STRUCTURAL CHANGES OCCURRING IN MUSCLE TISSUE DURING REPEATED FREEZING AND THAWING

by

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INTRODUCTION

Along with the advent of frozen meats came the study of the resulting effects of freezing on these products. Frozen meats up until a few short years ago, were quite unpopular with the average consumer. This was partly due to ignorance, and partly due to the fact that frozen meats were not processed in the correct manner.

Efforts were made by the meat industry to correct the faulty opinions regarding frozen meats, but before this could be done, an intensive study was made by the industry and numerous research workers in an effort to correct some of the mistakes in freezing meat which had been made in the past. It was also necessary to carry out extensive research on the subject so that those concerned could convince the wholesaler, the retailer, and the consuming public that properly processed frozen meats were as good as fresh chilled meats in all respects.

Continued studies with frozen meats will be absolutely necessary for the continued success of the meat industry, and for the safety and pleasure of the meat consuming public. The frozen food locker industry, which serves millions of people, will continue to rely on meat research workers to help build and maintain this important enterprise.

Of all the studies which have involved meats, histology is one of the most recent and least explored. The importance of histological observations is evidenced by the fact that this

phase of research has been included as an integral part of some meat investigations during recent years. Various histological studies have revealed some important facts concerning the behavior of muscle tissue of meat animals after slaughter. This study reveals additional data on the histological changes occurring in frozen meats.

REVIEW OF LITERATURE

The field of histology of meats has not been explored until recently and then only to a limited extent. The study of the changes occurring in muscle tissue as a result of repeated freezing and defrosting has scarsely been touched, but other histological studies have been made and inasmuch as they are more or less related, they will be considered here.

A strong pressure builds up inside meat when it is frozen because the water freezes first according to observations made by Richardson and Scherubel (1908). The ice crystals as they form around the muscle cells must press against the separating cell walls and such pressure exerted against a delicate membrane may possibly cause abrasions and ruptures. The sarcolema as it is frozen and thawed becomes permeable to proteins and they are thereby pressed out with the juice. Photomicrographs show a considerable amount of difference in the appearance of muscle fibers which have been thawed slowly as compared to those which have been thawed rapidly. In rapid thawing, the fibers did not return to their normal shape. The authors

pointed out that to be successful in making sections of frozen tissue, an investigator must take the microtome, microscope, and other accessories into the freezer with him. Only minor changes are expected to occur in frozen meats as they increase in age in the freezer. The principal changes occur during the period of freezing.

Pennington (1908) in examining muscle tissue of a chicken found cross striations more distinct in sections which had been frozen at 14° F. for one month than in fresh sections. He also observed a slight separation of muscle bundles and early degeneration. The theory that changes in muscle tissue held at 14° F. may be partly enzymatic and partly due to freezing was advanced at this time.

The fact that shrinkage of muscle fibers is associated with the storage interval was noted by Shrewsbury et al. (1942) during a study of pork muscle tissue. The individual muscle fibers were noted to become more condensed as the storage interval increased. Detailed observations of the stored meat indicated a progressive break down of the muscle tissue.

Ramsbottom and Koonz (1939) felt that it was apparent that a number of factors may influence the amount of drip and that all of these factors should be taken into consideration when histological studies are made.

Moran and Hale (1932) found that there was less drip from frozen beef muscle stored at minus 10° C. at the end of a 7 day period than there was at the end of a 145 day freezing

period. It was also pointed out that a rise in temperature in frozen muscle could increase the size of the ice crystals because of the regrouping of the molecules at the plane of contact of two smaller crystals.

Muscle globin loses part of its power to hold water probably because it undergoes some form of denaturation by the action of the concentrated salt solution in the partly frozen muscle.

Koonz and Ramsbottom (1939) found that the rate of freezing affects the size, number, and location of ice formations. Minute, evenly distributed ice columns within the fibers were produced in nearly instantaneous freezing. Proportionately slower freezing produced larger crystals smaller in number, and finally water was frozen outside of the fibers.

In a study of the effect of freezing and ice formation in chicken muscle, Carlin (1947) found that the tenderness of those birds aged six hours or less is definitely increased by freezing and thawing. It was also noted that the size and location of ice crystals are determined by the rate of freezing, the extent of the aging period, and the histological structure of the muscle tissue at the time it is frozen.

The fewer waves and kinks present in the fibers at the time of freezing as a result of aging, the more prominent the cross striations and the greater the disintegration.

In a histological study of chicken muscle Stewart et al.

(1945) found vacuoles present in this tissue when it was frozen within 2 hours after slaughter. No vacuoles appeared in the muscle fibers of those chickens which were held 18 hours before freezing. It was assumed that the vacuoles occurred where ice crystals had previously been formed. These authors also thought that postmortem changes affected the formation of intrafibrillar ice crystals, as there was no intrafibrillar freezing in those chickens held 18 hours before being frozen.

Hiner et al. (1945) reported that when water freezes it crystalizes as pure water and pushes aside the substances which are dissolved or suspended in it. Slow freezing at 18° F. causes ice crystal formation between the muscle fibers. The water forming these crystals is drawn from juices within the fibers leaving them in a semi-dehydrated condition and formed in irregular groups. Lower freezing temperatures indicated that smaller ice crystals were formed, and a temperature of minus 114° F. showed intrafibrillar ice areas with fiber splitting. These investigators found that frozen sections cut with the freezing microtome showed crystal formation with the resulting fiber distortion very well. When the same blocks of tissue from which these sections had been cut were defrosted, fixed, mounted in paraffin, sectioned, stained, and examined they showed little or no difference in structure regardless of the temperature of freezing.

Ramsbottom and Koonz (1939) in some histological studies of beef muscle found that in comparing tissue from steaks which had been frozen at minus 50° F. and minus 20° F., respectively, and then defrosted, there was very little difference in the appearance of these sections and a control section which had never been frozen. It was also observed that larger pieces of meat when defrosted will have a greater opportunity to absorb water which has been squeezed out of the fibers than smaller pieces.

Hankins and Hiner (1944) after determining that freezing had a tenderizing effect on meat, made histological studies of sections of beef with which they had worked and found a splitting of muscle fibers commencing at a temperature of minus 10° F. This effect became more apparent as the temperature was lowered. They attributed the increased tenderness to the splitting of the fibers.

In a study of beef muscle tissue Harrison (1947) concluded that it is probable that the tenderness of beef is associated with its histological structure. Disintegration of the muscle fibers started after 10 days of storage when the product was held at 34° to 36° F. and became more evident as the storage period progressed. Destruction of the striae in the fibers resulting in fragility of the tissue was caused by the disintegration.

Ramsbottom et al. (1945) stated in their study covering the comparative tenderness of different muscles that muscles

vary in the amount of collagenous and elastic tissue according to the size of the muscle bundles. A great variation in the texture of muscles was noted which was determined by the size of the bundles of fibers (fasciculus) and the amount of connective tissue (perimysium) surrounding the bundles.

In a histological study of beef muscle, Brady (1937) observed a finer texture in large muscle fiber bundles. Further study also proved that muscles with large bundles of fibers were more tender. It was also noted that in muscles containing a large number of bundles, the bundles were smaller and the fibers were larger. This led to tougher meat because a larger amount of connective tissue is found associated with small bundles.

Paul et al. (1944) in the study of beef muscle found disintegrated areas and breaks were quite numerous at the end of 9 days' storage and were about the same at the end of 18 days' storage, when held at 35° F.

Two types of breaks were noted by these investigators. One was a sharp fracture, the other a disintegration of the muscle protoplasm over a fairly long area within the fiber. The disintegrated areas were noted more frequently in the portions of the fiber between two nodes of contracture. Fractures occurred chiefly in the krinkled or kinked fibers.

Carlin (1947) stated that the changes in muscle fibers which occurred as a result of degeneration appeared to be correlated with changes in tenderness of the muscle.

The significance of microscopic nodes and internodes in

striated muscle is unknown according to Carey (1940 a). Evidence has been presented to show that the contraction node of heat rigor is not the result of mechanical approximation of previously noncontracted striations but rather an increase of new fine and closely placed striae continuous with the coarse inactive ones. Different degrees of muscle twiches, contraction, and contracture with and without increase in the number of striations has been observed. Structural changes associated with heat rigor may be linked up with chemical changes which take place in the fibers.

Paul et al. (1944) reported nodes to be very dense and resistant since they persisted through an 19 day storage period an experiment with beef muscle held at 35° F. Examination of this tissue in the fresh state showed the fibers poorly differentiated and straight to wavy, while after one days storage the fibers showed contracture nodes and crinkles of rigor.

Active and passive rigor was described. In the active change the appearance of alternate zones of condensation and rarefaction was noted, while a passive change was found in the crinkled fibers which were not contracted themselves but were thrown into waves from outside stresses.

Stewart et al. (1945) found both passive and active rigor nodes in the muscle tissue of fresh frozen chicken. The waves and kinks of passive rigor were nearly always found near connective tissue as though the contractures might have been caused by shrinking of the connective tissues.

The grade and age of cattle have very little bearing on the connective tissue content of muscle according to Mitchell et al. (1928). These investigators did however find a significant difference in the amount of collagen and elastin contained in different muscles. The percentage of elastin was in most instances an insignificant fraction of the portion of collagen.

Paul et al. (1944) found that cooked collagenous connective tissue would not take the acid fuchsin stain but was colored yellow by picric acid. Cooked connective tissue was broken and somewhat granular, whereas the uncooked tissue was fibrous.

Carey (1940b) reports that heat accelerates the appearance of lactic acid during muscle heat rigor, and that internal structural changes are associated with the chemical reaction of the muscle. When muscle tissue was subjected to a sudden elevation in temperature from 18° to 40° C. for 30 seconds or more in Locke's solution, there was a loss of the cross striated pattern and a granular stippling of the cytoplasm due to the turbulence produced by molecular thermal agitation.

HISTOLOGY

Muscle

The subject of histology deals with microscopic or minute structures of plant and animal tissues, and any evidence of abnormal appearance. The histological study of muscle involves the preparation of thin muscle sections which are to be observed and studied with the aid of a microscope. This study was made

on skeletal muscle and the connective tissues which are closely associated with it.

The muscles attached to the skeleton are known as skeletal or voluntary striated muscles. A muscle such as the longismus dorsi is properly classified as a striated voluntary muscle, the structure of which will be described.

A muscle is made up of parallel fibers which are held together by connective tissue (Fig. 1). Deep fascia which is composed of fibro-elastic connective tissue surrounds the muscle. This outer covering is called epimysium. Branching from the epimysium, connective tissue sheaths penetrate the muscle as perimysium, dividing and sub-dividing groups of muscle fibers into primary, secondary and tertiary bundles. Connective tissue known as endomysium surrounds individual muscle fibers or cells. These sheaths are branches from perimysium.

The muscle fiber itself bound by a membrane called the sarcolema (Figs. 2 and 3) (cross and longitudinal sections) is composed principally of long parallel thread like nonbranching structures known as myofibrils. The myofibrils are partially separated into groups called Cohnheim areas. These areas are separated by a substance known as sarcoplasm. The fluid substance is removed by fixation thus leaving clear areas in the stained sections. Sarcoplasm also separates individual myofibrils in the Cohnheim areas. Cross striations in muscle fibers which appear as alternately light and dark staining

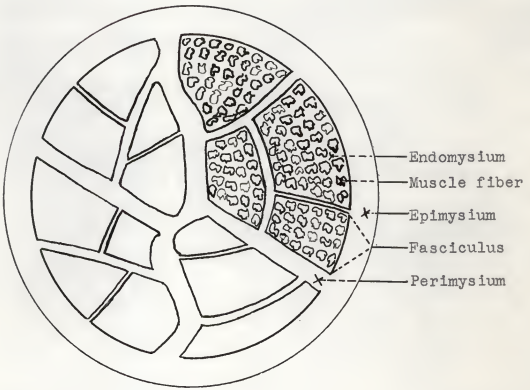


Fig. 1. Diagram showing cross section of a muscle.

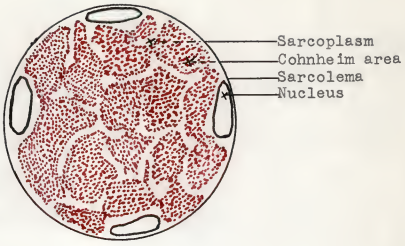


Fig. 2. Diagram showing cross section of muscle fiber.

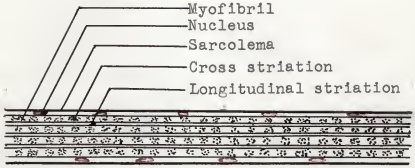


Fig. 3. Diagram showing longitudinal section of muscle fiber.

areas are characteristic of striated muscle. The myofibrils contain bodies called disks which stain alternately light and dark. These bodies lay adjacent to each other in the different myofibrils and produce the cross striated effect. Longitudinal striations in muscle fibers are produced by the numerous myofibrils which lie parallel to each other. The nuclei of a muscle fiber or cell are located at the periphery of a mature fiber, but are centrally located in the undeveloped or dark fibers. The number of muscle fibers in a given muscle will remain the same throughout life, but a change in the number of myofibrils will increase or decrease the size of a muscle. This will manifest itself in the individual in many ways; e.g., normal growth, exercise.

Connective Tissue

Connective tissue as it is associated with the longismus dorsi muscle is included in this study. The number of connective tissue fibers and the compactness of the fibers are recognized as important factors influencing the tenderness of meat. The age of the individual and the rate of the development of the muscle and connective tissue also has a good deal of bearing on tenderness. The greater the amount of exercise or usage of a muscle, the denser the connective tissue fibers, and the tougher the meat.

Connective tissue consists of cells and much intracellular substance (Fig. 4). The intra-cellular substance

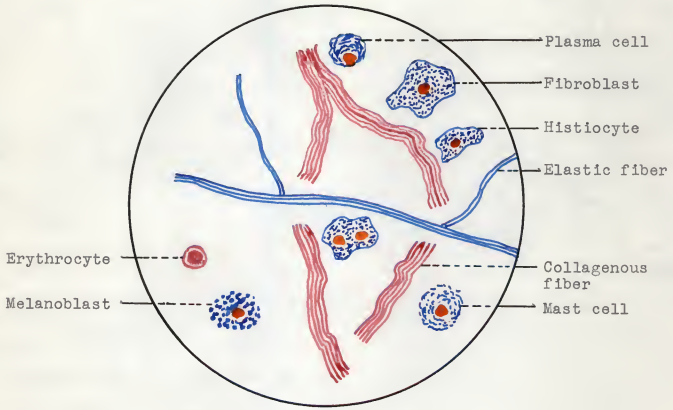


Fig. 4. Diagram of areolar connective tissue.

consists of tissue fluid or matrix and fibers. Classified according to composition there are several types of connective tissue, and the one which is of interest in this study is known as loose or areolar connective tissue. This tissue functions as a stroma or framework for muscle fibers, and carries blood vessels, lymph vessels, and nerves. In addition to this, areolar tissue furnishes protection both from a physical standpoint and by assisting with the body defense mechanism.

The fibers associated with areolar connective tissue are white or collagenous, and yellow or elastic. The elastic fibers as the name implies are quite elastic in nature. They are comparatively fine and are outnumbered by collagenous fibers. Since they are scarce in aerolar tissue they play only a small part in causing toughness in muscle tissue proper. Collagenous fibers however do play an important part in toughness. They are thick, nonelastic, flexible, pale staining, anastomosing fibers made up of fine fibrils which do not branch or anastomose. Depending on the density of the tissue, the fibrils are laid down anywhere from very loose to very close together. When the fibrils are more closely packed the fiber is tougher than one with loosely arranged fibrils. Therefore the density of the individual collagenous fibers and the number of collagenous fibers in a tissue determine its toughness to a very great extent.

MATERIALS AND PROCEDURE

Pork

The pork used in this study was obtained from the carcass of a hog which was raised and fattened at the Kansas State College farm. The hog was slaughtered in the meats laboratory at the College and immediately placed in the freezer and frozen solid at 5° F. The live weight was 250 lbs., the hot dressed weight 213 lbs., and the frozen weight 211 lbs.

After the carcass had been allowed to remain in the freezer for 10 days, it was placed in a chill room at a temperature of 30° F. and allowed to defrost. The carcass was then cut into wholesale cuts. The loins which were used in this study were cut into three sections as shown in Fig. 5. The anterior end was removed from each right and left section so that these sections commenced with the 5th rib. The first sections ended at a point between the 10th and 11th ribs. The second sections commenced at the above mentioned point and extended back posterior to the 1st lumbar vertebra. The third sections included the remainder of the loins minus the posterior portions which were removed just anterior to the hip bones of each loin. The sections removed from the extreme ends of each loin were discarded.

The first chops taken for histological examination were removed immediately after the loin had been divided into

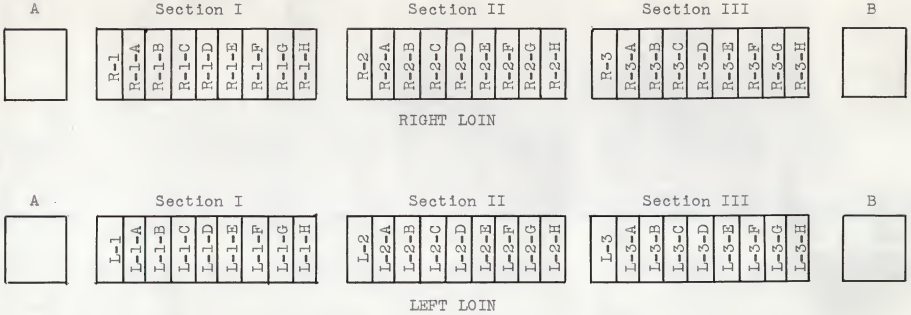
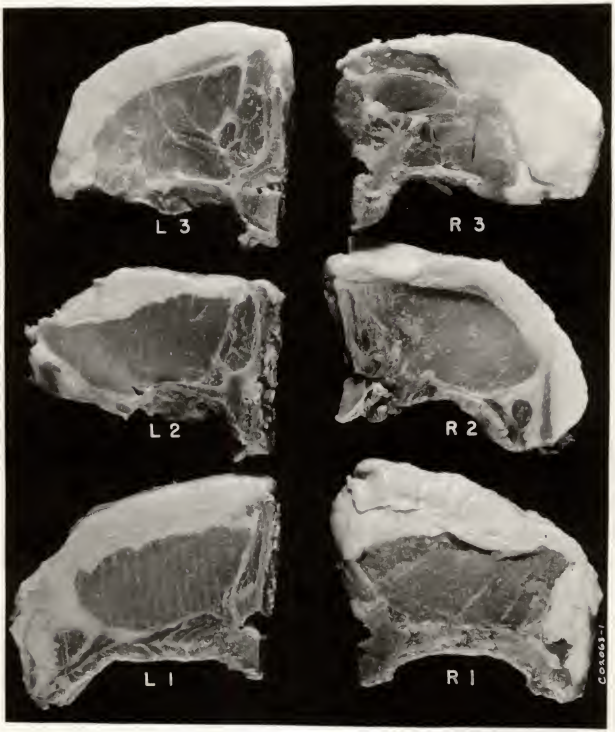


Fig. 5. Division of the pork loins into sections, and into chops from which the blocks for histological study were taken. Anterior and posterior sections designated as A and B respectively were discarded.

EXPLANATION OF PLATE I

The pork chops as they were removed from each of the six sections.

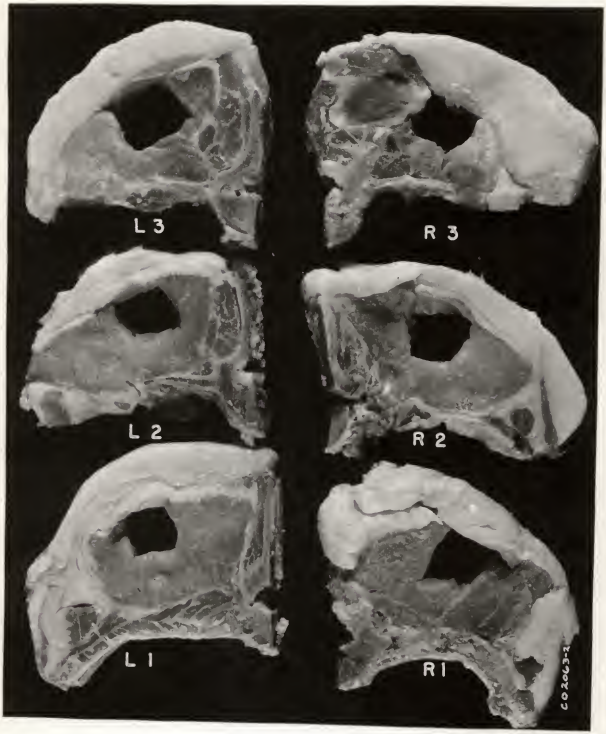
PLATE I



EXPLANATION OF PLATE II

The pork chops from each of the six sections showing areas from which the samples for histological study were taken.

PLATE II



sections. The thickness of each chop was approximately 1½ cm. Small samples approximately 3 cm on a side were removed at the same place from each chop, tagged, and placed in the fixing solution. The chops before and after the samples were removed are shown in Plates I and II. As soon as the samples were cared for, the three sections of each loin were double wrapped with one sheet of cellophane and one sheet of waxed paper and placed in the freezer.

A total of nine chops were removed from each section of both loins and labeled as shown in Fig. 5. Chops were cut from each section when the loins were removed from the freezer and again after they had been allowed to defrost. The defrosting, refreezing, and sampling process was repeated until all sections of the loins were expended. Samples for histological study were taken from each chop in a manner previously described, and illustrated in Plate II.

Detailed treatment of the different sections prior to obtaining each of the samples for histological study is shown in Table 1.

Beef

The beef used for this study was obtained from the right loins of two Angus steers one of which was a "double muscle" type and the other a normal animal.

When 6 months of age, the steers were placed on a full ration of grain and remained on this ration until they were 18

Table 1. Treatment of the pork loin sections prior to obtaining the different samples for histological study.

Phase	Sample no.	No. of days in freezer*	Temp. of freezer degrees F.	No. of days defrosting*	Temp. of defrost room degrees F.	Total no. of times defrosted	Total no. of times frozen or refrozen
1	R&L 1,2,3	11	5	4	28	1	1
2	R&L 1,2,3, A	2	-20	0	not defrosted	1	2
3	R&L 1,2,3, B	0	not frozen	3	38	2	2
4	R&L 1,2,3, C	2	-28	0	not defrosted	2	3
5	R&L 1,2,3, D	0	not frozen	2	38	3	3
6	R&L 1,2,3, E	3	-28	0	not defrosted	3	4
7	R&L 1,2,3, F	0	not frozen	5	38	4	4
8	R&L 1,2,3, G	7	-25	0	not defrosted	4	5
9	R&L 1,2,3, H	0	not frozen	3	38	5	5

* Number of days between each refreezing and defrosting process. When samples were taken without defrosting or without refreezing, it is so indicated.

months of age, at which time they were slaughtered. During feeding, both animals progressed satisfactorily so there was no reason to believe that either had an advantage that might have favorably influenced its performance in the feed lot. The steers were fed at the college farm, and slaughtered in the Animal Husbandry meats laboratory. The carcass weight of the double muscled steer was 650 lbs., and that of the normal steer was 693 lbs.

After aging for one week in the cooler, the carcasses were broken down into wholesale cuts. It was noted at this time that the muscle of the double muscled steer was coarse in texture, darker in color than the normal and showed very little marbling.

Ten steaks 2½ cm in thickness were removed from each of the right loins beginning with the first lumbar vertebra, and continuing posteriorly to the sixth lumbar vertebra.

Samples for histological study were immediately removed from each steak and placed in the fixing solution. Four samples from the longismus dorsi muscle of each steak, and four samples from the psoas major muscle of steak number nine were removed during the study from points shown in Fig. 6.

The steaks beginning with the anterior end of each loin were numbered consecutively from 1 to 10. The normal animal was identified as No. 1 and the double muscle animal was identified as No. 2 so that each sample carried the number of

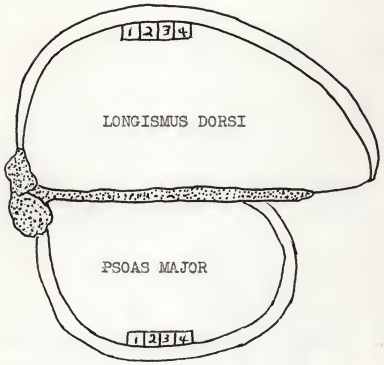


Fig.6. Diagram of steak showing areas from which samples for histological study were taken.

Table 2. Treatment of the steaks prior to obtaining the different samples for histological study.

Phase	Samples of normal and double muscle	Number of days in freezer	Number of days defrosting*	Temp., degrees F. of freezer	Temp., degrees F. of defrost room	Total no. of times defrosted	Total no. of times refrozen
1	1 to 10 L.dorsi #9 psoas	0	0	not frozen	not frozen	0	0
2	1 to 10A L.dorsi #9A psoas	95	1	0 to -5	38	1	1
3	1 to 10B L.dorsi #9B psoas	6	4	0 to -5	38	2	2
4	1 to 10C L. dorsi #9C psoas	5	2	0 to -5	45	3	3

* Number of days between each refreezing and defrosting process.

the animal, followed by the number of the steak. The first series of samples taken carried no letter, but samples 2, 3 and 4 were further identified with the letters A, B and C respectively. For example, the third sample taken from the longismus dorsi muscle of steak No. 8 from the loin of the double muscle animal was labeled "2-8-B". The samples from the longismus dorsi muscle were not further identified, but the samples from the psoas major were marked "psoas".

The treatment of the beef muscle was generally the same as that of the pork. The object was to defrost and refreeze, and obtain representative samples so that the effect of this processing on muscle and connective tissue could be studied. Detailed steps concerning the treatment of the beef is shown in Table 2.

Fixing, Mounting, Sectioning, and Staining

The histological study of meat consists of a series of carefully managed steps that prepare the tissue in sections which are thin enough to be studied under a microscope. These steps consist generally of the selection of a suitable section of tissue, killing and fixing it, solidifying it so as to permit cutting thin slices, mounting on slides and staining, and then the observation and tabulation of results.

Samples from the same point in the longismus dorsi muscle were removed from each pork chop as illustrated in Plates I

and II. Samples were also removed from the same region of the longismus dorsi muscle in each steak, and the psoas major muscle of the steak number nine as shown in Fig. 6. All samples were identified and placed in the fixing solution as soon as they were removed. This was necessary in order to stop enzyme and bacterial activity, thus preserving the tissue.

Two fixing solutions were used, namely ten per cent formalin (10 per cent formaldehyde in distilled water) and Zenker's solution. While both of these fixing solutions produce the same effect, they were both used on different samples at the beginning of the study to determine which one would be the most convenient. Since tissue placed in Zenker's solution can become over fixed if not taken out at the proper time, the use of it was abandoned in favor of formalin which does not cause over fixation during a period of at least three months. For this reason tissues were left in formalin for varying periods up to one month when time did not permit the immediate preparation of the slides.

Following fixation the samples were carefully cut and trimmed so as to produce a longitudinal and cross section from each specimen.

The next step was to dehydrate and harden in alcohol. The tissue was first placed in 80 per cent alcohol for 24 hours, then 95 per cent for 24 hours and finally in 100 per cent for 8 hours. Following this step, the tissue was cleared in oil of cedar for 14 hours.

The next step was to infiltrate and embed in Tissuemat. The infiltration was for 24 hours, but during this period the Tissuemat was changed twice in order to remove the oil of cedar. When the odor of oil of cedar could no longer be detected the tissue was embedded in Tissuemat, chilled, and then mounted on wooden blocks.

Obtaining thin sections on the rotary microtome was accomplished next. A ribbon containing about 20 sections 8 to 10 microns in thickness was cut from each of the cross and longitudinal sections of the muscle tissue samples. The two best sections of each ribbon were selected, removed, and secured one to each slide by the use of phenolized egg albumen. In this manner two slides of each longitudinal and cross section were prepared for future study.

The system of using the freezing microtome was attempted, but it was abandoned in favor of the Tissuemat embedding. Both direct freezing and embedding in gelatine was tried, but the muscle tissue had become so "mushy" as a result of repeated freezing and defrosting toward the end of the study that sections were cut with great difficulty.

Two staining procedures were followed. One set of slides was stained by the Hematoxylin - Eosin method, and the duplicates were stained by the use of Weigert's stain, Hematoxylin, and Van Geison's stain in the order named. Both methods require the passing of the slides thru xylol to dissolve the Tissuemat, and then through a series of alcohols to remove the xylol from

the tissues. After passing through the stains, the slides were again passed through a series of alcohols to dehydrate the tissues and then through xylol until the stained sections were clear. The last step was to mount the tissue in Clarite and affix a cover glass. The tissues will keep for an indefinite period of time with this type of processing. The reason for following two different methods of staining was to prepare the tissues for a more detailed study.

It is realized that if each step in preparation of the tissue is not carefully executed, some undesirable changes such as shattering may occur. Every effort was made to follow the same technique throughout the study so that any changes produced as a result of fixing, sectioning, or staining would undoubtedly be uniform in all of the finished slides.

Shrewsbury et al. (1942) observed that shrinkage in varying degrees was prevalent throughout the histological study of pork muscle when the paraffin embeding technique was used. It was thought that the shrinking was due in part to the methods employed in fixation and embeding.

Microscopic Examination of Slides

As was previously mentioned, two longitudinal sections and two cross sections were made from each sample of pork and beef. There was a total of 216 slides from pork and 320 from beef.

Each slide was carefully examined under high and low power, and the following points were observed for changes.

Cross striations	Intracellular vacuoles
Longitudinal striations	Intercellular vacuoles
Swelling of fibers	Granular disintegration
Natural fragmentation	U waves
Mechanical fragmentation	V waves
Rupture of sarcolema	Fibrillar waves
Density of collagenous fibers	Cohnheim areas
Waves in collagenous fibers	Rigor ridges
Tissue fluid	

The majority of the above listed terms are more or less self explanatory. The standard histological terms have been illustrated in diagrams, but inasmuch as some nonstandard terms were used, an explanation of these conditions will be given.

Natural fragmentation is the breaking or shattering of muscle fibers caused by the aging, freezing, and thawing of the meat. Mechanical fragmentation is that which is caused by fixing and handling the tissues in the laboratory, including improper function and operation of the microtome.

Intracellular vacuoles are small openings within the muscle fibers which are caused as a result of ice crystal formation and the subsequent melting of the ice particles. Intercellular vacuoles appear as circular openings or indentations between fibers.

Granular disintegration is the disappearance of striations and the subsequent appearance of granular material in that portion of the fiber which is affected.

The long sweeping waves involving an entire muscle fiber are called U waves. The waves which are more sharply defined are classified as V waves.

Fibrillar waves are observed in straight muscle fibers when the myofibrillae are thrown into long sweeping curves.

Rigor ridges are small ridges within the muscle fiber which run parallel to the cross striations. It is a bunching together of a few cross striations brought on by the action of rigor mortis.

Tissue fluid is a light staining substance found between the muscle fibers. It is presumably drawn from the fibers.

Gradations from one to four were used in determining the condition of the previously mentioned histological features as shown in Tables 3 and 4. All slides were thoroughly examined and the results recorded separately for each phase of treatment described in Tables 1 and 2. An average or composite figure was obtained for each point observed and recorded in Table 3 for the pork, and Table 4 for the beef.

Table 3. Composite values for each phase of treatment of the pork.

Points observed	Phase numbers 1	2	3	4	5	6	7	8	9	Gradations
Cross striations	1.00	1.00	1.16	1.00	1.16	1.83	1.00	1.00	1.00	Distinct 1-->4 Indistinct
Longitudinal striations	1.00	1.00	1.00	1.00	1.00	1.50	1.00	1.00	1.00	Distinct 1-->4 Indistinct
Swelling of fibers	3.00	3.00	2.16	2.16	2.50	2.50	2.50	2.83	2.16	Abundant 1-->4 Absent
Natural fragmentation	3.83	3.83	3.00	3.00	3.00	2.83	3.00	3.00	2.83	Abundant 1-->4 Absent
Mechanical fragmentation	1.66	2.00	2.16	2.66	2.16	2.50	2.00	2.16	1.83	Abundant 1-->4 Absent
Rupture of sarcolema	3.83	3.83	3.00	3.00	3.33	3.16	3.00	3.00	3.00	Abundant 1-->4 Absent
Density of col. fibers	2.50	3.00	2.50	2.66	2.66	3.00	2.33	2.66	2.50	Loose 1-->4 Dense
Waves in col. fibers	2.66	2.83	2.83	2.16	2.83	2.66	3.50	2.66	2.33	Abundant 1-->4 Absent
Intracellular vacuoles	4.00	3.50	3.66	3.83	3.16	3.33	3.16	3.33	3.00	Abundant 1-->4 Absent
Intercellular vacuoles	4.00	4.00	4.00	4.00	3.00	3.00	3.16	3.16	3.00	Abundant 1-->4 Absent
Granular disintegration	3.83	3.83	3.00	3.00	3.00	2.83	3.00	3.00	2.83	Abundant 1-->4 Absent
U waves	2.83	2.66	2.66	2.66	3.00	3.00	3.16	3.16	2.66	Abundant 1-->4 Absent
V waves	4.00	4.00	4.00	4.00	4.00	4.00	4.00	4.00	3.33	Abundant 1-->4 Absent
Fibrillar waves	3.16	3.00	2.83	2.50	3.00	3.33	3.16	3.00	3.00	Abundant 1-->4 Absent
Cohnheim areas	2.20	1.66	1.66	1.00	1.66	2.00	2.00	1.00	1.00	Distinct 1-->4 Indistinct
Rigor ridges	3.83	4.00	4.00	4.00	4.00	4.00	4.00	4.00	4.00	Abundant 1-->4 Absent
Tissue fluid	4.00	4.00	4.00	4.00	4.00	4.00	3.33	3.33	3.50	Abundant 1-->4 Absent

OBSERVATIONS AND DISCUSSIONS

Pork

Gross Appearance. It was intended during this study to subject the pork tissue to more severe treatment than that which would be found under ordinary field conditions. Temperatures were never allowed to go high enough to produce a heavy bacterial growth. This was evidenced by the fact that samples taken from the chops during the last phase showed a very low bacterial plate count.

The chops removed during the last phases were soft and mushy, and appeared to have very little body. From 10 to 50 ml of drip loss was observed at the end of each defrosting period depending on the amount of meat involved. The increase in drip loss became greater in proportion to the amount of meat as the experiment progressed.

Striations. Both the longitudinal and cross striations appeared to be fairly distinct throughout the experiment except for the changes noted in phase 6 when the striations became slightly indistinct. No explanation can be offered for this change, but the numerical ratings shown in Table 3 indicate that the granular disintegration became more apparent during the same phase. The loss of the striated appearance is frequently associated with disintegration. This is particularly true of the cross striations.

The cross striations were straight and practically no turbulence was noted. The principal abnormality observed was the fading of these linear markings. The alignment was good except at the broken ends of those fibers showing degeneration.

Swelling of Fibers. A few swollen fibers appeared in each field, and were particularly noticeable on cross section. The fact that they took a lighter stain indicated that they were undergoing a retrograde process. These large fibers showed indistinct striations and Cohnheim areas. Table 3 shows a gradual but irregular increase in the number of swollen fibers as the experiment progressed. It is believed that these swollen fibers are a form of degeneration. Swelling of fibers is illustrated in Plate III, Fig. 1.

Fragmentation. Splitting and breaking of the fibers was prevalent throughout all phases of the study. Every effort was made to differentiate the natural fragmentation caused by freezing and thawing, from the breaking and splitting caused by other mechanical means. It was felt that sharp, jagged breaks were caused by cutting, fixing, and errors in technique, while the breaks with rounded edges were caused by the treatment given the muscle during the study. An increase in granular material, and a loss of striations was invariably associated with the natural breaks. A loss or breaking up of the nuclei was likewise associated with natural fragmentation. Table 3 shows that little natural fragmentation was found during the first two phases but that it progressed as the study was

EXPLANATION OF PLATE III

Fig. 1. Cross section through sample of pork chop R-1-B showing several large muscle fibers. It is believed that the large fibers were undergoing some sort of a retrograde process. X 125.

Fig. 2. Longitudinal section through sample of pork chop L-3-A showing a large amount of mechanical fragmentation. Sharp breaks may be observed in this photo. X 150.

PLATE III

Fig. 1.

Fig. 2.

carried through to the end.

Mechanical fragmentation did not vary a great deal through out the nine different phases of treatment, but remained more or less constant. Individual sections did at times show a considerable amount of shattering. This was thought to be due to excessive dehydration because many unsuccessful attempts were sometimes made before a suitable section could be cut with the microtome. An example of mechanical fragmentation is illustrated in Plate III, Fig. 2.

Rupture of Sarcolema. The sarcolema which is the thin membrane surrounding each muscle fiber was examined for natural breaks and not for mechanical ruptures. Breaks in this membrane were noted occasionally along the sides of unbroken muscle fibers as the study progressed. There were almost no ruptures during the first two phases of treatment, but they prevailed to a larger extent at the end of the study. Later ruptures were associated with the disintegration and breaking of fibers, and the collection of tissue fluid between fibers.

Connective Tissue. Very few elastic fibers were observed in the pork muscle, so consequently no recordings were shown in Table 3 for this component of the connective tissue. The elastic tissue which was found associated with the pork muscle was in the form of finely divided particles.

The collagenous connective tissue was observed principally for its density and waviness. The density varied slightly from one phase to another but the end result proved that there was

no significant change. The numerical ratings for the waves indicated that the changes for this feature were just about as insignificant as the density. The waves displayed fewer kinks in phase 7, but no explanation can be offered for this.

No breaking, swelling, or granular appearance was noted during any phase of the study. Staining was normal during all phases.

Vacuoles. Intracellular vacuoles which may be considered the result of intracellular ice crystal formation, gave some indication of the amount of this type of freezing within the fibers. Table 3 indicates an unsteady increase in the number of intracellular vacuoles as the study progressed. No explanation can be offered for this, but it does show that intrafibrillar freezing can take place during repeated freezing and defrosting.

Intercellular vacuoles formed between the muscle fibers followed about the same pattern as did the intracellular vacuole formation, except that this type of freezing was not observed during the first four phases of the study. Intracellular vacuoles are illustrated in Plate IV, Figs. 1 and 2.

Granular Disintegration. There was an increase in the amount of granular disintegration as the study progressed. In the early phases small granular fragments were noted at the points of breaks in the fibers, and later on small amounts of this type of degeneration spread to other parts of the cell. Earlier breaks were sharp, but a granular substance was seen

EXPLANATION OF PLATE IV

Fig. 1. Cross section through sample of pork chop R-3-A illustrating large intracellular vacuole at center of photo. X 235.

Fig. 2. Diagonal section through sample of pork chop L-1-D showing intracellular vacuole with some granular disintegration. Vacuole is located above and to the left of center. X 125.

PLATE IV

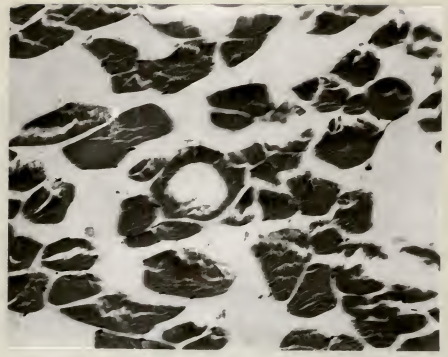


Fig. 1.

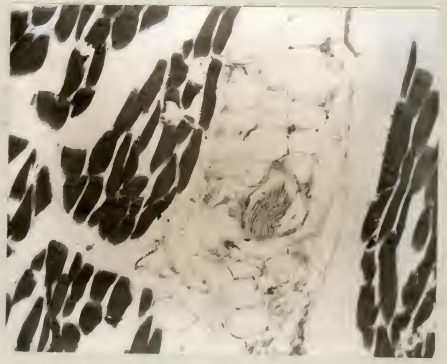


Fig. 2.

to ooze from the fibers at the points of the rounded breaks in the later phases of the study. Disintegration showed up well on cross section and was usually seen near the edge of the fiber. As was previously mentioned the disintegration was usually associated with the disappearance of striations in the longitudinal sections. Inasmuch as the temperatures were low and the periods of time short during the various phases of the study, it is not believed that much of the degenerative action could be autolytic in nature. The granular degeneration could not be considered extensive at any time during the study, and most of the causes for those changes which did appear could be attributed to the mechanical action of the freezing and thawing processes.

Waves. Three types of waves were observed, namely "U", "V" and fibrillar. In the U and V waves the fibers showed a curved or wavy appearance, and the fibrillar waves involved the myofibrils within individual fibers. The fibers containing fibrillar waves were straight. The frequency with which the U and fibrillar waves appeared was rather irregular, and not a great deal of variation was noted from the first to the last phase. The V waves were almost entirely absent except for the last phase.

The almost complete absence of rigor mortis, and the lack of elastic connective tissue fibers are considered good reasons for lack of waviness among the fibers. The changes observed during the different phases are considered to be insignificant.

Cohnheim Areas. The Cohnheim areas observed in the cross sections became more distinct as the study progressed. A small

amount of disintegration did from time to time, obliterate the clear spaces separating each Cohnheim area, but generally speaking the areas were quite clear. It is expected that the sarcoplasm decreased as more fluid was withdrawn from the fibers as the study progressed. The decrease in the amount of sarcoplasm separating the fibrillae within the Cohnheim areas increased the density of the groups of fibrillae thus causing the Cohnheim areas to stand out more distinctly.

Rigor Ridges. No rigor nodes whatsoever were observed during the study. A few rigor ridges were seen in the sections of the first phase, but the remaining phases were entirely without ridges. Inasmuch as the hog carcass was frozen immediately after slaughter, the rigor node formation must have either taken place while the carcass was freezing, while it was frozen, or perhaps the nodes were never present because of the unusual treatment given to this carcass.

Tissue Fluid. The tissue fluid appeared between muscle fibers in varying amounts during the last three phases of the study. Some slides displayed small amounts, and in others larger areas were formed. The substance was granular appearing and was undoubtedly derived from the sarcoplasm of the muscle fibers. Its presence indicates a gradual breakdown of the muscle fibers with a subsequent rupturing or disolution of the sarcolema. The tissue fluid is illustrated in Plate V, Figs. 1 and 2.

EXPLANATION OF PLATE V

Fig. 1. Longitudinal section through sample of pork chop R-1-E showing tissue fluid between the muscle fibers. X 125.

Fig. 2. Diagonal section through sample of pork chop R-1-H showing tissue fluid between the muscle fibers. X 125.

PLATE V

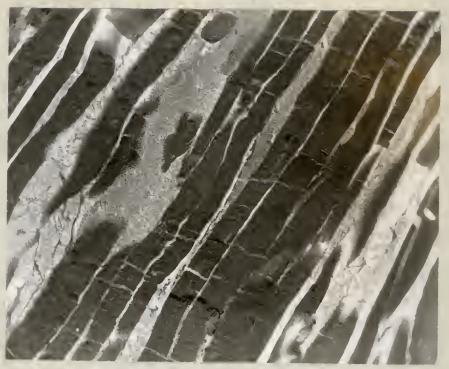


Fig. 1.

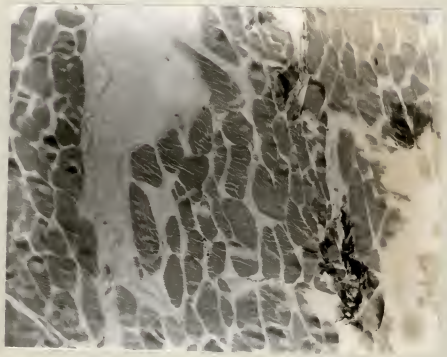


Fig. 2.

Beef

Gross Appearance. The treatment and system of collecting samples was somewhat different in the beef study than for the pork study, but the principle was the same. The beef did not undergo as many phases of freezing and thawing as the pork, and the macroscopic appearance of the beef at the end of the last phase did not indicate that any great change had taken place. The drip loss however did increase toward the end of the study.

Striations. Cross striations of the muscle fibers of the normal steer became slightly indistinct during the second and third phases, but were unchanged and quite distinct during the last phase. There was a loss of cross striations in the muscle of the double muscled steer beginning in the first phase, and a gradual increase was observed throughout the remaining phases until the end of the study was reached. This would indicate that the degenerative changes were greater in the double muscle than in the normal.

The longitudinal striations remained quite distinct in the muscle of the normal and double muscled steers throughout all phases of the study.

Swelling of Fibers. The swollen fibers increased in number as the study progressed in both the normal and double muscle. The number and rate of increase was found to be greater in the double muscle. This again indicates that the degenerative process is greater in the double muscle than in the normal. The swollen fibers invariably took a lighter stain.

Table 4. Composite values for each phase of treatment of the beef.

Points observed	Animal	Phase numbers 1	2	3	4	Gradations
Cross striations	Normal	1.00	1.50	1.30	1.00	Distinct 1--> 4 Indistinct
	Dbl. mus.	1.50	1.70	2.11	2.25	
Longitudinal striations	Normal	1.00	1.00	1.00	1.00	Distinct 1--> 4 Indistinct
	Dbl. mus.	1.00	1.10	1.00	1.00	
Swelling of fibers	Normal	3.89	3.30	3.00	3.00	Abundant 1--> 4 Absent
	Dbl. mus.	3.22	2.70	2.30	1.66	
Natural fragmentation	Normal	3.22	3.30	3.00	3.00	Abundant 1--> 4 Absent
	Dbl. mus.	3.00	2.70	2.30	1.66	
Mechanical fragmentation	Normal	3.00	2.30	2.60	1.80	Abundant 1--> 4 Absent
	Dbl. mus.	2.88	2.40	2.30	2.50	
Rupture of sarcolema	Normal	3.77	3.40	3.33	3.40	Abundant 1--> 4 Absent
	Dbl. mus.	3.22	3.10	3.00	3.00	
Density of col. fibers	Normal	3.22	3.00	2.40	3.80	Loose 1--> 4 Dense
	Dbl. mus.	2.44	2.70	2.40	2.33	
Waves in col. fibers	Normal	2.22	3.33	2.60	2.60	Abundant 1--> 4 Absent
	Dbl. mus.	3.00	2.50	2.60	2.33	
Intracellular vacuoles	Normal	4.00	4.00	3.80	3.80	Abundant 1--> 4 Absent
	Dbl. mus.	4.00	3.10	3.50	3.66	
Intercellular vacuoles	Normal	4.00	3.80	3.70	3.40	Abundant 1--> 4 Absent
	Dbl. mus.	4.00	3.00	3.25	3.16	
Granular disintegration	Normal	3.33	3.33	3.00	3.00	Abundant 1--> 4 Absent
	Dbl. mus.	3.11	2.70	2.20	1.66	
U waves	Normal	2.55	2.50	2.30	3.00	Abundant 1--> 4 Absent
	Dbl. mus.	2.87	3.00	2.33	2.66	
V waves	Normal	3.44	3.20	2.40	3.60	Abundant 1--> 4 Absent
	Dbl. mus.	3.75	3.60	2.55	3.33	
Fibrillar waves	Normal	3.44	3.20	3.80	4.00	Abundant 1--> 4 Absent
	Dbl. mus.	3.87	3.20	3.11	3.50	
Cohnheim areas	Normal	1.22	1.33	1.00	1.00	Distinct 1--> 4 Indistinct
	Dbl. mus.	1.11	1.40	1.33	1.25	
Rigor ridges	Normal	4.00	4.00	4.00	4.00	Abundant 1--> 4 Absent
	Dbl. mus.	4.00	4.00	4.00	4.00	
Tissue fluid	Normal	4.00	3.90	3.60	4.00	Abundant 1--> 4 Absent
	Dbl. mus.	4.00	3.80	3.70	3.66	

Fragmentation. Natural fragmentation increased slightly from the first to the last phase in the normal muscle. More natural fragmentation was observed in the double muscle in each phase of the study, and the rate of increase was greater in the double muscle than in the normal. This indicates that the degenerative changes are greater in the double muscle. Mechanical fragmentation increased in an irregular manner in the muscle tissue of both steers in such a way that this histological feature was considered to be insignificant. Plate VI, Fig. 2 shows that cross striations are clear at the points of breaks in mechanical fragmentation.

Rupture of Sarcolema. A small number of breaks was observed in the sarcolema of the muscle fibers of the normal steer. There was a slight increase as the study progressed. The double muscle showed a larger number of breaks with about the same rate of increase from the first to the last phase as was observed in the normal.

A few breaks were observed along the sides of intact muscle fibers, but they were more commonly observed in connection with areas of natural fragmentation and granular degeneration.

Connective Tissue. A few long elastic fibers were observed from time to time in the beef muscle tissue, but most of the elastic fibers were broken up. No record of the elastic connective tissue was made for incorporation into Table 4 because of the fact that only a small amount of this tissue was observed.

The density of the collagenous fibers was greater during

EXPLANATION OF PLATE VI

Fig. 1. Longitudinal section of longismus dorsi of steak No. 5 of the double muscle removed at the end of phase 2. Photo illustrates a small amount of disintegration at the ends of the broken fibers. Most of the cross striations have disappeared or are indistinct. X 450.

Fig. 2. Longitudinal section of longismus dorsi of steak No. 2 of the double muscle removed at the end of phase 2. Photo illustrated some sharp breaks which are called mechanical fragmentation. The cross striations of the large center fiber appear quite clearly even though the structure is shattered. X 450.

PLATE VI

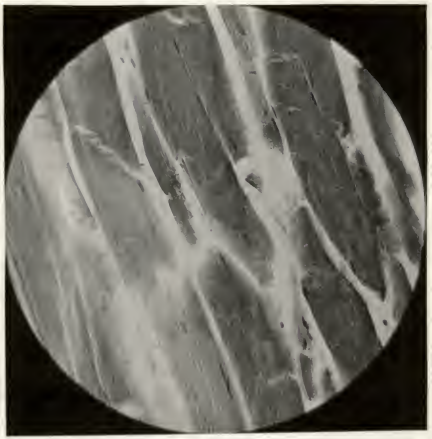


Fig. 1

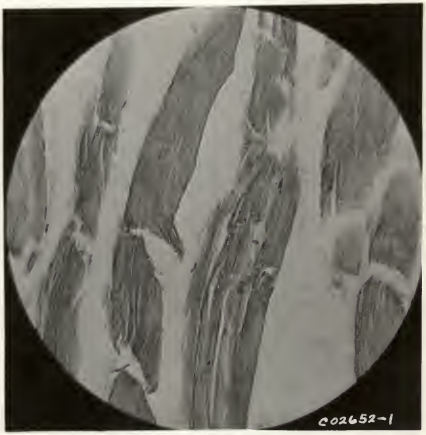


Fig. 2

phase 4 than during phase 1 in the muscle of the normal steer. The density was slightly greater in phase 1 than in phase 4 for the double muscle. The changes in density in both the normal and double muscle were considered to be insignificant because of the fact that the numerical ratings indicated no definite pattern for this histological detail.

The waves and kinks in the collagenous fibers decreased in phase 2 but increased in phases 3 and 4 in the muscle of the normal steer. These changes were considered to be insignificant.

The waviness of the collagenous fibers of the double muscle decreased as the study progressed. This may have been due to a slight degenerative process in this tissue.

The epimysium which is illustrated in Plate VII, Fig. 2 was observed to have a good deal of yellow color mixed with the red in the muscle tissue of both steers during the last phase of the study. This is considered to be significant because Paul et al. (1944) found that the collagenous connective tissue would not take the acid fuchsin stain but was colored yellow by picric acid. It is assumed that a change in this tissue similar to that produced by cooking had begun to take place in this tissue. This tendency to stain yellow was not observed in the perimysium. Plate VII, Fig. 2 shows some breaking of the epimysium, but no granular formations.

Vacuoles. Few intracellular vacuoles appeared in the muscle tissue of the normal steer. They were observed during the last two phases only. In the double muscle the vacuole

EXPLANATION OF PLATE VII

Fig. 1. Longitudinal section of longismus dorsi of steak No. 10 of the normal removed at the end of phase 2. Photo illustrates U and V waves in the muscle fibers. The cross striations appeared to be quite distinct in this section. X 450.

Fig. 2. Cross section of the epimysium covering longismus dorsi of steak No. 9 of the normal removed at the end of phase 3. Instead of taking the usual bright red stain, a definite yellowish tinge was observed. A small amount of shattering can be seen. X 450.

PLATE VII

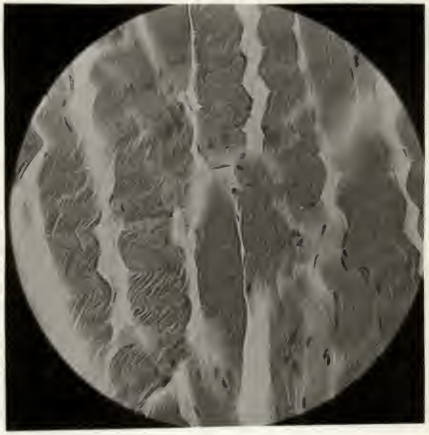


Fig. 1

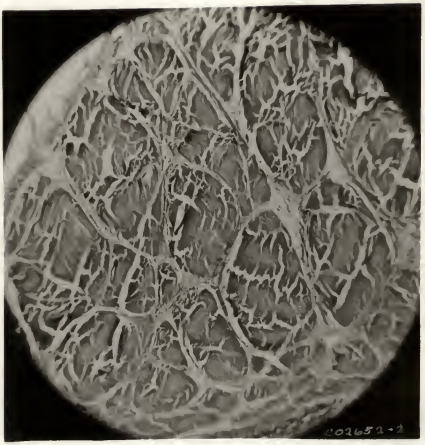


Fig. 2

formation was fairly heavy during the second phase but it decreased during the last two phases. The larger amount of vacuole formation in the double muscle indicates that intrafibrillar freezing takes place more easily in this tissue than it does in the normal.

Some intercellular vacuoles were observed during the last three phases, with the greatest number appearing in the double muscle. The increase and decrease in numbers of vacuoles observed in Table 4 presented an irregular pattern for the different phases, so the changes from one phase to another were considered to be insignificant.

Granular Disintegration. The areas of granular disintegration usually appeared around the ends of broken fibers as is shown in Plate VI, Fig. 1. As the study progressed the disintegration spread to other parts of the fibers. Disintegration was frequently associated with the loss of striations in the muscle fibers as is illustrated in Plate VI, Fig. 1.

The muscle of the normal steer showed a small amount of disintegration during the first two phases and an increase during the last two phases. The double muscle displayed more disintegration than the normal in all four phases. In addition to this the rate of increase in the degenerative process of the double muscle progressed steadily from the first to the last phase. This indicates that the degeneration was greater in the double muscle than in the normal.

The time and temperature of storage was not favorable for autolysis, so it is believed that the changes observed were due to freezing and thawing.

Waves. Three types of waves namely U, V, and fibrillar were observed during the beef study. The amount of waviness varied irregularily from one phase to another in both the normal and double muscle. Generally speaking all of the different types of waves increased from the first to the third phases and then decreased during the fourth phase. Shrinking of elastic connective tissue no doubt produced U and V waves as the tissue became more dehydrated at the end of the study. Dehydration of the muscle fibers probably caused the formation of fibrillar waves as well as some of the U and V waves. The U and V waves are illustrated in Plate VII, Fig. 1.

Cohnheim Areas. The Cohnheim areas of the muscle of the normal steer appeared to be very distinct during the last two phases and slightly less distinct during the first two phases. The Cohnheim areas of the double muscle followed an irregular pattern, with the first phase showing the most distinct areas, and the second phase showing the least distinct areas during the four phases of the study.

No explanation can be given for the irregular behavior of this histological feature because it is assumed that the loss of tissue fluid as a result of freezing and thawing would produce more distinct Cohnheim areas. Inasmuch as the areas were

PLATE VIII

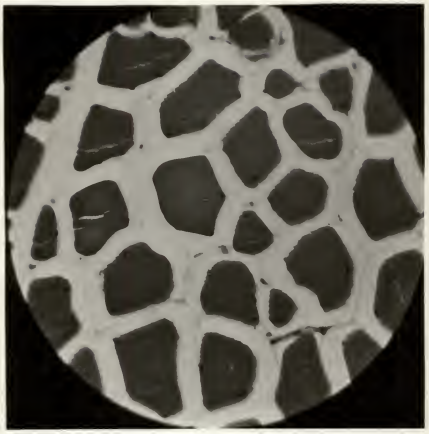


Fig. 1

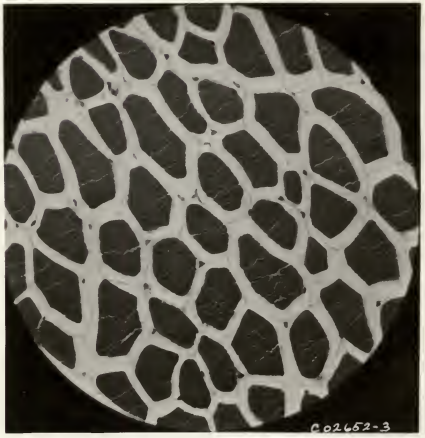


Fig. 2

less distinct in the double muscle than in the normal during the last three phases, it is expected that the double muscle lost more fluid.

Rigor Ridges. No rigor ridges were noted at any time during the study of the beef muscle tissue. A few scattered rigor nodes were observed in the psoas muscle during the third phase. This indicates that a few rigor nodes can be present after freezing and thawing, but this feature is considered insignificant due to the small number of nodes involved.

Tissue Fluid. No tissue fluid was observed during the first phase in either the normal or double muscle. A small amount was noted in each of the remaining phases except the last phase of treatment for the normal when no fluid was found. The double muscle displayed a steady increase in tissue fluid during the last three phases. This indicates that the breakdown of the double muscle was a trifle faster than the normal.

Size of Fibers. One of the most significant features observed during the beef study was the difference in the size of the muscle fibers as is illustrated in Plate VIII, Figs. 1 and 2. The fibers of the double muscle were generally larger than fibers of the normal. This characteristic prevailed throughout the study in all steaks and in all phases.

Some investigators have attributed the double muscle abnormality to a hyperplastic condition of the fibers, but this study clearly indicates that the majority of the muscle fibers of the double muscled steer are larger in size than those of the normal steer.

SUMMARY AND CONCLUSIONS

Several changes occurred in the muscle tissue as a result of repeated freezing and thawing. Mechanical changes from fixation and the microtome knife were also observed.

The various degenerative processes such as loss of striations, swelling of fibers, natural fragmentation, rupture of sarcolema, and granular disintegration increased during each step of freezing and defrosting with the exception of the striations of the pork, and normal steer muscle. The striated appearance of these muscles was lost in an irregular manner and then only to a limited extent.

Both intracellular and intercellular ice crystal formation contributed to the fragmentation of the fibers. The study indicated that intrafibrillar ice crystal formation could occur during repeated freezing and thawing.

The connective tissue remained about the same throughout the study, except for the changes observed in the epimysium where it formed the outer covering of the longismus dorsi of the beef carcasses. The staining tendencies of this tissue, along with the small amount of shattering observed indicated some degenerative changes were taking place during the last two phases of the study.

The freezing and thawing caused fluid to be withdrawn from the tissues with a resulting drip loss. This caused the Cohnheim areas to become more distinct and produced some

waviness in the muscle fibers. A granular like tissue fluid was observed between the muscle fibers during the last phases of the study. Many muscle fibers presented a soft crumbled appearance at the broken ends.

The muscle of the double muscled steer appeared to undergo more degeneration during each phase of treatment than the normal.

The muscle fibers of the double muscled steer appeared to be larger than the fibers of the normal steer.

ACKNOWLEDGMENT

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LITERATURE CITED

Brady, Daniel E.
Some histological and chemical factors affecting the texture and tenderness of beef. Unpublished Ph.D. Thesis. Minneapolis, Minn. University of Minnesota Library. 1937.

Carey, E. J.
Microscopic structure of striated muscle in heat rigor. Arch. Path. 30:881-892. 1940a.

Carey, E. J.
Effects of experimental variations in temperature and of microcapillarity on the cross striations in muscle. Arch. Path. 30:1041-1072. 1940b.

Carlin, M. A. F.
Effect of freezing on tenderness and on ice crystal formation in poultry after various periods of aging. Unpublished Ph.D. Thesis. Ames, Iowa. Iowa State College Library. 1947.

Hankins, O. G., and R. L. Hiner.
Evidence strong that freezing tenderizes meat. Quick Frozen Foods. 7:5:37. 1944.

Harrison, D. L.
Histological, physical, and organoleptic changes in three grades of beef during aging. Unpublished Ph.D. Thesis. Ames, Iowa. Iowa State College Library. 1947.

Hiner, R. L., L. L. Madsen, and O. G. Hankins.
Histological characteristics, tenderness, and drip losses of beef in relation to temperature of freezing. Food Res. 10:312-324. 1945.

Koonz, C. H., and J. H. Ramsbottom.
A method for studying the histological structure of frozen products, I. Poultry. Food Res. 4:117-128. 1939.

Moran, T., and H. P. Hale.
Rapid freezing temperature of storage. Soc. Chem. Indus. Jour. 51:20T-23T. 1932.

Mitchel, H. H., T. S. Hamilton, and W. T. Haines.
Some factors affecting the connective tissue of beef muscles. Jour. Nutr. 1:165-177. 1928.

Paul, Pauline, Bell Lowe, and B. R. McClurg.
Changes in histological structure and palatability of beef during storage. Food Res. 9:221-233. 1944.

Pennington, M. E.
A chemical, bacteriological, and histological study of cold-stored poultry. First Intern. Congr. Refrig. II, 216. 1908. (From Advances in Food Research, Vol. I, Mrak and Stewart p. 234-235. Academic Press, New York, N. Y. 1948).

Ramsbottom, J. M. and C. H. Koonz.
Freezing temperature as related to drip of frozen-defrosted beef. Food Res. 4:425-431. 1939.

Ramsbottom, J. M., E. J. Strandine, and C. H. Koonz.
Comparative tenderness of representative beef muscles. Food Res. 10:497-509. 1945.

Richardson, W. D., and Erwin Scherubel.
The deterioration and commercial preservation of flesh foods. Amer. Chem. Soc. Jour. 30:1515-1564. 1908.

Shrewsbury, C. L., L. W. Horne, W. Q. Braun, R. Jordan, O. Milligan, C. M. Vestal, and N. E. Weitkamp.
Chemical histological and palatability changes in pork during freezing and storage in the frozen state. Purdue Agr. Exp. Sta. Bul. 472, Lafayette, Indiana. May, 1942.

Stewart, G. F., H. L. Hanson, B. Lowe, and J. J. Austin.
Effects of aging, freezing rate, and storage period on palatability of broilers. Food Res. 10:16-27. 1945.