METHODS FOR STUDYING THE WATER-HOLDING CAPACITY OF PROTEINS IN MEAT

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Rather than speak specifically on new techniques on protein as in the printed program, I would like to spend a few minutes indicating to you some of the methods we have found satisfactory in our meat program. At the outset I should like to say that as you observed the data and the curves which I showed this morning very little was said on the methods for obtaining these data. It is only fair to say that in order to get good methods which are reproducible is not always easy, and this is doubly so for some of our meat work. For example, Dr. Wierbicki spent almost two years working on our method for determining hydroxyproline. Almost a year was spent in developing a satisfactory technique for measuring actomyosin in meat. We should also make the point that sometimes the methods we use are not entirely original with us, but may be modifications of methods found by workers in other fields. Methods have been developed by muscle biochemists and physiologists who are interested in understanding the action of muscle in situ. Methods by such workers are not directly adaptable to meat. Again referring to the hydroxyproline method, the original was suggested by Logan, yet we found it very difficult to apply in meat.

In the newer methods which have been developed for the studying of proteins, such as electrophoresis, ultra centrifugation, index of refraction, and dielectric constant method, all require work in relatively dilute solutions and are not particularly applicable to meat. All of these have been used in one way or another for the study of protein hydration, but I think we must admit that hydration in very dilute solutions is quite difficult for hydration in meat, which runs better than 20% protein. We are, therefore, faced with the problem of developing methods which in one way may be considered empirical, but which may give useful information nevertheless.

I would like to show you two slides of the type of information which we have been able to obtain and which have made possible the interpretations we gave this morning. In the case of the second slide I would like to call your attention to the radical changes in tenderness and water-holding capacity which have been brought on by modification of the ionic atmosphere in muscle. First I would like to say that one method which we have found most satisfactory which we have indicated in this slide is the modification of meat by means of the infusion of the vascular system. I do not know what we would do without Dr. Cahill who has been so helpful in this part of our program. We have been using infusion techniques for almost eight years. More recently we have been studying the effect of certain ions by using matched rounds of beef. The infusion technique is very simple when one talks about it, yet I hasten to add that it does take a bit of practice in order to apply efficiently. For whole animals we modify by bleeding by the jugular vein and carotid arteries.
and infused by way of the carotid artery. For excised parts we infuse simply by pumping the available arteries. Whereas in the intact animal fairly even distribution may be obtained by using pressures equivalent to normal blood pressure, in the excised portions above blood pressure may be necessary, perhaps fifteen pounds or more.

In studying the water-holding capacity of meat and its relation to tenderness and shrinkage we gave considerable thought to developing satisfactory methods which would give us sufficient data in a reproducible manner to permit the drawing of valid conclusions. Our first evidence to the relationship of shrinkage to tenderness was obtained using fairly large samples of meat in centrifuge bottles. This could be done, but it was cumbersome and time consuming. We developed these tubes which I show here which permit 25 gram meat samples to be used. You will note that in the upper portion of the tube we put the sample resting on a loose fritted glass disc. The smaller part of the tube is graduated so that direct visual measurement of the liquid may be made. It was necessary to establish the proper centrifugal rate, manner of heating to assure reproducible results. In this instance this did not prove to be a great stumbling block. A weighed portion of either ground or unground meat is placed in the top portion of the tube, stoppered with Bunsen valve or capillary and heated in a water bath at the temperature under study. Heating is continued for thirty minutes. The tube is cooled to about 30-35° C. and then centrifuged at 170 times gravity for ten minutes. While using a magnifying glass and estimating any fat in the liquid, juice expressed can be determined with an error of ± or one-tenth millimeter. We have found that for studying the drip of fresh meat or frozen and defrosted meat the sample should be equilibrated to 40° C. This is to prevent solid fat from interfering with the determination. The tubes that I am showing you here were made for us by the Corning Glass Works. We pay approximately Ten Dollars each. From the number of requests that we have had, it appears that other laboratories are using them and I would hope that the price would drop, but I have no information on that.

A simplified method for determining water-holding capacity of meat proteins has been developed in the German Institute of Meat Research at Kulmbach. The method was developed by Drs. Hamm and Grau and has been studied also in England. The method uses filter paper. Approximately four to six hundred milligrams of meat sample is placed on the filter paper which is then placed between two lucite plates. Five hundred pounds per square inch pressure is applied for one minute. The amount of water released is approximately sixty milligrams per each square inch of wetted surface. These authors have applied this technique to frozen beef, fresh beef and some pork products. We have compared this with the tube method in our laboratory and find excellent agreement on fresh meat. However, the tube method and the German method do not agree on cooked meat. I have two samples here which indicate how the method works. By using a pencil one outlines the periphery of the moisture ring and also of the protein ring. These then are measured by a planimeter. This difference between the two rings is a measure of water or juice expressed. It is very interesting that the area of the paper under the protein film does not contribute to the juice figure. Why this is so we do not know. I would like to share with you and I have drawn on the board a sketch of our results using the method of Hamm and Grau. This work was begun before Dr. Wierbicki left our laboratory and I am grateful to him and the
Rath Packing Company for letting him complete this work and share these results with you. I would like to have you observe the linear relationship between the area of free moisture and the grams of free moisture. This filter paper method has the advantage that it can be applied to a very large number of samples and requires less time than the tube method. Consequently it may be of great value in studying raw and frozen meat. Since, however, it does not correlate well with the tube method for cooked meat we feel that we are obliged to use only the tube method. The tube method has the further advantage that two usable fractions are obtained for future study. In our work, as you have seen, we have done considerable study of the various ions in the juice fraction and also of the meat or protein coagulum fraction. We feel that these tubes show great promise in studying the chemistry of meat during cooking.

You have noticed this morning and again this afternoon that we have made many references to the sodium, potassium, calcium and magnesium contents of meat or fractions thereof. The properties of proteins and particularly their water-holding capacity is a function of pH and the ions on the protein or in the protein solution. In order to study the effect of certain ions it became necessary to develop a technique for measuring them with some degree of rapidity. Twenty years ago one would be considered almost fool-hardy to embark on a study of these ions because of the difficulty of their determination. However, with the advent of chromatography this has not proved difficult. In studying the ion balance in milk, Professor E. F. Almy and one of his students, Dr. William Sutton, modified several techniques of other workers into a simplified scheme. The basis of their method is the chromatographing of the chlorides on ion-exchange resins and eluting with varying strengths of hydrochloric acid. By calibrating the column it became possible to take aliquots of certain fractions, evaporate to dryness, and titrate the residual chloride. From this the cations could be computed. We have adapted this to meat and its fractions. We find this more satisfactory than flame photometry because the ratios of sodium and potassium are more optimal for this technique.

Before concluding, I should like to say that in no way do I want to discount some of the more elegant techniques for studying protein. Indeed they have very great potential in studying meat.

I put in your hands of those of you who are interested reprints of our work which gives the details of the development of these procedures I have discussed. If any of you are interested in any of the other methods we are using in our laboratory we will be most happy to share them with you.

DR. SCHWEIGERT: We certainly appreciate your sharing with us some of these newer approaches and newer technics applicable to protein and its interrelationship with other vital constituents of meat.
We are indebted to our good friend, Dr. Hall, at Kansas State, for suggesting the next speaker to the Research Methods Committee, and in circulating the committee for their comments they were very much interested in what Dr. Clegg would have to share with us today. He will describe some of their recent protein research. I am sure that some of the approaches that he has will be of interest to us.

It is my real pleasure to present Dr. Clegg at this time who will review for a similar period their research. (Applause)