

MEAT ENZYMES

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Well, to go ahead and explain some of the fundamentals of enzymes, I would say I have about 15 volumes on my desk right now, and I don't think I can do it in this short period, but I should like to review and to discuss the nature of enzymes and some of the physical properties of them; secondly, I should like to discuss the significance of enzymes with relation to the meat industry, and if time permits I should like to review some of the work we have been doing at the American Meat Institute Foundation with respect to proteolytic enzymes in muscle.

To attempt to understand the existence of living things it becomes imperative to delve within these living things and study the chemical changes which take place. Some living organisms, for example, green plants and certain bacteria, are able to utilize energy from sunlight and to synthesize complex organic substances of high energy content from very simple starting materials, such as water, carbon dioxide, nitrates and phosphates. Other living organisms, on the other hand, are able to decompose and utilize the energy which is released from these complex substances for their bodily functions -- growth, locomotion, reproduction, etc.

It is known that many of the chemical reactions occurring in living matter or under its influence would, *in vitro*, occur far too slowly or require temperatures intolerably high for most organisms on this planet. A most striking characteristic of living organisms is the rapidity and precision with which the chemical changes necessary for their existence are carried on. How then can a living system obtain energy and utilize it to synthesize various chemical compounds? What controls the rate of complex biochemical reactions through the so well defined pathways? The answer is that living organisms possess numerous natural entities which speed up chemical reactions to the rates achieved in biological systems. Whether we consider digestion, metabolism, locomotion, fermentation, or putrefaction, literally hundreds of chemical changes are going on, and these chemical changes are catalyzed through the mediation of natural catalysts called enzymes. Enzymes, therefore, make it possible for numerous essential biological reactions to occur at the temperature and under the other mild conditions of the organism itself.

Enzymes are found in living systems; they are produced by the living cell; no living thing can exist without them; yet enzymes themselves are not living. It seems likely from evidence at hand that a majority of all the chemical reactions which occur in organisms are enzyme-controlled.

Long before written history, enzymatic reactions were used by man. Fermentation processes to produce wines, the making of cheese, the leavening of bread, and the manufacture of vinegar are enzymatic processes which stem from ancient times. They are all enzymatic processes which man has used for hundreds of years. These practical applications of enzyme chemistry have occupied a throne in the history of enzymology.

Now that we know that enzymes are around us, that they are within us, exactly what are enzymes? In brief, enzymes are defined as proteins whose biological function is the catalysis of chemical reactions in living systems. In short, they are catalytic proteins. A catalyst may be defined as a substance which accelerates a chemical reaction in which it is not involved in the reaction. In other words, it is not consumed in the reaction. From inorganic chemistry we have learned that platinum has been used as a catalyst for the synthesis of water from two simple chemicals, hydrogen and oxygen. The important characteristic of such reactions is that the amount of catalyst bears no stoichiometric relationship to the quantity of substance altered. The catalytic efficiency of enzymes is extremely high; this can be seen from the fact that pure enzymes may catalyze the transformation of as much as 10,000 to 1,000,000 moles of substrate per minute per mole of enzyme.

It should be remembered that catalysts in general cannot cause reactions to take place; they can only increase the rate of the reaction. In the case of enzyme catalysis, the rate of the uncatalyzed reaction is frequently so slow as to be negligible, and from all appearances it seems that enzymes themselves initiate the reaction. This is not essentially true because the reaction must occur itself, but usually the reaction occurs so slowly -- in other words, the reaction is so slow we cannot tell by any of the products that are formed.

In two respects enzymes excell in comparison with most, of not all, non-biological catalysts: They are unusually effective catalysts, and they are much more specific.

Enzymes, as compared with non-protein catalysts, have a highly specific action. They are extremely selective in their action on a group of closely related substances. The substances on which enzymes act specifically are usually referred to as the substrates of the enzymes.

One last fact I should like to point out is that enzymes as catalysts do not act by their mere presence. They take part in reactions, they change during the reaction, but they describe a cycle and reappear in their original form and, therefore, are unchanged in the overall reaction.

The classification of an enzyme is difficult. Enzymes are usually named in terms of the reaction which is catalyzed. The customary practice is to add the suffix "ase" to the name of the substrate. For example, the enzyme which attacks urea is called urease. That enzyme which attacks tyrosine is called tyrosinase, and in the same way the enzyme which attacks arginine is referred to as arginase. Nevertheless, much of the older nomenclature has persisted and we find such names as pepsin, trypsin, rennin, etc.

Enzymes may also be classified by groups, and in common usage these are such classes as the proteinases, lipases, oxidases, etc. There are many exceptions and certain disadvantages to the system of enzyme classification, but it would be out of place to discuss them during this session.

There are a number of factors which affect the rate of an enzyme catalyzed reaction, such as enzyme and substrate concentration, the effect of temperature and pH.

(Slide) Michaels and Menten, in 1913, postulated the enzyme-substrate theory whereby they assumed that the enzyme and substrate form and are in equilibrium with a dissociable complex, which may also break down to yield product plus enzyme, as we have in the first equation up there. The enzyme substrate complex in turn, since it is dissociable, will form the product and enzyme. Now this enzyme, as I said, may go in a cycle, but it will always end up in the reaction the original substance. On the basis of these assumptions they showed that the velocity of a catalyzed reaction at a given enzyme concentration is proportional to the concentration of the substrate.

(Slide) This next slide shows a diagram of the effect of the substrate concentration on enzyme activity. As we increase the substrate concentration the activity increases until a plateau is reached or a portion where all the enzyme itself is tied up in this enzyme substrate complex and no matter how much more substrate we add the activity will not increase because, as you can see, the substrate must wait its turn because there is just so much enzyme present.

(Slide) In the presence of an excess of substrate the velocity attained is proportional to the concentration of the enzyme. If we have an excess of substrate present in the reaction and vary the concentration of enzyme, there is a direct relationship between the concentration of enzyme and the activity.

Now what is the influence of temperature? Most enzyme reactions, like most chemical reactions, are influenced by temperature, the reaction velocity increasing with rising temperature and decreasing as the temperature decreases. However, because enzymes are susceptible to heat, there is a maximum point reached where the higher the temperature becomes the less enzyme activity we obtain.

(Slide) Here as the temperature is increased the enzyme activity increases until we reach an optimum temperature, and then as the temperature is further increased the activity decreases. This is most likely due to an inactivation of the enzyme by the heat. The optimum temperature of an enzyme, therefore, is that temperature at which the greatest amount of substrate changes per unit time. It should be mentioned that the optimum temperature for any enzyme changes not only in relation to time but also may vary with changes in pH concentration and purity of the enzyme solution.

(Slide) Enzymic reactions are markedly affected by pH, and each enzyme is found to function maximally at a certain pH or hydrogen-ion concentration. This bell shaped curve is very similar to the temperature curve, but as we increase the pH of the enzymic catalyzed reaction, the activity increases until we reach an optimum pH. Characteristically, each enzyme has a pH value at which the rate is optimal and on either side of this optimum the rate is lower as shown on this slide.

The pH optimum of an enzyme may, and most likely does, change according to the conditions of the experiment. For example, the substrate, the source of enzyme material, the element of time, and the buffer solution employed affect the optimum pH of an enzyme reaction.

Some physical properties of enzymes are closely related to proteins. This is mainly because an enzyme itself is a protein.

The solubility of enzymes vary greatly. In general, most enzymes are readily soluble in water; others are soluble in dilute neutral salt or buffer solutions, and still others are quite insoluble in water. In any case, the solubility of enzymes is characteristic of proteins. They are precipitated from solution by protein-precipitating agents, such as concentrated alcohol, ammonium sulfate, and trichloroacetic acid and, like most proteins, are least soluble in aqueous solutions at their isoelectric point.

Enzymes in general differ from other catalysts in that they are characterized by a high degree of specificity. The presence of an enzyme is indicated only by its action on a suitable substrate. If no substrate is known, the presence of the enzyme cannot be proven. In order to get information on the specificity of an enzyme, the enzyme must be introduced into a sufficiently large number of systems or substrates which are thermodynamically capable of reaction and which differ in one or another of their molecular groups. It then becomes possible to determine the specificity of the enzyme by observing the selectivity in its effect on the rates of the systems under study; therefore, by enzyme specificity, we mean the relationship between enzyme action and chemical structure of the substrates.

There are certain substances which activate enzyme activity and they are known as activators. Activators are referred to as substances of non-specific nature, generally of small molecular weight which appear to be necessary for or enhance the activity of an enzyme. The mode of action of an activator is still not clear.

Within this category fall many of the metal ions. Some enzymes, such as arginase, hexokinase, phosphatase, and certain peptidases, are activated by bivalent metallic ions, such as cobalt, magnesium, manganese, and in many cases nickel. None of these activators function universally, but each has a partial specific enhancing effect.

Other enzymes, such as the amylases, require certain anions, especially the chloride ion. It has been suggested that some enzymes may be regarded as metallo-proteins, and that the combination of these enzymes and their substrates occurs partly through metallic ions. In other cases, it has been proposed that the activation by metallic ions is really a reactivation of inactivated proteins. In some cases, though, it has been found that the metallic ions merely protect the enzyme under study from inactivating agents.

The coenzymes, in contrast to activators, are molecules of very specific action and organic constitution. They are usually larger molecules with molecular weights varying from 100 to several hundred. The prosthetic group of the conjugated enzyme is called the coenzyme. Coenzymes can be split off from the protein portion of the enzyme and upon recombination with the protein portion the activity of the whole enzyme is restored.

I will not discuss the inhibition of enzyme activity to any great extent, mainly because, like the kinetics of an enzyme reaction, the topic

would become rather lengthy. But a great deal of information regarding the nature of enzymes and their mode of action can be linked to studies dealing with enzyme inhibition. Almost all enzymes are inhibited by substances which precipitate or denature proteins, such as the heavy metals -- mercury, lead, silver, etc. Many enzymes are inhibited by products of their own activity, for example, glucose, a product in the action of the enzyme invertase on sucrose is also found to inhibit the reaction. Many more enzymes are inhibited by substances structurally related to their substrates. This inhibition occurs due to the blocking of the active centers on the enzyme.

A number of enzymes which contain essential metal ions are inhibited by substances which form complexes with the metal. Hydrogen sulfide and hydrogen cyanide inhibit enzyme activity by complexing with the iron atom of those enzymes containing the iron-porphyrin complex, such as catalase and peroxidase. It should be mentioned that any factor which changes the protein molecule itself, such as heat or irradiation, acts as an inhibitor of enzyme action.

Now we come to the enzymes which are of specific importance to the meat industry. I will essentially mention three groups, the glycolytic, the proteolytic, and the fat hydrolyzing enzymes, although there are many more we could discuss.

The glycolytic process involved in slaughtered muscle tissue is of great importance to the meat industry. After slaughter the carcass is dressed and chilled during which process there is a solidification of fat and simultaneous development of rigor mortis, accompanied with the formation of lactic acid and other acids from tissue glycogen. The conversion of muscle glycogen through a sequence of enzymic reactions to form lactic acid in muscle tissue is called anaerobic glycolysis. The actual sequence of reactions involves numerous glycolytic enzymes, but the overall reaction is shown in the next slide.

(Slide) There are essentially about 30-some reactions involved in the reaction but this is the overall picture where we have glycogen plus 3ADP plus 3 phosphate plus $H_2O \rightarrow 2$ lactic acid plus 3ATP.

One of the chief factors involved in glycolysis in meat is the production of lactic acid with a concomitant decrease in pH. The pH of normal living muscle tissue is approximately 7.2 to 7.4, but after rigor mortis, the pH drops to approximately 5.8. The chief asset of lowered pH in meat is its ability to restrain the growth of certain bacteria. Another factor which is of importance is that at a lowered pH the activity of proteolytic enzymes associated with tenderizing during the process of aging is enhanced. Some workers feel that glycolysis and, therefore, the lowered pH, directly affects the color and texture of meat. Work on dark cutting beef indicates that such tissue is often depleted of muscle sugar and, therefore, the amount of lactic acid produced is decreased with a concomitant higher pH than in normal beef. The sticky condition of dark cutting beef has been considered by some to be associated with the relatively high pH. Whether the dark color and sticky texture is a direct effect of a higher pH is questionable.

The second group of significant meat enzymes is the proteolytic enzymes.

The fact that meat undergoes physical and chemical changes during storage has long been noted. The process of aging meat, which has been practiced for many years has, in general, directly affected the tenderness qualities of meat.

Aging of meat is the process by which the meat is held at temperatures higher than normal for specified periods of time to enhance the quality of tenderness. It has been suggested that the tenderizing effect of aging is brought about by the action of naturally occurring proteolytic enzymes in muscle tissue. Very little is known about these natural entities which degrade the protein molecule into smaller units. At the present time, studies are in progress which may lead to the identification and characterization of these enzyme systems in muscle tissue. The information obtained through these studies may suggest new approaches to the problem of meat tenderization.

Proteolytic enzymes from other sources, such as glandular tissues, bacteria, fungi, and plants have been added to meat as a means of tenderizing and have gained much popularity. It should be mentioned that artificial tenderizers do not, at the present, indicate completely efficient tenderizing action in practical application, since many problems, including those associated with penetration of the tenderizers, uniform action, and flavor changes still have much to be desired.

The third group of significant meat enzymes is the fat hydrolyzing enzymes.

The fat splitting enzymes in meat are probably more of a detriment than an asset. Lipases hydrolyze fats to form free fatty acids and glycerol. If meat is stored without some control over the lipolytic enzymes, the fats will be considerably altered. The free fatty acids will increase progressively on storage and, depending upon the product, the change may or may not be considered spoilage. For example, we may tolerate a considerable amount of free fatty acid in summer sausage while a similar amount in fresh meat would be considered a sign of spoilage. The lipolytic process can be slowed down considerably by chilling the carcass during storage.

In the production of good quality edible fats, lard, etc., free fatty acids should be as low as possible. Cold storage prior to rendering minimizes the lipase activity and heat during the rendering process inactivates the lipases so that a minimum amount of free fatty acids are produced by enzymatic action.

I should like to describe very briefly one of the many phases which has been undertaken at the American Meat Institute Foundation which is directed toward basic studies of the natural tenderizing entities inherent in muscle tissue per se. These studies were initiated to determine, isolate and define the proteolytic enzymes naturally present in beef muscle.

A simple and sensitive method for determining total proteolysis in meat or meat extracts was developed based on a modification of Anson's method of hemoglobin digestion, whereby the acid soluble tyrosine liberated from the substrate by the enzymes was determined spectrophotometrically. Extraneous interfering materials which were extracted from the meat along with the enzymes and which absorbed in the range of tyrosine were removed by dialysis of the extracts prior to the assay of the enzymes. The data also indicates that proteolytic activity increases after dialysis.

Information has been obtained regarding the optimal conditions of temperature, incubation time, and pH. Using denatured hemoglobin as the substrate whereby the final concentration of the reaction mixture was 1 per cent hemoglobin in .1 molar acetate buffer, the optimal conditions of the assay were found to be at a pH of 4.4 if incubated for four hours at 37 degrees centigrade.

(Slide) We have a series of curves, but since time is getting so short I will just present this one. Under these conditions we found out that the maximum or the optimum time was at four hours at 37 degrees, and on this pH curve we found out the optimum pH conditions with a pH of 4.4. Evidence from the kinetic experiments indicates that a number of enzymes are involved in the over-all proteolysis.

Various extraction and isolation procedures have been investigated and highly active concentrates of the enzymes have been obtained from .6 saturated ammonium sulfate fractions. Cellulose exchangers have been employed in the fractionation and purification of the active ammonium sulfate fractions. By means of an anion exchanger, diethyl aminoethanol-cellulose, several fractions have been obtained containing proteolytic activity.

Studies in progress at the present time are focused toward isolating and defining the various natural proteolytic enzymes in these fractions and to study their mode of action, so that new approaches to the problem of effectively tenderizing meat may be suggested.

I realize I tried to cover a most broad subject in a very short time, but I tried to touch briefly upon a few of the more pertinent aspects of enzymology and to show the importance of some of these enzymes to the meat industry.

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Thank you. (Applause)

MR. SCHWEIGERT: Thank you, Bob.

I hope this gives you some idea of the tools and the technics, as indicated in a few specific applications at the end as well as some

of the broad principles, since it may have been a few years since some of you have had enzymes in physical chemistry.

To lead off the discussion we have asked Al Pearson and Joe Kastelic to indicate a few remarks, either to elaborate on some aspect that Bob may have mentioned or to tell of some experiences of their own laboratories relating to enzyme research applicable to the meat research program. So we will ask Joe Kastelic to lead off with his remarks. (Applause)

MR. J. KASTELIC: Thank you, Bernie.

It goes without saying that I am glad to be with you today, even though I find myself now somewhat around the backdoor of meats interests.

I am not certain how we ought to define our subject "meat enzymes" as it may be related to the activities of the "Research Methods Committee," the aegis under which we are presumably operating; I shall not therefore make any attempt to do this.

It is sufficient to say that enzymes can be employed as reagents for the assay of substrates and for the determination of the nature of the changes wrought in substrate molecules by enzymes. Such techniques are applicable to meats research. This is not a new idea by any means, for Kjeldahl over 70 years ago proposed the use of an enzyme as an analytical tool (invertase for sucrose determination).

Until a few years ago work done on connective tissue was almost entirely confined to the approaches of classical histology and morphology. Thus descriptions of connective tissue fibers, tendon, cartilage, the scales and fins of fish, the fibrils in earthworm cuticle had little or nothing in common. In more recent years the outlook about the nature of connective tissue has been substantially unified by the discovery of a limiting molecular framework for the edifice of connective tissue. This knowledge has come to us from those who have used x-rays and the electron microscope to examine the architecture of connective tissue and from chemists who have analyzed connective tissue. Notwithstanding, we are still abysmally ignorant about connective tissue as a biological entity and its role in meat tenderness.

It is certainly proper to state that the main burden and responsibility of research done on connective tissue must have its setting in the procedures and results of extraction. The necessity of disentangling and of fractionating connective tissue into its component parts is obvious if we are to come to any useful conclusion about its architecture and identifying reactive characteristics. Yet it is in this endeavor that we can come to grief all too quickly. There is little to be gained from investigations done on isolated fractions of connective tissue so altered and modified by unselective and drastic extraction or fractionation procedures that all we really have is tissue garbage to work with. It occurs to me that the use of selective enzymes reactive towards the various components of connective tissue offers a more

exciting and exacting approach. Studies of the biosynthesis of connective tissue would provide a marvelous approach to an understanding of the nature of the various forms of these tissues, for it would provide information about how they are elaborated and given their final form. The present view is that such studies lie beyond current research horizons. The alternative approach to an understanding of the properties of connective tissue is to use enzymes which would degrade it in specific ways.

There are many descriptions of elastin--morphological, histological and chemical; all show it to be heterogeneous. What we would like to know is its biological properties and how they are associated with its chemical structure. Many biological entities are enormously complex chemically; yet they may possess but one specific activity, for example, a protease which splits the peptide bonds of protein. Let us consider briefly what has been learned about elastin from enzymatic studies.

The biochemical properties of elastin have been discussed by several workers who have studied the effect of bovine pancreatic enzymes on elastin. Banga and Balo (1957) and Hall and Gardiner (1955) have shown that the enzymes of beef pancreas act on the outer amorphous mucoprotein as well as on the inner fibrous component of elastin in rather specific ways. Their work introduced the possibility that the outer casing of elastin acts as a stabilizer in the sense of a protective sheath against the action of the enzyme which degrades the inner or fibrous portion of elastin. Partridge and Davis (1955) have shown that one of the components of the elastase enzyme complex is a proteolytic enzyme. Banga and Balo (1956) succeeded in the isolation from pancreas of two mucoproteinases which split specifically the mucoproteins bound to connective tissue but which do not digest the polypeptide chain. One of these shows its greatest specific activity on the elastomucin component of elastic fiber; the second enzyme splits one component of collagen fiber. These mucoproteinases usually occur as contaminants in elastase preparations. Banga and Balo prefer that the name of elastase be reserved for the proteolytic enzyme which dissolves elastin fibers and with good reason.

If we can assume that tenderness of meat can be associated at least in part with connective tissue, it seems rather obvious that we need to know something about the factors which determine the composition of connective tissue and its reactivity in relation to nutrition, age, and the biochemical events which describe changes occurring post mortem. It has been shown, for example, that the mucoprotein composition of collagen fibers varies according to age.

While my experiences in this area of research interest does not qualify me to be authoritative, I am convinced about the promise held in the biochemical approach. The use of enzymes as analytical tools has been established as a powerful adjunct to research done on animal tissue. If there is anything to be said in a concluding way, it is that the meats worker has been much too conservative in his approaches to problems he would like to solve.

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University of Illinois, Urbana.

MR. SCHWEIGERT: Joe is a real scholar, isn't he?

Al Pearson has been pioneering in many ways. Those of you at the AMIF meetings may have heard about some opening wedges that he and his associates are putting into the flavor research field and they are also getting into some work in which enzymes may broaden the horizons in meat research. I should like to call on Al at this time to share a few of these thoughts with us. (Applause)

MR. A. M. PEARSON: Thank you, Bernie.

Members of the Conference: I am afraid you have greatly over-rated our work on enzymes, Bernie. Our interest in enzymes has been strictly in relation to another problem and, therefore, we are certainly not qualified as experts.

I might state that when I start to think about the subject of enzymes my thoughts go back to the great old enzyme chemist, J. B. Sumner, whom I had the good fortune and the misfortune, perhaps, to take a class under while in graduate school. The story used to go around that three boys went over and took the course together and that if you added their scores together they never had 100. (Laughter)

But I remember very clearly J. B. Sumner's story of urease which he told in this class. Urease, as you may or may not know, was the first enzyme that was catalyzed -- I think that this is one of the outstanding properties of our enzymes, one thing that we can think well of, and I presume that another is that they can be crystallized and give us the possibility of studying them and their properties in pure solutions or relatively pure solutions.

There was a lot of bitterness in J. B. Sumner. He crystallized urease in 1926. I went to Cornell in 1946 and he had just been awarded the Nobel prize for the work that he had done 20 years earlier and he had kind of mellowed and you never had to add more than one person's score to mine to make it 100. He felt rather good now, but he did tell this story in class. I remember distinctly that he stood up and he always looked at the ceiling as he lectured, and he said that he had crystallized this

enzyme and when it came time for the Nobel prize to be awarded one of the members of the committee, a German, said that it could not be crystallized and that as a result of this he was withheld recognition by the Nobel Committee until 20 years had elapsed and this man had died or he would never have received such recognition.

The moral of that story is if you cannot whip your enemies maybe you can outlive them.

But J. B. Sumner told how he had submitted the article to journal after journal and was turned down on the basis of this man's claiming that enzymes could not be crystallized, and in conclusion Sumner said that he submitted the article to the Journal of Education, which he said was a second rate journal, and they graciously accepted it for publication and so he took a lifetime subscription.

So if you ever have trouble publishing something that you think is worthwhile, I think you might reflect back on his experience.

I think probably one of the classical pieces of work on meat enzymes was done in the U. S. Department of Agriculture about 1918, and it was published, I believe, in Miscellaneous Publication by Hoagland, McBride and Boak. For this study they had a very difficult time in obtaining sterile meats so that they could actually follow the enzymatic changes. I think it is quite an interesting study and it would well bear looking into, if you are interested in this problem.

More recently, Pluton, at Harvard, has been very prolific in the field of enzymes which cause the natural catalytic changes and breakdown of the tissue after death. Of course, they are very closely allied to the problem which we are interested in. At the time that I took biochemistry the cathepsins were classified all into one group. Nowadays they are classified into about four or five different fractions and each one of them is specific and acts only upon certain substrates, as Mr. Sliwinski has brought out; so therefore this problem has become more complicated as we have learned more about it. This makes it somewhat more difficult to attack it.

Mr. Sliwinski indicated that certain enzymes may be undesirable, and I think I would like to elaborate a little on that, because we are interested in some of the flavor changes that take place during irradiation. I think that during the early history of the irradiation projects of the Quartermaster Corps they failed to recognize such changes. Most of the meat was irradiated in the raw state. It was held at temperatures for long periods of time and during these periods of time we got a natural breakdown of the tissue due to enzymatic changes, not sterile but still these enzymatic changes went on.

Perhaps this method may be a method of following the changes that occur. We can sterilize it much easier than the early workers could when they did their work. We have found, in our work at least, that amino nitrogen continues to increase very rapidly at room temperature with natural meat enzymes. We have recently initiated a study and we

have been adding cathepsin, that is, the crude cathepsin, the whole cathepsin, not trying to isolate its different components, and we find that we still get increases in the amino nitrogen which are even beyond those when you just have the raw meat; so that this indicates that this is quite a problem in research and one that we could well expand to find out just what is happening here.

More recently at the AMIF meetings a rather interesting problem was posed by some flavor chemist from the Evans Research Corporation in New York City. He mentioned the fact that each of the plants has certain enzymes present in its tissues. If you take the flavor enzyme, for example, in the cabbage, you can develop the flavor in the cabbage by adding an enzyme from mustard, but if you add the cabbage enzyme back to the cabbage you can greatly increase the amount of flavor you will get. It seems that the natural enzymes help to develop the flavor better than when you use enzymes from another source. I think that this is of some significance and certainly would concern us so far as flavor work is concerned in meats.

I think that the situation in the use of commercial tenderizers well needs some explanation and some thought. Currently there is a considerable amount of this going on in the industry, but Mr. Schweigert is much better qualified to tell you how much and what it means at this time than I am. All I know is -- well, I understand there is a lot of feverish activity and we do know that there is a lot of interest in enzymes in industry. Some of it, as I understand it, is being fed, and in some cases it is being injected. There are various other approaches. Some of it is by infusion into cuts. I hope that Mr. Schweigert, when I have sense enough to sit down in a minute or two, will elaborate on some of these developments and the knowledge that has been gained.

However, I want to throw out one more thing. Much of our work has been done on the basis of added enzymes. I recently visited a small meat establishment in East Lansing. I had visited them two years ago but I had not been back since, and I was surprised to find them preparing large amounts of steaks and selling them very cheap made from low grade carcasses dipped in enzymes and the steaks were sold in eating houses at a price that people would pay. It seems to me that natural meat enzymes is an approach that we might well think about.

Mr. Sliwinski also mentioned activators and inactivators. We have certain activators and certain inactivators as he so ably showed us and I think that these might possibly bear exploring in the meat industry.

Thank you. (Applause)

MR. SCHWEIGERT: I thought I was just to moderate the discussion, but he threw me a curve there. Before commenting on that I should like to throw out a couple of other comments. I hope you have seen that Mr. Sliwinski has developed the broad base of this whole discussion of new research approaches to enzymes, and Mr. Kastelic and Mr. Pearson have indicated some specific applications and opportunities as

we see the broad basis for research in meats become more widely used and thought of on these levels, but I should like to throw out a couple of others.

You know that meat was muscle in a living animal, and much research on experimental animals dealing with enzyme chemistry and physiology can be very useful in concept and, in fact, when you begin to think about the ramifications of all of these enzymatic changes in a meat cut, a piece of meat is not dead from the standpoint of the respiration and catalytic processes that are going on starting with rigor mortis and one can learn a great deal.

Joe Kastelic touched on collagen. I should like to emphasize that there is much activity in collagen diseases in man and a lot of very excellent enzyme work is going on which we can draw on in meats research. Some of you may not have thought to look in those places, if you are interested in mucopolysaccharides or mucoproteins as substrates or connective tissue substrates in some of the work you are doing.

I had better handle this little challenge from Al before throwing the floor open for general discussion on meat tenderizing enzymes.

We have been doing our best to assist in developing some fundamental principles by which such enzymes might be used on a more sound basis than an empirical system. George Wellington got the full treatment on the empirical system this noon. I believe that about 10 of us had enzyme tenderized steaks in this hotel, but I guess Lyman Bratzler was smart enough to order something else. The rest of us thought the steaks were fairly good for \$1.50, but there is much to be done in getting this on a better basis. Many smaller companies without the technical know-how may be using them and not in the best way, but it is a real development that you can help with and develop better because it is very important.

There is much experimental work on injecting enzymes and other technics, none as yet in commercial production in interstate commerce. As you know, the U.S.D.A. have approved the use of enzyme dipped procedures prior to freezing and distribution through their meat inspection division.

May I also remind you of the discussion by George Wilson last year on high temperature beef aging in which accelerating these natural enzymes by aging at higher temperatures was also mentioned. This ties into the discussion that Al Pearson referred to of enhancing the inherent activity of these enzymes, and also the discussion by Mr. Wang, of our staff, here in previous years, pointing out the specific modes of action, structural and on the taste panel of certain of these tenderizing agents, these again being experimental.

I hope that we have achieved our objective with the committee's report; that is, to broaden the potentials in your minds of where meat research can go in your own institutions. Also some of the people you may wish to contact if you are considering developing some of this research.

I would caution you to look with a rather critical eye on some of this enzyme work relating to flavor enhancement. Again it may be quite empirical and need very careful attention before news releases become realities and it is something we should watch.

Mr. Chairman, I think we have a few minutes for general discussion by the members of the conference and I will ask these experts to comment.

MR. ADAMS: I should like to know if you would want to venture a guess as to the amount of commercial tenderizing agents that is being used.

MR. SCHWEIGERT: Well, I suppose I might attempt to handle this one. This information is not available to us or to anyone else. We have been asked a great many times. The best public statement I have seen was in the January 6 issue of the Wall Street Journal in which it was stated, I believe, 20,000 gallons of meat tenderizing enzymes are used per month. Is that right? Does anybody remember that specifically?

MR. JACK T. GESLER (Polytechnic College): Either the last issue of Food Engineering or one of the subsequent issues made that statement plus the fact that \$10 million per year is spent for this material.

MR. SCHWEIGERT: That is the other figure that is available, that approximately this amount is being sold. You can appreciate that companies are not now quite so reluctant to state that they are using these for their steaks from strip loins of cows of commercial grade. But the volume of these enzyme tenderizers is somewhat of an empirical thing anyway because the enzymes are not pure and the solution is formulated differently in different places. It is very small in the sense of the total beef production but quite significant with certain processor relationships with restaurants, etc., small establishments I think in the main.

MR. HENRICKSON: Bernie, is it a common thing to call it a flavorizer rather than a tenderizer among the commercial trade?

MR. SCHWEIGERT: Are you thinking of those that are being sold as meat tenderizers?

MR. HENRICKSON: Yes, the Flame down there on State Street refers to it as a flavorizer rather than a tenderizer.

MR. SCHWEIGERT: That is a new one on me. I have not seen anybody that stated on the menu or otherwise that they had a really special steak.

MR. HENRICKSON: I won't say it was on the menu but when we asked him about it he said it was a flavorizer.

MR. SCHWEIGERT: That is news to me.

How about any of you men?

MR. KASTELIC: They call it sodium glutamate.

MR. SCHWEIGERT: That is something different, of course. People's understanding of what is done is in need of great improvement. Sometimes the restaurant itself is doing it. I should mention this. They are not all bought from the packing plant, frozen and delivered to the restaurant.

I did not expect to get into this. We have three experts up here on enzymes. Does anybody have any new enzyme research or some problems or some concepts that he is developing at his institution that he would like to share with us briefly?

MR. HALL: I think that Mr. Sliwinski mentioned various possibilities of the use of enzymes as analytical tools. One of the things that we have been trying is selective use of enzymes for separation of various protein fractions, the protease which will attack one type of protein and avoid another. We have been attempting to use that to isolate or to disentangle connective tissue by a protease that will attack the other protein fractions in the meat and leave the connective tissue undisturbed.

This is one of the great difficulties in the attack on the collagen problem, that is, to isolate it from the other protein, particularly the water-soluble fractions which will eventually wind up with the gelatin fraction and give a high value for your exposed collagen.

MR. SCHWEIGERT: Excellent, Mr. Hall. Bob, would you like to add anything to that?

MR. SLIWINSKI: No, I don't think so. The only thing I can say is that this is a really good method, because I think right now the only way they are separating them is by histological methods, just separating the individual fibers.

MR. SCHWEIGERT: I could add that in some of our work histologically we have had some degree of specificity of certain enzyme preparations on either elastin or collagen or the muscle tissue fibers. But Mr. Hall is pointing out one of our problems, that the enzyme preparations we have are by no means pure and, therefore, the activity of any given preparation isn't all on one and none on the other.

Would anybody else like to make a comment?

MR. DEATHERAGE: I have no comment. I would just like an elaboration of a remark made by Mr. Sliwinski, questioning the usually accepted explanation for dark cutting. I should like to know what he is questioning.

MR. SCHWEIGERT: Well, you have your head in there now, Robert.

MR. SLIWINSKI: I know that this has been brought up with me before, but as I see it in a dark cutter you have less lactic acid produced; therefore, the pH remains high, and because of this high pH you also have -- in other words, let us put it this way: in dark cutters, I should not say in general but ordinarily you find a sticky texture to the meat, and the consensus is that it is due to the higher pH rather than to some other factor which would be plausible, because increased pH with these proteins would make them more soluble and they would get that sticky feeling. This has been refuted by a number of people and that is why I mentioned it was questionable.

MR. SCHWEIGERT: We don't always agree in our own laboratories as to what is going on in dark cutting beef, which is probably healthy. Fred, would you like to throw in a little philosophical explanation of it?

MR. DEATHERAGE: I am just asking for information, and I am afraid I am not getting it.

MR. SLIWINSKI: I am afraid I don't have it either.

MR. SCHWEIGERT: I believe that we are ready to turn the meeting back to Vern, and I want to thank these men and the other members of the committee for their fine suggestions. I think it was a very worth while program. Thank you. (Applause)

CHAIRMAN CAHILL: We thank you.

Should we take a stretch now?

(Recess)

CHAIRMAN CAHILL: I am grateful to you for your promptness in returning after these breaks. Before we get into the program, George Wilson, are you prepared at this time to make announcements concerning the short course?

(Mr. Wilson made some announcements concerning the short course.)

CHAIRMAN CAHILL: Thank you very much, George.

Going to the Personnel Committee, we are approaching a committee that probably has more contact with all of us throughout the year than any other group of the conference. I can assure you from personal observations that this has been a very active, hard working committee and I was also cognizant of the fact that at least one member of the committee has gone all out in order to make news for the next News Letter, when I read that letter from Jerry Wanderstock a little while ago.

Now I should like to turn the discussion on personnel activities over to Professor Larry Kunkle.

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