I wish to make it clear at the onset that the topic which has been assigned to me does not presume any great knowledge of analytical chemistry on my part. Indeed, this branch of chemistry is a highly specialized field in itself. The observations which I wish to make are therefore of rather limited perspective but, I trust, of sufficient interest to stimulate discussion on this important phase of meat research.

You are quite aware, I am sure, that our knowledge of meat chemistry is limited. That it may at times appear otherwise is probably true only in retrospect. Certainly there is a close relationship between our knowledge of meat chemistry and the methods which are practically available to the meat research laboratory.

There is, however, no intent on my part to underestimate the important technological advances which are being made. They offer increasingly greater opportunities through chemistry and related sciences to discover the truths for which we search. Let us not minimize the great distance that we must travel. I believe the point that we are most apt to lose sight of is that a cut of meat which appears so commonplace and which is often taken for granted is the handiwork of the master craftsman. Here is a product of the class of Mammals; the end result of a series of complicated biological reactions involving not only the effects inherent in the germplasm but also the cumulative effect of many environmental changes during the animal's life and subsequent to slaughter.

Is it not surprising also how few of us really appreciate the great value of a trained sense of perception? These senses when used to evaluate complex changes in meat characteristics are usually much more efficient, comprehensive and adaptable than any other method of evaluation. Certainly, our chemical methods have been developed as a tool to supplement and correlate with our sensory perceptions giving us increased objectivity. It must be admitted that many of our chemical and physical chemical techniques, while not as precise as we might like, do give us also an increased knowledge of the "whys" and "wherefores" and that our results gain in objectivity, a characteristic much to be desired.

I feel sure that your program committee did not expect me to give a resume of a rather larger number of chemical methods and an evaluation of each. This would not be possible even disregarding the time limitation. I do wish to mention, however, a few analytical procedures which have been shown to be applicable to meats work.

Methods for Moisture Analyses

There are a number of methods available for determining moisture quantitatively. As you know, meat contains water in different forms. The most common form is free water as differentiated from bound water. Bound water serves as the dispersions medium for the colloidal material. The
lyophilic colloids which characterize meat possess a great affinity for water. The determination of the moisture content of meat is purely an empirical procedure determined by the three variables, time, temperature, and pressure. Thus, it is impossible to define the moisture content without defining the conditions under which the drying took place since the various moisture methods only determine more or less of the "moisture" in the meat. Therefore, standard or official methods should be used unless only comparative results are desired in which case any standard method may be used.

There are two official methods of the A. O. A. C. (1) Drying to constant weight at 95-100°C. under pressure not to exceed 100 mm. of Hg. and (2) drying over H2SO4 in a vacuum desiccator at a pressure of not more than 10 mm. of Hg. to a constant weight.

There are also a number of methods for determining bound water. Here again, the relationship of free, bound and total water must be expressed in relationship to the method used in the determination.

Methods for Fat Analyses

In order to remove fat from tissue it is necessary that the tissues be dried and this drying must take place in such a manner that there is no oxidation. Drying in air is not to be recommended. The A. O. A. C. method, however, calls for drying at 101°C to 102°C. for 16 to 18 hours or at a temperature of 125°C for 2 to 3 hours followed by extraction with anhydrous ether for 16 hours.

This is one reason why it would seem desirable to give special consideration to the method for fat analysis now being used by the Kansas Agricultural Experiment Station. Dr. Hall informs me that this method was developed at the University of Kentucky. It is essentially a modification of the Babcock butter fat test. A somewhat similar method was described by workers at Oscar Mayer's in 1945. The time required for a determination when one blender is used is reported to be about 30 minutes. Very close agreement is reported between this method and the A. O. A. C. Soxhlet method of extraction. With both the Kansas and Oscar Mayer methods the samples are put through the Waring Blender.

Free Fatty Acid Determination

The determination of free fatty acids is of value in estimating the amount of deterioration due to hydrolysis. This hydrolysis may be caused by the natural enzymes or by fat splitting microorganisms. It is a measure of hydrolytic rancidity. The free fatty acids are titrated against sodium hydroxide. This determination is a good indication of how fats and especially pork fats have been handled. Pork fats with even a small amount of hydrolysis will render out lard with a low smoke point. An increase, for example, in free fatty acid from 0.14 percent to 2.20 percent will lower the smoke point from 370°C to 271°F. Free fatty acid values are generally expressed as percent oleic acid.

The Iodine Absorption Value is a good indication of the degree of unsaturation. It has been used widely to indicate firmness of pork fat. This method measures directly the total concentration of double bonds and is therefore but an estimation of the degree of hardness. It would appear
desirable that a satisfactory method of physically determining the hardness be developed. Workers at the U.S.D.A. and others have shown that there is excellent agreement between iodine values and refractive index and that they both serve as a satisfactory measure of firmness of the adipose tissue.

Recently there has been a great deal of interest in oxidative rancidity in processed and stored meat products. Dr. Beadle of the American Meat Institute has pointed out and I quote "No one chemical test has been devised which can accurately measure and correlate all the factors which act simultaneously to produce the odors and flavors we call rancidity. Of the various tests which have been submitted for the detection of oxidative rancidity, the determination of 'active oxygen' or peroxide content seems to give rather good correlation of the data. During the oxidation of the fat certain oxygen compounds are formed which are 'active' in the sense they are capable of liberating iodine from potassium iodide. The iodine can be quantitatively determined and thus be a measure of rancidity." The peroxide compounds are themselves intermediate compounds and are not responsible for rancid flavors and odors. I would like to emphasize that peroxide values can only be proportional to rancidity as long as peroxides are being formed at a rate which is faster than they are being decomposed. It is also true that the temperature coefficient of peroxide formation is greater than that of peroxide decomposition so that with increasing temperatures the values become increasingly less reliable as indicators of rancidity development. When using peroxide tests under a new set of conditions it is always first necessary to be sure a correlation is obtained with organoleptic rancidity. Erroneous results or conclusions are also quite often the result of comparing peroxide values obtained under one set of conditions with those secured under another set of conditions.

As stated previously, rancid odors are not due to the peroxides themselves but to the breakdown products resulting in part from their action. The rate of breakdown is dependant in part upon the temperature of the tests. Therefore, the peroxide numbers will be much higher for the same degree of rancidity in the case of shelf storage tests than in the case of accelerated tests. It is also true that rancidity develops at very low peroxide values where certain antioxidants are used.

As C. H. Lea states "Basically all of the methods which have been employed in the determination of susceptibility (to oxidation) are similar in principle. Oxidation is accelerated under carefully controlled conditions while still preserving as far as possible the original relations between the various samples until the time necessary for the development of spoilage has been reduced from months or weeks to days or hours. .... The progress of accelerated oxidation is then followed by smell and taste, by direct measurement of the weight or value of oxygen absorbed, by changes in some physical characteristic of the fat, or by chemical estimation of the products of reaction. It is extremely important with all accelerated methods to calibrate the method with the particular type of fat studied."

The accelerated methods which are commonly used are: (1) Schaal Oven Method; (2) Swift Active Oxygen Method; and (3) Barcroft-Warburg Method. Time does not allow to discuss the relative merits of each but a good discussion by B. W. Beade can be found in OIL AND SOAP, 23, 33-35 (1946). In addition to these methods, Major and Watts report a simple accelerated method which consists of incubating the samples at 30°C. for 60 hours. Chloroform is used with the ground meat to prevent bacteriological decomposition.
There are a number of methods for determining peroxides including those of Lea, Wheeler and Stansby. We have used Stansby's almost exclusively. In general, all of the methods consist of using fat in a highly acidified solvent, adding potassium iodide and titrating the liberated iodine with sodium thiosulfate using starch as an indicator.

The Kreis test has also been used as a method for determining fat stability. It has a number of limitations since fats which are not rancid may give the test. Curves, however, may be obtained which are useful in studying stability. Watts and Major have shown that peak values obtained by Kreis tests varied greatly while peroxide numbers were quite constant. Both peroxide and Kreis values go to a maximum during prolonged oxidation of the fat. The peroxide peak, however, comes long after the Kreis peak. High temperature also has a depressing effect on Kreis values when compared with the peroxide values.

Methods for Nitrogen Compounds

There are a number of methods available in connection with proteins and other nitrogenous products. These include specific tests such as the Millon reaction for tyrosine; the color is specific for the phenol group. There are also tests for the aromatic nuclei which are easily nitrated such as in tryptophane and tyrosine and also for other groups. Unfortunately, these tests are not generally quantitative although a few have been adapted for quantitative determination of such amino acids as tryptophane and tyrosine. Perhaps the best quantitative method for amino acids is the Van Slyke method. By the apparatus of the same name it is possible to determine the amino and carboxyl groups in amino acids or groups of amino acids. By Sorenson's Method carboxyl and amino acid groups can be titrated. There are also methods for group analyses such as Hausmann's for ammonia nitrogen, basic nitrogen and non-basic nitrogen.

Methods which have been especially adapted to meat studies include collagen and elastin determinations. These methods were developed originally at Illinois to study tenderness. Dr. Hall of Kansas tells me that his method for collagen is to be published shortly in FOOD RESEARCH. There are several nitrogen determinations that are of value. These include water soluble nitrogen, heat coagulable water soluble nitrogen, non-protein nitrogen and free amino nitrogen. The latter is particularly valuable for studies of autolysis. Increase in sulfhydryl content are also characteristic of the early stages of denaturization and can be determined quantitatively.

Absorption Spectroscopy Measurements

One type of analysis that would appear to be adapted to certain phases of meat work is absorption spectroscopy. As you know, it is concerned with the amount of light absorbed or transmitted by a solution of the substance at each wave length throughout the spectrum. It is not only adapted to measuring color changes such as occur in the oxidation of hemoglobin to methemoglobin but also to other changes such as occur in the oxidation of fats. So far no great adaptation has been made to meats work. The accuracy and precision of spectrophotometric quantitative methods is high at various concentrations. For very low concentrations it is really more accurate than the ordinary analytical methods. It suffers by comparison, however, at high concentrations. One of the chief advantages it has is the speed in which the determinations can be made.
I should like to make one closing observation and that is that a chemical method is but another tool. The effectiveness of this tool is in no small way related to its adaptability to the task at hand and the accuracy of the worker who uses it. Unfortunately, there are many of us who do not have the time or the inclination to study these various methods, their adaptations and limitations. I believe the discussion on peroxide values should have brought out this point. There are many perhaps who feel that this method is not satisfactory for determining rancidity. They had hoped it could be used with the various types of fat handled by different methods and with various antioxidants in a straight forward manner. Certainly, no such claims have ever been made for the method. Given a rather standardized set of conditions it can, like other chemical methods, be very useful in giving reproducible results supplementing and correlating with other less objective methods.

CHAIRMAN BUTLER: Thank you very much, Dr. Brady. And now we will have a discussion of Dr. Brady's paper, with Professor Farwell of Michigan State College leading.

PROF. FARWELL: I know that Dr. Brady's paper has reviewed some of these chemical methods in your minds. Many of you have worked with them, and some of the rest of you probably have read a good deal about them.

I think that the Program Committee is to be complimented on the timing of these papers. I think that yesterday we began to get a few ideas in the chemistry of meats in our minds, and last night certainly, Dr. Newton's paper drove home a few things. The subject not covered in Dr. Brady's paper, that might stimulate a lot of work and a lot of thinking, would be the possibilities of using some means of chemistry in the tenderizing of meats, and there probably will be a lot of advance in that field in the future.

Do any of you have any questions you would like to direct to Dr. Brady now?

PROF. NELSON: Dr. Brady, you were mentioning a moment ago the protein decomposition products. You mentioned tryptophan and some of the amino acids. Have you tried the microbiological methods of assay for those?

DR. BRADY: No, I have not.

PROF. NELSON: They are quite sensitive and pretty adequate for about ten or twelve of the essential amino acids.

DR. BRADY: Quantitative?

PROF. NELSON: They are quite quantitative. They work very nicely.

DR. BRADY: That is a thing we are just getting into, so we are very much interested in your remarks.

PROF. NELSON: I have not worked any on meat recently, but I know it will work, very similar to your vitamin assay procedures.

PROF. FARWELL: Are there any other questions?
PROF. R. C. MILLER: I have a comment. I think Dr. Brady has handled this subject in a very creditable manner. A chemist is up against one more proposition in his chemical methods, though they are very useful. The meat man hands over his material to the chemist, and the chemist is qualified to apply the methods. However, between those two points there arises another question, namely the question of sampling, which is a very serious one in many cases and may have a marked effect on the results a chemist obtains.

I do not know whether that is a problem for the meat man or for the chemist. I merely suggest that it might be a suitable topic for discussion at another time.

PROF. FARWELL: I think that goes back to Dr. Newton's talk last night, and also Dr. Brady mentioned it. It is a matter of team work. Certainly, it was brought out in Dr. Brady's discussion that the value of the peroxide test, in a lot of cases, is a matter of team work and correlating what the chemist needs with what we can give him.

Are there any other questions?

PROF. BUTLER: I want to comment. The quantitative Kreis Test, as developed by Watts and Major, should be more effective in estimating rancidity as it measures one of the end products. In some work on pork sausage, I used that and used it along with the peroxide test -- Wheeler's method -- for 32 samples on two separate occasions, and the correlation between the quantitative Kreis Test and the peroxide test was almost perfect in both cases. In one case it was .96; in the other case, .95. Apparently they both have a pretty severe handicap, because neither one of them correlated too highly with the organoleptic test on the same sample.

PROF. FARWELL: Are there any other questions or discussions?

If not I will turn the meeting back to the chairman.

CHAIRMAN BUTLER: Thank you, Professor Farwell.

Now we are going to have a paper on "Methods Used in Studying Bacteriogenic Problems in Curing and Canning Meat", by Professor Willard O. Nelson, who is now Assistant Professor of Dairy Bacteriology at the University of Illinois. He was with Wilson & Co. for several years, and was a Captain in the Medical Corps. Mr. Bull says that he is a meat man who went to the bad and went over into the dairy department.

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