A Histological Study of the Various Tissue Components of Raw and Cooked Longissimus Dorsi Muscle in Pork

Dorothy E. Deethardt

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A HISTOLOGICAL STUDY OF THE VARIOUS TISSUE COMPONENTS
OF RAW AND COOKED LONGISSIMUS DORSI
MUSCLE IN PORK

BY

DOROTHY E. DETHARDT

A thesis submitted
in partial fulfillment of the requirements for the
degree Master of Science, Major in Food
and Nutrition, South Dakota State University
1966

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A HISTOLOGICAL STUDY OF THE VARIOUS TISSUE COMPONENTS
OF RAW AND COOKED LONGISSIMUS DORSI MUSCLE IN PORK

This thesis is approved as a creditable and independent investigation by a candidate for the degree, Master of Science, and is acceptable as meeting the thesis requirements for this degree, but without implying that the conclusions reached by the candidate are necessarily the conclusions of the major department.

Thesis Adviser

Date

Head, Food and Nutrition Department

Date

1-20-66

1-20-66

1-20-66
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INTRODUCTION

Consumer demand for lean pork has changed breeding, management and nutritional programs in hog production. Resultant changes in body composition of the hog may be responsible for the higher frequency of soft, watery pork and a less tender pork. The consumer has found this meat less acceptable.

There is no entirely satisfactory and reliable indicator for determining meat tenderness while the animal is in the feed lot nor can it be determined in the meat market. Other avenues of approach must be undertaken to evaluate tenderness.

Muscle fibers and connective tissues are known to affect tenderness. The partitioning of these two structures was first attempted by Lehman as early as 1897 (Wilson, et al., 1954). Since that time chemical and histological work has been done on beef muscle to study the quality and quantity of the connective tissue. Only recently has the connective tissue of pork muscle been examined extensively to relate tissue components to tenderness.

This study was undertaken to evaluate: (1) the effect of fixatives on (a) the reticular, collagenous, and elastic connective tissue of raw and cooked pork muscle and (b) the fiber diameter in the raw state; (2) the type and amount of connective tissue in pork muscle and the effect of heat on the various components; (3) the influence of weight, breed, and sex of hog on the various connective tissues in pork muscle.
This study is a portion of a larger investigation involving several aspects of pork quantity and quality evaluation.
It is reasonable to assume that the sensory evaluation of meat is influenced by the physical and chemical properties of each of the components, their relative proportions and distributions. Muscle fiber characteristics, connective tissue constituents, and degree of marbling are some of the factors of muscle tissue that have been studied in relation to eating quality. Sensory testing includes tenderness, juiciness, and flavor. Of these, tenderness seems to be the most important to the consumer.

For many years the quality of tenderness has been associated with connective tissue, particularly collagen and elastin. Relatively little attention has been given to the reticular tissue and ground substances.

**Characteristics of Connective Tissue**

Connective tissue binds the many tissues of the body together. It is composed of a network of non-living intercellular substances and living cells. These cells, fibroblasts and mesenchymal cells, aid in the production of fibers and ground substance, the non-living intercellular portions. These intercellular substances may be classified morphologically as follows (Ham, 1957):
The amorphous intercellular substances (ground substance) are carbohydrate in nature and of a jelly-like consistency. These substances were thought to have little or no effect on the tenderness of meat until the recent work of McIntosh (1961). She found that the ground substance contained mucoproteins (conjugated proteins containing a carbohydrate group) which may influence the relationship of connective tissue to meat tenderness.

More attention has been given to the fibrous or formed type of connective tissue because of its great tensile strength. The collagenous tissue or (white fibers) range from 1 micron to 12 microns in thickness and varies greatly in length. Collagenic fibers have the appearance of being cross-striated under an electron microscope, due to regular axial periodicity, thus giving an effect of a corrugated contour.

The reticular fibers are more delicate and are generally supported by collagen. They show the same axial periodicity as the collagenic fibers but are much smaller in diameter, and form a fine network by branching out in all directions. The nature of the protein is similar to that of collagen but the fibers react quite differently to histological stains.
Elastic tissue (yellow fibers) offers the most resistance to chemical and physical changes. The fibers are long, narrow and homogeneous in comparison to collagen. Elastic fibers stretch, contract and branch to give greater elasticity. They do not show an ordered structure in electron micrographs.

Methods of Studying Connective Tissue

The connective tissue content of muscle may be estimated chemically by a partitioning technique followed by a nitrogen determination of a hydrolysate. This method has limitations in that the distributional pattern of the connective tissue fibers, the fiber size and the physical properties brought about by physiological and functional changes are lost. Another chemical technique is the digestion of the collagenic fibers by pepsin in an acid solution, which are very resistant to any breakdown by trypsin in an alkaline solution. Reticular fibers resemble the collagenous fibers by resisting digestion with trypsin in an alkaline medium, but unlike collagen they do not swell or break down in a dilute acid medium. Elastin may be analyzed as the insoluble portion after exhaustive extraction with dilute acid or alkali following chemical determinations for the collagenous material (Maximow and Bloom, 1957).

A more recent chemical determination of collagen is the photometric-histochemical determination which shows distributional patterns of the connective tissue as well as the percent volume of the collagenous fibers (Kirkner and Auerback, 1960). By this method the
stained collagen can be measured for its light transmittance and simultaneously can be classified into structural groups.

Wilson, et al. (1954) made a study by chemical analysis of collagen and elastin in the longissimus dorsi of 88 beef carcasses. They found considerable variation in the collagen and elastin content within grades of each age group, but no significant difference among grades. There was no apparent difference between the old cows and steers, but both groups contained less collagen and elastin than veal. Veal is thought to be a more tender meat than that from more mature animals. This would seem to indicate that the percentage of collagen and elastin in the longissimus dorsi is not a critical measure of tenderness of meat. Wilson, et al. advanced the theory that the absolute amount of connective tissue remains relatively constant throughout life, and that the small connective fibers coalesce into stronger fibers with age and become more tough and less affected by heat during cooking.

Minner, et al. (1955), using nine muscle samples from various locations, made histological studies on 52 beef animals of varying ages and grades. They evaluated the size, distribution and frequency of the elastic and collagenic fibers. Their study indicated that there were more and larger fibers of elastin in the more frequently used muscles than in the less used ones. Collagen was rated about the same in all muscles, with a looser network of fibers where there were more fat deposits. They concluded that connective tissue did affect
tenderness, but that the function of many inter-related factors was involved and the control of just one would have little effect on the tenderness of meat.

Histological Methodology

Fixatives: There is no single ideal fixing reagent. A fixing agent which adequately preserves one substance may dissolve another, or it may be a good mordant for one element and interfere with the staining of another desired element in the same sample. A 10% formalin solution is a general preservative but often needs additional materials for special mordanting purposes. Acetic acid is a good preservative but has a tendency to cause swelling in the tissue. Alcohol rapidly dehydrates tissue and causes shrinkage. Picric acid penetrates well and fixes small objects rapidly but must be processed within 1 or 2 days. Chromates are good fixatives for certain tissues such as nerve tissue and adrenal glands, but the fixation time is very short.

Carpenter, et al. (1963) reported that picric acid (1.5%) was superior to 10 percent formalin for fixation of muscle tissue because of less distortion and tissue shrinkage and greater speed of fixation. They found that reaction time was critical and varied with the structure of the muscle.

Staining: The basic methods for demonstrating collagenous and reticular connective tissue involve various combinations of stains, such as silver impregnation, acid aniline dyes in acidic solutions,
periodic acid-Schiff method and phosphomolybdic or phosphotungstic acid-Hematoxylin. Staining methods used for elastic fibers include Weigert's (Gray 1954), Puchter and Sweat's (1960) resorcin-fuchsin, and Krutsky's (1962) permanganate resorcin-fuchsin.

Lewis and Jones (1951) developed a staining technique for demonstrating reticulin, collagen and elastin in the same section. It was based on the use of silver for reticulin, orcein for elastin and picro-aniline blue or fast green for collagen. Humason and Lushbaugh (1960) modified the Lewis and Jones procedure which resulted in a better defined and more easily interpreted stain for the three connective tissue components. In this procedure pyridine was used to extract the lipids and to improve silver impregnation. Mercuric chloride was used as a mordant but had to be cleared of any deposit. The clearing was done with an iodine-alcohol solution, followed by a sodium thiosulfate wash. Lillie (1954) suggested a five minute bath of 0.5% iodine in 80% alcohol in place of the usual 80% alcohol step.

In the Humason and Lushbaugh method periodic acid replaced the potassium permanganate oxidation used by Lewis and Jones. The former proved to be more selective in its action and produced a better histological picture of connective tissue. Even the smallest fibers were well defined.

A modification of Foot's (1929) ammoniacal silver carbonate solution was used for sharp impregnation of the reticular fibers. A
buffered 5% formalin solution was used to prevent complete reduction of the silver (Humason and Lushbaugh). According to Davenport (1960): gold toning made the sections more transparent and controlled the final intensity of the stain.Treating collagen with phosphomolybdic acid before aniline blue strengthened the specific staining reaction. Oxalic acid combined with the aniline blue intensified staining of the collagen. The final treatment with glacial acetic acid increased the transparency and enhanced the brilliance of the stain.

**Connective Tissue and Meat Tenderness**

Methods used to study beef tenderness have given less than desirable results. Lowe and Kastelic (1961) found little relationship in tenderness between age and fatness of beef animals. Cover (1959) attempted to score tenderness of cooked meat with the three components of connective tissue - softness, friability and tenderness. She reported that one sensory score for tenderness was inadequate in attempting to account for the chemical and physical variations. Later Cover, et al. (1962a,b,c) made up a theoretical framework of components of tenderness related to the physical and chemical variations in muscle tissue as follows: amount and kind of connective tissue, softness factors of feel on the tongue and cheek, tooth pressure, ease of fragmentation across the grain, mealiness and apparent adhesion between fibers.

The thicker backfat covering has been thought to indicate more marbling in the muscle. Murphy and Carlin (1961) found that the degree
of marbling increased only slightly as the thickness of backfat increased. The amount of backfat on the carcass did not have a significant effect on the tenderness, juiciness, or flavor of the longissimus dorsi. Batcher, et al. (1962) obtained similar results but found also that muscles with high marbling scores had more intramuscular fat and less moisture. Tenderness and juiciness, however, were not related to the marbling score or to the intramuscular fat content. Saffle and Bratzler (1959) indicated a positive, significant relation between backfat thickness and palatability, while Weir, et al. (1962) showed a relationship between fat content as a whole (external, intermuscular and intramuscular fat) and the tenderness and juiciness in broiled pork chops. Some histological studies have been made of the fat distribution in both beef and pork. Wang, et al. (1954) studying the effect of heat on fat of broiled beef, postulated that the fat droplets dispersed along the path of the degraded collagen gave the effect of sensory tenderness. Carpenter, et al. (1963) making histological observations on the longissimus dorsi of pork from five age groups of animals, found no significant difference between tenderness measurements and total connective tissue. The kind and amount of connective tissue did affect tenderness. The young animal had less elastic tissue but the older carcasses contained significantly thicker and coarser collagenic fibers. He also worked with pork loins of varying intramuscular fat content and theorized that the fat content within
the connective tissue, between muscle bundles and within muscle bundles, served as a "lubricant" for the fibers and enhanced the juiciness and tenderness of the cooked muscle. He concluded that many underlying differences are responsible for the complexity of tenderness.

Clark and Mullins (1962) believed that the cellular constituents of connective tissue played an important role in meat tenderness. They studied the mast cells which are found in groups about blood vessels and in connective tissue. These cells have a water holding capacity which may influence tenderness by the number of cells present. Connective tissue from the *longissimus dorsi* was examined for the number of mast cells in relation to shear value, and for the degree of degranulation. Freshly slaughtered animals and seven day aged carcasses with high and low shear value were studied. No significant difference in mast cell numbers could be detected.

**Effect of Cooking on Connective Tissue**

Upon heating, the collagenous fibers first swell, then shrink and finally disintegrate to a gelatin-like substance or animal glue. The gelatinous material is not a factor in tenderness but the residual collagen in the connective tissue may be. In some areas heat treated fibers seem to merge or fuse, tending to appear straighter and less distinct than unheated fibers. Stability to heat has been studied in purified collagen by Ritchey, et al. (1963) to determine thermal shrinkage, or the temperature at which contraction of the collagenic fiber occurs. The thermal shrinkage of collagen may explain the
tightening of connective tissue around a muscle during cooking. This is observed in the curled appearance of broiled chops and steaks, and the plumpness of oven roasts. Any relation of the thermal shrinkage of collagen within muscles, either to the loss of collagen or the tendering of connective tissue, is not yet clear.

The influence of heat on connective tissue has a direct effect on the eating quality of the product. Winegarden, et al. (1952), found that both collagen and elastin were softened when heated in water at sufficiently high temperature. Cover, et al. (1962a) studied the tenderness of connective tissue in 2 beef muscles heated to 61°, 80° and 100° C. At all three temperatures the panel rated the connective tissue in the longissimus dorsi muscle as tender. In the biceps femoris muscle the connective tissue was rated tough at 61° and 80° C but tender at 100° C.

Tuomy, et al. (1963) used six different internal temperature intervals, ranging from 60.0° to 98.9° C, on steaks from inside rounds of canner/cutter grade carcasses. The initial effect of heat on the meat was toughening in direct proportion to the increase in temperature up to 71.1° C. As the temperature was increased to 82.2° C, a tenderizing effect was noted in direct relation to both the amount of time and the increase in temperature.

Shear versus heating time curves set up by Draudt, et al. (1964), while studying the semitendinosus and longissimus dorsi of beef, indicate an increase in shear value in direct proportion to temperature.
increase from 40° to 50° C. They suggested that the toughening reaction may be related to the cooking loss observable in this range. In the temperature range of 56° to 59° C, there was a marked decrease in shear value which appeared to be related to the collagen shrinkage reaction. Another decrease in tenderness was noted at the 65° to 75° C increment of temperature with a "hardening" reaction not related to cooking loss. The second tenderizing factor became apparent at this higher temperature and with a longer heating time.

Wier, et al. (1963) worked with pork roasts cooked at four oven temperatures (148.9°, 162.8°, 190.6° and 204.4° C) and two internal temperatures (76.7° and 85.0° C). They found that these treatments had no significant effect on tenderness and flavor but did affect juiciness and yield.

Heat does not appreciably alter the structure, the staining affinities or the physical properties of elastic fibers. This may explain some of the inability to correlate meat tenderness with cooking procedures. Collagen yields gelatin on boiling, but it is not known whether the reticular tissue does or not. If it can be established that it does, the importance of reticular tissue to meat tenderness may be more clearly defined. The yield of gelatin from the collagenous fiber is in relation to the time and temperature used for cooking the meat.

**Muscle Fiber Diameter**

One of the characteristics of meat which is thought to influence tenderness is muscle fiber diameter. A muscle fiber is difficult
It is generally agreed that there is an increase in the diameter of muscle fiber with the increase in age of an animal, and with changes in animal nutrition. Studies are not in agreement on diameter difference between fibers of diverse muscles. Satorius and Child (1938) report little variation in average fiber diameter of four muscles (triceps brachii, adductor, longissimus dorsi and semitendinosus) from thirteen animals. While Hiner, et al. (1953) found significant diameter differences among fibers of nine muscles studied.

Tuma, et al. (1962) worked with the longissimus dorsi and semitendinosus muscles from 33 beef animals of five different age groups. They reported a gradual increase in fiber diameter within the longissimus dorsi muscle with increasing age of the animal. The fiber diameter of the semitendinosus muscle was found to increase during the earlier months of growth but leveled off with age. The effect of fiber diameter on tenderness appeared to be an animal-age-fiber diameter relationship. That is, the muscle fiber increased in size with age of the animal and tenderness decreased with an advance in animal age. Whether this same relationship holds true for pork muscle is not known, as the animal reaches the slaughter age much earlier.

Muscle fiber diameter in the longissimus dorsi of the pork loin was studied by Carpenter, et al. (1963). They reported that muscle
fiber diameter was affected by the age of the animal and was associated positively with the thickness of the connective tissue and bundle size. The older animal produced a larger muscle fiber. There was a trend for increased fiber diameter with the increase of muscular fat content. They also observed that the panel tenderness score decreased as the maximum fiber diameter increased in the longissimus dorsi muscle.
EXPERIMENTAL PROCEDURE

Experimental Material and Design

The pork loins used in this study were from 32 hogs of known history, produced and slaughtered at South Dakota State University in the summer of 1962. There were eight animals in each of four weight groups (150, 180, 210, and 240 pounds). Two breeds were represented, Duroc and Yorkshire-Hampshire cross, with equal numbers of barrows and gilts in each weight group.

The carcasses were boned and trimmed according to the procedure of Fletcher (1964). The backfat was trimmed to 0.25 to 0.30 of an inch. Chops were cut 1.2 inches thick by measurement.

All data were transferred to IBM cards and subjected to the analysis of variance on the electronic computer. Duncan's (1955) multiple range test was applied to determine differences among means. All correlations were calculated on a within weight group basis.

Histological Technique

Fixative:

Raw Sample: Chop 3 (Figure 1) of the anterior end of the loin was used for raw histological studies of reticulin, collagen, and elastin in connective tissue. A one-inch core, taken from the center of the longissimus dorsi muscle of the chop, was cut into four slices. One of the slices was put in each of three different fixatives. The fourth slice was put in one of the three fixatives at
Figure 1. Boned pork loin. Chop 3, raw histological study; chop 4, cooked histological study; chop 5, cooked tenderness evaluation.
random. The fixatives used were:

I. 10% formalin
II. F. A. A.
III. 10% formalin and saline solution

All formulas are given in the appendix.

The samples were allowed to remain in the fixative for 24 hours, then drained and fresh fixing solution added. The sample jars were well capped and placed in the refrigerator until ready for additional fixing and dehydration.

The extra sample or fourth slice was used for preliminary work. The one-inch core slice was again reduced by the use of a half-inch core. This sample, with its identifying number penciled on a small strip of bond paper, was carried through the procedure in a polyethylene tissue capsule.

Additional fixing materials help to provide a linkage or bond between a tissue component and a dye, or increase the affinity of a tissue for a stain (Davenport, 1960). Salts of mercury and chromium (Zenker's fluid or Helly's fluid) are often used for additional fixing. These two solutions plus saturated mercuric chloride were tried in the preliminary work. The color from the chromate of Zenker's fluid seemed to dull the colors in the stained section. Saturated mercuric chloride gave a more uniform and better differentiation of color in the connective tissue than when stained without the additional fixative.
Fixing procedure: Fresh sample into fixative 24 hours
Drain, add fresh solution Refrigerate
(4.5°C. until ready for additional fixative. Minimum 24 hours)
Saturated mercuric chloride 5 hours

Cooked Samples: By random selection, paired and adjacent chops, No. 4 and 5 (Figure 1), were heated to three different internal temperatures (37.8°, 65.6° and 85.0° C). Right and left chops of the same animal were cooked to the same temperature. Three animals were selected from each weight group for each temperature. Chop 4, after cooking and cooling, was sampled for histological work as previously described. Only one fixative was used, that of 10 percent formalin with a minimum of 18 hours fixation time. A section from chop 5 was trimmed out of the longissimus dorsi muscle for tenderness evaluation on the L.E.W. Kramer shear press. The force necessary to shear the sample was recorded on an electronic recorder. The area or work load was measured with a planimeter in square centimeters, divided by the weight of the sample and multiplied by 100, giving the force to shear a 100 gram sample. The lower the value the more tender the meat.

Dehydration:

Raw Sample: The dehydration of muscle tissue is important in setting the pattern for proper embedding. Alcohol is a good dehydrant, but may cause the tissues to harden and the sections to crumble. The timing of the alcohol baths is critical, however, tissues may be left in a 70% alcohol solution without damage to the material.
Following a five hour additional fixation period in saturated mercuric chloride, the dehydration method of Venable (1962) was used:

- 50% ethyl alcohol 1 hour
- 70% " overnight
- 80% " 1 hour
- 90% " "
- Absolute " "
- " and xylene 1:1 v/v ½ "
- Xylene 1 "
- Xylene and tissuemat ½ " (Fisher tissuemat M. P. 52.0°C.)

Cooked Sample: The samples from cooked meat are more granular and are more delicate to handle, therefore excessive dehydration has to be avoided. A dioxane method (Paul, 1963) for dehydration of muscle tissue and connective tissue was used on the cooked sample. Because dioxane is poisonous the work was done under an exhaust hood.

The cooked tissue sample was put in a capsule and placed in distilled, deionized water overnight to remove the formalin. Distilled, deionized water was used throughout the procedure wherever distilled water was indicated. From the water bath the procedure was as follows:

- Dioxane I 1 hour
- Dioxane II "
- Dioxane III 1½ "
- Dioxane and tissuemat 1:1 v/v ½ " (Fisher tissuemat M. P. 55.0°C.)

Embedding

Raw Sample: Care was used just to melt the embedding tissuemat and thus avoid distortion by over heating of the tissue at the time of impregnation. Tissuemat with a melting point of 52.0°C
was used throughout the processing of the raw tissue samples. The embedding process was started with the xylene and tissuemat mixture and it was in this media that the sample was held over night. The xylene-tissuemat mixture was carefully melted. The tissue capsule was taken from the mixture and placed in a jar of melted tissuemat M. P. 52.0° C with a cork and fittings for light vacuum and a capillary tube for the air flow. The sample was processed for six hours. Only one bath was needed with this method as the xylene was drawn off by the vacuum and air wash.

**Cooked Sample:** The embedding process (Paul, 1963) for cooked samples was as follows:

<table>
<thead>
<tr>
<th>Bath</th>
<th>Tissue Mat</th>
<th>M. P.</th>
<th>Time</th>
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<tr>
<td>Bath I</td>
<td>Fisher tissuemat</td>
<td>55.0°C</td>
<td>½ hour</td>
</tr>
<tr>
<td>Bath II</td>
<td>&quot;</td>
<td>&quot;</td>
<td>1½ &quot;</td>
</tr>
<tr>
<td>Bath III</td>
<td>&quot;</td>
<td>&quot;</td>
<td>2½ &quot;</td>
</tr>
<tr>
<td>Block</td>
<td>(Bond paper boats)</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

The higher temperature tissuemat (M. P. 55.0° C) was used for embedding the cooked tissues because these samples were not as apt to be distorted by the heat of the tissuemat. The first bath of tissuemat replaced most of the dioxane and therefore was discarded each time. The third bath was always fresh tissuemat.

**Blocking:** Blocking was done in bond paper boats. Fresh melted tissuemat of the same melting point as that used for embedding was poured into the boat. The embedded tissue was placed in position with warm tweezers; the boat then filled with the tissuemat was quickly cooled in ice water. As it cooled, the identifying number was placed
on the top so that it could be read after hardening had occurred. The blocks were stored in a refrigerator.

**Sectioning:** The hardened tissue block was removed from the paper boat, and trimmed with a sharp blade or knife so that just a small rim of tissue was left around the tissue. Trimming of the block was then done on the microtome so that all the tissue was exposed. It was found desirable to rehydrate the trimmed block of tissue in water in the refrigerator. A rehydration time of 30 minutes for the raw tissue and 1 hour for the cooked sample was used.

With the Bausch-Lomb sliding microtome, sectioning to 4 microns was possible although ribboning was not. The sections were cut and transferred to the water float individually. Occasionally a very crumbly block was cut at 10 microns to get a section to mount. In this way no samples were completely lost.

**Raw Sample:** After rehydration, the blocks were again adjusted to the microtome. Sections were cut and floated out on a water bath of 45 to 47°C, and then mounted on clean slides (75 x 25 mm). A drop of Mayer's albumen was placed on the slide, spread, and wiped smooth with the side of the thumb. The mounting medium was allowed to dry a few minutes. Then prepared slide was dipped into the water float at an angle so that the desired section could be guided onto the slide with a dissecting needle and positioned. Two sections were mounted on each slide and three slides were made for each sample. The slide was placed on a heated drying tray with the heat regulator set
to hold the temperature between 37 and 40°C. When the moisture had disappeared, the slides were placed flat on trays in a drying cabinet to completely dry before staining.

**Cooked Sample:** Freeze sectioning was tried on the fresh cooked sample but a mounting medium was not found that would withstand the long staining process. The embedded cooked samples, cut with the Bausch-Lomb sliding microtome, disintegrated when put into the water float. Mounting was accomplished by placing sections directly on the air-dried albuminized slides. These were placed on the drying tray set at 46.0°C. With a camel's hair brush, a drop or two of distilled water was placed on the section to cause it to spread out naturally.

**Section Evaluation:** The effects of the different fixatives used were evaluated as the embedded blocks were sectioned. Numerical ratings were used to indicate ease of cutting on the microtome, the way the section could be transferred to the float and how it floated out. The numerical values used and the descriptive terms in determining the values were as follows:

<table>
<thead>
<tr>
<th>Term</th>
<th>Value</th>
<th>Sectioning Evaluation</th>
</tr>
</thead>
<tbody>
<tr>
<td>Best</td>
<td>6</td>
<td>Six out of seven sections usable for neat and easy mounting.</td>
</tr>
<tr>
<td>Exceptionally Good</td>
<td>5</td>
<td>More than seven sections needed for neat mounting.</td>
</tr>
<tr>
<td>Very good</td>
<td>4</td>
<td>More sections needed than above for neat or satisfactory mounting.</td>
</tr>
<tr>
<td>Good</td>
<td>3</td>
<td>Many sections required for satisfactory mounting.</td>
</tr>
<tr>
<td>Fair</td>
<td>2</td>
<td>Sections of 4 microns only after brushing with water.</td>
</tr>
<tr>
<td>Poor</td>
<td>1</td>
<td>Sections of 10 microns only after second rehydration.</td>
</tr>
</tbody>
</table>
Staining:

Raw Sample: Time adjustment for the various stains was necessary in order not to over or under stain the desired tissue to be studied. Steps to prepare the tissue for effective staining and timing were adjusted. The detailed staining procedure, similar to Humason and Lushbaugh (1960), was as follows:

1. Xylene I 3 minutes
2. Xylene II dip to wash clean of any paraffin that might be left in the tissue
3. Absolute ethyl alcohol 1 minute
4. 90% " 1 minute
5. Pryidine and 90% ethyl alcohol 1:1 v/v 15 minutes
6. 90% ethyl alcohol 2 to 3 seconds
7. Running water 5 minutes
8. 0.5% iodine 1½ minutes
9. Wash in running water
10. 5% sodium thiosulfate ½ minute
11. Running water 5 minutes
12. 0.5% periodic acid 15 minutes
13. Running water 5 minutes
14. Distilled water 37°C. 5 minutes
15. Ammoniacal silver 37°C. 1½ hours (fresh each day)
16. Ammoniated distilled water 2 to 3 seconds
17. Buffered formalin 5 minutes (use fresh solution each time)
18. Running water 5 minutes
19. Gold toning 6 minutes
20. 10% sodium thiosulfate 5 minutes
21. Running water 5 minutes
22. Orcein 37°C. 20 minutes
23. 70% ethyl alcohol 2 to 3 seconds
24. Distilled water 2 to 3 seconds
25. Phosphomolybdic acid 15 minutes
26. Distilled water 2 to 3 seconds
27. Aniline blue 3 minutes
28. Distilled water 2 to 3 seconds
29. Acidulated water 5 minutes or more
30. 90% ethyl alcohol 1 minute
31. Absolute ethyl alcohol 1 minute
32. Xylene until covered
33. Cover glass
Using the preceding staining sequence gave the following connective tissue colors:

- Reticulin - black
- Collagen - blue
- Elastin - red

Glass staining dishes and carriers were used so 18 or 19 slides were stained at one time. The slides were sorted in such a way that only one slide from each set of three for a sample was stained on the same day.

**Cooked Sample:** The same staining procedure was used on the cooked sample as for the raw sample, omitting steps 8 through 11. It was found that mercuric chloride did not improve the staining qualities of the cooked tissue.

**Covering:** A No. 1 cover glass with a thickness of 0.13 to 0.17 mm was used as a cover slip. The samples were mounted with a non-water soluble mounting media, "Permoun," and allowed to dry before cleaning and marking for identification.

**Connective Tissue Evaluation**

The finished slides were evaluated on a microscope with a 10X eye piece and a low power objective (10X), or at a magnification of 100. Each section was evaluated at five points as shown in Figure 2, and each area was given a numerical rating for the amount of reticular, collagen and elastin in each field. The numerical ratings used followed that of Ramsbottom, *et al.* (1945):
Muscle Fiber Diameter Measurement

A small piece from the previously fixed raw (chop 3) sample of the longissimus dorsi was prepared for examination according to the procedure of Tuma, et al. (1962). The effect of the fixatives was studied on the muscle fiber and data were recorded as to the straight or wavy condition of the fibers and the length after teasing.

The fiber diameter measurements were recorded in units from an eyepiece micrometer and these units later converted to microns.

Photography

The photography was done on a microscope with an automatic camera attachment. The microscope had a 10X eyepiece, a 10X objective and one and one fourth inch diameter lens which gave a magnification of 125X. The film used was Black and White Panatomic X, fine grain, FX 135-20 and ASA of 40 (instrument setting 50).
DISCUSSION AND RESULTS

Histological Technique

The effect of three fixatives (10% formalin, F.A.A., and 10% formalin with saline) on the reticular, collagenous and elastic connective tissue of raw and cooked pork muscle was studied. A representative area of a section of tissue as evaluated under a microscope is shown in Figures 3 and 4. Magnification 125x.

Figure 3 shows an area from a raw section of the longissimus dorsi muscle, chop 3 (Figure 1), taken from a 180 lb. animal. The white line around the muscle fibers and the fat cells represent reticular fibers. The encircled dark spots are small fragments of collagen. The arrow in the right lower corner shows a small blood vessel with a dark line of elastin near the center of the vessel. This section was preserved in fixative II.

Figure 4 shows an area from a sample of meat heated to an internal temperature of 65.6°C. This sample was from chop 4 of a 240 lb. animal. Granulation of the reticular tissue where the fat cells had been is evident. Separation of the muscle fiber bundles with thin lines of reticulin around the bundles can be seen. The arrow indicates collagenous fibers which seemed to have cohered to form a thicker band between the muscle bundles. The preservative was fixative I with dioxane as the dehydrating agent.

Fixative Evaluation: The type of fixative used did not significantly influence the evaluation rating of the sections. Those made
Figure 3. Stained raw pork sample

Figure 4. Stained cooked pork sample
with fixatives I and II were rated good. Those made with fixative III were evaluated between fair and good.

**Muscle Fiber Diameter:** The effect of the fixatives on muscle fiber diameter was evaluated. The use of fixative II (FAA) resulted in a significantly smaller fiber diameter than occurred from using the other two fixatives (Tables 1 and 2). The dehydration effect of the alcohol in fixative II may have overshadowed the swelling effect of the acetic acid present. Saline helps to avoid dehydration and is reflected in the larger fiber measurement when fixative III was used.

Table 1. Analysis of variance for muscle fiber diameter.

<table>
<thead>
<tr>
<th>Source</th>
<th>df</th>
<th>MS</th>
</tr>
</thead>
<tbody>
<tr>
<td>Total</td>
<td>47</td>
<td></td>
</tr>
<tr>
<td>Fixative</td>
<td>2</td>
<td>413.80*</td>
</tr>
<tr>
<td>Side</td>
<td>1</td>
<td>101.62</td>
</tr>
<tr>
<td>Weight</td>
<td>3</td>
<td>1002.94**</td>
</tr>
<tr>
<td>Fixative X side</td>
<td>2</td>
<td>2.51</td>
</tr>
<tr>
<td>Fixative X weight</td>
<td>6</td>
<td>11.58</td>
</tr>
<tr>
<td>Side X weight</td>
<td>3</td>
<td>30.91</td>
</tr>
<tr>
<td>Fixative X side X weight</td>
<td>6</td>
<td>15.91</td>
</tr>
<tr>
<td>Residual</td>
<td>24</td>
<td>55.31</td>
</tr>
</tbody>
</table>

* * P < .01.
Table 2. Means of muscle fiber diameter x fixative

<table>
<thead>
<tr>
<th>Fixativea</th>
<th>Fiber diameter in microns</th>
</tr>
</thead>
<tbody>
<tr>
<td>III</td>
<td>61.12</td>
</tr>
<tr>
<td>I</td>
<td>56.98 b</td>
</tr>
<tr>
<td>II</td>
<td>51.00</td>
</tr>
</tbody>
</table>

a/ (I) 10% formalin (II) F.A.A. (III) 10% formalin and saline.
b/ Underlined means do not differ significantly.

Muscle fiber color differences were observed, with a darker color for fixatives I and II and a much lighter coloration in the fibers when fixative III was used. The difference in degree of hydration of fixative III may have caused the color variance. Dehydrated meat tends to turn darker in color, which agrees with these results. More short, broken fibers were observed when fixative III was used, making these samples difficult to measure.

Evaluation of Connective Tissue: In an analysis of variance for the numerical ratings of amounts of different types of connective tissue, more variance was found among animals in the amount of reticular than in the collagenous and elastic fibers present in the longissimus dorsi muscle (Table 3). This difference may not be of biological value due to the small error term. All numerical ratings of the amount of connective tissue on the finished slides followed the procedure used by Ramabottom, et al. 1945.

For the various connective tissue components, differences between sides of animals (Tables 3 and 4) were statistically significant. This may be due to the large number of samples and small
Table 3. Analysis of variance for the amount of reticular, collagenic and elastic connective tissue in raw pork muscle by animal, side and fixative

<table>
<thead>
<tr>
<th>Source</th>
<th>df</th>
<th>Reticulin MS</th>
<th>Collagen MS</th>
<th>Elastin MS</th>
</tr>
</thead>
<tbody>
<tr>
<td>Total</td>
<td>1151</td>
<td>11.45*</td>
<td>5.22</td>
<td>0.25</td>
</tr>
<tr>
<td>Animal</td>
<td>31</td>
<td>11.45*</td>
<td>5.22</td>
<td>0.25</td>
</tr>
<tr>
<td>Side/animal</td>
<td>32</td>
<td>1.73**</td>
<td>1.74**</td>
<td>0.12*</td>
</tr>
<tr>
<td>Fixative/animal</td>
<td>64</td>
<td>2.76**</td>
<td>2.81**</td>
<td>0.17**</td>
</tr>
<tr>
<td>Side x fixative/animal</td>
<td>64</td>
<td>2.28**</td>
<td>1.78**</td>
<td>0.15**</td>
</tr>
<tr>
<td>Residual</td>
<td>960</td>
<td>0.69</td>
<td>0.67</td>
<td>0.08</td>
</tr>
</tbody>
</table>

* P < .05.
** P < .01.

Table 4. Means for the amount of reticular, collagenic and elastic fibers x side

<table>
<thead>
<tr>
<th>Tissue</th>
<th>Side b/</th>
</tr>
</thead>
<tbody>
<tr>
<td>Reticulin</td>
<td>4.37 (2)</td>
</tr>
<tr>
<td>Collagen</td>
<td>2.44 (1)</td>
</tr>
<tr>
<td>Elastin</td>
<td>1.16 (1)</td>
</tr>
</tbody>
</table>

a/ Higher numbers indicate larger amounts
b/ (1) right side; (2) left side
error term. Biologically, the paired sides of each animal are considered equal in muscular structure and connective tissue components. However, some researchers have theorized that an animal may develop one side or the other by use, as in human development of right and left handed persons.

The three types of connective tissue were affected quantitatively by the fixatives used (Table 3). With fixative III a smaller amount of reticular and collagenic tissue was evident (Table 5) than when the other two fixatives were used. The saline solution of fixative III apparently did not dehydrate the tissues as much as fixative I and II or else staining was not distinct.

Table 5. Connective tissue x fixative interaction of reticular, collagenic and elastic fibers

| Tissue   | Fixative  
<table>
<thead>
<tr>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>A</td>
</tr>
<tr>
<td>Reticulin</td>
<td>4.38 (II)</td>
</tr>
<tr>
<td>Collagen</td>
<td>2.48 (I)</td>
</tr>
<tr>
<td>Elastin</td>
<td>1.19 (I)</td>
</tr>
</tbody>
</table>

a/ (I) 10% formalin (II) F.A.A. (III) 10% formalin and saline
b/ Underlined means do not differ significantly

The quantity of elastic fibers appeared to be significantly different in the several areas studied (Table 3). However, when the value of "1" represented "no elastin" in scoring the amount present in a section, the analyses were of little value. In other words, the
longissimus dorsi muscle has little elastin present. This coincides with the work of Carpenter, et al. (1963) who, while studying the connective tissue elements of the longissimus dorsi muscle in pork, found many carcasses in the 4 to 7 month age group devoid of elastin.

**Effect of Cooking on Connective Tissue**

Connective Tissue: The pan broiled chop No. 4, from 12 different animals, was used to study the amount of connective tissue. The same numerical score was used to determine the amount of connective tissue in the cooked sample as was used with the raw sample.

Heat treatment affected the reticular and collagenous fibers (Table 6). Reticular tissue appeared to have increased in amount as the temperature was elevated from 37.8° to 65.6° C (Table 7). As the temperature was increased to 85.0° C, or the well-done stage, the visible amount of reticular tissue then decreased quite noticeably. Draut, et al. (1964) theorized that the first increase in amount of reticular fibers could be explained by the observable cooking losses during the early cooking period, or that the tissue had swelled on initial heating. With the increase in temperature, the reticular tissue may disperse to a gelatinous material. This has been previously suggested but not established to be a true factor of disintegration of the tissue. The gelatinizing of the reticular and collagenic fibers with rising temperature may change the chemical structure of the fiber and decrease the staining qualities.
Table 6. Analysis of variance for the amount of reticular, collagenic and elastic fibers of cooked pork muscle

<table>
<thead>
<tr>
<th>Source</th>
<th>df</th>
<th>Reticulin MS</th>
<th>Collagen MS</th>
<th>Elastin MS</th>
</tr>
</thead>
<tbody>
<tr>
<td>Total</td>
<td>143</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Temperature</td>
<td>2</td>
<td>7.12**</td>
<td>14.57**</td>
<td>0.07</td>
</tr>
<tr>
<td>Weight</td>
<td>3</td>
<td>4.69**</td>
<td>11.76**</td>
<td>0.56**</td>
</tr>
<tr>
<td>Side</td>
<td>1</td>
<td>0.06</td>
<td>1.87</td>
<td>0.11</td>
</tr>
<tr>
<td>Temperature x weight</td>
<td>6</td>
<td>8.60**</td>
<td>1.47*</td>
<td>0.10</td>
</tr>
<tr>
<td>Temperature x side</td>
<td>2</td>
<td>0.36</td>
<td>0.05</td>
<td>0.08</td>
</tr>
<tr>
<td>Weight x side</td>
<td>3</td>
<td>0.61</td>
<td>1.80*</td>
<td>0.13</td>
</tr>
<tr>
<td>Temperature x side x weight</td>
<td>6</td>
<td>0.77</td>
<td>1.09</td>
<td>0.09</td>
</tr>
<tr>
<td>Residual</td>
<td>120</td>
<td>1.08</td>
<td>0.58</td>
<td>0.06</td>
</tr>
</tbody>
</table>

*P < .05.
**P < .01.

Table 7. Influence of heat on the amount of connective tissue of pork muscle

<table>
<thead>
<tr>
<th>Connective tissue</th>
<th>Temperature</th>
</tr>
</thead>
<tbody>
<tr>
<td>Reticulin</td>
<td>4.58 (2) 4.41 (1) b/ 3.84 (3)</td>
</tr>
<tr>
<td>Collagen</td>
<td>2.74 (1) 1.80 (2) 1.78 (3)</td>
</tr>
<tr>
<td>Elastin</td>
<td>1.08 (3) 1.01 (1) 1.01 (2)</td>
</tr>
</tbody>
</table>

\(a/\) 37.8° C. (2) 65.6° C. (3) 85.0° C.

b/ Underlined means do not differ significantly
The amount of collagen appeared to decrease as the temperature increased from 37.8° C to 65.6° C, then little change took place in the observed amount of collagenous tissue as the temperature increased to 85.0° C. It would appear that the collagen is dispersed at a lower temperature than the reticulin. Tuomy, et al. (1963) made similar observations on the study of connective tissue in beef.

Elastin apparently was not affected by heat as applied in pan broiling. Among the three temperatures used, there were no significant differences (Table 7) in the apparent small amount of elastic fibers present in the longissimus dorsi muscle.

The visible amount of connective tissue in the cooked samples was definitely related to the weight group of the animals from which the sample was taken. The means of the evaluated amount of connective tissue for both reticular and collagenic fibers (Table 8) indicate no difference between the 150 and 210 lb. weight classes, but the means are significantly larger in amount for the 150 and 240 lb. weight classes. There may be an age factor, as well as weight, involved in the amount of connective tissue and its development with animal growth.

The mean of the evaluated amount of reticular and collagenous tissue in the raw samples (Table 8), taken from the same animals as the cooked samples, followed a similar trend in the apparent amount of connective tissue. The 150 lb. weight class of animals had the most reticular and collagenic fibers evident and the 180 lb. weight
group had the least amount. In general the weight groups nearest 200 lbs. appeared to have less connective tissue than the lighter or heavier weight animals. This followed the findings of Wilson, et al. (1954) in relation to the amount of collagen and elastin in veal and beef. Veal had as much connective tissue as the older animal.

Carpenter, et al. (1963), working with the longissimus dorsi muscle of pork, found that the older carcasses, as compared to the younger, contained thicker and coarser collagenc tissue. He also noted that not many elastic fibers were found in this muscle. These findings coincide with this report in that elastin is apparently not present in the longissimus dorsi of pork except in the blood vessels.

The interactions of heating temperature by animal weight for reticular and collagenic fibers (Table 6) were significant (P = .01 and .05 respectively). Duncan's multiple range test of mean differences (Table 9) for reticular fibers showed a varied sequence in the amount of reticulin present. The interaction for the collagenous fibers (Table 9) followed a pattern for the lower cooking temperatures, but the animals from the 240 lb. weight group and the higher cooking temperature (85.0° C) had more collagen present.

The animal weight class by side interaction was significant (P = .05) for collagen (Table 6). As shown in Table 10 the animal side sequence was reversed for the collagenic fibers in the 180 and 210 lb. weight groups. This is unexplainable when sides are theoretically alike.
Table 8. Connective tissue x weight class interaction for the amount of reticular, collagenic and elastic fibers in cooked and raw pork samples (12 animals)

<table>
<thead>
<tr>
<th>Connective tissue</th>
<th>Weight classa</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cooked sample</td>
<td></td>
</tr>
<tr>
<td>Reticulin</td>
<td>4.78 (1) 4.28 (4) 4.09 (3) 3.95 (2)b</td>
</tr>
<tr>
<td>Collagen</td>
<td>2.35 (1) 2.24 (4) 1.76 (3) 1.57 (2)</td>
</tr>
<tr>
<td>Elastin</td>
<td>1.16 (1) 1.10 (4) 0.99 (3) 0.87 (2)</td>
</tr>
<tr>
<td>Raw sample</td>
<td></td>
</tr>
<tr>
<td>Reticulin</td>
<td>4.58 (1) 4.53 (4) 4.35 (3) 4.15 (2)</td>
</tr>
<tr>
<td>Collagen</td>
<td>2.66 (1) 2.55 (3) 2.30 (4) 1.97 (2)</td>
</tr>
<tr>
<td>Elastin</td>
<td>1.29 (3) 1.23 (4) 1.17 (1) 1.15 (2)</td>
</tr>
</tbody>
</table>

a/(1) 150 lb. (2) 180 lb. (3) 210 lb. (4) 240 lb.
b/ Underlined means do not differ significantly

**Objective Measurement of Tenderness**

Objective measurements were made on chop No. 5 to evaluate tenderness of the longissimus dorsi muscle in relation to the weight of the animals and the internal temperature of the cooked chop. L. E. E. Kramer shear values, as calculated from the two measurements on a Westronic recorder, indicated a highly significant difference among animal weight classes and internal cooking temperatures (Table 11).
Table 9. Temperature x weight class interaction for the amount of reticular and collagenic fibers in cooked pork

<table>
<thead>
<tr>
<th>Tissue</th>
<th>Temperature</th>
<th>Weight class&lt;sup&gt;a&lt;/sup&gt;</th>
</tr>
</thead>
<tbody>
<tr>
<td>Reticulin</td>
<td>37.8° C.</td>
<td>5.23 (4) 4.77 (3) 2.88 (2) &lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
<tr>
<td></td>
<td>65.6</td>
<td>5.43 (1) 4.40 (2) 4.40 (4) 4.07 (3)</td>
</tr>
<tr>
<td></td>
<td>85.0</td>
<td>4.57 (2) 4.13 (1) 3.45 (3) 3.22 (4)</td>
</tr>
<tr>
<td>Collagen</td>
<td>37.8° C.</td>
<td>3.82 (1) 2.80 (4) 2.40 (3) 1.95 (2)</td>
</tr>
<tr>
<td></td>
<td>65.6</td>
<td>2.73 (1) 1.80 (4) 1.52 (3) 1.13 (2)</td>
</tr>
<tr>
<td></td>
<td>85.0</td>
<td>2.13 (4) 2.00 (1) 1.63 (2) 1.35 (3)</td>
</tr>
</tbody>
</table>

<sup>a</sup> (1) 150 lb. (2) 180 lb. (3) 210 lb. (4) 240 lb.

<sup>b</sup> Underlined means do not differ significantly

<sup>c</sup> There is reason to doubt this low value

Table 10. Weight class x side interaction for the amount of collagenic fibers in cooked sample of pork muscle

<table>
<thead>
<tr>
<th>Tissue</th>
<th>Side</th>
<th>Weight class&lt;sup&gt;a&lt;/sup&gt;</th>
</tr>
</thead>
<tbody>
<tr>
<td>Collagen</td>
<td>right</td>
<td>3.58 (1) 2.38 (4) 1.73 (2) 1.57 (3) &lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
<tr>
<td></td>
<td>left</td>
<td>2.50 (1) 2.11 (4) 1.94 (3) 1.41 (2)</td>
</tr>
</tbody>
</table>

<sup>a</sup> (1) 150 lb. (2) 180 lb. (3) 210 lb. (4) 240 lb.

<sup>b</sup> Underlined means do not differ significantly
The shear values for the two heavier animal weights (Table 12) were significantly greater than the values for the two lighter weight groups of animals. The higher the shear value the less tender the meat.

Table 12. Means for L. E. E. Kramer shear values per 100 grams of cooked pork loin muscle x weight class

<table>
<thead>
<tr>
<th>Shear value</th>
<th>178.5 (3)</th>
<th>158.2 (4)</th>
<th>126.3 (2)</th>
<th>123.8 (1)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Weight a/</td>
<td>150 lb.</td>
<td>180 lb.</td>
<td>210 lb.</td>
<td>240 lb.</td>
</tr>
</tbody>
</table>

a/ Underlined means do not differ significantly
A significant change in muscle tenderness occurred with change in internal cooking temperature of the longissimus dorsi. Shear value was highest when the internal temperature of the meat was taken to 65.6° C, intermediate at 85.0° C and least at 37.8° C (Table 13).

The relation of animal weight and internal cooking temperature to shear values was investigated. As shown in Table 14, the meat from the 240 lb. weight class of animals reacted differently to heat in relation to tenderness than the meat from animals in the other weight groups. This may be due in part to the greater amount of reticular tissue which was evident at the 65.6° C internal temperature of the meat, and the possibility that the heavier animals have a thicker and coarser connective tissue which does not gelatinize readily at temperatures below boiling.

Table 13. Means for L. E. E. Kramer shear values per 100 grams of pork loin cooked to three internal temperatures

<table>
<thead>
<tr>
<th>Temperature</th>
<th>Shear value</th>
</tr>
</thead>
<tbody>
<tr>
<td>37.0° C</td>
<td>175.9 (2)</td>
</tr>
<tr>
<td>65.6° C</td>
<td>157.6 (3)</td>
</tr>
<tr>
<td>85.0° C</td>
<td>106.8 (1)</td>
</tr>
</tbody>
</table>

Muscle Fiber Diameter: There is a general feeling that the size of the muscle fiber has a direct relation to tenderness of the cooked product. The correlation between muscle fiber size and shear values of the longissimus dorsi was a positive 0.336 but it was not
Table 14. Weight class x temperature interaction for shear values per 100 grams of cooked pork loin

<table>
<thead>
<tr>
<th>Shear value</th>
<th>Weight</th>
<th>Temperature$^a$</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>150</td>
<td>185.0 (2)</td>
</tr>
<tr>
<td></td>
<td>180</td>
<td>168.5 (2)</td>
</tr>
<tr>
<td></td>
<td>210</td>
<td>207.5 (2)</td>
</tr>
<tr>
<td></td>
<td>240</td>
<td>224.5 (3)</td>
</tr>
</tbody>
</table>

$^a$ (1) 37.8° C. (2) 65.6° C. (3) 85.0° C.

$^b$ Underlined means do not differ significantly.

significant. Further work needs to be done in this area before conclusive results can be reported.

**Effect of Weight, Breed and Sex**

A study was made of the influence of weight, breed, and sex of hogs on the various connective tissues in the raw pork muscle, and of the effect these factors may have on the diameter of the muscle fiber.

*Connective Tissue:* The amount of reticular and collagenous tissue in the samples from the longissimus dorsi muscle was significantly different between breeds of hogs (Table 15). The apparent amount of collagenous fibers appears to be influenced by two factors, breed and sex. How much effect sex has on connective tissue has not been determined.
Table 15. Analysis of variance for the amount of reticular, collagenic and elastic connective tissue in raw pork muscle by weight, breed and sex

<table>
<thead>
<tr>
<th>Source</th>
<th>df</th>
<th>Reticulin MS</th>
<th>Collagen MS</th>
<th>Elastin MS</th>
</tr>
</thead>
<tbody>
<tr>
<td>Total</td>
<td>31</td>
<td>5.64</td>
<td>1.7</td>
<td>0.36</td>
</tr>
<tr>
<td>Weight</td>
<td>3</td>
<td>85.15*</td>
<td>27.45*</td>
<td>0.01</td>
</tr>
<tr>
<td>Breed</td>
<td>1</td>
<td>8.96</td>
<td>2.26</td>
<td>0.52</td>
</tr>
<tr>
<td>Sex</td>
<td>1</td>
<td>0.14</td>
<td>2.25</td>
<td>0.02</td>
</tr>
<tr>
<td>Weight x breed</td>
<td>3</td>
<td>7.77</td>
<td>5.95</td>
<td>0.24</td>
</tr>
<tr>
<td>Weight x sex</td>
<td>3</td>
<td>0.14</td>
<td>2.25</td>
<td>0.02</td>
</tr>
<tr>
<td>Breed x sex</td>
<td>1</td>
<td>2.96</td>
<td>17.94*</td>
<td>0.12</td>
</tr>
<tr>
<td>Weight x breed x sex</td>
<td>3</td>
<td>3.36</td>
<td>1.28</td>
<td>0.16</td>
</tr>
<tr>
<td>Residual</td>
<td>16</td>
<td>12.95</td>
<td>5.06</td>
<td>0.30</td>
</tr>
</tbody>
</table>

*P < .05

The amount of reticular and collagenous fibers was significantly greater in the Duroc breed samples under study than the Yorkshire-Hampshire cross (Table 16). This is an area that may warrant further investigation. Due to unequal numbers of animals used for the cooked portion of the study, tenderness differences between breeds was not analyzed.
Table 16. The amount of connective tissue in the raw longissimus dorsi of different breeds of hogs

<table>
<thead>
<tr>
<th>Connective tissue</th>
<th>Duroc</th>
<th>Yorkshire</th>
<th>Hampshire Cross</th>
</tr>
</thead>
<tbody>
<tr>
<td>Reticulin</td>
<td>4.59</td>
<td>4.05</td>
<td></td>
</tr>
<tr>
<td>Collagen</td>
<td>2.56</td>
<td>2.25</td>
<td></td>
</tr>
<tr>
<td>Elastin</td>
<td>1.16</td>
<td>1.16</td>
<td></td>
</tr>
</tbody>
</table>

Muscle Fiber Diameter: The muscle fiber diameter as measured in the samples was significantly (p < .01) affected by the animal weight classes as given in Table 1. The measurements indicated an increase in size of the fiber as the animal weight increased (Figure 5). These data are in agreement with Tuma, et al. (1962) from work with beef, and Carpenter, et al. (1963) from porcine muscles. Breed differences were not included in this study due to unequal numbers but may be an influential factor in the size of the muscle fiber.
Figure 5. Increase in muscle fiber diameter with increase of weight in pork muscle.
SUMMARY

A histological study was made of reticular, collagenic, and elastin connective tissue components and muscle fiber diameters of the raw and cooked longissimus dorsi muscle in pork. The effect of these factors upon muscle tenderness was also considered.

Under the conditions of the experiment, the type of fixative for the preservation of the sample was found to affect the quantity of stained connective tissue, the measurements of muscle fiber diameter, the color of the fibers and the amount of breaks in the fibers. The fixatives did not significantly affect the sectioning of the tissues.

Differences among animals were significant in the amount of stained reticular tissue but not in the amount of collagen and elastin. Differences between sides of animals were significant for all three connective tissue components. This is probably not of biological value. The amount of elastin in the longissimus dorsi muscle in pork was very small.

Heat treatment was found to affect reticular and collagenous fibers. The greatest amount of stained reticular tissue was evident in the meat when heated to 65.6°C internal temperature. A decrease in the visible amount of this tissue was evident when the meat was heated to 85.0°C. Upon heating the meat to 65.6°C, the amount of stained collagenous fibers was markedly decreased. A slight reduction
in amount occurred with continued heating. No significant difference in amount of elastic fibers due to heat was found.

Sections of cooked samples taken from the *longissimus dorsi* muscle from the lightest and heaviest weight classes of hogs had significantly more evident reticular and collagenous connective tissue fibers than the intermediate weight classes. The trend was similar for sections from the raw samples taken from the same animals.

L. E. E. Kramer shear values for the two heavier animal weight classes were significantly greater than values for the two lighter weight groups. For all except the heaviest weight class of animals, shear values were significantly higher when the meat had been cooked to an internal temperature of 65.6° C, intermediate in value when the meat was heated to an internal temperature of 35.0° C and lowest in value when the internal temperature was 37.8° C. The 240 lb. weight group was least tender (highest shear value) at the 35.0° C internal temperature.

The amount of reticular and collagenous fibers in the *longissimus dorsi* muscle appeared to be greater in the Duroc breed than in the Yorkshire-Hampshire cross.

Measurements of muscle fiber diameter indicated a positive correlation of fiber size and animal weight.
The results of this study indicate a need for further investigation of the dehydration and the paraffin embedding of raw pork samples for histological study of porcine tissue.

More information also is needed on the effect of heat on the connective tissue in pork in relation to tenderness, the effect of muscle fiber diameter to tenderness, and the effect of breed and sex differences on the amount of connective tissue.
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Cover, Sylvia, 1959, Scoring for three components to tenderness to characterize differences among beef steak. Food Research 24: 364.


Paul, Pauline, 1963, Personal communication, University of California, Davis, California.

Puchler, Holde, and Faye Sweat, 1960, Commercial resorcin - fuchsin as a stain for elastic tissue. Stain Technol. 35:347.


Venable, J. H., 1962, Personal communication, Oklahoma State University, Stillwater, Oklahoma.


APPENDIX

Reagents

Fixatives:

I. 10% formalin
   Formalin
   Distilled water
   10 cc
   90 cc

II. F.A.A. (formalin, alcohol and acetic acid) (Brauer 1958)
   70% ethyl alcohol
   Formaldehyde
   Glacial acetic acid
   90 cc
   10 cc
   2 cc

III. Saline solution (Venable 1962)
   40% formalin
   Distilled water
   Sodium acetate
   Sodium chloride
   10 cc
   90 cc
   2 gm
   2 gm

Additional fixative (Davenport 1960)

Helly's Fluid or Zenker's Fluid
   Potassium dichromate
   Mercuric chloride
   Distilled water
   2.5 gm
   5.0 gm
   100.0 ml

To this solution add 1 - 2 ml of formalin for each 10 ml of solution used (Helly's fluid). Or 0.5 ml of glacial acetic acid for each 10 ml of solution used. Add either the formalin or acetic acid at the time of using.

Saturated mercuric chloride
   Mercuric chloride
   Distilled water
   20 gm
   100 ml

Warm solution until dissolved, thoroughly cool then filter. Add 1 ml of concentrated formalin for each 10 ml of solution when ready to use.

Mounting fluid

Mayer's albumen (Brauer 1958)
   White of egg
   Glycerine C. P.
   Thymol
   50 parts
   50 parts
Beat white of egg well, let set overnight. Drain off liquid and measure, add equal amount of glycerine and a few crystals of thymol to preserve the mixture. Filter under vacuum. Will keep for several months when stored in refrigerator.

Staining reagents:

A. 0.5% periodic acid 0.5 gm/100 ml distilled water
B. Ammoniacal silver (Humason and Lushbaugh 1960)
   Silver nitrate (0.6 M) 10.2% 10.4 gm/100
   (AgNO₃  F. W. 169.888)
   Sodium carbonate (0.3 M) 3.1% 1 gm/100

   To 10 ml of 10.2% (0.6 M) silver nitrate add ammonium hydroxide (concentrated) until the precipitate which forms is almost, but not completely, redissolved. Add 10 ml of 3.1% (0.3 M) sodium carbonate (no precipitate forms) and distilled water to make 100 ml. Filter.

C. Ammoniated distilled water

   1 to 2 drops of ammonium hydroxide to 100 ml of distilled water.

D. Buffered formalin
   1% aqueous Na₂CO₃  1 ml
   5% formalin  50 ml

E. Sodium thiosulfate 10% 10 gm/100 ml

F. Orcein solution (Humason and Lushbaugh 1960)
   Orcein 1.0 gm
   70% ethyl alcohol 100 ml
   Hydrochloric acid C.6 - 1.0 ml (concentrated)

   Dissolve orcein in alcohol, filter then add HCl. Mix thoroughly.

G. 55% phosphomolybdic acid 5 gm/100 ml

H. Aniline blue solution (Humason and Lushbaugh 1960)
   Aniline blue-water soluble C. I. 42755 0.1 gm
   Oxalic acid 2.0 gm
   Phosphomolybdic acid 15.0 gm
   Distilled water 100.0 ml

   Thoroughly mix until dissolved. Filter.
I. Acidolated water
   Glacial acetic acid 1 ml
   Distilled water 100 ml

J. Iodine solution 0.5% (Gurr 1956)
   Iodine 0.5 gm
   70% ethyl alcohol 100.0 ml

K. Sodium thiosulfate 5%
   5 gm/100 ml

Mounting media