The purpose of adopting histological methods in meat research is to explore and establish relationships between the structure and quality of meat and its by-products. With a microtome, certain indispensable biological stains and a microscope, the histologist prepares, examines, and compares the detailed structure of muscles and other materials either in their normal state or under experimental conditions. His methods of analysis sometimes may be qualitative, but more often quantitative or both, depending on the nature of the problem. For example, in an extensive study undertaken by several divisions at the Foundation not long ago, characteristic structural differences associated with carcass grade and weight were both qualitatively and quantitatively appraised. Such histological entities as the muscle fiber diameter, muscle fiber bundle size, fat, collagen and elastin content from thousands of samples were determined by methods (1,2), which were considered at least no less reliable than corresponding chemical methods of determination. In fact, the histological techniques possess the unique attribute of being capable of preserving the distributional patterns of any measured element whether it is fat, collagen or elastin.

Similarly, we have studied beef and pork casings, pointing out their basic structural differences and disclosing the function of each of the operating processes in casing preparations (3,4). The hog dehairing process was experimentally studied and the adverse effect of overscaling was structurally demonstrated (5). The histological manifestation of polyoxyethylene monostearates (bread-softener) and of vitamin B_{12} deficiency on the vital organs of the rat were determined (6,7,8). Cooked beef was studied for the fate of fat with some interesting results (9). In short, the basic histological technique renders itself well to any situation requiring the examination of the structure of tissues or organs.

A second basic histological technique involves the isolation of muscle tissue into its components and their subsequent individual experimental treatment. The isolation is accomplished by beating up instead of cutting up small pieces of meat in a Waring blender at a much reduced speed and with a completely dulled knife (12). I will devote the rest of this talk to the problem of tenderness and discuss with you some of the results obtained with both these histological methods.

About two years ago we started a program of studying the effect of proteolytic enzymes on the structure of meat. Our primary assumption has been that the structural alterations brought about the action of enzymes might provide a basis for evaluating the potential tenderizing properties of these enzymes, thereby enabling us to better understand the real nature of tenderness (11).

One of the first results obtained from the isolated muscle fibers was a demonstration of the muscle fiber membrane, a compound structure
consisting of an inner ultra-thin sarcolemma and an outer fibrous envelope immediately surrounding the former (13, 14). Furthermore, it was shown that the true sarcolemma is obtainable only from raw tissue. But when meat is cooked or/and chemically fixed, then the sarcolemma and the fibrous envelope become separated together from the muscle fiber protein upon blending. This separation is due to a shrinkage of the actomyosin as a result of moisture loss. The significant thing is that the sarcolemma and the envelope both are not affected in any way by either cooking or aging. It may be inferred from this that these structures might have something to do with tenderness as will be presently shown to be so.

We have developed a procedure for measuring "muscle fiber extensibility" in isolated single muscle fibers. This property represents an additional distance to an initial 5 mm. length of the fiber, at which the fiber breaks under the tension. It has been shown previously by us (12) that this property is not only related to carcass grade and weight, but is actually correlated with tenderness rating of the cooked meat, i. e., the greater the muscle fiber extensibility, the less tender is the meat. Furthermore, it was conclusively shown that muscle fiber extensibility decreased during aging and increased on cooking.

In order to further establish a relationship between muscle fiber extensibility and tenderness, single muscle fibers were immersed in an enzyme solution and immediately afterwards, their extensibility was determined. It was shown that the enzyme treatment had reduced their extensibility. Moreover, the degree of this reduction appeared to be visibly correlated with the extent of structural degradation which the fibers had undergone, i. e., the more highly degraded a muscle fiber is, the less extensible it would be. Coupled with these results, our data show that frozen-dried beef steaks after being rehydrated in a suitable concentration of an enzyme which is known to affect the muscle fiber protein were rated more tender than control steaks not treated with an enzyme. These findings have given us a working hypothesis that tenderization may be achieved through decreasing the extensibility of muscle fibers.

For treating whole muscle tissue with an enzyme we have developed a technique for quick uniform penetration by rehydrating pieces of frozen-dried steaks in an aqueous enzyme solution (10,11). Enzymes or enzyme preparations tested this way have revealed other specific effects on the muscle tissue components. For instance, Age-it, a preparation containing salt, papain and a hydrolyzed vegetable protein is capable of disintegrating the sarcolemma and the muscle fiber envelope and reducing the endomysial collagen into a granular material; the muscle fibers, on the other hand, were not affected to any appreciable degree. Beef steaks treated with Age-it or with enzymes known to attack connective tissues of meat were consistently rated higher in tenderness than the controls. Thus, it appears that another way of achieving tenderization is through the breakdown of the muscle fiber membrane and the endomysial collagen filling the spaces between the muscle fibers.

Other enzymes tested have indicated very potent action on the connective tissues, collagen and elastin, and much less, if any, on the muscle fibers. Papain, Bromelin and Picin, all of which are of tropical plant origin, belong to this class. Any one of these, when applied to steaks in exceedingly low concentration, was shown to be capable of
tenderizing the meat. However, mushiness can easily result from a slight over treatment. There is no doubt that a third way of achieving tenderization is through hydrolysis or gelatization of the perimysial connective tissues, consisting of relatively larger amounts of collagen.

We plan, next, to apply enzymes directly to connective tissue elements as well as to the muscle fiber envelopes in the same manner as the isolated muscle fibers referred to above. There can be no more direct way than this approach of obtaining information with regard to the specific effect or effects of an enzyme on each of the definitive tissue components. It is hoped that these results coupled with those obtained from whole tissues treated with these enzymes will eventually lead us to a better understanding of the mechanism of tenderization and also provide better practical means of tenderizing lower grades of meat to meet the rising demand.

REFERENCES


CHAIRMAN PEARSON: We want to thank you, Dr. Wang, for your very interesting presentation.

Dr. Breidenstein will lead the discussion.

DR. BREIDENSTEIN: Thank you, Mr. Chairman.

I should like first of all to thank our two speakers for their very excellent presentations of their respective papers, and to compliment our Chairman this year on his recommendations of speakers.

Taking the papers in the order in which they were presented, Dr. Ayres' paper certainly should promote an awareness of sources of contamination of our products. We certainly have become more aware of the numerous sources of such contamination and the possibilities for multiplication and growth of microbial flora.

I should like to direct to Dr. Ayres a couple of subjects for comment. I don't know whether I can put them in the form of
questions or not. I should like to hear his comments on the use of antibiotics in the preservation of our normal meat animals, both intraperitoneal induction and infusion. Also I would like to hear his comments on the claims of the people who manufacture the Turbo-Chill cooler in that they contend that they can keep meat for such an extended period of time.

DR. AYRES: Well, in reverse order, I don't feel that I am equipped to say much about the Turbo-Chill cooler as yet. But concerning the use of antibiotics on meat, they have one problem there that is a little different from its use on chicken. With chickens you can destroy all of the antibiotics by heating the product afterwards, and with meat the way that some of us eat it -- and especially the way the group I was with last night were eating it -- it is little more than warmed up. So that there would be the problem of the residual antibiotic that would be present in the meat if it were injected into the animal.

Some evidence is being accumulated to indicate that the breakdown of the tetracycline is such that these products are harmless, and when that material appears in the literature, if and when it does, I think that one might have a little different view concerning its use.

I would, however, wonder about the use of any preservative or any antibiotic unless it was necessary to improve and prolong the storage life of the product and otherwise it would be impossible. I would not in any sense condone the use of an antibiotic where it was a substitute for cleanliness.

DR. BREIDENSTEIN: Are there questions that anyone would like to direct to Dr. Ayres?

Dr. Wang's paper certainly again demonstrates the possibility for at least another approach to some of the subjects that were covered yesterday, namely, the important one in the quality of meat and even perhaps the most important single factor contributing to quality, namely, tenderness. It seems that these studies may eventually point the way to major technological advances in meat processing and, as he mentioned, may make some of our lower grade products much more acceptable to the consumer.

Do you have any questions to direct to him?

CHAIRMAN PEARSON: I should like to ask Dr. Wang concerning the enzyme preparations he used, whether he attempted to standardize them as to activity or just exactly how he handled the situation.

DR. WANG: So far we have been using per cent solutions. In other words, enzymes supplied to us by the different manufacturers. Each enzyme is labeled and the units are given according to the chemical assay methods that they determined in their own plants and at our own laboratory we have not tried to standardize them.
For instance, I used, say .015 of a solution for those immersions of isolated single fibers, and so on. Those concentrations have been carefully tried. If you use too high a solution those muscle fibers will go completely. A critical range is established by the trial and error method. We have been spending time determining the range that is usable for determination of the fiber extensibility, and then you have to determine the concentration range for actual use on the steaks. We have not so far but we may in the future attempt to standardize the potency of those enzymes.

DR. BREIDENSTEIN: Are there other questions?

MR. WANDERSTOCK: What are your current recommendations relative to the use of tenderizers?

DR. WANG: We have quite a bit of data on these things, but I don't know. We have tried a dozen or more different enzymes. You noticed that I grouped the three that are derived from proteolytic enzymes, Papain, Bromelin and Ficin, since their properties are very similar. I am speaking only of frozen dried steaks because the concentrate will be very different if you use it on raw meat. We have evidence that when you use the enzyme on frozen dried it gets into the tissue because the steak loses three-quarters by weight on dehydration. If you want to apply those enzyme values I will give you a recommendation of .002 per cent for those tropical enzymes. That is very dilute. But on raw tissue it is an entirely different problem. The reason we have not used raw tissue in this program is simply the fact that we do not get even penetration.

If you cut sections of a piece of raw meat treated with the enzyme by immersion and you get emulsion on the surface, indicating that the enzyme has over-acted on the surface but in the middle you get nothing or very little, there is a gradient of the enzyme penetration. So that for this fundamental study we definitely wanted to apply the enzymes to frozen dried meat.

We also tried other sources of enzymes, largely, say, from rhizyme 11, rhoyzme 48 and protease 15, all derived from fungi, and their properties are different from the Papain, Bromelin and Ficin. Their action seems to be more on the muscle fiber protein and little or nothing on the connective tissue. In between you have some other enzymes that are fairly gradual, in that they act relatively more on the fibers and less on the connective tissue and at the opposite end you have enzymes that act more on the connective tissue and much less on the fibers and you have all the gradients in between.

DR. BREIDENSTEIN: We have time for one more question, and we have two men with their hands up, Bob and Lyman. I think we will take Bob.

MR. HENRICKSON: I presume that it would be much more desirable if we had natural enzymes to use rather than artificial enzymes.
DR. WANG: By natural you mean of animal origin?

MR. HENRICKSON: Right.

DR. WANG: Such as from the pancreas.

MR. HENRICKSON: What progress have we made toward isolating the enzymes from meat and using them?

DR. WANG: We have made no attempt to isolate enzymes from meat. You can obtain it in crystal form from wheat, but we have not used wheat. The only natural enzyme that we have used is a product called Viocase, which is a whole pancreas powder. We used it the same way and we didn't find it as desirable as some of the enzymes that are of pancreas origin. We have tried it but without any too fine results.

MR. HENRICKSON: Have you tried actually injecting actin or myosin?

DR. WANG: We have not done anything on it. These are all applied to samples of meat.

DR. BREIDENSTEIN: I think we will turn this back to our chairman at this time. Thank you.

CHAIRMAN PEARSON: We wish to thank you, Dr. Breidenstein, for leading the discussion, and the gentlemen who have participated in the Research Methods Committee's program. Also we particularly thank the committee. They set up a very fine program which was enjoyable for all of us.

(Following announcements, the meeting recessed at 12:10 o'clock.)