is possible that the globular actin that is extracted so readily with water is polymerized in the presence of

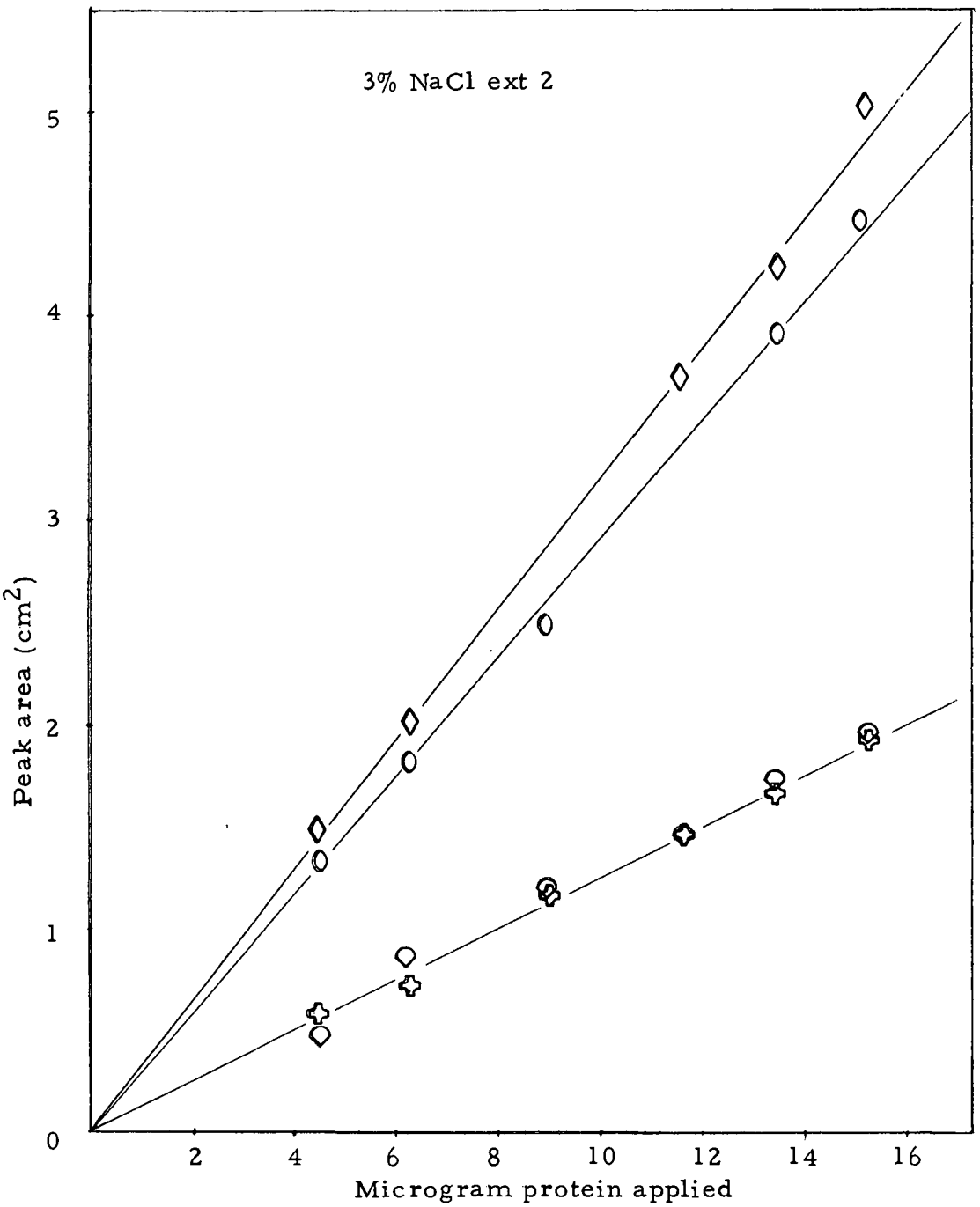


Figure 14. Dependence of densitometric peak areas on the protein concentration of the 3% NaCl extract 2. The peak areas for each of the proteins are plotted as a function of total applied proteins of 3% NaCl extract 2. (O-O) Myosin HC; (◇-◇) actin; (○-○) troponin-T; (✣-✣) tropomyosin.

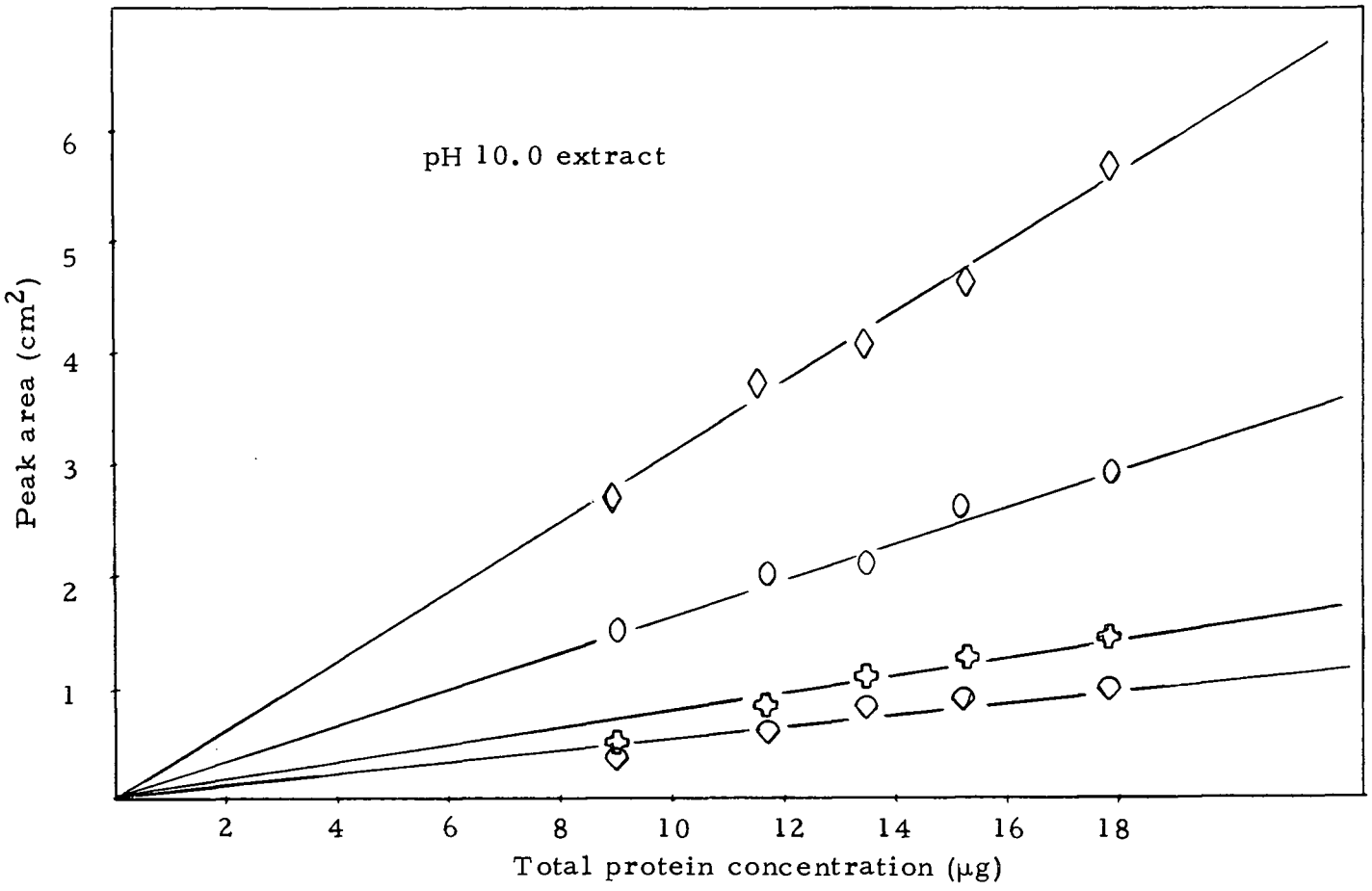


Figure 15. Dependence of densitometric peak areas on the protein concentration of the pH 10.0 extract. The peak areas for each of the proteins are plotted as a function of total applied proteins of the pH 10.0 extract. (○-○) Myosin HC; (◇-◇) actin; (○-○) troponin-T, (✣-✣) tropomyosin.

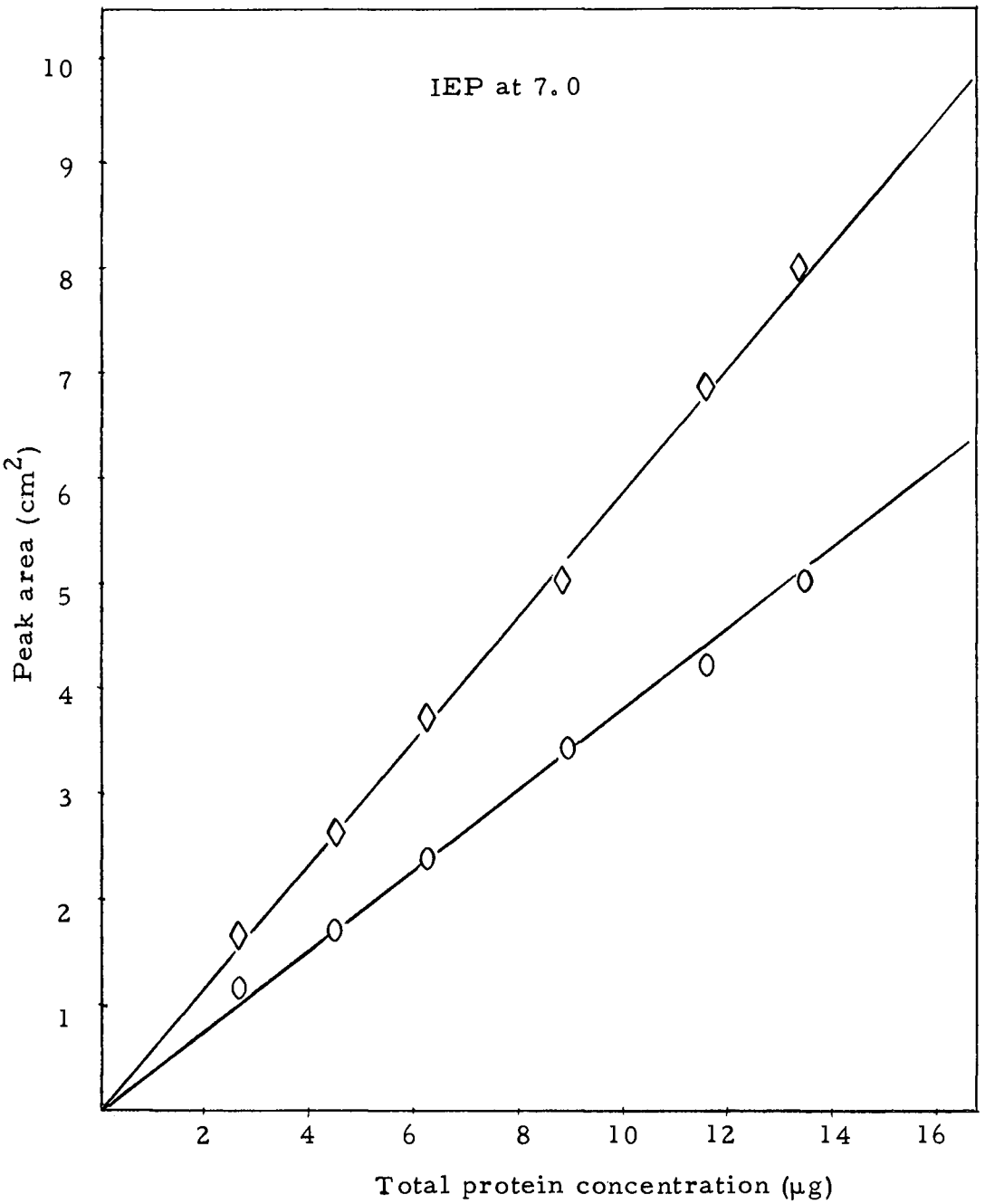


Figure 16. Dependence of densitometric peak areas on the protein concentration of the isoelectric precipitate at pH 7.0. The peak areas for each of the contractile proteins are plotted as a function of the total applied proteins of the IEP at pH 7.0. (◊-◊) Myosin HC; (◊-◊) actin.

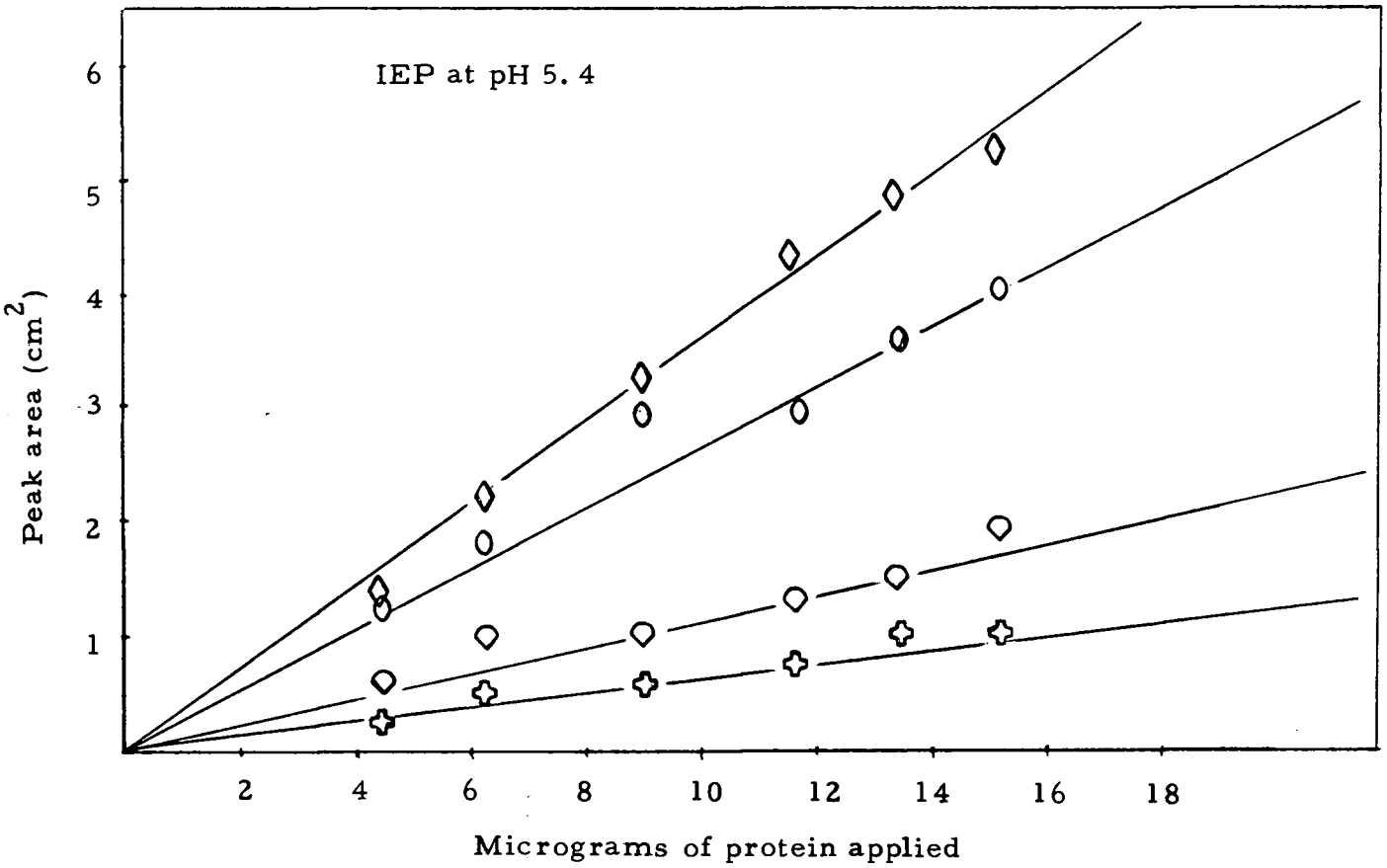


Figure 17. Dependence of densitometric peak areas on the protein concentration of the isoelectric precipitate at pH 5.4. The peak areas for each of the contractile proteins are plotted as a function of the total applied proteins of the IEP at 5.4. (○-○) Myosin HC; (◇-◇) actin; (○-○) troponin-T; (✧-✧) tropomyosin.

Table 9. Peak areas and weight percentages of myosin, actin, troponin and tropomyosin in various extracts and isolates of rumen tissue.

	H_2O extract			3% NaCl extr. 1			3% NaCl extr. 2			pH 10.0 extr.			IEP at pH 7.0			IEP at pH 5.4		
	area (cm^2)	wt (μg)	(%)	area (cm^2)	wt (μg)	(%)	area (cm^2)	wt (μg)	(%)	area (cm^2)	wt (μg)	(%)	area (cm^2)	wt (μg)	(%)	area (cm^2)	wt (μg)	(%)
Myosin	0.65	0.50	5.0	1.50	1.15	11.5	2.92	2.18	21.8	1.65	1.20	12.0	3.80	2.80	28.0	2.65	2.00	20.0
Actin	4.05	4.50	45.0	2.00	2.25	22.5	3.20	3.60	36.0	3.10	3.50	35.0	5.80	6.45	64.5	3.40	4.00	40.0
Troponin	2.15	2.20	22.0	0.35	0.60	6.0	1.25	2.10	21.0	0.55	0.95	9.5	---	---	---	1.15	1.90	19.0
Tropomyosin	1.40	2.00	20.0	---	---	---	1.25	2.10	21.0	0.80	1.30	13.0	---	---	---	0.65	1.10	11.0

NaCl, thus decreasing its original solubility. The second NaCl extraction sobilized almost twice more myosin, one and a half times more actin and three times more troponin than the first NaCl extraction.

It was interesting to note that the first isoelectric precipitate at pH 7.0 of the pH 10.0 extract did not contain any troponin or tropomyosin (refer to Table 9, Figures 10 and 16). Similar results were obtained when the pH was lowered to 6.5. Below this pH however, both troponin and tropomyosin began to precipitate along with myosin, actin and a host of other unidentified proteins. The precipitation of the four major contractile proteins was complete when the pH reached 5.4, below which no further precipitation was observed.

SDS gel electrophoresis of the supernatant obtained after the last isoelectric precipitation at pH 5.4 showed a strong band corresponding to a molecular weight of 67,000 in addition to several other minor bands. It was noted that there were no electrophoretic bands corresponding to the contractile proteins in the supernatant. This indicates that the precipitation of the contractile proteins was probably complete at pH 5.4.

Figure 18 compares the SDS gel electrophoretic patterns of IEP at pH 5.4 and the supernatent obtained from this precipitation. From the relative migration data of the prominent band in Table 8,

it was suspected this band to be a serum albumin.

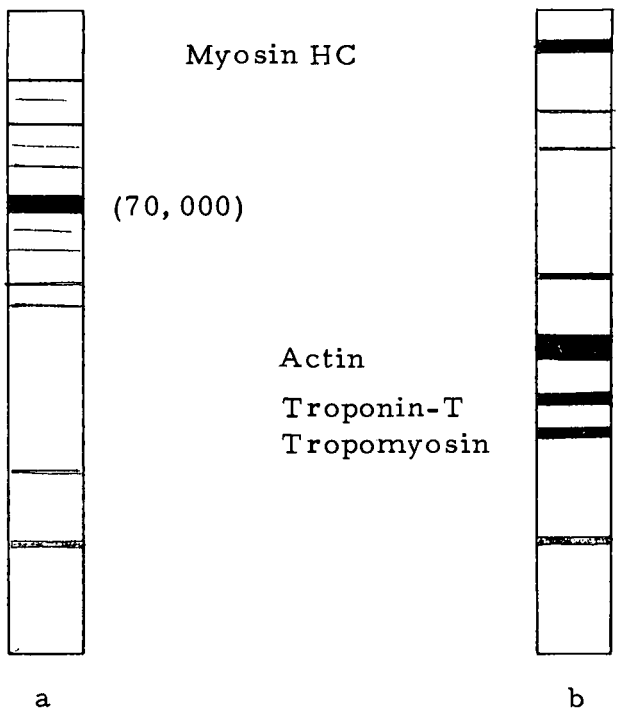


Figure 18. SDS gel electrophoretic patterns of the supernatant from (a) the second isoelectric precipitation at pH 5.4, and (b) the isoelectric precipitate at pH 5.4.

Referring to the solubility data presented in Figure 2, it can be seen that a pH 10.0 about 92% of the total protein present in the rumen tissue is solubilized. It appears safe to assume that under these conditions the insoluble connective tissue proteins such as collagen, elastin, etc., probably account for the remaining 8% of the total protein. Thus recalculating the percentage of contractile proteins for the pH 10.0 extract in Table 9, on the basis of 92% protein extraction, the percentages of these proteins in the intact

rumen muscle can be estimated. Table 10 summarizes the percentages of various contractile proteins calculated as shown above for rumen muscle and those values reported in literature for skeletal muscle.

Table 10. Comparison of percentages of various proteins in fresh bovine rumen and rabbit skeletal muscle.

	Rabbit muscle (%)	Bovine rumen muscle (%)
Sarcoplasmic proteins	34 (a)	28
Myosin	34 (a)	11
Actin	14 (b)	32
X-Protein (troponin and tropomyosin)	7 (a)	21
Stroma protein (residue)	11 (a,b)	8

(a) Values obtained by Hasselbach and Schnider, reported by Weber and Portzehl (1952).

(b) Values obtained by Huxley and Hanson (1957).

Amino Acid Analysis

Table 11 summarizes the amino acid analysis of myosin and actin purified from rumen and skeletal muscles. The amino acid composition of rumen and skeletal myosins resemble to each other closely. Some of the notable differences were the lower amounts of lysine and histidine in rumen myosin. Also, the ratio of basic

Table 11. Amino acid composition of bovine skeletal and rumen muscle myosin and actin expressed as residues per 10^5 gm protein.

Amino acid	Bovine skeletal myosin	Bovine rumen myosin	Chicken gizzard myosin[a]	Bovine skeletal actin	Bovine rumen actin	Bovine carotid actin[b]
Lys	93.7	83.0	88.0	68.3	82.2	45.1
His	17.9	15.4	14.0	22.5	12.6	18.2
Arg	50.1	49.9	48.0	44.5	49.5	42.8
Asp	99.1	85.0	89.0	91.4	90.9	78.3
Thr	45.2	45.0	44.0	53.9	34.5	56.3
Ser	42.0	43.3	47.0	43.2	41.0	56.8
Glu	186.7	189.9	166.0	110.5	202.4	99.9
Pro	31.1	35.9	25.0	50.8	20.8	43.8
Gly	44.4	64.4	48.0	70.4	41.8	66.3
Ala	89.9	88.9	76.0	82.5	97.9	69.6
Val	43.0	49.1	41.0	58.9	44.9	43.6
Met	15.3	25.1	20.0	21.3	23.7	31.7
Ileu	45.3	37.3	36.0	67.0	31.9	66.5
Leu	89.5	70.7	87.0	77.3	79.3	64.6
Tyr	11.2	24.4	16.0	21.2	15.3	33.4
Phe	47.3	31.2	27.0	36.1	18.6	27.4

a
Barany et al. (1966)

b
Carsten (1963)

amino acids to acidic amino acids in rumen myosin was found to be slightly lower than that of the skeletal myosin. Thus, rumen myosin should have a higher rate of electrophoretic migration than the skeletal myosin. In these properties rumen myosin resemble other smooth myosins studied. Another notable difference was the higher content of methionine in rumen myosin than in skeletal myosin. Similar results were noted for actin of both muscle types. Young and Lawrie (1975) reported an overall higher methionine content for rumen protein extracts than for skeletal extracts.

The amino acid composition of rumen actin seems to differ rather significantly from that of skeletal actin as well as from other smooth muscle actins reported in literature. This difference cannot be explained on the basis of present knowledge. On SDS gel electrophoresis, both skeletal and rumen actins showed identical relative migrations and they consistently gave essentially a single band even at high protein concentrations.

Emulsifying Capacity and Emulsion Stability

The values for the emulsifying capacity (EC) for the various extracts and isolates are given in Table 12. The EC of the water extract, pH 7.0, was the least. It has been shown that the water-soluble proteins of skeletal muscle have a lower emulsifying capacity than the salt-soluble proteins (Carpenter and Saffle, 1965). The

spherical shape of the water-soluble proteins as opposed to the fibrous, elongated nature of the salt-soluble proteins has been proposed to be the reason for this difference in the emulsifying capacity (Saffle, 1968). The EC of the rumen proteins was generally lower than those of skeletal muscle. With the exception of the second NaCl extract, all others gave similar values for EC. This means that in the case of rumen tissue there is no obvious distinction between the water-soluble proteins and the salt-soluble proteins insofar as the emulsifying capacity is concerned. In a way such a conclusion could be justified from the results obtained by gel electrophoresis earlier. It was found that the water extract, pH 7.0, also contained a large amount of contractile proteins. Emulsifying capacities of purified muscle proteins were determined by Tsai et al. (1972) and Neelakantan and Froning (1971). Even though there are some discrepancies in their findings, probably due to the differences in the procedures employed, they found myosin to have the highest EC, closely followed by actin. Sarcoplasmic proteins gave the lowest EC at neutral pH.

The emulsion stability data are shown in Table 13. It is shown that the emulsion stability of all preparations were similar except that of cheek meat which was lower than the others. Visual observations of the consistency of the emulsions are also recorded in the table. Cheek meat emulsions were very thin while the protein

Table 12. Emulsifying capacity of various rumen tissue extracts and isolates.

Protein preparation	Emulsion capacity[a] (g oil/100 mg protein)
Water extract, pH 7.0	57.2 ± 1.7
3% NaCl extract 1	60.1 ± 2.3
3% NaCl extract 2	80.2 ± 0.2
IEP at pH 7.0	64.2 ± 1.1
IEP at pH 5.4	60.0 ± 0.1
Cheek meat (bovine)	93.8 ± 1.8
Skeletal meat (bovine)	97.5 ± 0.7

[a] Mean of three replications ± standard deviation.

Table 13. Emulsion stability of various rumen tissue extracts and isolates.

Extract	Stability rating[a]	Consistency[b]
Water extract, pH 7.0	94.5	Thin
3% NaCl extract 1	94.4	Moderately thick
3% NaCl extract 2	94.3	Thick
IEP at pH 7.0	97.8	Very thick
IEP at pH 5.4	97.9	Very thick
Cheek meat (bovine)	92.9	Thin
Skeletal meat (bovine)	98.7	Very thick

[a] Stability rating of 100 refers to a perfectly stable emulsion.

[b] Visual observation.

isolates obtained at pH 7.0 and 5.4 gave very thick emulsions. It may be that these two isolates and the second NaCl extract had a greater percentage of actin and myosin (refer to Table 9), which are known to give thick emulsions (Neelakantan and Froning, 1971).

Foaming Ability

The foam over-run which is a measure of the foaming ability, with an increase in protein concentrations, for the various extracts and isolates is graphically presented in Figure 19. In the protein range studied, most of the isolates and extracts behaved similarly. Below a protein concentration of 2 mg/ml, the water extract, pH 7.0, gave the highest value for foam over-run. An effective foam could not be formed from the other preparations below a 2 mg/ml protein level. At higher concentrations the pH 10.0 extract gave the highest value for foam over-run. The values obtained for egg albumen were the lowest for all of the protein concentrations studied. This is difficult to explain. However, at higher levels of protein, egg albumen usually forms excellent foams. But under the conditions of this experiment, rumen tissue proteins had better foaming properties than egg albumen in the range of protein concentrations studied.

One interesting observation is that the three protein extracts, namely, the water extract, pH 7.0, the 3% NaCl extract and the pH

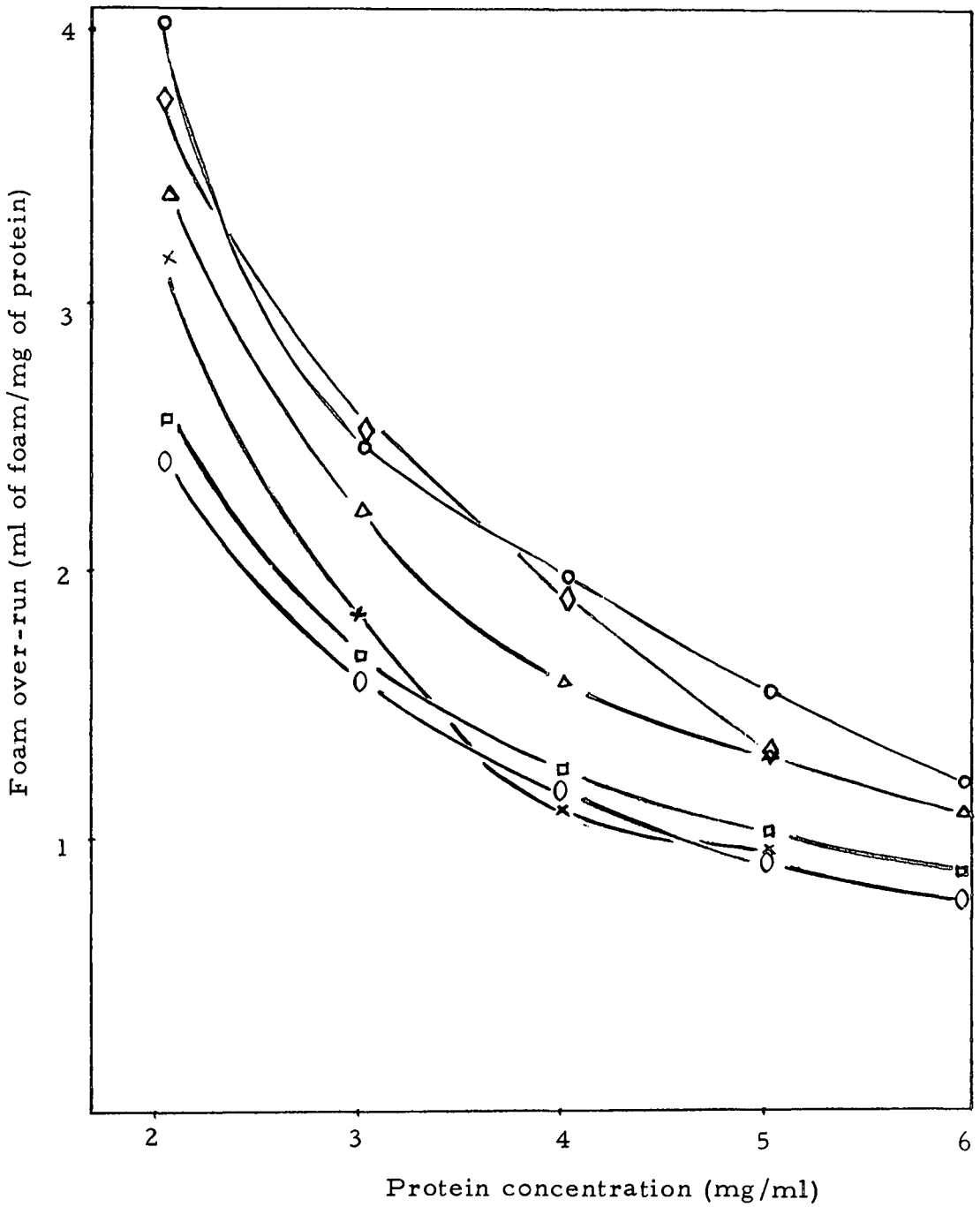


Figure 19. Variation of foam over-run with protein concentration. (Δ-Δ) 3% NaCl extrac; (o-o) pH 10.0 extract; (□-□) IEP at 7.0; (x-x) IEP at 5.4; (◇-◇) water extra ct, pH 7.0; (0-0) egg albumen.

10.0 extract had higher foam over-run values than the isolates obtained at pH 7.0 and 5.4. Referring to Table 9, it can be seen that these extracts have a lower content of myofibrillar proteins than the isolates obtained at pH 7.0 and 5.4. Thus, it can be concluded that non-myofibrillar proteins are the major contributors to the foaming ability of a protein preparation. The water soluble proteins, albumins and globulins, of cereal flours also exhibit considerable foaming ability (Pomeranz and Shellenberger, 1971).

Data concerning foam stability as measured by the volume of liquid drained from 100 ml of foam at various time intervals are shown in Table 14. The protein content of each of the preparations was 5 mg/ml. These stability data were very similar from one protein to another. The proteins obtained from rumen muscle seems to have slightly better foam stability than that of egg albumen.

These studies indicate rumen tissue proteins have very good functional properties. However, because of the unavailability of more definitive methods for measuring functionality, it is difficult to compare these values with those obtained for other proteins (Kinsella, 1976). Therefore, the final test would be to use the rumen proteins in an actual food systems to determine how these functionalities are affected in a multicomponent system.

Table 14. Foam stability expressed as mililiters of liquid collected from 100 ml of foam[a, b].

Protein preparation	Time		
	30 min	60 min	90 min
Water extract, pH 7.0	18.0	21.0	21.8
3% NaCl extract 1	17.5	19.0	19.8
pH 10.0 extract	15.1	16.3	17.0
IEP at pH 7.0	14.0	17.8	18.8
IEP at pH 5.4	10.7	16.2	18.0
Egg albumen	24.0	26.9	27.5

[a] Protein concentration used was 5 mg/ml.

[b] Higher the volume of liquid collected lower the stability of the foam.

One of the most important aspects that has to be resolved before rumen tissue protein can be utilized in the food industry is adverse odor. Odor plays an important part in the consumer acceptance of a food product. Hence, it is important to identify these odor causing factors and develop a means of eliminating or minimizing them.

Another aspect that needs investigation is the collection of these proteins with a minimum of microbial contamination. Since rumen tissue is difficult to be removed without some contamination with the rumen contents, the microbial load of the tissue may be

quite high. Thus, it would be important to learn more about the microbial flora found in the tissue, their fate during processing and handling, and ways of eliminating or minimizing them.

No study of a new food protein is complete without investigating the basic nutritional value. Determining the Protein Efficiency Ratio (PER) with respect to a standard protein such as casein is essential in the final assessment of the protein.

SUMMARY AND CONCLUSIONS

The major objective of this study was to gain basic information about the type, nature and functional properties of the proteins of bovine rumen tissue in relation to those of skeletal muscle. The solubility characteristics of the rumen tissue proteins in various ionic environments were studied initially. Studies pertaining to the isolation, purification, partial characterization and quantitation of the various contractile proteins were carried out next. Finally, an evaluation of some of the functional properties essential in the food processing industry were completed with certain rumen protein fractions.

Data of the solubility studies indicated that the rumen tissue proteins had unusual properties in comparison to those of skeletal muscle. The four major contractile proteins of the rumen tissue were soluble in distilled water provided the tissue was thoroughly homogenized initially. This property appears to be highly specific for the smooth muscle contractile proteins since it was not observed with any of the skeletal muscle contractile proteins. Results of the solubility studies also showed that two distinct protein isolates could be obtained by the isoelectric precipitation of an extract initially prepared at high pH. These findings were later confirmed by quantitative analysis of the isolates.

Many difficulties were encountered in the purification of rumen tissue myosin. Most of the problems were due to contamination with ill-defined components. Once purified, however, it behaved similarily to skeletal myosin in terms of solubility in high ionic strenghts, elution patterns on DEAE Sephadex A-50 columns and relative migration (R_m) during SDS polyacrylamide gel electrophoresis. A distinct difference was noted in the ATPase activity between rumen and skeletal myosin. At high ionic strengths, the Ca^{++} ATPase activity of rumen myosin was higher than that of skeletal myosin.

Estimations of the molecular weights of the purified rumen tissue proteins as determined by SDS gel electrophoresis were identical to the corresponding skeletal muscle proteins.

Results of studies concerning the quantitative estimation of the various proteins in rumen tissue showed that actin was the predominant contractile protein while the myosin content was only about one-third of the actin concentration. Conversely, myosin is the major protein in skeletal muscle with actin always occurring at a much lower content.

The amino acid analysis of the purified rumen myosin showed many similarities to that of skeletal myosin. However, one of the more notable differences was the smaller ratio of basic to acidic amino acids in rumen myosin compared to skeletal myosin. The

histidine content also was found to be lower in rumen myosin. This has been reported to be the case for all smooth muscles thus far studied. The amino acid composition of rumen actin was found to be considerably different from that of skeletal muscle and to that of some of the other smooth muscle actins reported in the literature. The glutamic acid content found in the rumen actin in this study was higher than any thus far reported in the literature regardless of origin.

In spite of the above differences in amino acid composition, the SDS gel electrophoretic patterns of the contractile proteins purified from rumen tissue were identical to those purified from skeletal muscle.

The salt-soluble protein extracts of the two protein isolates prepared from rumen tissue show emulsifying capacities and emulsion stabilizing properties comparable to those obtained with skeletal muscle proteins. The whippability and foaming properties of the various extracts and isolates prepared from rumen tissue were similar to those obtained with purified egg albumen. Results of the functionality testing indicate that the rumen tissue proteins possess properties that might be appropriately utilized in many food systems.

In summary, it can be stated that the rumen tissue contains most of the same proteins found in skeletal muscle although in

different proportions. Also, some intrinsic differences occur between the same proteins obtained from the two different types of muscle. Data of the present study indicate that the rumen tissue proteins have the necessary functional properties that may be readily adaptable in many food systems. The relatively simple isolation procedures employed to extract rumen tissue proteins enhances the feasibility for the development of a commercial venture. However, since the rumen tissue cannot be obtained without contamination from its contents, a thorough microbiological evaluation must be completed before the product can be used universally throughout the food industry.

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