Histology deals with the study of the microscopic appearance of cells and tissues. So the study of the histological structure of meat involves preparation of sections thin enough to be studied microscopically, and the observation and interpretation of the results obtained.

Histological technics have been in use for many years, but it is only within the past 10 or 15 years that these technics have been applied to observation of material from the food standpoint. The earlier work dealt principally with anatomical details. I propose to review for you some of the basic technics, and discuss briefly the types of results which can be obtained.

The principal steps in histological study consist of killing and fixing the tissue, solidifying it in some way to permit cutting it into very thin slices, cutting the sections, staining and mounting on slides, and studying the results. Fixing the tissue is usually necessary in order to stop the activity of the cells and preserve the material for future study. Ten percent formalin (4% formaldehyde in water) is frequently used for killing and fixing. The tissue blocks can be preserved in formalin for several months if time does not permit immediate preparation of the slides. However, for some studies the material cannot be handled in this manner. In Dr. Beard's work, where he was studying the distribution of mobile fat droplets (not fat deposits) within and around the cells, he found it necessary to work with unfixed material and prepare the sections immediately. If one wishes to study glycogen distribution in the muscle tissue, it is necessary to fix the blocks in 95% alcohol, and the tissues must be sectioned within a short time as prolonged exposure to higher concentrations of alcohol will shrink and harden the tissue so that it is extremely difficult to section.

There are three common technics for embedding or hardening the tissue so that it may be sectioned. When you realize that for most problems it is necessary to cut the sections 10 microns (1/2500 inch) or less in thickness, you will understand that ordinary slicing with a sharp knife will not do.

Paraffin embedding consists of dehydrating the tissue, infiltrating with paraffin, and embedding in a block of paraffin. Dehydration may be carried out in a number of ways. The most common methods are to use (1) a graded series of alcohols of increasing concentration, (2) dioxane, or (3) chloroform. The point is that the water must be removed from the tissue, and replaced with a solvent miscible with paraffin, so that when the tissue is placed in paraffin, the paraffin will penetrate all the way through, not just form a framework around the tissue. If this happens, the tissue will differ in hardness from the paraffin, and will not cut properly on the microtome.

The microtome is a device for cutting very thin sections and consists principally of a very sharp knife coupled to a device for advancing the block any desired distance after each section is cut. This enables one to cut uniform sections of only a few microns thickness.
Paraffin blocks are usually cut on a rotary microtome. In this device, the block moves while the knife is stationary. Since the paraffin sections will adhere to each other, it is possible to obtain the sections in ribbons. This makes it possible to make serial sections through a whole block of tissue if one wishes to observe changes with depth. Another advantage is that the paraffin blocks can be preserved almost indefinitely. Also, the paraffin sections can be mounted on the slides and then stained, which is usually easier than staining the sections and then mounting them.

There are also some disadvantages to the paraffin method. The solvents used to dehydrate and infiltrate the tissue dissolve out the fat, so this technic is not suitable for studies of fat distribution. Also, the dehydration and the heat used in infiltration tend to shrink and distort the muscle tissues, so if one wishes to measure size of muscle fibers, for instance, another technic must be used.

The second technic for cutting tissues involves freezing the block with CO₂ and cutting on a freezing microtome. This microtome is arranged so that the knife travels across the block, cutting the section. As the knife is returned, the tissue carrier advances to raise the block for the next section. A connection to a CO₂ tank is provided so that CO₂ under pressure is released just under the tissue block to freeze it. The fixing solution must be thoroughly washed out of the tissue before freezing so that it will freeze evenly. Tissues for frozen sections may or may not be embedded. With cooked meat, embedding helps, as the tissue is so loose that the sections tend to disintegrate when put in water. Ten percent gelatin is usually used for embedding. In this case, it is not necessary to infiltrate the tissue completely, just to form a secure frame around the block. This can usually be accomplished by holding the block in the gelatin solution for an hour or so at a temperature just high enough to keep the gelatin melted. Prolonged exposure of the blocks to heat must be avoided, as this also hardens the tissue and makes it difficult to cut.

The frozen sections are removed from the knife with a small brush and placed in water. Then they may be mounted directly on slides and covered with celloidin, then stained, or they may be transferred through the stains in a small basket, then mounted after staining.

Slides prepared from frozen sections are well adapted to studies of fat distribution, size of muscle fibers, and such items. However, it would be very laborious to obtain serial sections. Also, much more handling of the sections is required than with paraffin sections, and this increases the difficulty as the sections are very fragile.

The third technic for preparing and cutting the blocks involves embedding in celloidin and cutting the sections on a sliding microtome similar in action to the freezing microtome, but without the freezing attachment. Celloidin embedding gives a firm block which is easily cut, and causes much less shrinkage and distortion than paraffin embedding. However, again it is difficult to obtain serial sections. Also, this method is very time consuming as it requires 10 to 20 days to properly infiltrate and harden the blocks.

The methods of staining and mounting to be employed depend on the things for which you are looking. To study just the muscle tissue, one of the hemotoxylin stains is usually employed. Harris haemotoxylin is the one we use, as it can be prepared and used the same day. However, it improves with age up to a certain point. Regular haemotoxylin must be aged 6 months to a
year before it can be used. For connective tissue, either Weighert's or Mallory's triple stain is usually employed. In the Weighert sequence, one uses haemotoxylin to stain the Nuclei blue, picric acid to stain the muscle tissue yellow, acid fuchsin to stain the collagenous tissue red, and Weighert's to stain the elastic tissue dark green. Mallory's stains give a color combination of red, orange and bright blue.

Sections for study of fat distribution are often stained with haemotoxylin and Scarlet R (Sudan IV). This gives blue muscle tissue and red fat deposits. Fat can also be stained with osmic acid fumes, if you wish to study material which cannot be soaked in any solutions. However, osmic acid is extremely poisonous, so must be used with caution.

The study of distribution of minerals usually involves micro incineration of the section on the slide, followed by micro chemical tests of the ash for the various minerals. It has been possible by this method to demonstrate the concentrations of various elements in the different regions of the cross striations of muscle.

The material to be used in affixing the cover slip is chosen in accordance with the final solution in which you have the sections. If they are in water, glycerine jelly is usually used. This is a solution of glycerine and gelatin in water and must be put on warm, as it sets on cooling. If the sections can be dehydrated, they usually are brought up through a graded series of alcohols into xylene, then mounted with Canada balsam or one of the synthetics such as clarite. These are solutions in xylene, and harden by evaporation of the solvent. This requires a longer time than does the glycerine jelly to set, but usually gives a more permanent mount. Some stains fade badly in glycerine jelly. On the other hand, if you wish to study fat deposits, the sections cannot be dehydrated without dissolving out the fat.

You will be interested in some of the uses to which these technics have been put, with relation to the use of meat as food. A number of studies have been made, and are being made, on the amount and distribution of connective tissue, and the changes in connective tissues which take place on cooling. These are being related to the tenderness of the various samples. Another area of interest is in relation to amount and distribution of fat as affected by factors such as feed, grade, age, sex, and method of cooking. The problem we are studying involves changes in histological structure as related to tenderness of meat with aging. Other studies have been concerned with size and number of muscle fibers per bundle, and size of bundles, as related to tenderness of various cuts. A number of studies have been done on the size and distribution of ice crystals in frozen meat.

I have tried to give you a very brief summary of the technics employed in making microscopic sections of meat, and the types of problems to which these technics have been applied. This is by no means a complete coverage of the field. The list of references attached gives some of the standard reference works in this field, and some articles to show the applications. Again, this is not a complete coverage. I have just tried to indicate some of the places one might start in acquiring information along these lines.

One very interesting study which should contribute materially to our information concerning connective tissue is the work now being done at M.I.T., where they are attempting to relate results obtained with the electron microscope and with X-ray diffraction patterns. Another area which may be
fruitful is the use of the phase microscope for studying unstained material. It is necessary to recognize the possibility that each additional step in preparing materials for observation may cause changes in the tissue from what it was originally. Therefore, a device which tends to eliminate any of these steps may yield results more indicative of the true state of the cells under observation.

Histological work is very slow and time consuming. It is not a tool suitable for use with studies of short duration. It requires considerable investment of time and effort, and also a fairly large investment of money for equipment. However, in work where there is a continuity from year to year, it may be profitably employed to study changes which definitely affect the palatability of meat, and which cannot be observed macroscopically. It enables one to get at some of the basic causes for the changes which occur in meat due to various treatments such as freezing, aging, and cooking, and due to production aspects such as feed, age and sex.

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CHAIRMAN BUTLER: Thank you very much, Dr. Paul.  

Professor Wanderstock will please conduct the discussion on this paper.

PROF. WANDERSTOCK: I am sure all of us enjoyed Dr. Paul's very fine review of the histological techniques involved in meat research. I think she did an excellent job of summarizing the methods of imbedding tissue, and the slides were certainly helpful to most of us.

There is a new method that she did not mention, the so-called "freezing-drying" method of tissue preparation which involves very, very little chemical change. She mentioned that all of these methods involve a degree of chemical change. The freezing-drying technique is one that involves very little chemical change.

I thought you might be interested in seeing some pictures of what the electron microscope shows, and so I have a reprint here of some English work that I will pass around, and you can see this while we discuss Dr. Paul's paper.

Do any of you have any questions you'd like to direct to Dr. Paul?

PROF. BLUMER: What method do you use in labeling muscle tissue after it comes out of the picture? It is rather difficult to know whether you can get a cross-section or not in the imbedding of the tissue.

DR. PAUL: When we are collecting our samples, we cut long strips about as big as my little finger, and put them right in formalin as we are cooking, or whatever the treatment is that we are doing. Then those are allowed to sit over-night, or 24 hours. They are then cut down to a smaller size. We tie them up in cheesecloth and put a label on them, and then they can be put in big bottles to save time and space. Otherwise, you are going to have your whole laboratory or all your space, covered with little bottles, because they accumulate terrifically.
Then that material is very easy to work with. It is good and firm, and from those strips we cut our blocks before we start imbedding them. We cut very carefully either along or across the fibers. It takes considerable training to learn just how to do it, and then, of course, you look at the first section, and if it did not turn out to be what it is supposed to be, you cross it out and start with another block.

We routinely collect sufficient material so that we can go back and start over again several times, if we need to, because you can get into all kinds of difficulty. If you do not have something to go back to, then it is not too easy to go back and start with another animal. It is a good idea to get plenty while it is there, and then you have something to work with.

PROF. BULL: Professor Wanderstock, several of us are just poor, dumb meat men, and a few of us do not hesitate to show our ignorance at times, but what is a Phase microscope.

DR. PAUL: Well, I have only seen one, and I will try to tell you about it. It is so arranged that the light comes up underneath the stage through a very small, very carefully defined circle. It is sort of on the order of a dark field microscope, but the light comes through this little circle, it hits your material, and then everything is blocked out except just this one special ray. Up and above they have another circle. Anyway, something about the optics of the system puts the light out of phase, so that part of it comes through it at one peak or point in the wave, part of it comes through at another point, so that you get your contrast, your dark and light, in your material without having to stain it.

You see, you stain it so you can see it, and this means of putting the light out of step in some way so that you can see it just with the light. You do not have to have any stains in it. The Phase microscopes are fairly new. They have just come out in the last couple of years. I think both Bausch & Lomb and Spencer have them now, and they have a lot of information on how they work. I am not a good enough physicist to know.

PROF. BULL: Thank you. Nelson tells me he has one.

PROF. WANDERSTOCK: Just one other thing on the Phase microscope: It actually enables us to see structures that we could not see with the ordinary light microscope. In other words, it brings out some of the very minor constituents; things that we have been calling artifacts; things that we cannot describe and we guess at, and it has enabled the histologist to actually see what those are.

PROF. NELSON: I have a question on that with reference to the ability to get your light through your tissue sections. I have used the Phase microscope in connection with protozoa and micro-organisms, but there is a question in my mind as to the thickness of the tissue and the amount of light you can get through in order to get differentiation.

DR. PAUL: I have not seen any work where it has been applied to meat. I do know they have techniques for cutting sections for a tenth of a micron.

PROF. NELSON: That certainly is thin enough.
PROF. WANDERSTOCK: Thin and transparent.

PROF. NELSON: That is what I wanted to find out.

DR. PAUL: I do not know how to do it, but there are articles in the literature asserting that it can be done.

PROF. WANDERSTOCK: Are there any other questions? I think we are running a little late, and so I will turn the meeting back to Professor Butler.

CHAIRMAN BUTLER: Thank you.

Now, we will have a paper on "Observations on Chemical Methods Used in Meat Research", by Dr. Dan Brady of Missouri. Dr. Brady.

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