Symposium on Chemistry of Meat Quality and Processing

INTRODUCTION

Meat supplies high proportions of many of the essential nutritive elements in the average American diet. To maintain the high nutritional level of our people in this era of a rapidly increasing population, we must make every effort to keep our consumption of meat and other animal food products at high levels. Since the American consumer continues to demand that all food items require a minimum of preparation immediately prior to serving—so-called "built-in" maid service—more and more meat items are subjected to some degree of processing before distribution. To maintain high quality and acceptability in these processed meat items, it is essential that we understand the basic chemistry of the compounds present in meat and establish processing and distribution procedures that will minimize any undesirable chemical reactions that may occur between the time of slaughter of a meat animal and ultimate consumption of the meat food.

Thus, this symposium is particularly timely. Each paper presented here is an up-to-date review of basic chemical, physical, and/or physiological properties of muscle tissue—particularly as these properties may be related to changes that occur during processing and subsequent storage and distribution. We hope that these papers will be not only a source of information in the important areas covered, but will also stimulate additional new research on the basic chemistry of meat and on factors that influence the quality of meat products.

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TENDERNESS AND COMPOSITION

Protein Composition and Functional Properties of Meat

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Studies of the role of protein composition of meats in the determination of functional properties such as tenderness, texture, water-binding capacity, and emulsion stabilization have been hampered by the lack of adequate analytical methods. Rudimentary understanding came from studies of meat composition in terms of muscle and connective tissue. Later, analysis of muscle tissue in terms of sarcoplasmic, myofibrillar, and stroma proteins gave further insight into the role these fractions play in determining functional properties. Most recently, better analytical techniques made it possible to attempt to correlate functional properties with the constituent proteins themselves. Many studies reviewed here have shown that protein compositions change while desirable functional properties decrease during rigor mortis. Preliminary studies of protein composition in meat sources known to give differences in functional properties indicate that sarcoplasmic protein fractions may vary with source, but that myofibrillar fractions are likely to vary more in amount than in composition.

In a protein system, functional properties might be understood and controlled by studying composition in an effort to attribute various functional properties to different components. This paper will review the state of such efforts as they apply to meat.

Tenderness and Connective Tissue

Perhaps the earliest attempts to relate functional properties of meat to protein composition involved the relationship between toughness and connective tissue content. In 1928, Mitchell, Hamilton, and Haines (*18*) attributed to Lehmann about 1900 the statement that, "It is the connective tissue fibers, rather than muscle fibers, to which the greater portion of toughness of meat is due." These authors studied the collagen content of a number of commercial meats by autoclaving them to convert collagen to gelatin and estimating the gelatin nitrogen. Unfortunately, they did not really try to relate toughness to connective tissue content but rather assumed it to be so related, although they did verify that cuts generally considered more tender showed generally lower collagen content than tougher cuts.

More refined studies along this line have since been carried out. In 1950, Husaini and coworkers (*13*) used the

amount of protein insoluble in $0.1M$ NaOH as a criterion of connective tissue content and found a good negative correlation with tenderness. In 1955, Hiner, Anderson, and Fellers (*11*) published a study of the relationship of the amount and character of connective tissue to tenderness using histological measurements. They concluded "... that collagenous and elastic fibers are factors influencing tenderness." In 1954, Wierbicki and Deatherage (*30*) developed a method for measuring connective tissue by hydroxyproline content and, in 1960, Loyd and Hiner (*17*) showed a significant correlation between hydroxyproline content of the alkali-insoluble protein of six beef muscles and two measurements of their tenderness.

Tenderness and Muscle Fibers

At the same time there are a large number of observations which indicate that a substantial part of the toughness of some meat must be due to muscle fibers. For example, in 1939, Steiner (*25*) found that both muscle fibers and connective tissue fibers contributed to toughness as he measured it. That connective tissue is only partially responsible for toughness can be inferred from the 1917 studies of Hoagland, McBryde, and Powick (*12*), who showed that increased tenderness on storage was almost entirely due to autolysis. In 1954, Wierbicki and coworkers (*31*) showed that, although tenderness increases, there is no change in the connective tissue content of meat on aging, and in 1963, Goll, Bray, and Hoekstra (*7*) published findings showing that, while tenderness decreases with the age of the animal, no significant changes in hydroxyproline content are found. An observation which somewhat mitigates this type of evidence is the 1935 statement of Bate-Smith (*1*) that collagen fibers swell on hanging of meat and contribute to increased tenderness by being more readily converted to gelatin.

Recently more attention has been given to the role of the muscle fibers and their protein constituents as they relate to tenderness and other functional properties. Many of the ideas in this area are the result of inference. For example, Ramsbottom and Strandine (*21*) reported in 1949 that tenderness in deep-fried steaks was originally high, decreased as rigor set in, and increased on subsequent storage. Paul and coworkers (*20*) confirmed this, but found that roasts never showed the initial high tenderness, apparently because they went into rigor during cooking. In the meantime, Szent-Gyorgyi (*28*) had proposed his now well known theory of muscular contraction. This was based on the identification of two contractile or myofibrillar proteins, myosin and actin. Since rigor is accompanied by a decrease in the extractability of myosin and a concomitant increase in actomyosin, an aggregate of these two proteins, it seemed that the state of the myofibrillar proteins had some significance in the regulation of tenderness. In fact, Wierbicki *et al.* (*31*), using an extraction system which should have removed all the proteins except actomyosin and stroma, the insoluble portion, showed a significant correlation between the nitrogen extracted and tenderness.

Muscle Protein Analyses

In an early attempt to study protein components, Dubuisson and Jacob (*4*) examined electrophoretically the protein extracted by a pH 7.4, ionic strength 0.35, phosphate buffer thus representing sarcoplasmic proteins and perhaps some myosin. Figure 1, which is taken from Jacob (*14*), shows differences found between extracts of normal, fatigued, and contracted muscles. Differences are observed which are not fully explained.

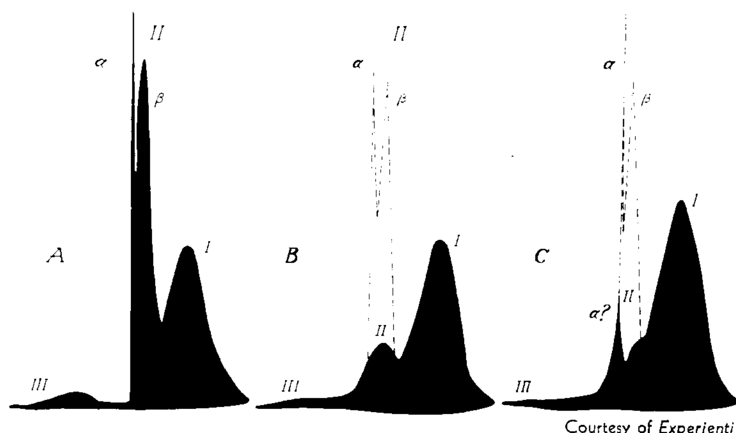
Myofibrillar Proteins

In 1955, Turner and Olson (*29*) applied for a United States patent based on the use of salt to extract myosin from prerigor muscle for improving sausage from emulsions processed in impermeable casings. These authors state that "Experiments have established that the binding property of meat in sausage is due to its content of myosin." However, they indicate that this is myosin B, or actomyosin. In effect, they contend that emulsion stability is regulated by the myofibrillar proteins. In 1960, Hansen (*9*) showed that an appreciable fat-globule membrane was formed only in the presence of proteins soluble in 7% sodium chloride, but not soluble in water. Figure 2 is reproduced from his article and shows the difference in the thickness of the fat-globule membrane in emulsions prepared with these proteins as opposed to the water-soluble proteins. Swift, Lockett, and Fryar (*27*), working along similar lines, established by their method of evaluating emulsifying ability, that the proteins soluble in $1M$ sodium chloride, but not in water, were more effective than the proteins soluble in water. They have used the ultracentrifuge as an analytical tool, especially for the salt-soluble proteins. Figure 3, taken from a report by Sulzbacher *et al.* (*26*), illustrates results obtained with this technique. It indicates that the myofibrillar proteins from four muscles have similar protein compositions, and that the major change on aging seems to be a loss of solubility, with perhaps the appearance of components in a different state.

In 1964, Saffle and Galbreath (*22*), using a 3% sodium chloride solution at pH 6 as an extracting medium, studied the extractability of nonactomyosin, nonstroma proteins from various meat sources, and attempted to relate this to the known effects of these meats in sausage emulsions. They found that, generally speaking, those meat sources considered to give superior emulsions tend to have higher amounts of protein extractable under these conditions.

In a study which has some bearing on both the functional properties of fresh meats, such as tenderness of steaks, and binding of meat emulsions, Borchert and Briskey (*3*) demonstrated in conjunction with their studies of storage at liquid nitrogen temperatures a marked decrease of solubility of myofibrillar proteins and a slight decrease of sarcoplasmic proteins in stored controls. These workers, following Helander (*10*), defined sarcoplasmic proteins as those extracted with pH 7.4, $0.03M$ potassium phosphate, while myofibrillar proteins are measured as those which dissolve in $1.1M$ potassium iodide, pH 7.4, $0.1M$ potassium phosphate, but not in the $0.03M$ phosphate buffer.

Khan and Van den Berg (*15*) and Scharpf and Marion (*23*) have made related studies for the proteins of poultry muscles. Khan and Van den Berg have



Courtesy of *Experientia*

Figure 1. Moving boundary electrophoretograms from Jacob (*14*)

A. Extract of normal muscle
B. Fatigued muscle
C. Contracted by sodium monobromacetate

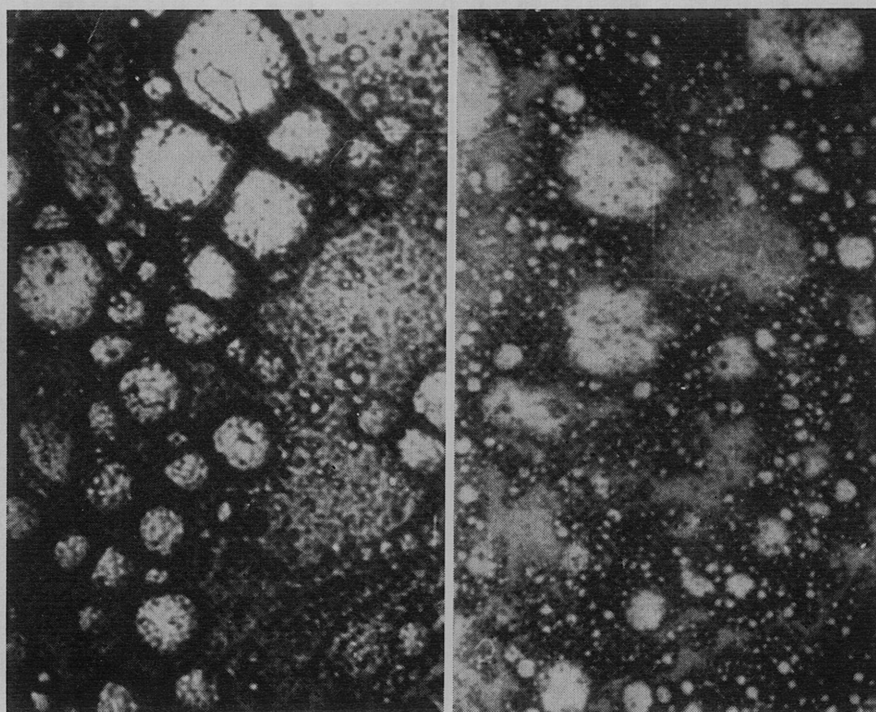


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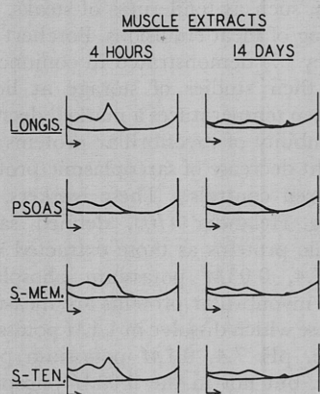
Figure 2. Micrographs of meat emulsions from Hansen (9)

Left. Effect of sarcoplasmic extract
Right. Appreciable fat-globule membrane formed in presence of myofibrillar proteins



Courtesy of the American Meat Institute

Figure 3. Ultracentrifugal patterns from Sulzbacher *et al.* (26)

Myofibrillar proteins for four different muscles at differing times of extraction

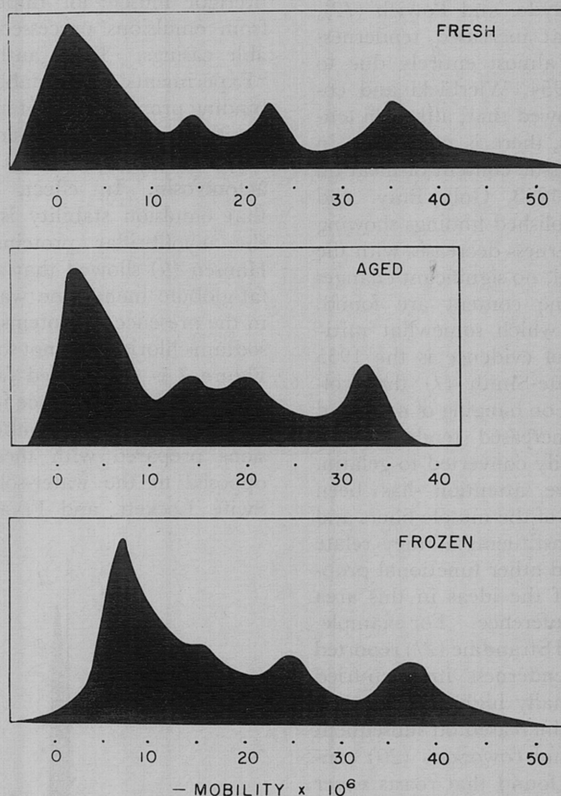


Figure 4. Electrophoretograms of sarcoplasmic extracts from Kronman and Winterbottom (16)

Migration is toward the right

attempted to classify the myofibrillar proteins as myosin and actomyosin on the basis of solubility *vs.* ionic strength, myosin being taken as that protein soluble in KCl–borate buffers at ionic strength 0.08 to 0.25, and actomyosin soluble from 0.25 to 0.5. The sarcoplasmic proteins are taken as those soluble at an ionic strength of less than 0.08. They noted increases in their myosin fractions and decreases in the sarcoplasmic fractions on storage for 7 weeks.

Sarcoplasmic Proteins

Thus, there is a great deal of evidence that myofibrillar proteins play a key role in the determination of functional properties of meat. However, it could be inferred, from the work of Bendall and Wismer-Pedersen (*2*) on pale, soft, exudative pork tissue, that the sarcoplasmic proteins also play a role in the loss of water binding in this type of tissue by being absorbed on the myofibrillar proteins, to which Hamm (*8*) ascribes 95% of the water-holding capacity of beef muscle proteins.

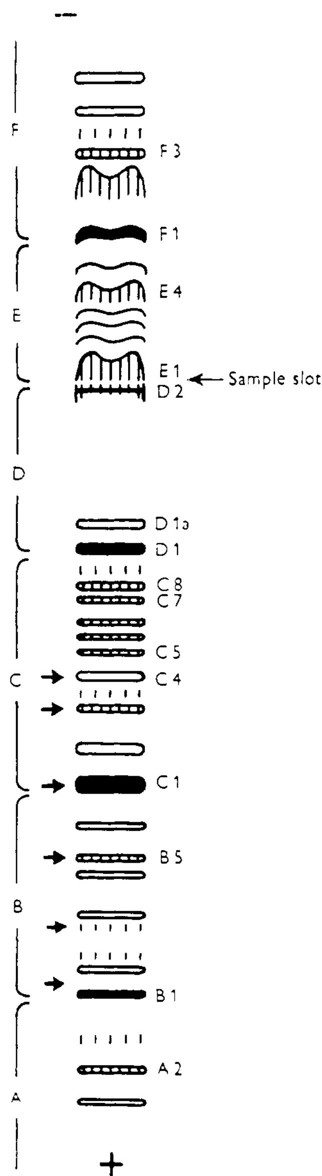
Kronman and Winterbottom (*16*) considered that the sarcoplasmic protein fractions would affect functional properties of meat if only because this fraction contains most of the enzymatic activities. These authors have shown differences in ultracentrifugal and electrophoretic patterns between extracts of the sarcoplasmic proteins of fresh beef, aged at 3° C. for 7 weeks and beef frozen for 5 weeks at a temperature of −20° C. Some of their electrophoretic results are shown in Figure 4, which is taken from their paper. These extracts seem to be qualitatively similar and quantitatively different. More marked differences have been shown for fractions obtained by ammonium sulfate precipitation. These workers speculated that creatine kinase might be lost on storage, since it is present in large amounts in rabbit muscle and since material with a similar sedimentation constant is shown to decrease.

In one of the most definitive studies of muscle protein composition, Scopes (*24*) has applied the technique of starch-gel electrophoresis to sarcoplasmic extracts from steer muscle. He has identified several components as may be seen from Figure 5. Here C1 is myoglobin; C4, metmyoglobin; D1, creatine kinase; B1, myoalbumin; E1, phosphoglyceraldehyde dehydrogenase; and F1, pyruvate

kinase. All the components designated with arrows had peroxidase activity. He found that the loss of creatine kinase was the only consistent change in sarcoplasmic protein composition associated with pH's below 6. Neelin and Rose (*19*) have used a similar method to identify components of poultry sarcoplasmic proteins, but have not found a clear pattern emerging.

Fischer (*5*) used chromatography on DEAE-cellulose to separate sarcoplasmic proteins and found one unidentified component, component III in Figure 6, taken from his paper, which increased with age of meat and with tenderness.

Fujimaki and Deatherage (*6*) have used chromatography on cellulose phosphate with stepwise changes in eluant pH and ionic strength to resolve several components of the sarcoplasmic protein, as shown in Figure 7, and have attempted to identify these with some of the enzymatic activities. They have noted quantitative differences arising on storage but have been hampered by finding similar activities associated with several peaks.



Courtesy of *The Biochemical Journal*

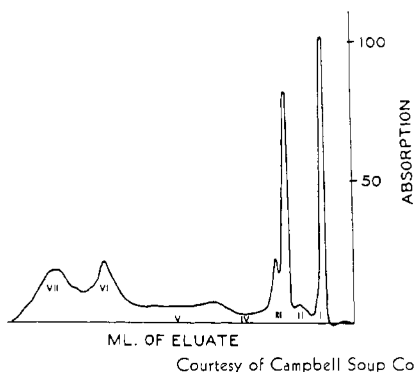
Figure 5. Starch-gel electrophoretic pattern of sarcoplasmic proteins from Scopes (*24*)

C1 identified as myoglobin, C4 as metmyoglobin, D1 as creatine kinase, B1 as myoalbumin, E1 as phosphoglyceraldehyde dehydrogenase, and F1 as pyruvate kinase. Arrows show compounds with peroxidase activity

Analytical Methods under Study

Experiments similar to those of Saffle and Galbreath (*22*), with attempts to subject the protein fractions to componential analysis, have been carried out in this laboratory. Rather striking differences between water extracts of heart muscle and semitendinosis by moving boundary electrophoresis at pH 8.5, 0.1*M* barbiturate buffer as shown in Figure 8 have been observed. Unsatisfactory electrophoretograms have been obtained in these red solutions since, as Figure 9 shows, they are partly obscured by myoglobin, and the possibility of using a helium neon gas laser as a light source has been investigated. This was feasible and relatively simple. A Spectro-Physics Model 130 laser has been used in conjunction with a short focal-length diverging lens (double concave, 10-mm. diameter, −13-mm. focal length) mounted in the plane of the usual collimating lens and a cylindrical lens (44-mm. focal length) which focuses the beam into a line in the plane of the usual slit. Figure 10 shows the pattern thus obtained with a pH 5.8 sarcoplasmic extract and shows no obscuring by myoglobin. Not enough samples have yet been investigated by this technique to determine the relationship of the electrophoretic components to protein components, or to verify the observations of Scopes (*24*) and others.

Procedures for analysis of the protein composition of the myofibrillar fraction are still unsatisfactory. The fraction soluble in ionic strength 0.55, pH 6.5 potassium chloride phosphate buffer but not in water has been taken, in this labora-



Courtesy of Campbell Soup Co.

Figure 6. Chromatogram of sarcoplasmic extract from Fischer (5)

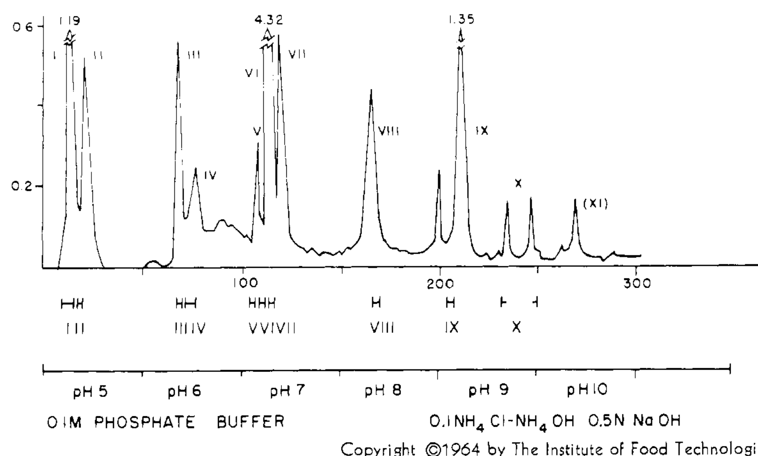


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Figure 7. Chromatogram of sarcoplasmic extracts from Fujimaki and Deatherage (6) showing stepwise changes of eluting buffers

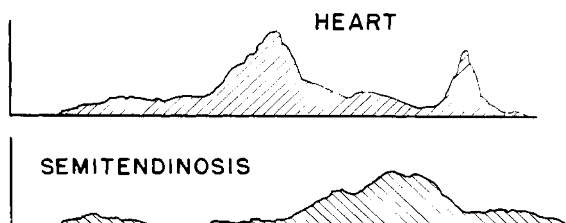

Figure 8. Electrophoretograms of sarcoplasmic extracts of heart and semitendinosis at pH 8.5, 0.1M sodium barbiturate

Migration is to the right

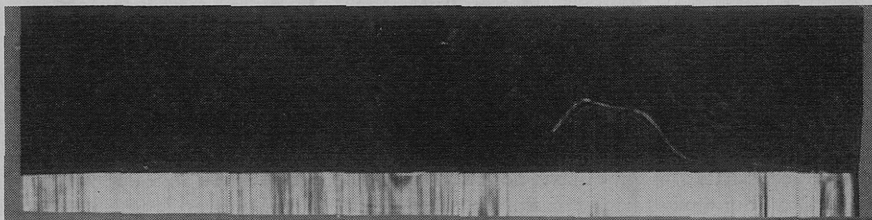

Figure 9. Electrophoretogram of sarcoplasmic extract of semitendinosis from which Figure 8 was made

Myoglobin obscures left side of pattern

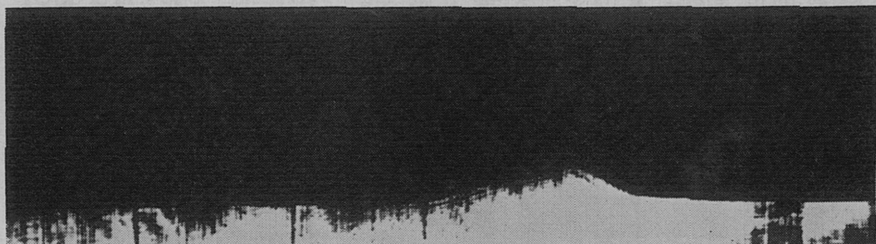

Figure 10. Electrophoretogram of pH 5.8 sarcoplasmic extract run at pH 8.5 in 0.1M sodium barbiturate, using laser light source

Myoglobin does not obscure pattern

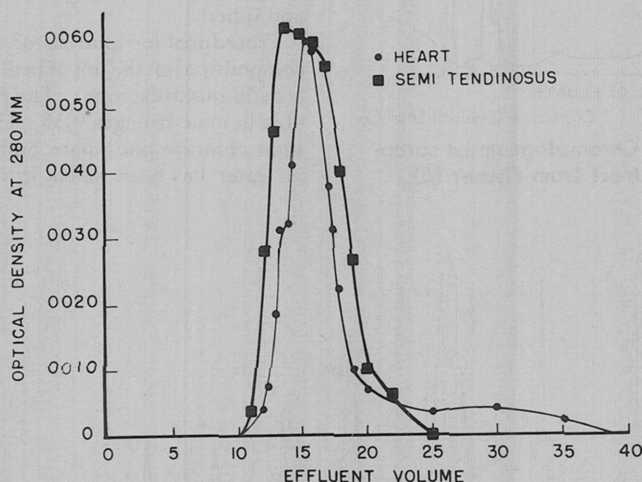

Figure 11. Chromatogram of myofibrillar proteins of heart and semitendinosis using S = 0.55, pH 6.5 KCl-phosphate solvent elution from DEAE-cellulose

tory, as representative of these proteins. The most reproducible results were obtained using solvent chromatography on DEAE-cellulose. Figure 11 shows chromatograms made from such extracts of heart muscle and of semitendinosis. In this case the myofibrillar proteins have been reprecipitated once by dilution with water. These chromatograms indicate that either this is not a sufficiently sensitive procedure to differentiate between these extracts or that no great differences exist between them. However, differences in the amounts of these proteins recoverable from heart muscle and from semitendinosis have been found. Preliminary tests on emulsion stabilization by these fractions indicate that the sarcoplasmic proteins do not aid in this functional property, but that myofibrillar proteins do.

The present state of knowledge indicates that the sarcoplasmic fraction of the muscle proteins may be studied well by electrophoresis or by chromatography. Methods for the chromatography of the myofibrillar fraction leave a great deal to be desired and probably the ultracentrifugal procedure is the most satisfactory present procedure. It does seem to be well established that collagen content has an appreciable bearing on tenderness, that the state of the myofibrillar proteins have some bearing on tenderness, that the amounts of myofibrillar proteins have a bearing on emulsion stabilizing ability of meat sources. Also, many leads have been developed showing the role of protein composition in determining the functional properties of meat but none of these has really been brought to a definitive conclusion.

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