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Changes in palatability, microscopic appearance and electrical resistance in beef during the onset and passing of rigor and during subsequent storage

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CHANGES IN PALATABILITY, MICROSCOPIC APPEARANCE
AND ELECTRICAL RESISTANCE IN BEEF
DURING THE ONSET AND PASSING OF RIGOR
AND DURING SUBSEQUENT STORAGE

by

Pauline Constance Paul

A Thesis Submitted to the Graduate Faculty
for the Degree of

DOCTOR OF PHILOSOPHY

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Approved:

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INTRODUCTION

It has long been the practice of the better-grade meat handlers in this country to age or ripen beef from one week to six months before selling it. This is done by hanging the dressed carcass in a storage room at temperatures just above freezing. The storage is intended to make the meat more tender and to develop a more desirable flavor and odor. However, not all beef is ripened before cooking. In many places, the necessary cold-storage facilities are not available; so the animals are killed and the meat used as soon as possible, to prevent loss by spoilage. This has given rise to the frequently heard statement that freshly killed beef is more tender than beef which has been ripened. Some people lay stress on the idea that the meat should be cooked before it has a chance to go into rigor.

This study was undertaken to check whether or not un-ripened beef actually was more tender than ripened, and to determine if it would be possible to handle the meat so rapidly as to cook it before it had gone into rigor. Studies on chickens had shown that even if the chicken were placed in the oven before rigor set in, the muscles were in rigor by the time the cooking was finished.

The specific objectives of this study were as follows:

1) to follow any changes in palatability with onset and passing of rigor and ripening of beef;

2) to ascertain any changes in cooking time and losses in volume of the meat with ripening;

3) to determine the histological changes during storage of beef;

4) to correlate any histological changes with changes in palatability, if possible; and

5) to check the comparability of the different muscles for each measurement employed.

The conclusions reached were based on both subjective and objective measurements. The subjective determinations included the grain and marbling of the raw meat and any development of undesirable odors or stickiness with storage, and the flavor, aroma, tenderness and juiciness of the cooked meat. The objective tests included electrical resistance and pH of the raw meat, shear and press fluid values for the cooked meat, and histological studies of both the raw and cooked meat. Supplementary studies were made on the effect of massage on the meat before it had gone into rigor and of sudden heat treatment in inducing histological changes.

REVIEW OF LITERATURE

Muscle

This discussion is concerned with the voluntary, cross-striated, skeletal muscles, since these are the ones which are chiefly used as food. The terms "striation" and "striae" refer to the cross striations of these muscles. When longitudinal striation is intended, this will be specifically indicated.

Cross structure

The voluntary muscles are made up of long cylindrical muscle fibers supported by a thin fibrous network of connective tissue, the endomysium. The fibers are grouped in bundles defined by layers of connective tissue known as the perimysium. The muscle itself is enclosed in a heavy connective tissue sheath, the epimysium. The size of bundle varies with the kind of animal, and with the age, muscle and activity of the specific animal, and determines the grain of the meat.

Microscopic structure

Fibers: Striated muscle fibers vary in length from 1 to 41 millimeters and in diameter from 10 to 100 microns, or more, the diameter depending not on the length of the fiber but on

the type and age of the animal, and the particular muscle (Maximow and Bloom, 81).

Each muscle fiber is an elongated, multi-nucleated cell made up of nuclei, sarcoplasm, and fibrils, and surrounded by the sarcolemma. The nuclei occur along the edges of the fiber just under the sarcolemma--the enveloping membrane. The sarcoplasm fills the spaces between the fibrils. The fibrils are long, parallel, rod-like structures extending the length of the fiber. They are considered to be the active contractile structures of the muscular tissue. They do not anastomose, but are believed to be held in fixed relation to one another by some kind of cross connection. Buchthal and Knappels (22) present microscopic evidence for the existence of such connections.

The fibers of voluntary muscles are cross-striated. The two most prominent bands or discs of the fiber are the A (Q) and I (J) discs. These arise from differences in the myofibrils, whose alternating layers are so arranged that the stripes appear to be continuous across the entire fiber. The A discs are dark and shining, stain deeply, and are anisotropic. The I discs are paler, do not stain as deeply, and are only slightly anisotropic--about 10 per cent as much as the A discs, according to Schmidt (109, 110).

The anisotropy of the A discs is supposed to arise from a more ordered arrangement of the long, thread-like myosin chains within them. When the muscle is at rest, the A and I

discs are approximately the same height, the ratio being given as 55 to 45 by Langelaan (71).

The I disc is bisected by a thin dark line--the Z (T) line. The Z line is generally regarded as continuous throughout the diameter of the fiber, being attached to the sarcolemma. During contraction, the A disc is bisected by a thin light line known as Hensen's disc. This disc is regarded as signifying a state of contraction or stretch as it is not found in relaxed muscle.

The muscle fibers are birefringent. Buchthal and Knappels (22) state that this birefringence is partly caused by a parallel arrangement of micelles within the fibrils, and partly by the crystalline structure of these micelles. Von Muralt and Edsall (131) agree with this theory.

Connective tissue: The connective tissue is made up of two components, white and yellow. The white, or collagenous, fibers are non-elastic, colorless, and under high magnification show distinct striations. They occur as long, straight or wavy threads or ribbons, made up of fine, uniform, parallel collagenous fibrils. They are very flexible, but show great resistance to a pulling force. The yellow, or elastic, fibers are very long and appear brilliant and highly refractile. They occur in cylindrical threads or flat ribbons, and are highly elastic.

Constituents

Fibers: The protoplasm within the muscle fibers is an aqueous mixture containing proteins, fats, carbohydrates and other organic and inorganic compounds. The proteins of muscle fibers, according to Smith (115), consist of 68 per cent myosin, 21 per cent globulin X, 10 per cent myogen, and 1 per cent myoalbumin. The myosin occurs chiefly in the myofibrils and the other proteins in the sarcoplasm.

Myosin is one of the most interesting constituents of muscular tissue, as it is believed to be the substance which actually contracts and relaxes during activity. It is a globulin, being insoluble in water, but soluble in neutral salt solutions. However, between pH 5 and 6 it has been found by Edsall (38) and Salter (103) to be insoluble in any of the salt solutions customarily employed in the study of proteins. Edsall (38) found a marked increase in solubility of myosin in solutions of ionic strength from 0.2 to 0.4. This increase was especially marked in the range from 0.25 to 0.3, which is probably very close to the ionic strength of muscle plasma.

Solutions of myosin were found by von Muralto and Edsall (130, 131) to exhibit the phenomenon of double refraction of flow, a property indicating an asymmetrical micellar shape. In fact, the myosin micelles are believed to be rod- or needle-shaped. However, it has proved impossible so far to obtain native myosin crystals, because of the high affinity of myosin for water. This affinity for water may arise, at least partly,

from the fact that about one-half the amino acids known to occur in myosin are either acidic or basic, making a highly polar compound.

X-ray studies of myosin by Astbury and co-workers (6, 9, 10) indicate that the micelles consist of long chains of amino acids arranged parallel to one another, being held together by cross linkages between reactive R groups, hydrogen bonds, and van der Waals' forces. These chains are considered to be folded in some manner, the folds being so arranged and held in position that under stress they can either stretch or become increasingly folded. Woods (135) and Boehm (17) proved that these x-ray pictures were those of the true elastic substance, not some secondary histological component.

Myosin is easily denatured by all the usual protein denaturing agents. Its heat denaturation is given by Mirsky (87) as occurring in two steps, one at 37°C and the second at 40-45°C, which correspond to reversible and irreversible thermal contraction in muscle. He found that native myosin contained some free sulfhydryl groups, that no additional sulfhydryl groups were liberated in the first denaturation step, but that all the sulfhydryl groups in myosin became detectable after the second denaturation step. Astbury (7), by x-ray studies, confirmed the two-part denaturation of myosin.

Moran (92) stated that denaturation decreased the amount of water which could be bound by protein. This may account

at least in part, for the increased ease with which fluid can be expressed from cooked meat as compared to raw meat.

Connective tissue: The collagenous fibers are largely made up of the albuminoid, collagen. It is insoluble in water and neutral salt solutions, but soluble in dilute acids and alkalis. Collagen is broken down into gelatin under the influence of heat and water, particularly in an acid medium. This is important in meat preparation as the conversion of collagen to gelatin is one of the chief factors in increasing tenderness of meat by cooking.

Astbury (5, 8), from x-ray studies, suggested a nearly fully-extended configuration for the collagen micelle, the only bends in the chain other than those incident to the preservation of normal bond angles being those arising from the necessity of accomodating the imino rings of the proline and hydroxy-proline residues. This normal stretched configuration he related to the facts that collagen sheared rather than stretched under tension, and that when heated, tendons contracted to approximately 20 per cent of their normal length. Schmitt, Hall and Jakus (111) agreed with Astbury that collagen fibrils consisted of parallel columnar units, but suggested that these linear elements were highly folded and that the greater density of the dark striations was produced by greater folding in these areas than in the light striations.

The yellow elastic fibers consist principally of elastin,

which, like collagen, is classed as an albuminoid. Elastin is characterized by being extremely resistant to solution, and is therefore little affected by normal cooking operations. However, where it occurs in bundles, the fibers may be separated by disintegration of the cementing substance by heat and water, or by enzymic action.

Elastin is classed by Astbury (5, 8) as having the β -keratin configuration, a moderately-folded form like that of myosin. This permits either extension by straightening the chains, or contraction by additional folding.

Activity of Muscle

Normal contraction

The normal contraction of muscle is of interest here in that the changes which take place may furnish some ideas as to the alterations found in rigor.

Physical changes: During contraction the muscle as a whole shortens and thickens, there is a decrease in birefringence and in solubility of myosin, the bending of the myosin chains is altered, and the location of the inorganic salts shifts.

Muscle, according to Fenn (42), has a high degree of extensibility with small coefficient of elasticity, shortens when warmed, gives off heat when stretched, and decreases in

volume on shortening. These responses are similar to those of slightly stretched rubber. Wöhlisch (134) explains the heat changes as follows. The elastic force is represented by the tendency of the oriented molecules to return to a more random distribution by thermal agitation. The heat given off on stretching is an orientation heat rather than a heat of crystallization. Bozler (18) agrees with this.

The changes in volume are attributed by Dubuisson (35, 36) to hydrolysis of phosphocreatine and adenosine triphosphoric acid, since the hydrolysis products have a smaller molecular volume than have the combined forms.

According to Bozler (18), contraction is due to folding of the long molecules in the actual contractile structures, this folding being produced by electrostatic forces between active side groups. Fischer (44) states that during isotonic contraction the ratio of the long to the short axis of the myosin micelles decreases from the normal value of about twelve to two or three. Dubuisson (35) states that diffraction spectra indicate that muscle fibers possess a more or less rudimentary crystalline structure which is reinforced by stretching and diminished by contraction or by heat. This is paralleled by the work on birefringence of muscle.

Fischer (43) found an increase in birefringence of smooth muscle under increasing tension, probably indicating a more nearly parallel arrangement of the micelles, and possibly also an elongation. Buchthal and Knappels (21) found a 26 per cent

drop in double refraction of normal striated muscle during a series of contractions, paralleled by a 315 per cent increase in lactic acid content. They found that in the resting fiber the micelle bundles were not completely parallel, but became so on stretching or on isometric contraction. Jordan (67) also attributed the decrease in anisotropy during contraction to disturbances in the orientation of the particles as found in the extended fibers.

Chemical changes: The chemical reactions which occur during the contraction and relaxation of muscle are exceedingly complex, and much work remains to be done on the pathway of the conversion of carbohydrate to energy. The most important factors are given by Sacks (102) as phosphocreatine, adenosine triphosphoric acid, the hexose-phosphates, glycogen-lactic acid, and enzymes.

Schaffer and Ronzoni (108) in a review of carbohydrate metabolism state that the production of lactic acid was formerly thought to be one of the most important steps in the contraction of muscle, the energy of its formation being regarded as the source of the work of contraction. Now, however, the production of lactic acid is regarded as merely an emergency anaerobic reaction by which the normal muscle bridges the gap in the oxygen supply when activity is first initiated, until the respiratory and circulatory mechanisms are stepped up sufficiently to supply the extra oxygen necessitated by such

activity. A recent review of work on this phase is given by Millikan (84).

Smith (117) gives the pH of living muscle as 7.0-7.5, and states that it may actually be slightly lower. Sacks (102) gives the normal pH of mammalian tissue as 6.6-6.7, while Voegtlin et al. (129) give the pH of living muscle as 7.55.

Dubuisson (35, 36) summarizes the main physical and chemical changes during contraction and relaxation as follows:

1. During contraction, hydrolysis of adenosine triphosphoric acid results in the initial heat of contraction, an increase in acidity, a rapid decrease in volume, and an increase in impedance.

2. At the maximum of contraction and beginning of relaxation, the hydrolysis of phosphocreatine causes the heat of relaxation, a decrease in acidity, a slow decrease in volume, an increase in impedance, and a decrease in transparency.

3. The production of lactic acid gives the anaerobic post-contraction heat, an increase in acidity, an increase in volume, and an increase in transparency.

Simultaneously with these changes, the molecules of myosin change in structure, these changes making possible the mechanical manifestations of muscular contraction. It has been suggested that contraction involves gelation of myosin, which returns to the sol state on relaxation. Smith (114), however, states that the natural environment of the protoplasm is such that at least 90 per cent of the myosin is in the gel

state in resting muscle. Dubuisson (36) suggests that the iso-electric point of myosin shifts during contraction, while Deuticke (34) states that contraction is accompanied by dehydration of muscle proteins, as indicated by the decreased solubility in certain salt solutions. These findings would suggest that myosin may be denatured during contraction, since loss of solubility is one of the criteria of denaturation, while Mirsky and Pauling (89) hold that the iso-electric point of proteins shifts toward neutrality upon denaturation.

There has been much discussion as to whether or not the changes in myosin which occur during contraction represent an actual denaturation of the protein. Part of the difficulty arises from the various definitions of denaturation, which may be based on solubility, availability of sulfhydryl groups, or biological activity. Kendall (69) suggests that some term other than denaturation be used to designate the changes which occur in myosin following activity of the muscle.

Mirsky (85) found that there was little or no denatured myosin present in freshly minced muscle. Smorodintzev (121) confirmed this finding. Saxl (107) first showed that prolonged activity decreased the solubility of muscle protein, attributing the change to myogen. Deuticke (33, 34), Mirsky (86), and Danielli (30) have shown that the change is in the solubility of myosin, not myogen. This loss of solubility is not a case of precipitation, as the myosin in resting muscle is probably not in solution. The change involves a modification

of myosin by activity such that when the structure of the tissue is destroyed the myosin is not soluble in salt solutions which will dissolve the myosin of resting muscle. Mirsky goes on to say that this is not a true denaturation, as no sulfhydryl groups are activated.

Whether or not this decrease in solubility is a true denaturation, it must be reversible. Anson and Mirsky (3, 4) have succeeded in reversing the denaturation of methemoglobin and of globin. Neurath, Cooper and Erickson (97, 98) found only an apparent, not a true reversal of denaturation of horse serum albumin and pseudoglobulin. However, these proteins are quite different from myosin, the micelles being globular rather than rod-shaped as the myosin micelles are. So far, the denaturation of myosin has not been reversed in vitro.

Marsland and Brown (80) suggested that the phosphorylation and dephosphorylation of myosin itself might be the final factor governing the actual contraction and relaxation, since the degree of phosphorylation of adenosine triphosphoric acid seemed closely related to myosin. Millikan (84) also stated that on contraction myosin liberated phosphorus, while during relaxation phosphorus was donated to myosin by adenosine triphosphoric acid.

Deuticke (33) found a definite relationship between the solubility of the muscle proteins and the ability of muscle brei to use inorganic $(PO_4)^{\equiv}$ in forming hexosediphosphate. Lundsgaard (75) also suggested a phosphorus compound as the direct

energy furnisher in muscle activity.

Needham et al. (96) suggest the following possible sequence for the contraction of muscle.

1. Stimulus allows the enzyme, myosin, and the substrate, adenosine triphosphoric acid, to come in contact.

2. Contraction occurs during linking up between the triphosphate and myosin, since both the $(PO_4)^{\equiv}$ and the purine-ribose ends of the former are linked to the enzyme, and the molecular length of the triphosphate is probably shorter than the length of the myosin chain between the groups to which the triphosphate must attach. This is in line with the general two-affinity theory of enzyme action.

3. Later splitting off of inorganic $(PO_4)^{\equiv}$ from the substrate provides the energy for relaxation and recharging of the myosin fibril.

Histological changes: The findings on the histological changes which take place during contraction and relaxation are difficult to coordinate because of the great variety of conditions employed in the various studies. Jordan (66, 67) and Speidel (124) speak of the formation of contraction bands, produced by the migration of the darkly-staining substance of the A disc away from the M line to a position near the Z line. This is termed reversal of striation. Speidel (124) states

The number of cross striations in a fiber does not vary during contraction, relaxation, stretching, or retraction. Shortening of the fiber is accompanied by shortening of individual sarcomeres, not by multiplication of sarcomeres.

Carey (26, 27), on the other hand, believes that during contraction there is an active multiplication of striae produced by micro-compressional waves within the muscle fiber.

Buchthal et al. (23) measured the length of the A and I discs in living muscle fibers at rest and during isometric and isotonic contraction. They found that in isometric contraction the A disc decreased and the I disc increased in length. Bozler (18) confirmed the finding that only the A discs contract actively, the I discs becoming longer. This bears out the theory that the myosin is concentrated in the A disc.

It was formerly held that the "all-or-none" principle applied to contraction, that is, that the stimulus had to be sufficient to cause the entire fiber to react before any response could be obtained. However, Gelfan (49, 50, 51) has shown that, with micro-equipment fine enough to localize the stimulus to a part of one fiber, the stimulated part reacts without affecting the remainder of that fiber or adjacent fibers. The "all-or-none" principle is still regarded as valid for diffuse stimuli, however.

Rigor

Rigor is designated as a case of contracture rather than contraction, as the changes which take place are not quickly reversible, and in many instances are actually irreversible. The chemical and histological changes accompanying rigor vary with the state of the tissue and the means employed for

killing the tissue. After the death of the animal, the tissues usually live on for varying lengths of time until the accumulation of metabolic products poisons them. Thus, the individual muscle fibers may die at varying times, resulting in slow death of the muscle. This leads to the onset of normal rigor. Various experimental forms of rigor may be produced in tissues killed by methods inducing sudden extensive changes in the protoplasm, such as heat, freezing, strong electric shock, exposure to volatile anesthetics, or injection of tetanus toxin.

Gross changes: Rigor is manifest macroscopically by a stiffening of the muscle substance, with increase in opacity. Parnas (101) states that

. . . all the familiar metabolic changes (viz., lactic acid formation, deamination, splitting-off of phosphorus from phosphocreatine and adenosine triphosphoric acid) are known to occur at a very fast rate in the traumatic and postmortal decompositions.

Smith (118) states that it is unusual for a muscle to shorten in normal rigor, but that occasionally it does so when the onset of rigor is exceptionally rapid.

The length of time before normal rigor sets in, and the duration of such rigor depends on the kind of animal, its nutritive state, and the amount of physical activity just prior to killing. It first affects the muscles of the head and then spreads backward over the body.

Smith (118) states that temperature has little effect on

the rate of onset of rigor, except at extremes. Temperatures of 45°C or above cause heat rigor, while freezing will either cause or delay rigor, depending on whether or not the muscle is still alive when frozen, and on the freezing temperature employed. In beef, normal rigor appears in from three to seven hours after death, is maximum in from twelve to twenty-four hours, and may persist from one to three or more days.

Mangold (77, 78, 79) studied the onset of rigor in mice, rats, and rabbits. He found that, in general, an isolated muscle went into rigor faster, took longer to attain maximum stiffness, but began to lose rigor more quickly than did the entire body of the animal. The muscles in rigor were approximately 67 per cent stiffer (more resistant to pressure) than they were immediately after death.

Chemical and physical changes: In the normal animal, the onset of rigor is paralleled by a drop in pH, an increase in lactic acid, a decrease in glycogen, and hydrolysis of nearly all the esterified phosphates to orthophosphates. There is also a decrease in double refraction. Liang (74) found no fundamental relationship between the change in double refraction and the increase in lactic acid content, but suggested a relation of the change in double refraction to the development of tension. This, he concluded, indicated that the decrease in double refraction arose from an alteration in the structure of the myosin micelles in the muscle fibrils. This would be analogous to the change occurring in normal

contraction of living muscle.

Smith (118) found that the onset of rigor in rabbit muscle increased the modulus of elasticity from 700-3000 to around 10,000. In the normal muscle containing not less than 0.7 per cent glycogen, this stiffening was paralleled by the conversion of glycogen to lactic acid, by the heat production accompanying this reaction, and by a drop in pH. The pH of freshly killed muscle varied from 6.6 to 7.6. In full rigor, the pH had dropped to 5.8. Above 6.2, the pH varied from point to point in the same muscle, but when this pH was reached, the muscle rapidly became uniform throughout. The time of onset of rigor in normal muscle varied from zero to seven hours or more, with violent activity immediately before killing hastening its appearance. Voegtlin et al. (129) found that normal rigor in rats set in at pH 6.85-6.68, at which time the lactic acid content was six times that of the living muscle. The lowest postmortal pH which they obtained was 6.21, corresponding to twelve times the normal lactic acid content. This was obtained by holding the animals at 6°C for one to two days.

As with normal contraction, the production of lactic acid was formerly thought to be the most important factor in the appearance of rigor, the stiffening of the tissue being attributed to the denaturation of myosin by increasing acidity. However, Wacker (132) found that in animals depleted of glycogen by starvation or extreme physical activity just before death, almost no lactic acid was produced. The muscles

remained alkaline, about pH 7.1, and went into rigor very quickly, taking from three to thirty minutes, while the normal muscle took one and one-fourth to two hours. Smith (118) and Hoest and Marks (65) confirmed this finding that rigor would occur in the absence of lactic acid in glycogen-depleted muscles. Lundsgaard and coworkers (61, 75) showed that muscles containing glycogen would go into rigor without production of lactic acid if the muscle were poisoned with iodoacetic acid, which prevents the conversion of glycogen to lactic acid. Voegtlin et al. (129) found that the pH of muscles so poisoned increased from 7.55 to as high as 7.96, with rigor setting in at about 7.68. Parnas (100) summed up these findings with the statement:

Rigor has nothing whatever to do with lactic acid formation or with changes brought about by the acidification of the tissue.

Smith (118) found that the change in pH during rigor was insufficient to have any effect on the elasticity or length of the collagen fibers, and concluded that the connective tissue played no part in the stiffening of the muscles in rigor.

It has been found by many investigators (33, 62, 86, 132) that as much as 70 per cent of the myosin of muscle became insoluble in neutral salt solutions during rigor. Mirsky (88) attributed this to a denaturation analogous to the first step in heat denaturation of myosin, since there was loss of

solubility without activation of sulfhydryl groups. Smith (116), however, was unable to confirm the loss of solubility unless the temperature of storage was high enough to produce heat denaturation. He attributed the change in apparent solubility to changes in the permeability of the membranes surrounding the fibrils, giving increasingly difficult extraction of the protein, rather than to any denaturation of myosin itself.

Histological changes: One of the common observations in microscopic studies of rigor is the variable response of the fibers. Some fibers develop the characteristic zones of contraction and rarefaction, others exhibit a wavy or kinked appearance which is interpreted by Carey (26) as indicating that these fibers are not under tension but are passively retracted by the contraction of the noded fibers. The appearance of the contraction nodes also varies with the means employed in inducing rigor.

Most of the studies on the histological aspects of rigor have been done on material where rigor was induced by experimental treatment such as heat, electrical stimulation, freezing, and so forth.

In muscle where rigor was produced by extreme heat, freezing, electrical stimulation, and so forth, the fibers showed irregular transverse bands of dense material having very fine striations, alternating with rarefied areas having

widely separated striations. Nageotte (95) explained this phenomenon as follows. The entire muscle, when killed suddenly, contracted from 60 to 80 per cent, became thicker, more opaque, and rigid. The fibrils might contract as much as 90 per cent. However, the total fibrillar contraction could not be greater than that of the muscle itself. This produced alternate zones of high contraction and of stretching in the fibrils. Where the fibrils were contracted to the maximum degree, the fiber appeared homogeneous and very dense. If the contraction was not quite so great (about 70 per cent), very fine striations were still apparent in these dense regions. The alternating sections were very much stretched by this contraction, and appeared thin with widely-spaced striations. The fibrils in the contracted areas were so swollen that all the liquid sarcoplasm between them was squeezed out into the stretched sections. The loss of liquid increased the density of the contracted areas, while the increased liquid content of the rarefied areas made them even more transparent. Nageotte concluded:

The complete homogeneity of the fibrils in a state of extreme contraction and the new characteristics which they acquire in that state lead one to believe that the striations are not produced by the periodic distribution of distinct substances, but by periodic distribution of distinct molecular states in the midst of a single substance; these states are susceptible to multiple variations during the course of the contraction. Thus it is possible to explain . . . the multiplicity of forms of contraction of muscle, depending on the means of excitation, which the physiologists have observed. (translation)

Carey (26) studied the heat rigor nodes produced in muscle by exposure to temperatures of 20 to 40°C. Such treatment produced long compact nodes in the contracted fibers. These nodes were longer and less compact than those found in rigor produced by electric current or freezing, and exhibited the fine striations characteristic of less than maximal contraction. They alternated with areas of coarse, widely-spaced striations indicating stretching. He believed that the fine striations of the contraction node arose from active multiplication of the striae by splitting.

Meneely (83) described contractures, similar to those studied by Carey, produced in living fibers stimulated by dilute caffeine solution or by the application of constant current. The nodes produced by gentle stimulation were swollen, with very crowded striations. The adjacent regions were stretched, with increase in distance from one Z line to the next. The strain sometimes caused rupture of the fiber. These nodes were local, reversible, and not propagated, and were formed by a crowding together of the striations. With prolonged, or increased stimulation, the nodes became homogeneous, refractile, and irreversible, indicating injury of the fiber. These latter nodes he believed to resemble those of Zenker's hyaline degeneration.

Hanson (58) found heat rigor nodes in chicken muscles that were cooked before the onset of normal rigor. She also found some evidence of rigor nodes in raw chicken muscles that

had gone into rigor under normal conditions.

In the stretched regions, rigor increases the prominence of the longitudinal striations of the fibers. There is also some slipping of the fibrils, so that the bands which give the cross-striated appearance are no longer perfectly aligned. This gives rise to irregularities in the striations, such as Vernier effects, zigzags, diagonal stripes, and so on.

Davenport et al. (31, 32) describe the myostatic contractions produced in situ by tetanus toxin or by section of one tendon of the muscle. This gave (1) increased diameter of the muscle caused by shortening, (2) more pronounced longitudinal striations due to greater evidence of the individual fibrils, and (3) blurred cross striations occasioned by disruption of the accurate transverse alignment of the myofibrils. Some fibers exhibited a wavy contour, similar to that attributed by Carey (26) to passive retraction. The fibers contracted by tenotomy showed irregular condensed areas akin to the extreme contraction bands found by Nageotte (95) in electrically stimulated and in frozen muscle.

Dissolution of rigor

Physical changes: If muscle in rigor is stored at temperatures above freezing, the gross aspects of rigor will pass off, the muscle becoming pliable and soft again. The time of passing will depend on the animal, the particular muscle, the

time of onset of rigor, and the temperature at which the muscles were stored. In normal rigor, the muscles which first go into rigor are the first to lose it. In the so-called alkaline rigor where no lactic acid is produced, Hoet and Marks (65) found that rigor passed off more rapidly than in normal muscles which became acid from increase in lactic acid content. Also, the higher the storage temperature, the more rapidly the state of rigor passes. Hanson (58, 59) found that the heat of cooking accelerated the passing of rigor.

Histological changes: Hanson (58, 59) found that holding chicken muscle after death produced breaks in the fibers, some as sharp cracks or ruptures in the fiber, others as long areas where the protoplasm of the fiber had disintegrated. Cooking hastened the appearance of these breaks.

Changes in electrical resistance: The electrical conductivity of any medium depends on the number and mobility of the ions present. It is usually measured in terms of resistance rather than conductance, the one being the reciprocal of the other.

Electrical conduction in meat takes place for the most part through the extracellular fluid around the fibers, but also partly through the intracellular material of the fibers themselves. However, the cell membranes impose a barrier to the passage of the current through the cells. And, in living tissue, the extracellular fluid is usually found in

discontinuous pools or layers, rather than as a continuous system. Therefore, the electrical resistance of living muscle tissue is usually fairly large, especially when measured across the fibers rather than along them.

Hemingway and Collins (60) studied the changes in resistance in dying rabbit muscle, using a 1000-cycle current. They found that the resistance increased 25 to 100 per cent over that of the living muscle in from two to ten hours after death of the animal, then steadily decreased, finally reaching a plateau at about the resistance of the cell sap. They considered that these changes might be attributed to one of two autolytic changes: (1) increase in the ionic concentration by liberation of ions normally bound to protein, or (2) changes in the permeability of the cell membranes. They concluded that the changes were in the cell membranes rather than in the ion concentration.

Osterhout (99) found that the increase in cell permeability which accompanied death was paralleled by a simultaneous increase in electrical conductivity. He thought at first that changes in resistance could be explained by changes in viscosity, but found that viscosity changes did not parallel changes in resistance produced by treating either living or dead tissue with various reagents.

Callow (24) investigated the changes in electrical resistance of pork muscle. He found that immediately following death, the resistance was greater across than along the fibers.

After storage for twenty-four hours, the difference was no longer detectable. He also found that the resistance decreased after death, the time to reach the minimum varying with the glycogen content of the muscles and the temperature of storage. He suggested that high resistance indicated a "closed" structure, the cell membranes being poor conductors, and that the decrease in resistance indicated the change to an "open" structure, the membranes becoming permeable. The formation of the "open" structure was favored by the increase in lactic acid obtained when the muscles had a normal glycogen content, and by slow cooling, too rapid cooling leading to persistence of the "closed" structure.

Hall (53), summarizing investigations on electrical resistance as a possible indication of tenderness and quality in beef, states that, in general palatable tender beef has a resistance of around 150 to 200 ohms, as compared to values up to 700-800 ohms for less desirable meat. However, he points out that this is a very variable measure.

Effect of massage: Callow (24) found that massage caused a very rapid drop in the electrical resistance, especially that across the fibers, the resistance decreasing as much as 50 per cent in thirty seconds. This he attributed to the effect of massage in separating the fibers and favoring the coalescence of the discontinuous interstitial fluid. The magnitude of this change depended, of course, on the amount of

fluid present in the capillary spaces. So the effect of massage was less shortly after death than after some storage.

Stewart (127) has shown that massage of chicken muscles will prevent the development of rigor or cause very rapid dissolution of rigor in muscles which have already attained that state.

Cold Storage of Meat

Purpose and practices

It is a common practice among meat handlers to chill meat to temperatures just above freezing immediately after the animal is killed and dressed. In the larger meat packing houses, the carcasses are usually placed in a chill room for about twenty-four hours until the exterior of the meat is cooled to about 35°F, when they are transferred to storage rooms for at least another twenty-four hours until the interior of the meat has cooled. Further storage will depend on the type of meat and the state of the market.

Beef may be held in cold storage from a few days to several months. It is generally considered that storage up to two to four weeks improves the palatability of the meat. However, if there is an unusually heavy demand, or refrigeration facilities are not available, the beef may be sold with little or no cold storage.

The changes which occur in beef held at temperatures just above freezing have two chief causes, one the autolytic action of the enzymes of the tissue, the other the action of various microorganisms with which the meat may be contaminated. The first series of changes is desirable, the second usually not.

The autolytic changes which occur involve the resolution of rigor and subsequent partial breakdown of the muscle fibers and connective tissue. These changes increase the tenderness and juiciness of the meat, and may improve the flavor.

The microorganisms which may grow on meat include bacteria, molds and yeasts. Pathogenic bacteria are, of course, undesirable. However, they usually are not a great problem, as most of them do not grow at temperatures below 5°C. Many other bacteria will, however, grow at lower temperatures, and may cause sliminess of the surface, bad odor, and eventually putrefaction of the meat. They grow principally on the surface of the meat, but may penetrate into the body along blood vessels, connective tissue, or bone. Molds and yeasts usually grow on the surface of the meat only. If not allowed to go too far, they can be wiped or trimmed off with very little damage to the meat itself. Yeasts, however, may cause fermentations which impart undesirable flavors and odors to the meat.

The two processes--ripening by autolysis and changes by microorganisms-- are independent of one another. The purpose

of cold storage is to discourage the growth of microorganisms while permitting the enzymic changes to proceed. Enzyme activity will continue in beef held just above freezing, but it is very much slower than at higher temperatures.

The cold storage life of beef depends on the character of the beef and the temperature and humidity of the storage room. In general, the higher the quality of the beef, the longer it can be stored. This is because high quality beef has a heavier, more nearly continuous covering of fat than does poor quality meat. This fat inhibits the growth of many microorganisms and prevents the meat from drying out. The temperature and humidity must be balanced between discouraging the growth of microorganisms on one hand, and lessening the quality of the beef on the other. The temperature must be near freezing to prevent rapid multiplication of microorganisms, yet too low a temperature will freeze the meat so that it must be sold as frozen rather than as fresh beef. Low humidity discourages the growth of microorganisms, but will also cause excessive drying of the meat with consequent loss of quality.

Studies on storage of meat

The studies on cold storage of meat prior to 1909 have been summarized by Emmett and Grindley (39). Since that time, quite a few studies have been made, centering chiefly on chemical and palatability changes. Very few histological studies have been made, however.

The following criteria for ripening performance are given by Hall and Mackintosh (54), for beef rib cuts:

1. Ends of cut should remain smooth and compact with a light covering of filamentaceous mold having a mild inoffensive odor; discoloration should not penetrate more than 1 centimeter.

2. The normal expectancy of shrink in the 6-12 rib cut weighing 10 kilograms is about 500 grams.

3. The electrical resistance should drop from 300-500 ohms for the fresh meat to 150-250 ohms for the ripened meat.

4. The reaction of the meat usually becomes about 0.1 pH unit more alkaline.

5. The collagen may be changed very little if the original content was below 4 per cent. When originally 8 to 10 per cent, the collagen may be reduced by half, and correspondingly less between 4 and 8 per cent.

The principal chemical changes which occur during ripening of beef include some evaporation of water, slow rise in pH, changes in the proportions of the nitrogen fractions, with increase in the soluble nitrogen, and, if ripened long enough, oxidation of the fat. The physical changes include increase in electrical conductivity and in tenderness, brought about by breakdown of the muscle fibers and connective tissue.

Changes in pH: It is usually considered that in meat obtained by normal practices, there will be a drop in pH, brought

about by production of lactic and other acids in the tissue, followed by a gradual increase in pH attributed to the activity of bacteria. This has been clearly shown in several studies (119, 122, 123). The higher the temperature at which the meat is stored, the quicker the rise in pH sets in and the more rapidly it progresses. However, Hoagland, McBride and Powick (64) found that in meat samples procured under rigidly aseptic conditions and stored so as to prevent bacterial contamination, there was a gradual increase in acidity throughout the storage time of seventy-seven days.

The lowest pH which will be attained by meat not held aseptically is a function of the glycogen content of the meat. Callow (25) spoke of this as the "ultimate pH," and stated that the higher the glycogen content of the meat, the lower the "ultimate pH" from formation of lactic acid.

Changes in proteins: There seems to be no appreciable change in the proteins of beef during the first few days of storage. There may be a decrease in solubility of myosin in rigor, but this has been challenged by Smith (116) as discussed previously. However, after a period of storage varying from six to twenty days, various investigators (39, 64, 73, 82, 120) have found evidence of actual hydrolysis of the tissue proteins, with increase in the total soluble nitrogen and in the amino-nitrogen fractions, at the expense of the protein nitrogen.

Changes in tenderness: Most investigators agree that one of the most important palatability changes during ripening of beef is increased tenderness. However, there is some disagreement as to the magnitude of the change in any given time, probably arising from differences in experimental materials and conditions.

Lehmann (73) found a 20 to 40 per cent increase in tenderness from the second to the eighth day of storage. Hoagland, McBride and Powick (64) found that unripened beef was tough and rubbery and that the greatest change in tenderness occurred in two to four weeks of storage, with flavor deteriorating after six weeks. Hiner and Hankins (65) found that tenderness increased up to thirty-five days, the greatest change occurring from the fifth to fifteenth day. Criswold and Wharton (52) found no increase in tenderness between the ninth and thirty-seventh day of storage, and Beard (15) obtained this same result in a comparison of ten and thirty days of storage. Moran and Smith (94) found the tenderness increased from the third to seventeenth day. The consensus seems to be that beef should be held in cold storage from ten days to two weeks to allow for increase in tenderness. Beyond this point, flavor changes occur which may be desirable or undesirable, depending on the market for which the meat is intended.

Ewell (41) and Criswold and Wharton (52) found that two to three days' storage at 60°F had the same tenderizing effect as three or more weeks at 34-36°F. However, careful handling

to minimize bacterial contamination was necessary to avoid heavy trimming and consequent economic loss.

There are two theories as to the mechanism of the tenderizing action during cold storage. Steiner (125, 126) states that the decrease in toughness is caused chiefly by changes in the muscle fibers, making them brittle and inelastic, while Ewell (41) holds that the principal changes occur in the connective tissue rather than in the muscle fibers. It seems reasonable to suppose that both changes occur, the magnitude of each depending on the make-up and condition of the meat.

Winkler (133) and Duisberg and Miller (37) have shown that the tenderness of meat increased with increase in pH above 5, with a pH above neutrality causing undesirable softening. They attributed this increased tenderness to changes in the state of the proteins and activation of proteolytic enzymes, rather than to actual hydrolysis of connective tissue during storage.

Changes in cooking losses: Emmett and Grindley (40), Moran and Smith (94), Alexander and Clark (1), and Beard (15) all agree that storage decreased the total cooking losses by decreasing the evaporation loss, giving more juicy meat. Storage also decreased the cooking time per pound in most of these studies, although Beard found no difference between ten and thirty days' storage.

Changes in palatability: The changes in tenderness and

juiciness have already been mentioned. The other principal factors in determining palatability are the flavor of fat and of lean. In these factors too long storage may be undesirable. Investigators are agreed that after about six weeks of storage, the lean of beef develops a "high" or "gamey" flavor. This is undesirable to most people although connoisseurs generally prefer well-ripened beef. Prolonged storage may also lead to rancidity of the fat. Australian workers (45, 46, 47, 48, 112) found that many of the microorganisms contaminating beef carcasses were capable of liberating fatty acids from the neutral beef fat. This was confirmed by Lea (72). Moran (93) states that rancidity in beef fat stems chiefly from the breakdown of the connective tissue framework of the fat by microorganisms.

Changes in microscopic structure and electrical conductivity: The changes in these factors during onset and resolution of rigor have already been discussed. Little work has been done on the effect of further storage on them.

Hoagland, McBride and Powick (64) found no noticeable change in histological structure of beef after seventy-seven days' storage. However, they did notice an increased ease in the separation of the muscle bundles. Hanson (58) has shown that there is a definite change in chicken muscle with storage, characterized by increase in the number of breaks in the muscle fibers. Brady (19, 20) found that the diameter of muscle fibers of beef triceps brachii, longissimus dorsi,

adductor and semitendinosus decreased during ten days' cold storage.

Cooking Studies

Palatability

The palatability of beef depends chiefly on the tenderness, juiciness, and the flavor of lean and fat. The ultimate criterion of any food is the reaction of the person who eats it. Therefore, any final judgment on the worth of various treatments of beef depends on the reactions of the judges who sample it.

Many attempts have been made to reduce the judging of meat to objective terms. So far, the only one which has been successful is the use of mechanical devices to measure tenderness. Juiciness apparently depends not only on the quantity of fluid in the meat, but also on the amount of fat, and the response of the salivary glands during chewing. This latter depends on the texture, flavor and aroma of the meat. Flavor, also, is difficult to gauge objectively, since it is compounded of many factors--taste, aroma, and "feel" in the mouth, the latter depending on texture and tenderness.

The literature on palatability has been summarized by Towson (128) and by Hanson (58).

Tenderness

Tenderness is considered as one of the palatability factors, but seems to warrant separate discussion, since it is one of the most important factors in the changes occurring during storage.

The inherent toughness or tenderness of meat seems to depend largely on the amount of connective tissue present. A high correlation between connective tissue content and tenderness was found by Lehmann (73), Mitchell (90), and Mackintosh, Hall and Vail (76). The connective tissue content is apparently intimately related to the size of the muscle bundles, or texture, as each muscle bundle is surrounded by a connective tissue sheath, so the smaller the bundles, the greater the amount of sheathing in the muscle. Beard (14) states: "The inherent properties of the endomysium contribute to the toughness of meat more than does the size of fiber." Hammond (55) and Brady (20) found that the grain (size of muscle bundle) was closely related to tenderness.

It has been suggested that the amount of intramuscular fat or marbling in beef may affect tenderness. Hankins and Ellis (56) found no evidence of such relationship. Beard (14, 15), however, found that tenderness increased with increasing intramuscular fat content.

In the cooking process, there are two opposing factors affecting tenderness. One is the change in connective tissue, the other the change in the fiber proteins. The elastic

tissue is not changed by the normal cooking processes, but part of the collagen is hydrolyzed, the extent of hydrolysis increasing with longer cooking time. Mitchell, Zimmerman and Hamilton (91), and Bell, Morgan and Dorman (16) have shown that the collagen content of beef decreased during cooking. This decrease was paralleled by an increase in tenderness. Cover (28, 29) found that long slow roasting of beef increased the tenderness. She attributed this to increased hydrolysis of collagen. However, Satorius and Child (105) found that the tenderness decreased if the meat were cooked to internal temperatures above 67°C. This they attributed to the toughening effect of heat in coagulating and hardening the fiber proteins.

Cooking losses

The effect of storage on cooking losses has already been mentioned. The other important factors in determining losses are the degree of fatness of the meat and the cooking time and temperature.

The effect of degree of fatness on cooking losses is twofold, and is principally governed by the thickness and extent of the external fat layer. A layer of external fat increases the dripping losses, since the melted fat collects in the pan. This fat layer, however, helps to decrease the evaporation losses, as it reduces the external area from which water can be lost.

It has been definitely established by many studies that high oven temperatures increase the cooking losses by speeding up evaporation of water and by increasing the amount of fat melted off the surface.

Comparability of Various Cuts

One of the major problems of meat research is that of obtaining comparable material with which to work. It has long been considered that similar cuts from the right and left sides of the same animal are comparable. This has been confirmed in such studies as those of Hankins and Hiner (57) and Alexander and Clark (2). However, the use of only two cuts per carcass for any given study would create a serious economic problem. Therefore, much consideration has been given to the comparability between different cuts and muscles of the same side of the carcass. Proper statistical design will assist in eliminating much of the difference attributable to variation between muscles, but to be able to consider the muscles as truly comparable would often simplify the working out of meat problems.

Hankins and Hiner (57), studying beef short loin, found that the cuts from the rear portion were more tender than those from the front portion.

Satorius and Child made extensive studies of the comparability of beef cuts. Working with the adductor,

longissimus dorsi, and triceps brachii, they obtained the following results (104):

1. There was no difference in press fluid among the three muscles.
2. The triceps brachii had greater total moisture than the other two, which did not differ from each other.
3. The adductor received the lowest judging scores for texture, tenderness, quality and quantity of juice. The longissimus dorsi and triceps brachii received about the same scores.
4. The adductor had the greatest cooking losses, and least fat--both internal and external.
5. The adductor required the greatest shearing force, had the smallest number of fibers per bundle, and the largest fiber diameter of the three. The other two were comparable in these factors.

They concluded from these findings that the longissimus dorsi and triceps brachii could be considered comparable for many tests, but that the adductor was quite different from the other two muscles.

In a study utilizing the different rib roasts of beef, the 7-8, 9-10, and 11-12 rib cuts, they found the following (106):

1. The 11-12 rib had higher press fluid and lower cooking losses than the other two.
2. All three were the same in tenderness.
3. The 7-8 rib cut had the highest content of ether-extractable material.

4. In total moisture, the 7-8 cut was highest, 9-10 next, 11-12 lowest.

They concluded that the 7-8 and 9-10 rib cuts could be considered comparable in tests of press fluid, tenderness, and cooking losses.

These studies illustrate the possibilities of variation between muscles and even between different positions in the same muscle, since the tests on the rib roasts were all done on the longissimus dorsi muscle. They also show that the comparability must be determined in terms of each measure to be employed.

Muscles Employed in This Study

Semitendinosus

The semitendinosus is a long narrow muscle, roughly triangular in cross section, which runs down the back of the thigh from the proximal end of the femur to the upper end of the tibia. It is an external muscle, so has a layer of fat on one side.

Semimembranosus

The semimembranosus is a large, thick muscle, lying on the medial side of the thigh. It is covered by the gracilis, so has no external fat. It is approximately rectangular in cross section, being nearly as thick as it is wide.

Biceps femoris

The biceps femoris is a very large external muscle which lies on the lateral surface of the thigh. Its upper end is attached to the sacral and coccygeal portions of the spine. It then curves down over the outside of the thigh and ends in attachments at the side and back of the joint between the femur and tibia. The biceps is rather rectangular in cross section, being much wider than it is thick.

Vasti

The vastus muscles include three divisions, the vastus lateralis, vastus intermedius, and vastus medialis. These muscles lie on the front of the thigh, partially surrounding the femur. They extend from the top of the femur to the patella, and are rather difficult to separate.

Gastrocnemius

The gastrocnemius is a thick-bellied muscle extending from the lower third of the femur to the point of the hock. It has two sections which surround the superficial digital flexor. This latter muscle is usually included with the gastrocnemius in cutting beef for cooking, and is very tough because of the large amount of tendinous material in it.

Adductor

The adductor is a small fleshy interior muscle, lying

close to and just in front of the semimembranosus. It extends from the pelvic bone to the lower part of the femur.

Psoas major

The psoas major is a fleshy muscle extending from the last two ribs to the pelvic region. It is somewhat triangular in cross section, and the proximal portion is smaller than the distal end.

A detailed description of the origin, insertion, action, structure, and relations of these muscles may be found in Sisson and Grossman (113).

MATERIALS AND METHODS EMPLOYED

Materials

History of animals

The meat used in this study consisted of a pair of rounds and a pair of psoas major muscles from a yearling steer obtained through the Animal Husbandry department. The steer had been on a feeding experiment for two months before slaughter, and was graded "good." The rounds weighed 48 and 50 pounds, and the psoas muscles 1-1/2 pounds each. It had been hoped to utilize the same cuts from at least two or possibly three animals, but the present rationing program and scarcity of beef made it impossible to obtain more than one animal.

The animal was killed on February 16, and was dressed immediately. The psoas major muscles and the rounds were cut off as soon as the animal was dressed. The roasts cooked without storage were cut off at once, and the remainder of the meat stored in the Animal Husbandry cooler at 34-36°C until the next day, at which time the rest of the muscles were separated and cut into roasts. Each roast was labeled and wrapped in pliofilm. Then the five cuts for each storage time were wrapped together and returned to the storage cooler.

Storage

The meat was stored in the Animal Husbandry meat cooler at 34-36°F. The cuts were so divided as to give five roasts for each storage time. One group was cooked without any storage. For these cuts, the time between killing the animal and putting the roasts into the oven was three hours. The storage times used for the other groups were one, two, four, nine, and eighteen days. It was originally planned to hold the last group for at least three weeks before cooking (longer, if possible), but, since the cutting and handling increased the rapidity of ripening and also the bacterial contamination, the cuts could not be held longer than eighteen days without loss of palatability through undesirable changes in the flavor of the lean and fat, especially the latter.

Methods

Statistical design

A balanced incomplete block design was employed, to yield as many tests as possible from the one animal. The following muscles were utilized: semimembranosus, semitendinosus, biceps femoris, the vastus group, gastrocnemius, adductor, and psoas major. The first four were large enough to yield three roasts from each muscle. The last three muscles were combined into one group. However, it was recognized that this last

group would not be as homogeneous as the first four, and allowance was made for this in certain parts of the analysis of data. These last three muscles were deliberately chosen to represent a range of tenderness, the psoas major or tenderloin being considered as the most tender muscle, the adductor as intermediate, and the gastrocnemius as one of the toughest muscles of the beef carcass.

The roasts were paired throughout; that is, the corresponding cuts from the left and right sides were matched for direct comparison. The assignment of treatment to the various roasts was made at random, giving the following pattern for the five muscles and six storage times.

Table 1. Statistical Pattern for Storage Times (in days).

| Muscle | Pairs | | | | | |
|-----------------|-------------|--------------|-------------|--------------|-------------|--------------|
| | <u>Left</u> | <u>Right</u> | <u>Left</u> | <u>Right</u> | <u>Left</u> | <u>Right</u> |
| Semitendinosus | 1 | 9 | 4 | 18 | 2 | 0 |
| Semimembranosus | 9 | 4 | 18 | 0 | 2 | 1 |
| Biceps femoris | 2 | 4 | 9 | 18 | 1 | 0 |
| Vasti | 4 | 0 | 1 | 18 | 9 | 2 |
| Gastrocnemius | 18 | 2 | | | | |
| Psoas major | | | 0 | 9 | | |
| Adductor | | | | | 1 | 4 |

The data were analyzed by analysis of variance. Adjustments were made for any possible difference between pairs, but these turned out to be unnecessary, so were omitted. The values for ranking the muscles in order of tenderness were adjusted for the gastrocnemius, adductor, and psoas major to

eliminate the effect of treatment, as these did not have all treatments for each muscle. This adjustment proved unnecessary for diameter of muscle fibers, as these values were not affected significantly by treatment.

Electrical conductivity

After removal from storage, the roasts were taken to the Physics building for measurement of the electrical conductivity of the raw meat. The conductivity was measured with a Wheatstone bridge, using a two-meter slide wire, a coil-type resistance box, a vacuum-tube oscillator supplying 1000-cycle current, a telephone receiver to detect the end point, and electrodes patterned after the probe electrodes used by Banfield (11).

The use of 1000-cycle current follows the work of Banfield and Callow (11, 12, 13, 24). Landis (70) found that the use of direct current when measuring the electrical conductivity of flesh permitted polarization of the electrodes. Banfield (11) found that a fifty-cycle current also gave irregular results due to excessive polarization, while Hemingway and Collins (60) found that with frequencies greater than 10,000 cycles the membrane resistance became negligible, the measured resistance representing that of the cell interior only.

The electrodes consisted of two brass points five millimeters long, mounted five millimeters apart in a circular plastic disc two and one-half centimeters in diameter and one

centimeter thick. The points were connected through the disc to brass binding posts, to which the connecting wires could be fastened. The points were coated with a layer of platinum black, to increase the surface and decrease polarization and reaction with the constituents of the meat. Brass was selected for the metal parts to prevent rusting, since the points had to be kept in water after platinizing, to avoid drying of the surface.

To measure the conductivity, the meat was unwrapped, and any surface moisture wiped off. A place free of obvious fat and connective tissue on the roast was selected, and the epimysium removed with a scalpel before the electrode points were inserted. This precaution was necessary, as fat and connective tissue have much lower conductivities than that of the muscle tissue. The electrode points were inserted into the muscle, being careful that they really penetrated into the meat. Then various ones of the resistance plugs were pulled out, and the contact on the slide wire adjusted until the sound of the current could no longer be heard through the receiver. The amount of resistance in the resistance box was adjusted to bring this point as near the center of the slide wire as possible, to minimize the per cent error.

The electrical resistance was measured both across and along the fibers. To measure the resistance along the fibers, the points were inserted so that the current flowed between them parallel to the direction of the fibers of the meat. The

resistance across the fibers was measured by turning the disc through ninety degrees and reinserting the points so that the current flowed between them at right angles to the direction of the meat fibers. The two resistances were measured at as nearly the same place in the meat as possible, to minimize the variation within the same cut.

This method of measurement did not give the absolute conductivity of the meat, since the meat surrounding the points would permit the transmission of the current in elliptical orbits between the two electrodes, as well as in a straight line between them. However, the results would be comparable as long as the same equipment was employed.

The temperature of the meat at the time of measuring the conductivity was determined by inserting a thermometer parallel to, and one-half inch below, the surface of the meat.

Cooking

After measuring the conductivity, the roasts were brought to the Home Economics building and prepared for cooking. Samples were removed for pH measurement and for histological sections. Then the roasts were weighed and measured. The measurements were used to determine the placement of the thermometer. Right angle thermometers registering from -10° to 90° C were used. The weighed thermometer was inserted so that the bulb was in the center of the muscle. Special care was needed with the gastrocnemius, since this muscle consists of

two heads which nearly surround the superficial digital flexor. In this muscle, the thermometer was inserted to reach the center of the medial head.

After the thermometer was in place, string was tied around each roast to facilitate handling in measuring the volume. The volume was determined by water displacement. A straight-sided, deep pan was used. The weight of the pan full of water was recorded; then the roast was completely immersed in the water, removed, and the pan and remaining water weighed. The difference between the two weights gave the weight of the water displaced and consequently the volume of the roast.

After measuring the volume, the roasts were placed on racks in open roasting pans, and roasted in gas ovens at 150°C until the internal temperature of the roasts was 65° to 66°C. The temperature of the oven and the roasts was checked every 15 minutes during the cooking process.

The semimembranosus, biceps femoris, and gastrocnemius were cooked to 65°C, as they were rather large cuts and at least partially covered with exterior fat, so that the internal temperature continued to rise after the roasts were removed from the oven. The highest temperature attained by these roasts varied from 65° to 67°C. The other cuts were small and, with the exception of the semitendinosus, had no exterior fat; so very few of these showed any temperature rise after removal from the oven. For this reason, these roasts were cooked to an internal temperature of 66°C. A few of them

showed a slight temperature rise after removal from the oven, but none went over 67°C.

When the roasts were removed from the oven, they were weighed, and the volume again measured. The same method of volume measurement was used for the cooked meat as for the raw, substituting boiling water for cold, to avoid sudden cooling of the meat. Since the boiling water had a lower surface tension than the cold water, the weight of the pan full of boiling water had also to be determined, and this value used when subtracting to determine the volume of the cooked roast.

The appropriate weights to give the weight of the raw meat, the cooked meat, and the pan drippings were taken. The difference between the raw and cooked weight gave the total cooking losses. The total cooking losses minus the weight of the drippings gave the loss by evaporation. The per cent losses were calculated on the basis of the raw weight of the meat.

When the cooked volume had been measured, the roasts were allowed to stand to permit any rise in internal temperature which might take place. After the internal temperature started to drop, the roasts were cut for judging and objective tests.

Palatability

The roasts were judged by four judges, Miss Belle Lowe of the Foods and Nutrition department, Mr. McClurg of the Animal Husbandry department, and Mrs. Soledad Payawal and Miss

Pauline Paul, graduate students in Foods. The roasts were judged for aroma, flavor of lean, juiciness and tenderness. Each factor was scored from 1 to 10, 10 being the highest possible score. For sample scoring sheet, see page 163.

A slice from one half of the roast was used for scoring, and the other half of the roast was reserved for shearing tests, press fluid determination, and histological studies.

The thermometer was removed and the roast cut through the thermometer hole at right angles to the direction of the meat fibers. A slice approximately one-fourth inch thick was cut from one side of the center. This slice was divided into four quarters by two cuts through the center at right angles to each other. Each judge was given one quarter, the pieces being selected from the same location for each judge. Whenever possible, the fat on the roasts was also judged. However, only the semitendinosus, the biceps femoris, and gastrocnemius had sufficient external fat for judging.

The scores were recorded each day, and the average of the four judging figures used as the expression of the factor being considered.

Objective measurements

pH: The pH of the raw meat was measured with a Coleman electrometer, style 200. A five-gram sample of the meat was minced, then triturated with five grams of washed sea sand. Forty cubic centimeters of distilled water were added and the

mixture transferred to a beaker, covered, and allowed to stand fifteen minutes, with frequent stirring. The supernatant liquid was then decanted and used for the pH determination.

Shearing test: A metal borer one inch in diameter was used to remove samples of meat for the shearing tests. The cylinders of meat were cut parallel to the direction of the meat fibers, and every effort was made to avoid obvious deposits of fat and connective tissue.

The cylinders of meat were sheared mechanically, using a modified form of the Warner-Bratzler shear stress machine. From three to five readings were obtained for each cylinder, the number depending on the length of the cylinder. At least six readings were obtained for each roast.

The measurements were made from the center of the roast outward and the readings recorded in that order to make possible a check on any effect of difference in position of the reading, since the exterior of the roast attained a higher temperature than the interior during the roasting process.

Press fluid: Two samples for press fluid determination were taken from the center of the roast next to the thermometer hole. Each sample was weighed, wrapped in canvas, subjected to 250 pounds pressure for five minutes, and reweighed. The difference between the two weights, divided by the original weight, gave the per cent press fluid.

Histological studies

The samples of meat for histological study, both raw and cooked, were placed immediately after removal from the roast in 10 per cent formalin, in which they remained for at least eighteen hours in order to fix the tissue.

Paraffin sections: For the raw tissues, a series of graded alcohols and xylol was used for dehydration and clearing. The dioxane method was used for the cooked tissues. Details of these methods are given by Hanson (58).

After imbedding and chilling, the blocks were trimmed, mounted on wooden carriers, and sections 10 microns in thickness cut on a Spencer rotary microtome. Both longitudinal and cross sections were made, the two samples of tissue being imbedded in the same block, sectioned, mounted and stained together. The sections were mounted on albuminized slides, and allowed to dry at least twenty-four hours before staining. The staining method employed was the same as that described by Hanson (58), using French's modification of Weigert's elastic tissue stain, Harris' alum hematoxylin, and van Gieson's mixture of acid fuchsin and picric acid.

The stained sections were mounted in Canada balsam. It was necessary to have the final series of alcohols completely cover the slides and to change the absolute alcohol frequently to prevent carrying any water over into the xylol, since any

traces of water remaining in the mounted sections caused the stains to fade.

The muscle tissue stained a dark yellow-orange, the collagenous connective tissue magenta, and the elastic tissue dark blue-green. In the cooked samples, some of the collagen had been so changed that it stained the same color as the muscle tissue.

Frozen sections: The fixed tissues were imbedded in gelatin before sectioning. The tissue blocks were immersed in 10 per cent Gelatin solution, held in the paraffin oven at 54-56°C for four hours, then removed and the gelatin allowed to harden. The blocks were then trimmed, leaving approximately one-sixteenth inch of gelatin around the tissue. The trimmed blocks were placed on a drop of Gum arabic solution on the carrier of the freezing microtome, and frozen with carbon dioxide under pressure. The longitudinal sections were cut 5 microns thick, and the cross sections 15 microns thick. The sections were removed from the knife with a moistened camel's hair brush and floated on distilled water.

For staining, the sections were floated into a small wire basket and run through the following set of solutions:

| | |
|---------------------------------|-------------|
| 70% alcohol | - 5 seconds |
| Herxheimer's Scarlet R solution | - 3 minutes |
| 70% alcohol | - 5 seconds |
| distilled water | -10 seconds |
| Harris alum hematoxylin | - 5 minutes |
| tap water | -10 seconds |

The sections were then floated on tap water, a slide immersed in the water and the sections oriented on the slide with a

brush. The gelatin mounting made it possible to stretch and flatten the sections with a teasing needle after removing the slide from the water. The excess water was then wiped off and the sections mounted in glycerine jelly. The cover slips were weighted with small vials full of shot until the jelly had set.

This treatment stained the muscle tissue blue and the fat red. The gelatin mounting also stained blue, but could be differentiated easily from the muscle by the difference in structure.

Fiber diameters and number of fibers per bundle: The measurements of fiber diameters and counts of the number of fibers per bundle were made on cross sections prepared on the freezing microtome. This method was chosen as it caused much less distortion of the fibers than did the processes involved in the preparation of paraffin-embedded sections.

The measurements were made with the aid of a bellows enlarging device. This consisted of an extendible camera bellows with a ground glass plate at the top, mounted on a vertical post with adjustable clamps so that the height of the glass screen could be varied. This was set over the microscope so that the beam of light coming through the microscope passed vertically through the bellows and was projected on the ground glass screen. A Bausch and Lomb photography lamp with a ribbon-filament bulb was used to provide sufficiently powerful illumination to compensate for the bellows extension.

With the aid of a stage micrometer, the ground glass plate was adjusted at such a height that the 16 millimeter microscope objective plus the eyepiece and bellows gave a magnification of exactly 100. Then the prepared slides were substituted for the micrometer slide, and the fiber diameters measured on the glass screen with a millimeter ruler. The magnification of 100 made the conversion of the measurements very simple, as 1 millimeter on the glass screen corresponded to 10 microns on the slide.

The diameters of fifty fibers of each sample were measured for both the raw and the cooked meat. The fibers were not truly round; so it was arbitrarily decided to use the longest dimension, avoiding any very distorted fibers. This is in accord with the practice of the Veterinary Anatomy department here. The fifty measurements were averaged and the resulting value used as the fiber diameter of that particular sample in the statistical analysis.

A ground glass plate ruled in one-inch squares was superimposed on the glass screen of the enlarging device to facilitate counting the number of fibers per bundle by dividing the field into smaller units. The number of fibers in a primary bundle was counted in each of ten bundles per sample. The counts were averaged to give the number of fibers per bundle for statistical analysis.

Changes in muscle fibers: Longitudinal and cross sections

of the meat samples prepared on the freezing microtome were inspected microscopically for changes in the fibers induced by storage and by cooking. The paraffin sections were also checked in the same manner.

Connective tissue: The sections made from paraffin-
imbedded samples were checked for kinds and relative amounts of connective tissue, as the stains used for these sections differentiated very clearly between muscle fibers, collagenous fibers, and elastic fibers. Large deposits of elastic tissue could be observed in the frozen sections, but it was sometimes difficult to distinguish between the collagenous material and the gelatin used in imbedding, as both stained the same color.

Supplementary studies

Effect of massage: The psoas major muscles were large enough to yield two roasts apiece. The distal sections were used in the storage series, and the proximal portions used to check the effect of massage on the meat before it had gone into rigor. The left proximal cut was cooked without treatment, while the right proximal cut was gently squeezed and rubbed for fifteen minutes before being cooked. These two cuts went into the oven one and one-half hours after the animal was killed. They were cooked and tested just as the roasts in the storage series except that the electrical conductivity was not measured on them.

Heat rigor: Pieces of neck muscle from a freshly-killed steer were obtained from the Animal Husbandry department and treated within one-half hour after the death of the animal, to check the effect of heat in producing rigor. The meat was cut into small strips approximately one centimeter square and three centimeters long. These strips were immersed in water at 70°C for two, five, fifteen, and twenty-five seconds. They were then placed in 10 per cent formalin and subsequently were imbedded in paraffin, sectioned and stained according to the method used for cooked meat. The slides were inspected microscopically for changes due to heat.

RESULTS AND DISCUSSION

Characteristics of Raw Roasts

Size

The size and weight of the roasts varied considerably from muscle to muscle. The variation from cut to cut of the same muscle was quite small. Table 2 gives the average measurements for the roasts from the different muscles.

Table 2. Average Weights and Measurements of Roasts

| Muscle | Weight gms. | Length cms. | Width cms. | Depth cms. | Volume cos. |
|-----------------|----------------|----------------|---------------|---------------|----------------|
| Semitendinosus | 365.3 | 9.2 | 6.2 | 5.8 | 433 |
| Semimembranosus | 884.3 | 9.5 | 13.3 | 8.3 | 919 |
| Biceps femoris | 1243.5 | 10.6 | 16.2 | 7.9 | 1279 |
| Vasti | 440.3 | 13.9 | 8.0 | 5.5 | 491 |
| Gastrocnemius | 1033.0 | 13.5 | 12.5 | 7.0 | 1047 |
| Psoas major | 369.5 | 11.0 | 6.0 | 5.5 | 438 |
| Adductor | 590.0 | 11.0 | 9.5 | 8.0 | 633 |

Appearance

External fat: The biceps femoris and semitendinosus had a layer of external fat on one side. The gastrocnemius had some external fat, but not a continuous layer. The other muscles had only occasional strips of external fat which had

been deposited along the connective tissue between the muscles.

Texture: The grain of the meat varied with the muscle, as shown in figures 1 to 7. These are pictures of slices cut from near the center of each roast. The psoas major had the finest grain. The gastrocnemius and vasti were next finest. The adductor and semitendinosus were medium in texture, and the semimembranosus and biceps femoris were coarse. The grain or texture was perhaps most obvious in the semitendinosus, figure 4, the bundles standing out very distinctly from one another. This may be related to the observation from the microscopic slides that this muscle was the only one which consistently showed large quantities of elastic connective tissue. The grain was least distinct in the psoas major, figure 1, the muscle having the lowest connective tissue content.

The shining dark stripe across the gastrocnemius, figure 3, is a collagenous insertion. The shorter dark line is an irregularity in the surface produced during the cutting of the slice.

Marbling: The amount of marbling or intra-muscular fat also varied with the muscle. The psoas major showed extensive marbling, the semitendinosus, semimembranosus, biceps femoris, vasti, and adductor slight to moderate, and the gastrocnemius very little marbling.

Slices of Meat Showing Grain
(magnification 3x)

Figure 1. Psoas major.

Figure 2. Vasti.

Figure 3. Gastrocnemius.

Figure 4. Semitendinosus.

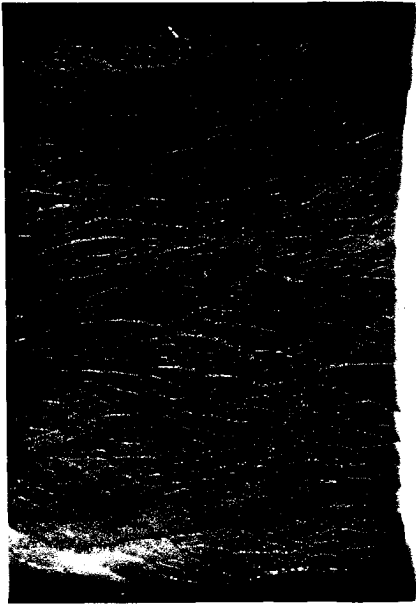


Figure 1.



Figure 2.

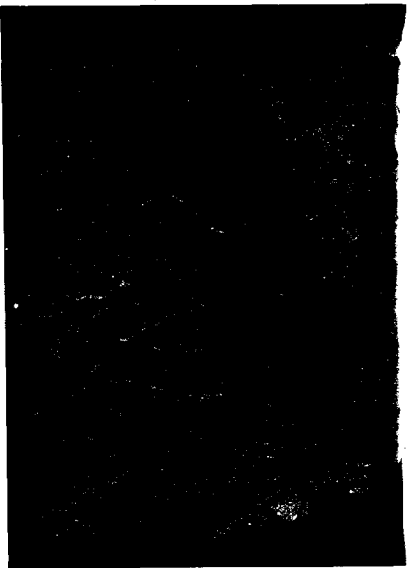


Figure 3.

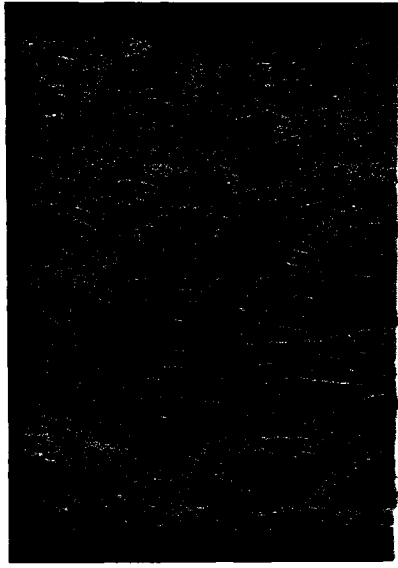


Figure 4.

Slices of Meat Showing Grain
(magnification 3x)

Figure 5. Adductor.

Figure 6. Semi-
membranosus.

Figure 7. Biceps femoris.



Figure 5.



Figure 6.



Figure 7.

Effects of storage

Gross changes: One of the most noticeable changes with storage was the hardening of the fat. The external fat on the roasts cooked without storage was soft and oily. After one day of cold storage, the fat was firm and brittle.

The amount of juice exuded from the meat varied with the storage. The roasts with zero and one day of storage were quite dry on the surface. Those with two and four days of storage were quite moist. By the ninth day, the surface of the meat was again fairly dry, but the juice had collected in the folds of the wrapping paper. The roasts stored eighteen days were sticky rather than wet. These changes indicated that the forces holding the water had changed rather extensively, and that the changes occurred fairly early in the storage period.

No signs of bacterial activity were observed until the eighteen-day group was tested. The semitendinosus at that time showed a small patch of blue mold. Another sign of growth of bacteria was the sticky surface of all the eighteen-day roasts. This was noticed on the external fat as well as on the lean surfaces. All these roasts had rather "high" aroma when raw, which persisted during and after cooking.

Rigor: With the exception of the psoas major, none of the zero-storage roasts had gone into rigor before being cooked, but they were definitely in rigor when taken from the

oven. The psoas major was stiff and resistant before it was cooked, but had lost the macroscopic appearance of rigor by the end of the cooking period. All the stored cuts were out of rigor when tested.

The very rapid onset and dissolution of rigor found in this study undoubtedly resulted, at least partly, from the extra handling the roasts received in the process of dissection and wrapping. In actual practice, it is customary to ripen beef as halves or quarters of the carcass, not as individual roasts. However, to insure comparability of treatment and to prevent excessive drying of the cut surfaces, it was necessary in this work to cut up and wrap the individual roasts early in the storage period.

The presence of rigor was very obvious, as the roasts in this condition were very stiff and hard, and difficult to cut--rather like trying to cut a rubber stopper.

Electrical conductivity

The measurement of electrical conductivity was only partially successful, due to difficulties with the apparatus. The data obtained are given in table 3. The measurements are given as resistance, which varies inversely with conductivity.

Table 3. Electrical Resistance of Raw Beef (in ohms)

| Muscle | Direction of Measurement | Time of Storage (in days) | | | | | |
|-----------------|--------------------------|---------------------------|------|----|------|------|-----|
| | | 0 | 1 | 2 | 4 | 9 | 18 |
| Semitendinosus | across | 1322 | 261 | 14 | 356 | 611 | 778 |
| | along | 901 | 267 | 13 | 325 | 581 | 565 |
| Semimembranosus | across | 1732 | 411 | 28 | 1186 | 515 | 626 |
| | along | 2825 | 392 | 23 | 712 | 505 | 587 |
| Biceps femoris | across | 2981 | 906 | 12 | 490 | 492 | 936 |
| | along | 2053 | 595 | 12 | 464 | 493 | 779 |
| Vasti | across | 2475 | 1463 | 54 | 1032 | 1554 | 546 |
| | along | 1542 | 878 | 39 | 736 | 1286 | 462 |
| Gastrocnemius | across | | | 66 | | | 818 |
| | along | | | 79 | | | 709 |
| Psoas major | across | 1130 | | | | 499 | |
| | along | 1608 | | | | 467 | |
| Adductor | across | | 412 | | 141 | | |
| | along | | 425 | | 148 | | |

It was obvious from inspection of the figures that part of the data were not accurate. The measurements for zero storage were probably fairly representative. And the general agreement among the figures for the nine- and eighteen-day storage periods suggested that these were all right. The figures which were usable indicated a decrease in resistance (increase in conductivity) with storage. They also illustrated the variability inherent in this type of measurement. Because of this, many more data would be required before any conclusions could be drawn.

pH

The changes in pH with storage are shown in table 4.

Table 4. pH of Raw Beef

| Muscle | Time of Storage (in days) | | | | | |
|-----------------|---------------------------|------|------|------|------|------|
| | 0 | 1 | 2 | 4 | 9 | 18 |
| Semitendinosus | 5.92 | 5.28 | 5.52 | 5.47 | 5.52 | 5.77 |
| Semimembranosus | 6.08 | 5.47 | 5.52 | 5.36 | 5.42 | 5.46 |
| Biceps femoris | 5.98 | 5.45 | 5.48 | 5.43 | 5.53 | 5.77 |
| Vasti | 6.30 | 5.92 | 5.87 | 5.32 | 5.78 | 6.10 |
| Gastrocnemius | | | 5.55 | | | 5.86 |
| Psoas major | 5.33 | | | | 5.52 | |
| Adductor | | 5.42 | | 5.35 | | |

Analysis of Variance: Treatments, $F = 5.62^{**}$;
Muscles¹, $F = 3.84^*$.

¹In this and all subsequent tables, the term "muscles" refers to the five major groups, the first four muscles forming one group each and the values for the last three muscles being combined for the fifth group.

The results showed that the change in pH with storage was great enough to be highly significant. The changes followed the pattern usually found in beef during storage--a drop followed by a slow rise.

The differences between the muscles were significant, and probably represented initial differences in glycogen content, in line with Callow's work (25).

It was observed that the vasti group, with the highest pH throughout of any of the muscles, had the strongest "high" odor of any of the aged raw roasts.

Characteristics of Cooked Roasts

Cooking losses

The cooking losses were made up of water, salts, exuded protein, and fat. The fat, protein, and salts constituted the dripping losses, while most of the water was lost by evaporation.

Total losses: The data on total cooking losses are given in table 5.

Table 5. Total Cooking Losses (in per cent)

| Muscle | Time of Storage (in days) | | | | | |
|-----------------|---------------------------|-------|-------|-------|-------|-------|
| | 0 | 1 | 2 | 4 | 9 | |
| Semltendinosus | 9.93 | 7.85 | 8.55 | 10.76 | 7.02 | 8.08 |
| Semimembranosus | 10.98 | 10.69 | 10.85 | 11.11 | 12.35 | 10.09 |
| Biceps femoris | 10.02 | 11.37 | 11.53 | 10.40 | 8.92 | 7.61 |
| Vasti | 11.67 | 9.62 | 7.14 | 8.00 | 9.83 | 7.33 |
| Gastrocnemius | | | 7.37 | | | 8.97 |
| Psoas major | 7.86 | | | | 9.76 | |
| Adductor | | 12.26 | | 13.39 | | |

Analysis of Variance: Treatments, $F = 1.4$;
Muscles, $F = 2.0$.

The changes attributable either to storage or to muscle were too small and too variable to be significant. It was noted, however, that the adductor had the highest total losses, in line with the results obtained by Satorius and Child (104).

Dripping losses: The dripping losses are given in table 6.

Table 6. Dripping Losses (in per cent)

| Muscle | Time of Storage (in days) | | | | | |
|-----------------|---------------------------|------|------|------|------|------|
| | 0 | 1 | 2 | 4 | 9 | 18 |
| Semitendinosus | 1.75 | 1.18 | 0.65 | 1.97 | 0.19 | 5.03 |
| Semimembranosus | 0.66 | 1.27 | 1.25 | 0.46 | 1.30 | 4.63 |
| Biceps femoris | 2.21 | 3.00 | 2.35 | 1.46 | 2.05 | 3.17 |
| Vasti | 0.64 | 1.09 | 0.52 | 0.02 | 0.67 | 0.86 |
| Gastrocnemius | | | 0.52 | | | 1.62 |
| Psoas major | 0.87 | | | | 0.44 | |
| Adductor | | 1.06 | | 1.33 | | |

Analysis of Variance: Treatments, $F = 4.63^{**}$;
Muscles, $F = 4.04^{**}$.

The amount of loss by dripping changed in a highly significant manner with treatment. The effect of treatment was obvious in the greatly increased losses after eighteen days of storage as opposed to the other storage periods. The relative amounts of fat and non-fat in the drippings were not determined. However, both fractions probably contributed to the increased loss with storage. Moran (93) stated that bacteria break down the connective tissue framework holding the fat, which would increase the ease with which the fat was melted off during cooking. Also, it is held that some of the proteins and the salts become more readily soluble with increased storage. This would increase the loss of these factors in the juice exuded from the lean during cooking.

The differences in dripping losses between the muscles were significant. The largest losses were found in the semitendinosus and biceps femoris, the only muscles which had

appreciable amounts of external fat. This was in line with the finding that the presence of external fat increased the dripping losses, as reported by many workers.

Evaporation: The losses due to evaporation are given in table 7.

Table 7. Evaporation Losses (in per cent)

| Muscle | Time of Storage (in days) | | | | | |
|-----------------|---------------------------|-------|------|-------|-------|------|
| | 0 | 1 | 2 | 4 | 9 | 18 |
| Semitendinosus | 8.18 | 6.67 | 7.88 | 8.79 | 6.83 | 3.05 |
| Semimembranosus | 10.32 | 9.42 | 9.30 | 10.65 | 11.05 | 5.46 |
| Biceps femoris | 7.81 | 8.37 | 9.18 | 8.94 | 6.87 | 4.44 |
| Vasti | 11.03 | 8.53 | 6.62 | 7.98 | 9.16 | 6.47 |
| Gastrocnemius | | | 6.85 | | | 7.35 |
| Psoas major | 6.99 | | | | 9.32 | |
| Adductor | | 11.20 | | 12.06 | | |

Analysis of Variance: Treatments, $F = 4.85^{**}$;
Muscles, $F = 2.41$.

The evaporation losses did not differ statistically among the muscles. In general, however, the higher losses occurred in the roasts which had little or no external fat to protect them.

The change in evaporation losses with storage was highly significant, the roasts stored eighteen days having, in general, lower losses than at any other time. This decrease in evaporation losses balanced the increase in dripping losses so that the total losses were not affected by storage.

Volume: The term shrinkage has been applied in meat cooking studies to the losses just discussed and to the change in volume. It is used in the latter sense here. The changes in volume of the roasts during cooking are given in table 8.

Table 8. Decrease in Volume during Cooking (in per cent)

| Muscle | Time of Storage (in days) | | | | | |
|-----------------|---------------------------|-------|-------|-------|-------|-------|
| | 0 | 1 | 2 | 4 | 9 | 18 |
| Semitendinosus | 14.95 | 8.84 | 23.31 | 27.02 | 6.14 | 11.68 |
| Semimembranosus | 19.74 | 21.05 | 19.77 | 23.74 | 23.21 | 14.25 |
| Biceps femoris | 16.23 | 9.33 | 14.50 | 18.53 | 17.39 | 11.05 |
| Vasti | 24.19 | 10.28 | 22.22 | 24.82 | 9.06 | 3.92 |
| Gastrocnemius | | | 14.74 | | | 12.93 |
| Psoas major | 22.10 | | | | 28.54 | |
| Adductor | | 23.66 | | 22.50 | | |

Analysis of Variance: Treatments, $F = 3.23$;
Muscles, $F = 1.81$.

The changes in volume, due either to treatment or to muscle, were too random to be significant. However, there was some indication that the shrinkage decreased with longer storage. This may reflect the small shrinkage and loss of water which had already occurred during the storage period.

Cooking time

The cooking times for the various roasts are listed in table 9.

Table 9. Cooking Time (in minutes per pound)

| Muscle | Time of Storage (in days) | | | | | |
|-----------------|---------------------------|-------|------|------|------|-------|
| | 0 | 1 | 2 | 4 | 9 | 18 |
| Semitendinosus | 72.1 | 71.2 | 77.4 | 70.4 | 94.6 | 102.3 |
| Semimembranosus | 44.7 | 56.3 | 52.1 | 56.4 | 48.9 | 53.9 |
| Biceps Femoris | 28.1 | 41.5 | 46.2 | 60.3 | 41.2 | 46.0 |
| Vasti | 57.0 | 119.2 | 60.1 | 54.2 | 81.6 | 75.6 |
| Gastrocnemius | | | 39.3 | | | 43.8 |
| Psoas major | 57.7 | | | | 85.9 | |
| Adductor | | 61.1 | | 69.2 | | |

Analysis of Variance: Treatments, $F = 1.26$;
Muscles, $F = 6.34^{**}$.

Contrary to the usual finding, the cooking time did not decrease with increased storage. The variation in cooking times between the different muscles was, however, highly significant, the larger, heavier muscles taking fewer minutes per pound to reach the given temperature. This could be attributed partly to the lower internal temperature to which the larger roasts were cooked, and partly to the fact that the distance to the center of the roast did not increase in direct relation to the increase in weight, so that while the larger roast might be twice as heavy as a smaller one, the heat did not have to travel twice as far to reach the center of the roast.

Palatability

The roasts were judged for tenderness, juiciness, flavor of lean, and aroma. Where sufficient fat was present, the flavor of the fat was also scored.

Tenderness: One of the main reasons for holding beef in cold storage before selling is the resulting increase in tenderness. Therefore, tenderness was one of the most important factors in this study. The judges' scores for tenderness are given in table 10.

Table 10. Average Tenderness Scores (maximum possible score, 10)

| Muscle | Time of Storage (in days) | | | | | |
|-----------------|---------------------------|------|------|------|------|------|
| | 0 | 1 | 2 | 4 | 9 | 18 |
| Semitendinosus | 4.25 | 5.75 | 6.75 | 7.25 | 6.75 | 7.5 |
| Semimembranosus | 2.75 | 6.0 | 5.25 | 6.5 | 8.0 | 6.75 |
| Biceps femoris | 1.75 | 4.25 | 6.5 | 6.25 | 8.25 | 7.25 |
| Vasti | 2.5 | 6.5 | 6.0 | 6.25 | 6.5 | 9.25 |
| Gastrocnemius | | | 8.5 | | | 8.0 |
| Psoas major | 6.25 | | | | 10.0 | |
| Adductor | | 6.25 | | 8.5 | | |

Analysis of Variance: Treatments, $F = 14.58^{**}$;
Muscles, $F = 5.13^{**}$.

There was a highly significant difference between tenderness for the various treatments. It was obvious that the tenderness increased up to the ninth day of storage, but the change between nine and eighteen days was variable, indicating that most of the increase in tenderness was complete in nine days. This was in line with the usual finding that the principal increase in tenderness takes place in from ten days to two weeks of storage. And, of course, in these small pieces, the ripening action was faster than it would be in the larger sections of beef such as quarters or halves. The continued

increase in tenderness of the vasti group was in line with the higher pH which it attained, since Winkler (133) and Duisberg and Miller (37) have shown that tenderness increased with increasing pH above 5.

All the roasts cooked without storage were very tough, even the psoas major. This does not bear out the statement of Ewell (41) that: "Meat is generally tender if cooked immediately after slaughter, before rigor has set in." Only the psoas major was in rigor before the roasts were cooked, but all were in rigor by the end of the cooking period. This was in line with Hanson's finding (58) that chickens cooked within as short a time as six minutes after killing were in rigor when cooked.

The tenderness of the roasts also varied significantly according to the muscle being judged. The mean scores and standard errors are given in table 11 for each muscle.

Table 11. Mean Tenderness Scores and Standard Errors

| Muscle | Mean Score | Standard Error |
|-----------------|------------|----------------|
| Group 1 | | |
| Psoas major | 8.85 | ± 0.718 |
| Gastrocnemius | 7.63 | ± 0.718 |
| Adductor | 7.28 | ± 0.718 |
| Group 2 | | |
| Semitendinosus | 6.38 | ± 0.358 |
| Vasti | 6.17 | ± 0.358 |
| Semimembranosus | 5.88 | ± 0.358 |
| Biceps femoris | 5.71 | ± 0.358 |

The muscles were separated into two groups, to indicate the difference in the number of treatments. In group two, since each muscle received all the treatments, no adjustment was necessary. In group one, however, since each muscle received only two of the six treatments, it was necessary to adjust for the effect of the treatment.

Within each group, the standard error of that group was used in assessing the significance of the differences between the muscles. Between the two groups, the standard error of ± 0.567 should be used. The "t" test was used to determine the significance or non-significance of these differences.

Within each group, there was no significant difference between the tenderness of the muscles. Between the two groups, the psoas major was more tender than any of the muscles in group two, the differences being highly significant in each case. The gastrocnemius was more tender than the vasti, semimembranosus, and biceps femoris, the differences being significant for the first two and highly so for the last one. The adductor was significantly more tender than the semimembranosus and the biceps femoris.

The fact that the psoas major was the most tender muscle of the lot was expected. However, most of the others were somewhat out of line, as the adductor and semimembranosus are usually thought to be about the same, with the semitendinosus, biceps femoris, and vasti less tender, and the gastrocnemius the toughest of all. Part of the toughness usually attributed

to the latter is undoubtedly caused by the inclusion of the superficial digital flexor, an extremely tough muscle, in the gastrocnemius. The avoidance of this flexor in judging may account for the higher score received by the gastrocnemius.

Juiciness: The average juiciness scores are given in table 12.

Table 12. Average Juiciness Scores (maximum possible score, 10)

| Muscle | Time of Storage (in days) | | | | | |
|-----------------|---------------------------|------|------|------|------|------|
| | 0 | 1 | 2 | 4 | 9 | 18 |
| Semitendinosus | 6.0 | 7.25 | 7.5 | 7.25 | 7.75 | 7.5 |
| Semimembranosus | 6.75 | 7.5 | 7.25 | 7.5 | 8.25 | 8.75 |
| Biceps femoris | 7.5 | 7.5 | 8.0 | 7.25 | 9.0 | 8.5 |
| Vasti | 7.75 | 8.0 | 8.5 | 8.75 | 8.3 | 8.75 |
| Gastrocnemius | | | 8.75 | | | 8.75 |
| Psoas major | 8.0 | | | | 9.0 | |
| Adductor | | 7.5 | | 8.0 | | |

Analysis of Variance: Treatments, $F = 8.09^{**}$;
Muscles, $F = 8.84^{**}$.

The juiciness of the roasts showed a gradual increase with aging, the change being highly significant. This checked with the finding in most storage studies that juiciness increased with storage.

The muscles also showed highly significant differences in juiciness, the semitendinosus, semimembranosus, and adductor being the least juicy and the psoas major the most juicy.

Flavor of lean: The average scores for flavor of the lean meat are given in table 13.

Table 13. Average Lean Flavor Scores (maximum possible score, 10)

| Muscle | Time of Storage (in days) | | | | | |
|-----------------|---------------------------|------|------|------|------|------|
| | 0 | 1 | 2 | 4 | 9 | 18 |
| Semitendinosus | 6.67 | 7.75 | 7.5 | 7.75 | 8.0 | 8.25 |
| Semimembranosus | 6.33 | 8.0 | 7.25 | 8.25 | 8.75 | 8.5 |
| Biceps femoris | 4.3 | 7.5 | 7.75 | 8.25 | 9.0 | 7.75 |
| Vasti | 6.0 | 7.75 | 8.0 | 8.0 | 7.5 | 9.25 |
| Gastrocnemius | | | 8.5 | | | 9.0 |
| Psoas major | 5.67 | | | | 9.0 | |
| Adductor | | 6.25 | | 8.5 | | |

Analysis of Variance: Treatments, $F = 12.27^{**}$;
Muscles, $F = 0.43$.

Variation in the time of storage gave highly significant differences among these scores. The flavor score increased steadily through nine days of storage, but showed no consistent change thereafter. This increase in desirability of flavor was in line with the usual finding that the flavor was improved with aging. The variability on the last storage set was principally attributable to individual tastes. After eighteen days of storage, the roasts were found to be rather "high" in flavor, and, while two of the judges liked this flavor, two of them did not, considering the meat to be slightly spoiled.

The flavor did not vary significantly among the different muscles.

Aroma: The average aroma scores are given in table 14.

Table 14. Average Scores for Aroma (maximum possible score, 10)

| Muscle | Time of Storage (in days) | | | | | |
|-----------------|---------------------------|------|------|------|------|------|
| | 0 | 1 | 2 | 4 | 9 | 18 |
| Semitendinosus | 7.75 | 7.0 | 7.75 | 8.0 | 8.5 | 8.25 |
| Semimembranosus | 7.75 | 7.25 | 7.5 | 8.25 | 9.25 | 7.5 |
| Biceps femoris | 7.75 | 7.25 | 7.5 | 8.0 | 8.5 | 7.25 |
| Vasti | 8.0 | 7.25 | 7.75 | 8.0 | 8.25 | 8.25 |
| Gastrocnemius | | | 8.25 | | | 8.25 |
| Psoas major | 7.75 | | | | 8.75 | |
| Adductor | | 6.5 | | 7.75 | | |

Analysis of Variance: Treatments, $F = 11.35^{**}$;
Muscles, $F = 0.4$.

The aroma scores followed the same pattern as the flavor scores, showing no significant variation between muscles, but a highly significant one between treatments. The aroma increased in desirability with storage up to nine days, then dropped a little on the eighteenth day. Here again, the decrease in score with eighteen days was at least partly due to individual preferences, the judges who disliked the "high" flavor scoring down on aroma also.

Flavor of fat: Since many of the roasts had insufficient external fat to allow judging its flavor, the figures were not complete enough to warrant statistical analysis. However, the scores which were obtained are given in table 15.

Table 15. Average Scores for Flavor of Fat
(maximum possible score, 10)

| Muscle | Time of Storage (in days) | | | | | |
|-----------------|---------------------------|------|------|-----|------|------|
| | 0 | 1 | 2 | 4 | 9 | 18 |
| Semitendinosus | 8.0 | 8.67 | 8.5 | 8.3 | 8.0 | 4.25 |
| Semimembranosus | --- | 7.0 | 8.0 | --- | --- | 4.25 |
| Biceps femoris | 8.0 | 7.0 | 7.67 | 6.5 | 8.67 | 4.5 |
| Vasti | --- | 8.0 | 7.3 | --- | --- | 4.0 |
| Gastrocnemius | | | 8.3 | | | 5.0 |
| Psoas major | 8.0 | | | | --- | |
| Adductor | | --- | | --- | | |

Inspection of the figures indicated that the desirability of the flavor of the fat dropped sharply between nine and eighteen days of storage. This was caused by the development of a definitely rancid flavor in the fat during this last storage period.

Objective tests

Shear: The force in pounds required to shear a cylinder of muscle one inch in diameter is given in table 16. Each figure represents the average of from six to ten readings.

The shearing force, like the tenderness score, varied in a highly significant way with storage, the force required decreasing with increasing storage. The very high shearing force for the roasts cooked without storage checked with the low tenderness scores for these roasts, since the higher the shear force, the less tender the meat.

Table 16. Average Shearing Force for Cooked Meat (in pounds)

| Muscle | Time of Storage (in days) | | | | | |
|-----------------|---------------------------|-------|-------|-------|-------|-------|
| | 0 | 1 | 2 | 4 | 9 | 18 |
| Semitendinosus | 93.0 | 20.35 | 25.52 | 19.77 | 22.52 | 17.22 |
| Semimembranosus | 82.92 | 21.85 | 20.47 | 17.50 | 25.78 | 19.93 |
| Biceps femoris | 72.08 | 28.67 | 23.03 | 16.60 | 18.80 | 19.61 |
| Vasti | 77.2 | 14.62 | 22.72 | 20.58 | 21.18 | 16.56 |
| Gastrocnemius | | | 18.50 | | | 12.10 |
| Psoas major | 17.6 | | | | 9.93 | |
| Adductor | | 16.69 | | 14.35 | | |

Analysis of Variance: Treatments, $F = 16.22^{**}$;
Muscles, $F = 2.67$.

The variation in shear between the different muscles was not significant for the unadjusted figures, but when the values for the gastrocnemius, psoas major, and adductor were adjusted for treatment, some of the differences were highly significant. The ranking of the muscles by shear force is given in table 17.

Table 17. Means and Standard Errors of Shear Force (in pounds)

| Muscle | Mean Shear | Standard Error |
|-----------------|------------|----------------|
| Group 1 | | |
| Psoas major | 8.00 | ± 7.87 |
| Adductor | 23.76 | ± 7.87 |
| Gastrocnemius | 24.43 | ± 7.87 |
| Group 2 | | |
| Vasti | 28.81 | ± 3.94 |
| Biceps femoris | 29.97 | ± 3.94 |
| Semimembranosus | 31.41 | ± 3.94 |
| Semitendinosus | 33.06 | ± 3.94 |

As with tenderness scores, the shearing averages were divided into two groups, with those of group one requiring adjustment for treatment. The standard error for comparison between the two groups was ± 6.22 .

Because of the small number of figures represented in the first group and the high standard error, the differences in shear between these three muscles were not significant, although the value for the psoas major was much lower than that for the other two. Also, there was no significant difference among the members of the second group.

Between the two groups, the psoas major was more tender than any of the ones in group two, the differences being highly significant. There was no significant difference between either of the other two muscles of group one and any of the muscles of group two.

The very low shearing force required for the gastrocnemius after eighteen days of storage was interesting because one cylinder of this muscle had a heavy strip of connective tissue in it. See figure 8. By histological study, this strip was found to be principally collagenous connective tissue. Apparently, storage and cooking had changed the collagen sufficiently so that it was no longer tough.

Shear force and tenderness score: The shearing force and tenderness score showed a rather high negative correlation, $r_{xy} = -0.784^{**}$. This was in line with the values obtained by



Figure 8. Sheared sample of gastrocnemius,
showing heavy strip of
collagenous connective tissue.
(magnification 3x)

other investigators. The negative sign came from the fact that as the score increased, the shear force decreased.

Variation in shear force with position in the roast: The shearing values were measured and recorded from the center to the exterior of the roast. Analysis of the individual values showed that the center part of the roast was more tender than the exterior, the differences being highly significant. See page 164 for data. The greater toughness of the exterior portion was probably due to the higher temperature attained in that region. Towson (128) found that the exterior of a roast reached temperatures 3 to 13°C higher than the center, and Satorius and Child (105) found that the tenderness decreased at internal temperatures above 67°C.

Press fluid: The figures for press fluid are given in table 18. Each figure is the average of two determinations.

Table 18. Press Fluid (in per cent)

| Muscle | Time of Storage (in days) | | | | | |
|-----------------|---------------------------|-------|-------|-------|-------|-------|
| | 0 | 1 | 2 | 4 | 9 | 18 |
| Semitendinosus | 50.55 | 54.65 | 50.25 | 44.17 | 46.80 | 59.64 |
| Semimembranosus | 53.24 | 53.47 | 50.33 | 40.03 | 36.29 | 60.33 |
| Biceps femoris | 53.77 | 52.16 | 53.73 | 44.51 | 44.16 | 68.24 |
| Vasti | 53.65 | 57.58 | 52.53 | 43.79 | 44.02 | 64.53 |
| Gastrocnemius | | | 54.62 | | | 60.10 |
| Psoas major | 55.64 | | | | 42.69 | |
| Adductor | | 56.95 | | 43.93 | | |

Analysis of Variance: Treatments, $F = 47.61^{**}$;
Muscles, $F = 2.64$.

The change in quantity of press fluid with storage was highly significant. The amount of expressible fluid dropped sharply from the second to the ninth day of storage, then rose to the highest values obtained with a further nine days of storage. These changes undoubtedly reflected changes in the water-holding powers of the tissue proteins and in the permeability of the connective tissue sheaths and cell membranes.

The muscles did not differ significantly in press fluid.

Press fluid and juiciness scores: The correlation coefficient between the press fluid and juiciness scores was only -0.084, a very small value. This was expected from the conclusion of Satorius and Child (106) that press fluid was not a good indicator of scoring juiciness.

Histological Studies

Size of fibers

The average diameters of the muscle fibers are given in table 19. Each value is the average of fifty measurements.

Cooking caused a definite shrink in the muscle fibers. This was as expected, and was in line with the decrease in volume of the roasts during cooking. The large muscle fibers seemed to shrink more than did the small ones.

Table 19. Muscle Fiber Diameters (in microns)

| Muscle | State | Time of Storage (in days) | | | | | |
|-----------------|--------|---------------------------|------|------|------|------|------|
| | | 0 | 1 | 2 | 4 | 9 | 18 |
| Semitendinosus | Raw | 58.8 | 45.4 | 51.8 | 49.7 | 56.2 | 47.4 |
| | Cooked | 53.3 | 43.4 | 43.1 | 47.4 | 49.9 | 48.5 |
| Semimembranosus | Raw | 58.3 | 64.5 | 61.6 | 56.9 | 48.7 | 54.6 |
| | Cooked | 43.8 | 50.2 | 52.5 | 49.9 | 47.1 | 47.4 |
| Biceps femoris | Raw | 55.1 | 58.8 | 63.5 | 67.0 | 60.7 | 70.7 |
| | Cooked | 50.1 | 57.7 | 53.5 | 53.9 | 48.0 | 54.2 |
| Vasti | Raw | 62.6 | 83.6 | 70.2 | 57.6 | 81.4 | 59.8 |
| | Cooked | 55.6 | 60.7 | 56.5 | 50.3 | 75.2 | 54.3 |
| Gastrocnemius | Raw | | | 79.5 | | | 61.8 |
| | Cooked | | | 52.0 | | | 47.0 |
| Psoas major | Raw | 55.8 | | | | 45.5 | |
| | Cooked | 43.7 | | | | 43.5 | |
| Adductor | Raw | | 56.7 | | 52.5 | | |
| | Cooked | | 46.2 | | 45.3 | | |

| | |
|---------------------------------------|--------------|
| Analysis of Variance: Raw vs. Cooked, | F = 26.67**; |
| For raw--Treatments, | F = 0.66; |
| Muscles, | F = 3.45*; |
| For cooked--Treatments, | F = 0.34; |
| Muscles, | F = 5.59**. |

Treatment caused no significant change in the size of the muscle fibers. This was at variance with the work of Brady (19, 20), who found that the fibers decreased in diameter during ten days of cold storage.

The fiber diameters varied from muscle to muscle, the differences being significant for the raw muscles, and highly

so for the cooked muscles.

The average fiber diameters for the various muscles, and their standard errors, are given in table 20. No adjustment for treatment was necessary, since treatment did not change the diameters significantly.

Table 20. Average Fiber Diameters and Standard Errors (in microns)

| Muscle | Raw | | Cooked | |
|-----------------|----------|-------|----------|-------|
| | Diameter | S. E. | Diameter | S. E. |
| Semitendinosus | 51.5 | ± 3.5 | 47.6 | ± 2.2 |
| Semimembranosus | 57.4 | ± 3.5 | 48.5 | ± 2.2 |
| Biceps femoris | 62.6 | ± 3.5 | 52.9 | ± 2.2 |
| Vasti | 69.2 | ± 3.5 | 58.8 | ± 2.2 |
| Gastrocnemius | 70.6 | ± 6.1 | 49.5 | ± 3.8 |
| Psoas major | 50.6 | ± 6.1 | 43.6 | ± 3.8 |
| Adductor | 54.6 | ± 6.1 | 45.7 | ± 3.8 |

The proper standard errors for evaluating the differences between the muscles having different standard errors were ± 5.0 for the raw and ± 3.1 for the cooked muscles.

Among the raw muscles, the biceps femoris, vasti, and gastrocnemius fibers were all larger than the semitendinosus, the differences being highly significant. Also, the biceps femoris, vasti, and gastrocnemius fibers were larger than those of the psoas major, the vasti and the gastrocnemius fibers were larger than those of the semimembranosus, and the gastrocnemius fibers were larger than those of the adductor, these differences being significant. Between the other

possible pairs, there were no significant differences.

After cooking, the vasti fibers were larger than those of the semitendinosus, semimembranosus, psoas major, and adductor, the differences being highly significant. With significant differences, the vasti fibers were larger than those of the biceps femoris and gastrocnemius, while the biceps femoris fibers were larger than those of the semitendinosus, psoas major, and adductor. None of the other possible pairings showed significant differences.

Throughout, the fibers could be somewhat arbitrarily grouped in size as follows: smallest, semitendinosus, psoas major, adductor; medium, semimembranosus, biceps femoris; largest, vasti. The gastrocnemius did not fit well in grouping, as the raw fibers were the largest found, but shrank the most during cooking.

Fiber diameter and tenderness: The correlation coefficient between fiber diameter for the cooked roasts and tenderness score was only -0.198 , too small to be significant.

Number of fibers per bundle

The data on the number of fibers per bundle are given in table 21. Each figure represents the average of ten counts. The variability between counts for any given section was quite high, due, at least partly, to difficulty in distinguishing between primary and secondary bundles.

Table 21. Number of Fibers per Bundle

| Muscle | State | Time of Storage (in days) | | | | | |
|-----------------------|--------|------------------------------|-------|-------|-------|-------|-------|
| | | 0 | 1 | 2 | 4 | 9 | 18 |
| Semitendinosus | Raw | 56.8 | 67.8 | 84.2 | 113.0 | 87.9 | 95.3 |
| | Cooked | 102.9 | 61.3 | 84.3 | 76.7 | 83.4 | 92.2 |
| Semimembranosus | Raw | 93.4 | 100.4 | 88.5 | 118.4 | 108.0 | 92.7 |
| | Cooked | 119.5 | 127.5 | 127.7 | 90.4 | 104.7 | 130.5 |
| Biceps femoris | Raw | 193.3 | 74.9 | 109.2 | 77.9 | 71.3 | 71.5 |
| | Cooked | 59.0 | 108.1 | 94.7 | 73.7 | 96.9 | 73.5 |
| Vasti | Raw | 89.0 | 25.3 | 73.6 | 117.8 | 58.1 | 88.6 |
| | Cooked | 135.6 | 73.4 | 81.0 | 139.2 | 63.4 | 97.4 |
| Gastrocnemius | Raw | | | 61.0 | | | 52.9 |
| | Cooked | | | 112.5 | | | 75.8 |
| Psoas major | Raw | 127.2 | | | | 181.7 | |
| | Cooked | 139.0 | | | | 162.8 | |
| Adductor | Raw | | 67.3 | | 100.8 | | |
| | Cooked | | 96.4 | | 118.6 | | |
| Analysis of Variance: | | Raw vs. Cooked, $F = 1.27$; | | | | | |
| | | Treatments, $F = 1.54$; | | | | | |
| | | Muscles, $F = 1.96$. | | | | | |

The number of fibers per bundle did not vary significantly for different treatments, for muscles, or between the raw and the cooked. This was at variance with the work of Brady (19, 20), who found significant differences between the muscles in this respect. However, the finding that the psoas major consistently had a large number of fibers per bundle was in agreement with Brady's work.

Fibers per bundle and tenderness: Brady also found a high correlation between the number of fibers per bundle and the tenderness of the meat. This was not borne out in this study.

Histological Observations

Effect of storage and of cooking

General: The general picture of the microscopic changes which occurred with storage was as follows. The freshly killed meat had very poorly differentiated fibers which were straight to slightly wavy. These showed the longitudinal striations more prominently than they did the cross striations. After one day of storage, the fibers were much more distinct, some of them showing contracture nodes, others the kinks or crinkles of passive retraction. The nodes seemed to be very dense and resistant, as they persisted all through the eighteen days of storage. The kinks and crinkles disappeared after four to nine days of storage. Breaks began to appear about the second day of storage, becoming more numerous as storage time increased. Figures 9 to 14 illustrate the changes in one muscle, the semimembranosus. These pictures are, in general, characteristic of all the muscles.

The change from a tightly-packed to a more open structure with aging could also be seen in the cross sections, as shown in figures 15 and 16.

Uncooked Semimembranosus, Showing Changes with Storage

Longitudinal Frozen Sections

(magnification 42x)

Figure 9. Zero storage.

Figure 10. One-day
storage.

Figure 11. Two-day
storage.

Figure 12. Four-day
storage.

Figure 13. Nine-day
storage.

Figure 14. Eighteen-
day storage.



Figure 9.

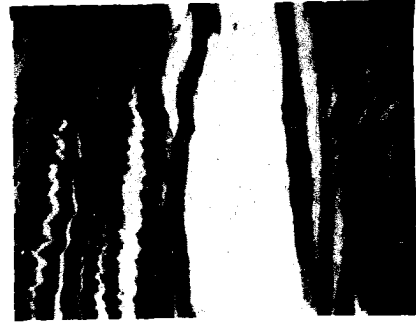


Figure 10.

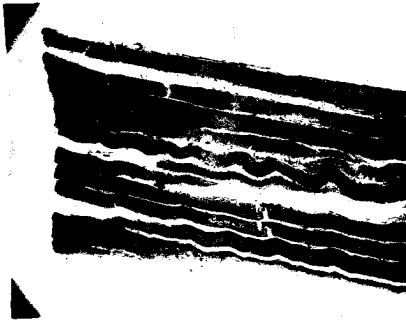


Figure 11.



Figure 12.



Figure 13.



Figure 14.

Raw Frozen Sections

(magnification 42x)

**Figure 15. Semitendinosus,
zero storage,
cross section.**

**Figure 16. Semitendinosus,
eighteen-day storage,
cross section.**

**Figure 17. Semimembranosus,
one-day storage,
longitudinal section.**

**Figure 18. Psoas major,
zero storage,
longitudinal section.**

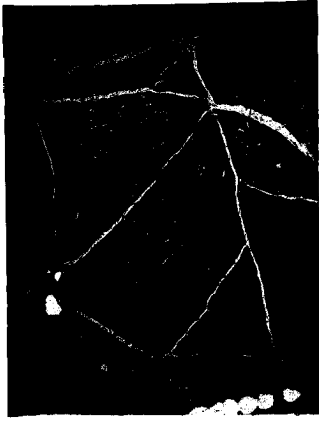


Figure 15.

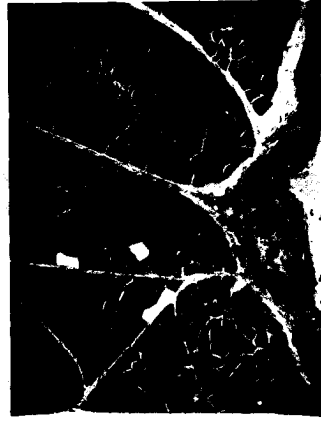


Figure 16.



Figure 17.

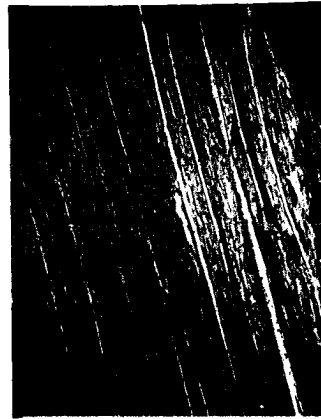


Figure 18.

The time of appearance of the breaks or ruptures in the tissue was variable, some sections showing them as early as the first day of storage. See figure 17. None were found, however, in the unstored tissue. Figure 18 shows the appearance of the tissue which was fixed first after the death of the animal, so represented the nearest to the living tissue. It was found that the fibers were straight, poorly differentiated, and showed no signs of nodes or breaks.

Changes in the semitendinosus with storage: The raw semitendinosus which was not stored presented very much the same picture as the other muscles with this treatment, as can be seen in figure 19. After one day of storage, however, a decided change in this muscle was noticed. The entire cut had contracted, throwing the fibers into pronounced waves much larger than the crinkles produced by differences in contraction among the individual fibers. These waves could be observed macroscopically. Figure 20 shows the appearance of a surface area of the roast, while figure 21 shows a microscopic section made from this same roast. This waviness made it difficult to prepare proper longitudinal or cross sections, as the waves were more or less spiral rather than in one plane. Figure 23 is a higher magnification of one section of the slide shown in figure 22, illustrating that, where a longitudinal section could be obtained, the characteristic nodes and kinks of rigor were visible. Figure 24 is a close-up of such a node. These

Semitendinosus, Showing Changes
with Storage

Figure 19. Uncooked longi-
tudinal frozen section,
zero storage.
(magnification 20x)

Figure 20. Cooked surface,
one-day storage,
(magnification 3x)

Figure 21. Cooked longitu-
dinal frozen section,
one-day storage.
(magnification 20x)

Figure 22. Cooked longitu-
dinal frozen section
eighteen-day storage.
(magnification 20x)



Figure 19.

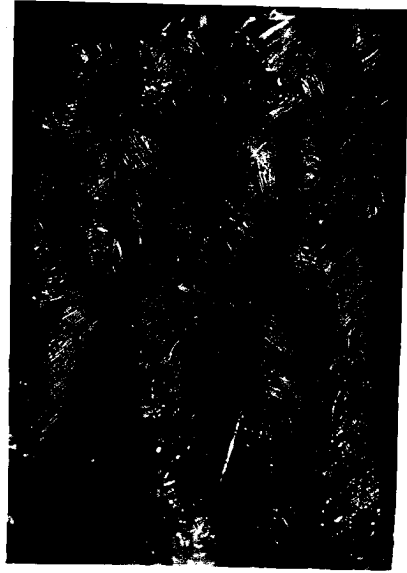


Figure 20.

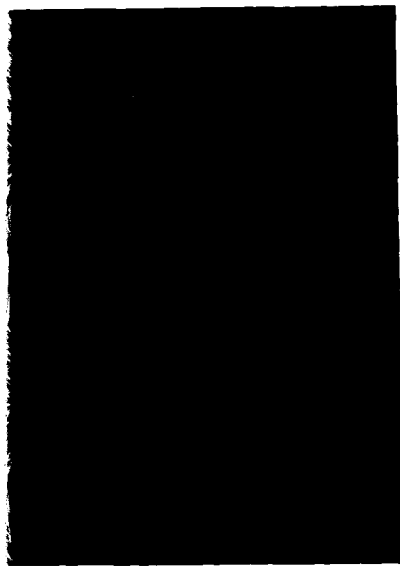


Figure 21.



Figure 22.

Figure 23. Cooked semi-
tendinosus,
longitudinal
frozen section,
one-day storage.
(magnification 42x)

Figure 24. Raw semi-
tendinosus,
longitudinal
frozen section,
one-day storage.
(magnification 200x)

Figure 25. Cooked semi-
membranosus,
longitudinal
frozen section,
one-day storage.
(magnification 42x)

Figure 26. Same slide
as figure 25.
(magnification 180x)

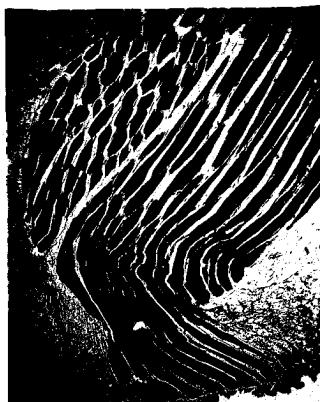


Figure 23.



Figure 24.



Figure 25.



Figure 26.

macro-waves were still present after four days of storage, but had disappeared by the ninth day. Figure 22 shows how the fibers straightened out. The broken appearance of the fibers was typical of ripened tissue.

The cause of these macro-waves could not be determined definitely, but it seemed likely that they were due to contraction of the connective tissue, since all the muscle fibers were waved, while the bands of connective tissue appeared to be more or less straight. Perhaps the elastic tissue was the causative agent, since the semitendinosus was the only muscle having large amounts of this type of connective tissue in all the sections, and the only one showing these macro-waves.

Effect of cooking: In general, the effect of cooking was to increase the microscopic manifestations of whatever state was observed in the raw roast. In the zero-storage roasts, cooking increased the definition of the fibers and produced signs of heat rigor. In the one- and two-day storage roasts, the indications of rigor were made more prominent by cooking. The roast stored four days showed little change after cooking, while those stored nine and eighteen days had more of the breaks characteristic of aged meat than had the raw samples.

The change in the collagenous connective tissue was one of the most interesting effects of cooking, and was readily observed in the muscles having heavy deposits of this material

around the muscle bundles. In the raw meat, all the collagenous tissue stained a bright magenta from the acid fuchsin of the van Gieson stain. However, much of this tissue in the cooked meat would no longer take this stain, being colored yellow by picric acid in the staining procedure. This yellow-stained connective tissue was broken and somewhat granular, while the magenta-stained was fibrous.

Rigor changes: There were two distinct microscopic changes produced by normal rigor--one active, the other passive. The active change was the appearance of alternate zones of condensation and rarefaction in the fibers under tension from contracture. The passive phase was found in the crinkled or kinked appearance of the fibers which Carey (26) said were not contracted themselves, but were drawn up by the contraction of the noded fibers.

The nodes which occurred in normal rigor were quite characteristic. They are illustrated in figures 25 to 32. These nodes were swollen and dense. Occasionally one was observed in which the striations could still be seen, as in figure 32. Such nodes appeared to be less completely contracted than the very dense ones. The completely contracted nodes were too dense to show any striations, as in figure 31.

The distance between the nodes varied. Sometimes two or more nodes occurred in a short space, while others were widely separated. Compare the nodes in figures 25 and 27.

Figure 27. Raw semi-
membranousus,
longitudinal
frozen section,
one-day storage.
(magnification 42x)

Figure 28. Same slide
as figure 27.
(magnification 180x)

Figure 29. Raw vasti, longitudinal
frozen section, nine-day
storage.
(magnification 200x)



Figure 27.



Figure 28.



Figure 29.

Figure 30. Raw semimembranosus,
longitudinal frozen
section, one-day storage.
(magnification 800x)



Figure 30.

Figure 31. Raw semimembranosus,
longitudinal frozen
section, one-day storage.
(magnification 1870x)

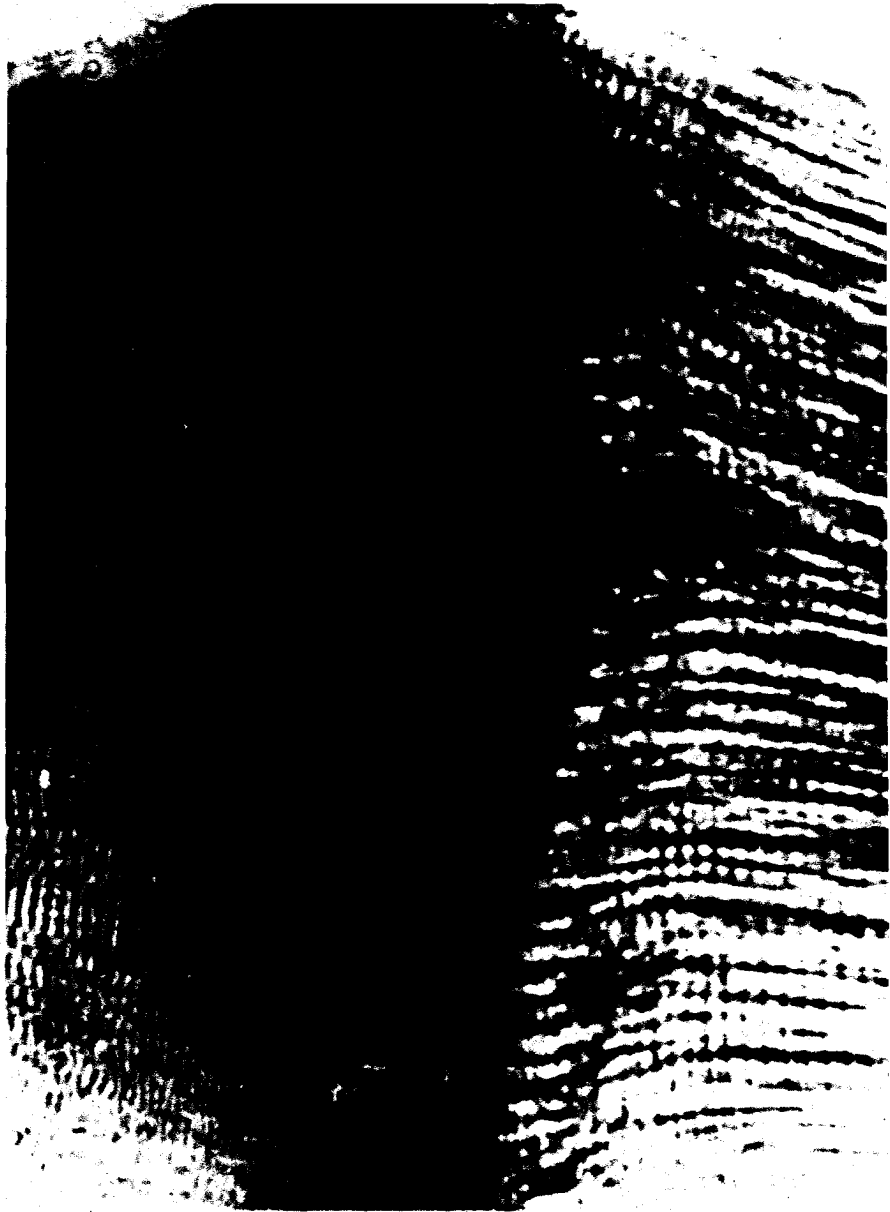


Figure 31.

Figure 32. Raw biceps femoris,
longitudinal frozen
section, nine-day storage.
(magnification 1870x)

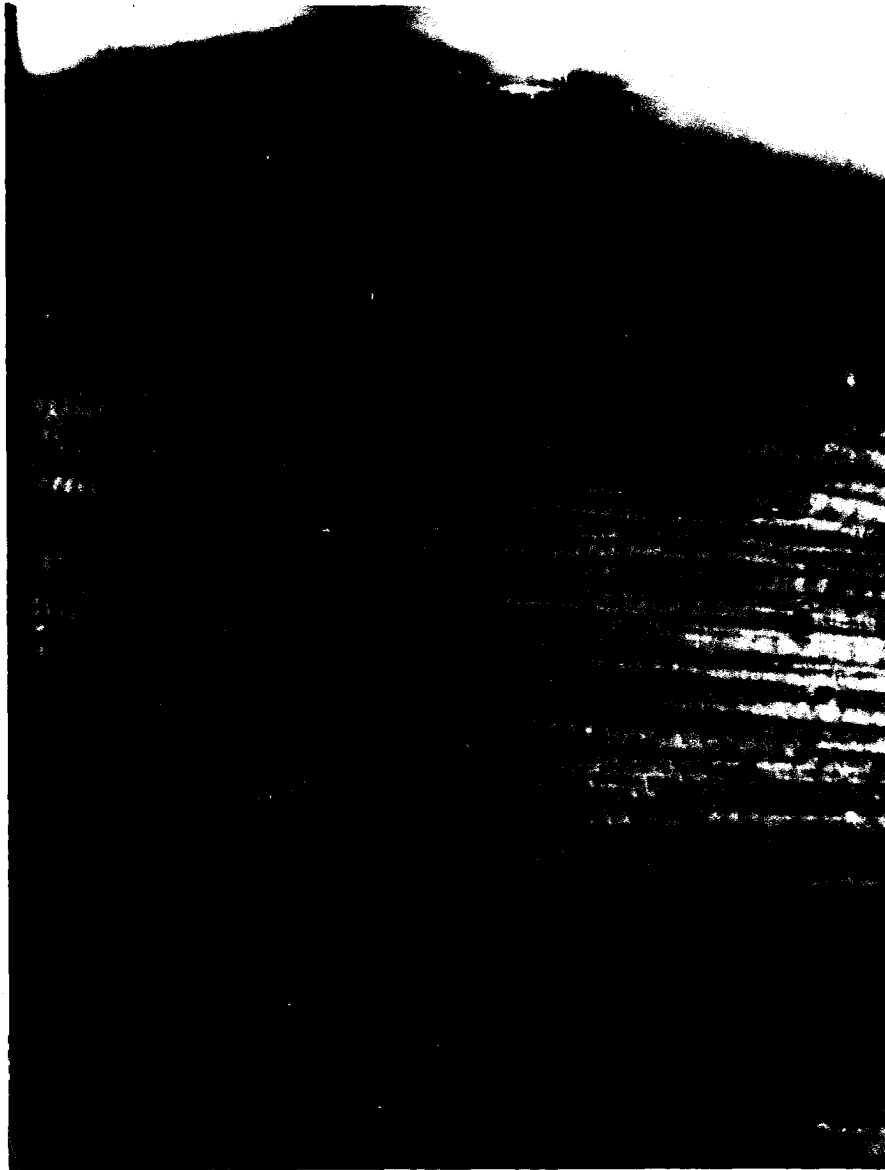


Figure 32.

The changes in the striations around the nodes were very definite. The stretched areas immediately adjacent to the nodes in the same fibers were narrower and stained less deeply than the nodes themselves, as shown in figure 26. These rarefied areas showed widely separated cross striations and prominent longitudinal striations, indicating stretching. The A discs often showed a light middle line, Hensen's disc, characteristic of fibers under tension. Close to the node, the striations became very fine and much closer together. See figure 30 for these changes. It was difficult to decide whether the nodes arose by condensation of the striae, as stated by Jordan and Speidel (68), or by splitting of the striae, as believed by Carey (26). However, the striations were very close together in the nodes, and the change in spacing was continuous from the contracted to the stretched regions, which would seem to be more in accord with the theory of Jordan and Spiedel than that of Carey. Also, where apparent splitting of the striations was observed, it could also be interpreted as being produced by the slipping of the fibrils so that the striations were no longer continuous. It seems likely that the actual formation of the node would have to be followed under the microscope to arrive at a conclusion on this point.

In the higher magnifications, figures 30, 31, and 32, the Vernier and zigzag effects produced by slipping of the fibrils are apparent.

The crinkles and kinks which occur in the passively

retracted fibers can be seen in figures 25, 26, 28, and 29. These are further illustrated in figures 33 to 38. The term "crinkle" is used to refer to the all-over wavy appearance of the fibers in figures 33 and 34, while "kink" refers to the tight S twist seen at intervals in some of the fibers in figures 35 to 38. It could be seen, especially in the higher magnifications, that there was no change in the thickness or spacing of the cross striations in these passively-retracted fibers, the striations merely shifting around to follow the twists in the fibers. In figures 34 and 35 the nodes of the contracted fibers which caused these bends in the uncontracted fibers can be seen. Figure 38 is interesting because it shows the microscopic appearance of rigor in the only zero-storage cut which went into rigor before it was cooked (storage series).

Heat rigor produced in zero-storage roasts by cooking:

The nodes produced by cooking roasts before they had gone into rigor are illustrated in figures 39 and 40. These differed from the normal rigor nodes in being less dense, but showed the narrowing of the cross striations and increased prominence of the longitudinal striations, with increased fiber diameter at the node. They appeared to be representative of an early stage in the formation of normal rigor nodes. Figure 41 shows another manifestation of heat rigor--alternate irregular areas of condensation and rarefaction. Figure 42 is a higher magnification of one of these areas, showing the closely-spaced

Effects of Passive Retraction
on Muscle Fibers

Figure 33. Raw semimem-
branosus, longi-
tudinal paraffin
section, one-day
storage.
(magnification 42x)

Figure 34. Same slide
as figure 33.
(magnification 180x)

Figure 35. Cooked vasti,
longitudinal
paraffin section,
nine-day storage.
(magnification 42x)

Figure 36. Same slide
as figure 35.
(magnification 180x)

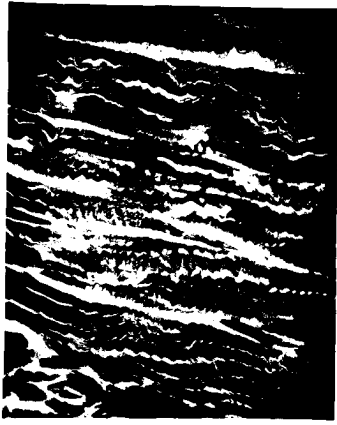


Figure 33.



Figure 34.



Figure 35.

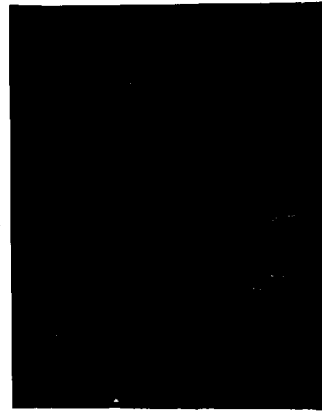


Figure 36.

Figure 37. Raw vasti,
longitudinal
paraffin section,
one-day storage.
(magnification 42x)

Figure 38. Raw psoas
major, longitu-
dinal frozen
section, zero
storage.
(magnification 42x)

Figure 39. Cooked semimembranosus,
longitudinal frozen
section, zero storage.
(magnification 400x)

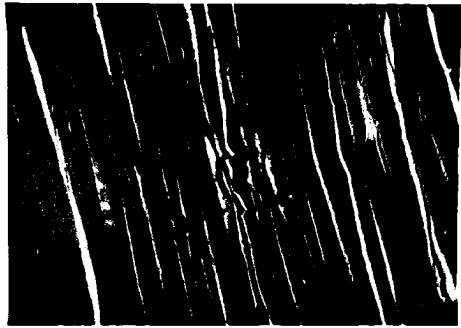


Figure 37.

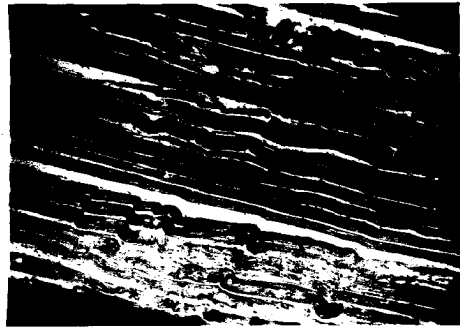


Figure 38.

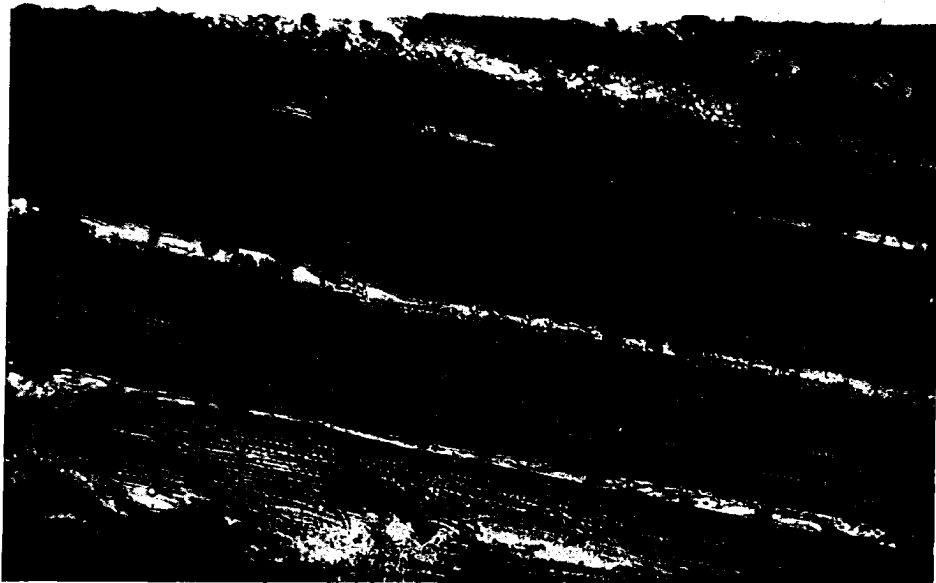


Figure 39.

Figure 40. Cooked biceps femoris,
longitudinal frozen section,
zero storage.
(magnification 400x)

Figure 41. Cooked vasti,
longitudinal frozen section,
zero storage.
(magnification 200x)

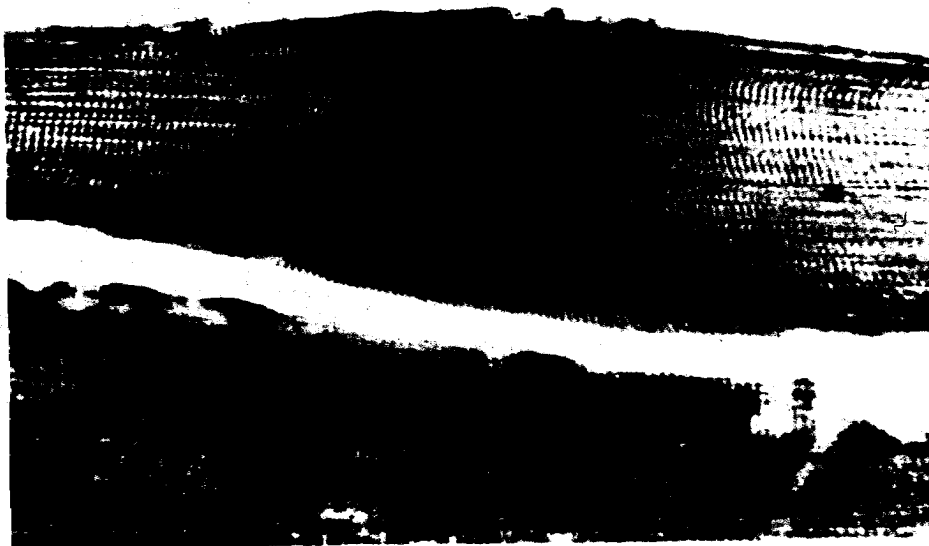


Figure 40.



Figure 41.

Figure 42. Same slide as figure 41.
(magnification 800x)

Figure 43. Cooked psoas
major, longitudinal frozen
section, zero
storage.
(magnification 42x)

Figure 44. Same slide
as figure 43.
(magnification 180x)



Figure 42.



Figure 43.



Figure 44.

striae of the condensed area and the stretched striae in the rarefied area. These fibers appeared to be in an early stage of the extreme heat rigor discussed later.

Figures 43 and 44 show the crinkles and kinks in fibers passively retracted by heat rigor. These were similar to those produced in normal rigor.

Breaks in the fibers: There were two types of breaks which appeared in the stored meat fibers, one a fracture, the other a disintegration of the muscle protoplasm over a fairly long area within the fiber. These disintegrated areas had lost both the longitudinal and cross striations, and the material remaining within the sarcolemma had a granular appearance.

The fractures seemed to occur chiefly in the crinkled or kinked fibers. In figure 44, constrictions can be seen along the periphery of some of the crinkled fibers. Figure 45 shows the progress of these constrictions to actual cracks in the fiber, and figure 46 shows an area having many cracks and breaks in the passively-retracted fibers.

The disintegrated areas were observed most frequently in the portions of the fiber between two nodes of contracture. Figure 47 shows the narrowing of such an area, while figure 48 shows a similar region where the rarefied part has disintegrated. Figures 49 and 50 show very clearly the long granular areas between the nodes. Figure 51 is a cross section from the same sample as figure 50. The few clear spots within the muscle bundles are places where the fiber fell out during

Figure 45. Raw biceps
femoris, longi-
tudinal frozen
section, nine-
day storage.
(magnification 200x)

Figure 46. Raw biceps
femoris, longi-
tudinal paraffin
section, four-
day storage.
(magnification 42x)

Figure 47. Raw vasti,
longitudinal
frozen section,
four-day storage.
(magnification 180x)

Figure 48. Same as
figure 47.

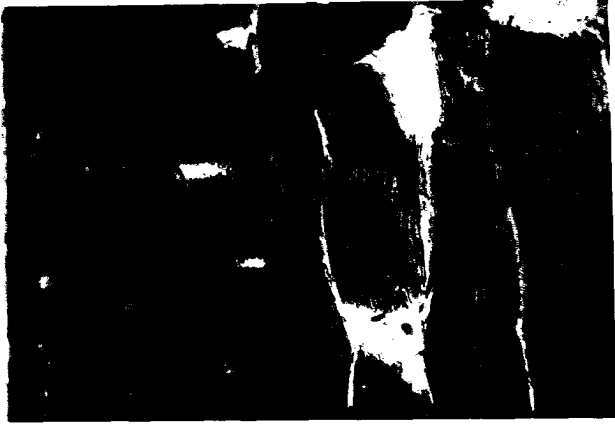


Figure 45.



Figure 46.

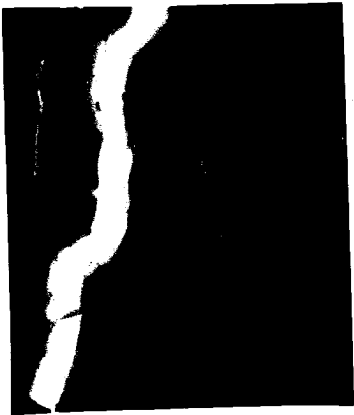


Figure 47.



Figure 48.

Slides Showing Powdered and Fractured Fibers

(magnification 42x)

Figure 49. Cooked biceps femoris, longitudinal frozen section, one-day storage.

Figure 50. Cooked semi-membranosus, longitudinal paraffin section, nine-day storage.

Figure 51. Same as figure 50 except cross section.

Figure 52. Raw biceps femoris, longitudinal frozen section, nine-day storage.

Figure 53. Raw biceps femoris, longitudinal frozen section, eighteen-day storage.

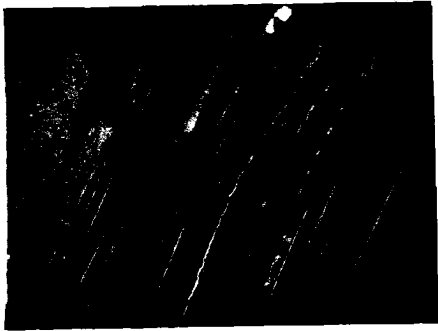


Figure 49.

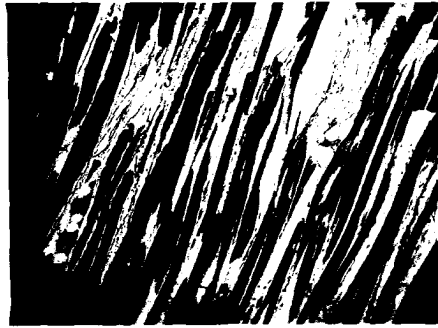


Figure 50.

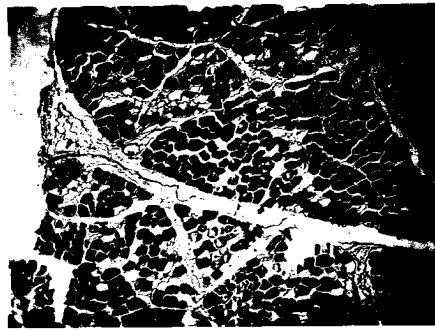


Figure 51.



Figure 52.



Figure 53.

preparation of the slide. There are many small granular areas surrounded by the endomysial reticulum which represent sections where the muscle protoplasm has disintegrated.

Figures 52 and 53 show both the fractures in the passively-retracted fibers and the areas of disintegration around the nodes in the same section.

The cause of these breaks in the fibers is unknown. There may be two possible explanations, one the autolytic action of the tissue enzymes, the other the physical stresses on the stretched and twisted fibers engendered by contraction in the noded fibers.

Shrinkage of fibers: Figures 54 and 55 illustrate the shrink in the fibers caused by cooking. In the cooked sections (figure 55) the endomysial reticulum which surrounds and supports each fiber is clearly visible.

Figure 56 shows the increased fiber shrinkage caused by the solutions used in making paraffin-embedded sections. If figure 56 is compared with figure 55, it becomes obvious why the frozen sections were used for the fiber diameter measurements, since they were more nearly representative of the true size of the fibers. Figures 54 and 55 also indicate some of the difficulties inherent in measurement of fiber size by the method employed. Since none of the fibers were truly round, it was perhaps a misnomer to speak of fiber diameter, but the term was used to correspond with the custom in this type of work.

Effect of Cooking and of Dehydrating Reagents
on Fiber Diameters. Cross Sections.
(magnification 42x)

Figure 54. Raw semi-
membranosus,
frozen section,
one-day storage.

Figure 55. Cooked semi-
membranosus,
frozen section,
one-day storage.

Figure 56. Same as figure 55
except paraffin
section.

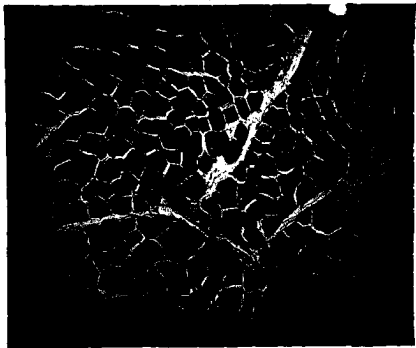


Figure 54.

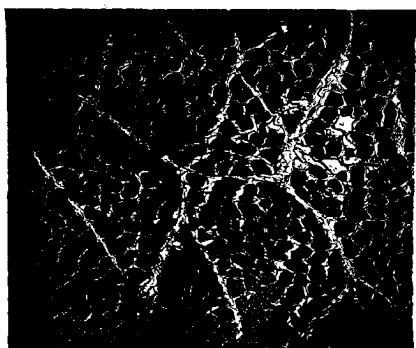


Figure 55.

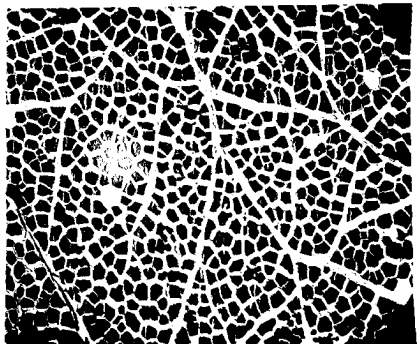


Figure 56.

Differences between muscles

Variation in size and shape of fibers: Figures 57, 58, and 59 illustrate the variation in size and shape of the fibers from the different muscles. Figure 57 is a cross section of the adductor, showing the many tiny fibers which result in the small average fiber diameter found for this muscle. Figure 58 shows the uniformly large fibers of the gastrocnemius. Figure 59 illustrates the elliptical shape of the fibers of the vasti group.

Variation in amount of connective tissue: The variation in amount of connective tissue could not be assessed on a truly quantitative basis from microscopic slides, since each slide represented only a very small area of the muscle, and the possibilities of variation were large. However, it was possible by this means to group the muscles rather broadly as having small, medium, or large amounts of connective tissue. The psoas major had the smallest amount, only a few fragments being visible in figure 60. The semimembranosus and biceps femoris showed heavy bands of connective tissue around the fiber bundles, as illustrated in figure 61. The other muscles might be classed between these two extremes as having moderate amounts of connective tissue, as shown in figure 62.

It was observed, during study of the slides stained to differentiate the two types of connective tissue, that only the semitendinosus consistently showed large deposits of

Cross Sections Showing Variation in Size of Fibers.

All raw; frozen sections.

(magnification 42x)

Figure 57. Adductor,
four-day
storage.

Figure 58. Gastrocnemius,
two-day
storage.

Figure 59. Vasti,
four-day
storage.

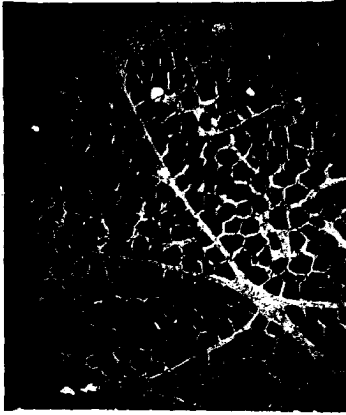


Figure 57.

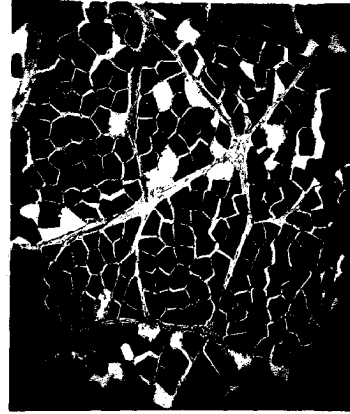


Figure 58.

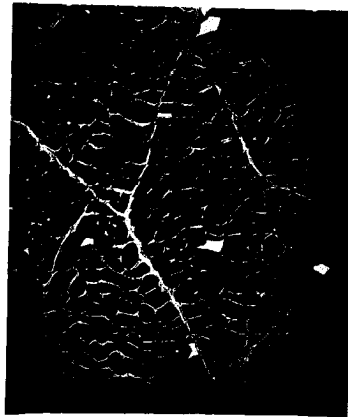


Figure 59.

Cross Sections Showing Relative Amount of
Connective Tissue.

Paraffin sections, zero storage.

(magnification 42x)

Figure 60. Cooked psoas
major.

Figure 61. Cooked semi-
membranosus.

Figure 62. Cooked semi-
tendinosus.

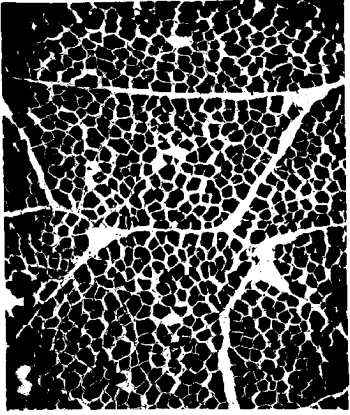


Figure 60.

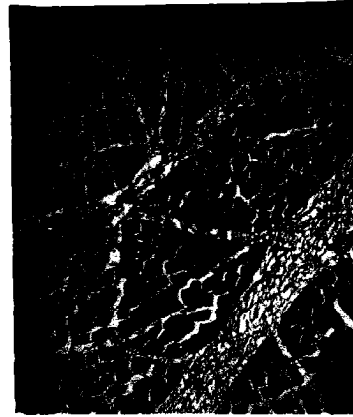


Figure 61.

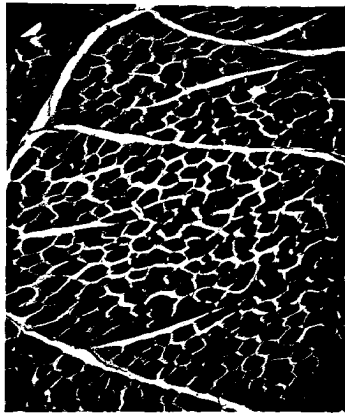


Figure 62.

elastic connective tissue. The semimembranosus, biceps femoris, and gastrocnemius showed a little elastic tissue in some of the sections. None was found in the other muscles. The elastic tissue content of the semitendinosus may account for the high force required to shear it, since elastin is considered to be unaffected by ordinary cooking operations.

Supplementary Studies

Effect of massage

Since only two roasts--one experimental and one control--were used in studying the effect of massage before the muscle had gone into rigor, the figures obtained could not be definitely evaluated. However, the data are given in table 22, as an indication of the effect of such treatment. The two roasts tested were the left and right proximal portions of the psoas major. Both were cooked without storage.

Inspection of the slides made from these two roasts showed that there was little difference in their microscopic appearance. The raw meat had poorly-defined fibers, most of them being straight or slightly waved. The massaged muscle had some signs of beginning rigor, with passively-retracted fibers surrounding straight fibers in which tension was indicated by the prominent appearance of Hensen's disc. The cooked sections had more clearly defined fibers, most of which

showed the tight wrinkle characteristic of passive retraction, with a few heat rigor nodes in the unmassaged muscle, and many in the massaged muscle.

Table 22. Data on Effect of Massage on Beef Not Yet in Rigor

| Factor | Control Cut | | Massaged Cut | |
|-----------------------------|--------------------|------------|--------------------|------------|
| | Raw | Cooked | Raw | Cooked |
| Condition before cooking | Soft, not in rigor | | Soft, not in rigor | |
| Condition after cooking | Not in rigor | | In rigor | |
| Cooking time | 80.6 min./lb. | | 95.4 min./lb. | |
| Total cooking losses | 8.71% | | 9.23% | |
| Decrease in volume | 22.47% | | 32.31% | |
| Tenderness score | 5.75 | | 2.5 | |
| Juiciness score | 7.0 | | 6.5 | |
| Flavor score--lean | 6.3 | | 6.67 | |
| Aroma score | 7.75 | | 7.5 | |
| Shear force | 27.18 lbs. | | 45.13 lbs. | |
| Press fluid | 47.71% | | 52.15% | |
| Fiber diameter | 52.2 μ | 47.1 μ | 55.8 μ | 55.6 μ |
| Number of fibers per bundle | 115.4 | 124.6 | 145.1 | 124.2 |

The results seemed to indicate that the control muscle was cooked quickly enough to prevent the full appearance of rigor before the proteins were coagulated by heat. However,

in the treated cut, the appearance of rigor was hastened by massaging so that the muscle had stiffened before the heat became great enough to stop the action.

Extreme heat rigor

Subjection of small samples of fresh meat to sudden heat (70°C) produced the maximum contraction bands of heat rigor discussed by Nageotte (95). The samples heated for two seconds showed this only around the edges, as seen in figure 63. After ten seconds' heating, these rigor bands were observable all through the sample, as in figure 64. Longer heating, up to twenty-five seconds, produced no further change.

The appearance of the nodes of alternating condensation and rarefaction can be better seen in figure 65. The higher magnification shows the density of the contracted areas, and the thinness and widely-spaced striations of the stretched portions.

Suggestions for Further Studies

Since it was only possible to obtain one animal for this study, it would seem advisable to have the work repeated in toto, to check on the animal-to-animal variation. Other questions which arose during the course of the investigation were as follows.

Longitudinal Paraffin Sections of Samples
Subjected to 70°C for Different
Lengths of Time.

Figure 63. Two seconds
at 70°C.
(magnification 42x)

Figure 64. Ten seconds
at 70°C.
(magnification 42x)

Figure 65. Ten seconds
at 70°C.
(magnification 180x)

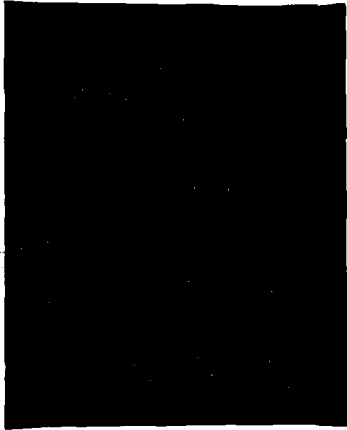


Figure 63.



Figure 64.

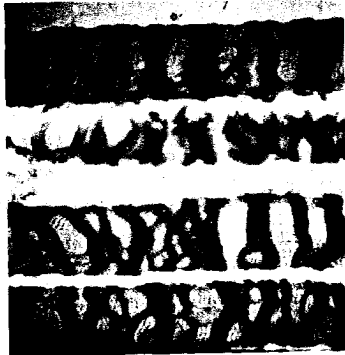


Figure 65.

1. What were the quantitative amounts of collagen and elastin present in the different muscles?

2. What was the actual extent of the change in collagen during cooking indicated by its failure to take the acid fuchsin stain?

3. Was the concentration of elastin in the semitendinosus characteristic of this class of beef animal or merely of this one animal? The more usual histological finding seemed to be that all muscles of the round contained some elastin.

4. What was the true histological basis of the formation of rigor nodes? Did it differ in heat rigor from that in normal rigor?

5. Was it possible to obtain a tender roast by cooking beef immediately after killing? There seemed to be some idea that this could be done, but no such result was obtained in this study.

6. How much of the breaking of the fibers during aging was caused by action of enzymes, and how much by the physical stresses arising from the contraction of some of the fibers? Was there any other reason for the occurrence of these breaks?

7. What changes occurred in the water-holding capacity of the proteins and the permeability of the cell membranes and connective tissue during aging and cooking?

The answers to these and other similar questions would afford a better foundation for the explanation of the changes which occur during aging and cooking of beef.

SUMMARY

A study was made of the effect of storage at 34-36°C on a yearling beef steer, and of the differences between the principal muscles of the round of this animal. The storage times used were zero, one, two, four, nine, and eighteen days. The muscles utilized were the semitendinosus, semimembranosus, biceps femoris, the three vasti, adductor, and gastrocnemius of the round, and the psoas major from the loin. A balanced incomplete block design was employed in assigning the storage times to the various cuts and in analyzing the data.

The muscles were separated, cut into roasts, wrapped in pliofilm and stored in the Animal Husbandry meat cooler. After the appropriate period of storage, they were taken out for testing. The raw roasts were inspected for any changes due to storage and for differences between the muscles. The electrical conductivity and the pH were determined for the raw meat. The cuts were then roasted in 150°C ovens. The maximum internal temperature of the roasts averaged 66°C, with a range of 65 to 67°C. The appropriate weights and measurements were taken so that the total, dripping, and evaporation losses, change in volume, and cooking time per pound could be calculated.

After the internal temperature started to drop, the roasts were sampled for judging and objective tests. The

palatability factors of tenderness, juiciness, aroma, and flavor of lean and fat were scored by four judges. The objective tests included the force required to shear a cylinder of meat one inch in diameter, and the amount of fluid expressible by 250 pounds pressure in five minutes.

Samples of both raw and cooked meat were made into microscopic slides for histological study. Cross and longitudinal sections were made of gelatin-imbedded tissues, using the freezing microtome, and of paraffin-imbedded tissues, using the rotary microtome. The frozen sections were stained to differentiate between the muscle fibers and the fat, while the paraffin sections were stained to show the muscle fibers and the two types of connective tissue--collagenous and elastic.

The slides were inspected for changes produced by cooking muscle not yet in rigor, by normal rigor, and by aging in cold storage. The frozen cross sections were used for measurement of the fiber diameters and counts of the number of fibers per bundle in the various muscles.

Supplementary studies were made on a pair of psoas major cuts to check on the effect of massage before the muscle went into rigor, and on small strips of neck muscle to check the effect of exposure to 70°C for various times in producing heat rigor.

Inspection of the raw roasts showed that the amount of leakage of juice was greatest during the second to fourth days of storage. By the eighteenth day, the roasts were

sticky on the surface and rather "high" in aroma, indicating bacterial activity. This was most noticeable in the vasti, which also attained the highest pH.

The grain and amount of external and internal fat varied from muscle to muscle. The psoas major was the finest in texture and showed the most marbling. The gastrocnemius and vasti were next in texture, the semitendinosus and adductor were medium grained, and the semimembranosus and biceps femoris were coarse. Only the semitendinosus and the biceps femoris had a continuous covering of external fat on one side. The gastrocnemius had some, and the other muscles very little external fat.

The electrical resistance of the raw meat dropped sharply with storage, while the pH first decreased, then increased as the storage time lengthened.

Only one of the fresh roasts, the psoas major, went into rigor before being cooked. It had passed through the stage of maximum stiffness by the end of the cooking period. The other fresh roasts were not in rigor before cooking, but were in rigor when cooked. All the stored roasts had passed out of rigor before being cooked.

The total cooking losses did not vary significantly with increased storage or between the muscles. The amount of dripping loss increased sharply from the ninth to the eighteenth day of storage, but this was offset by a corresponding decrease in the loss by evaporation. The muscles with large amounts of external fat had somewhat higher dripping losses

than those with little or no outside fat covering.

The volume of the roasts decreased with cooking. The cooking time was not changed by storage, but the smaller roasts required more minutes per pound than the larger ones.

The judging scores for tenderness, juiciness, flavor of lean, and aroma increased generally from zero to nine days of storage, then either remained about stationary or decreased somewhat. The decrease in scores for flavor of lean and aroma after eighteen days of storage was due to the development of "gaminess" in the meat which was considered definitely undesirable by some of the judges. Where judgments of the flavor of the fat were possible, the scores indicated little change up to nine days of storage, with a decided decrease in desirability after eighteen days of storage. This decrease was due to the development of rancidity.

The force required to shear the meat decreased with storage, the drop being especially marked from the fresh roasts to those stored one day. The press fluid dropped during the early part of the storage period, then increased decidedly during the last nine days.

The variation in tenderness between the different muscles as indicated by judging scores and by shear showed that the psoas major was the most tender muscle used, the gastrocnemius and adductor being next. The four large muscles of the round, the semitendinosus, semimembranosus, vasti, and biceps femoris, did not differ significantly from each other in tenderness.

The diameter of the muscle fibers decreased with cooking but not with storage. The variation between the muscles was quite large, the biceps femoris, vasti, and gastrocnemius having the largest fibers and the semitendinosus and psoas major the smallest. The number of fibers per bundle was too variable to show any consistent change with storage or between the different muscles.

Microscopically, the freshly-killed meat had straight to slightly wavy fibers, which were poorly differentiated. Storage for one day led to the appearance of rigor nodes and crinkled or kinked fibers. The nodes persisted throughout the storage time, but the crinkles and kinks tended to disappear, being replaced by sharp breaks in the fibers. The stretched areas of the fibers immediately adjacent to the rigor nodes frequently disintegrated, leaving a granular residue. Cooking brought on the appearance of rigor in the fresh meat, and increased the number of broken fibers in the meat stored nine and eighteen days. The rigor nodes produced in the fresh meat by cooking were not as dense as those found in the stored meat.

The change in collagen caused by cooking could be observed in the slides, as a loss of ability to take the acid fuchsin stain and a change from the normal fibrous state to a somewhat granular residue.

Massaging the freshly-killed muscle before cooking apparently speeded up the onset of rigor, but caused little

other change except in tenderness, the score of the massaged roast being 3.25 points lower, and the shearing force 18 pounds higher, than that of the control.

Exposure of small strips of fresh muscle to 70°C gave the typical picture of extreme heat rigor, the fibers showing alternate bands of maximum contraction and rarefaction. Two seconds exposure to this heat affected only the outer fibers, but within ten seconds the fibers were changed throughout the sample of tissue.

CONCLUSIONS

The conclusions listed below should be evaluated in terms of the definite limitation that the tests were made on only one animal. Biologic variability is too great to permit any definite statements concerning an entire class, based on only one animal. Within this restriction, the conclusions possible from this study were as follows.

1. The findings on the changes induced by storage indicated that the greatest increase in palatability in the individual roasts was obtained with a nine-day storage period. Further storage led to the development of "high" or "gamey" odors and flavors, and to rancidity of the fat. The handling of the fresh meat incident to cutting up the muscles into individual roasts was considered to hasten the appearance and dissolution of rigor. Therefore, beef hung as half- or quarter-carcasses would probably require a longer time in cold storage for the same changes to occur.

2. Rigor was shown histologically by the formation of dense nodes of contracture bordered on either side in the same fiber by areas of extreme stretch. Fibers which did not contract were drawn into waves and kinks by the shortening produced by the contracted fibers. Normal rigor produced denser nodes than those caused by the heat of cooking meat not in rigor.

The passing of rigor and progress of ripening was indicated by the appearance of breaks in the fibers. Sharp fractures usually occurred in the passively-retracted fibers, and granular areas and fractures appeared in the stretched portions of the fibers adjacent to the rigor nodes.

3. The comparison between the muscles indicated that, for many of the tests employed, the four large muscles of the round were quite comparable. These, the semitendinosus, semimembranosus, biceps femoris, and vasti, furnished six roasts per animal from each pair of muscles from the right and the left sides. There was no significant difference among these four muscles as to total cooking losses, tenderness (score or shear), flavor of lean, aroma, press fluid, or number of fibers per bundle. They also appeared to have about the same amount of marbling, but only the semitendinosus and biceps femoris had appreciable quantities of external fat. These last two also had greater dripping losses, balanced by lower losses from evaporation. The juiciness varied, the semitendinosus and semimembranosus scoring lower than the biceps femoris and the vasti. The latter two had larger muscle fibers than the former two. The other three muscles used, the psoas major, adductor, and gastrocnemius, differed significantly in many factors from the four large muscles of the round.

Despite the rather good comparability between the four large round muscles demonstrated for this experiment, it would

probably be advisable to retain statistical control of the muscle-to-muscle variation, at least until more animals have been tested.

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APPENDIX

Sample No. _____

Date _____

SCORE CARD FOR MEAT

| Factor | 10 | 9 | 8 | 7 | 6 | 5 | 4 | 3 | 2 | 1 | Remarks |
|---------------|------------------|-------------|--------|--------|---|-------|---|-------|------------|-----------------|---------|
| | Extremely good | Very good | Good | Medium | | Fair | | Poor | Very poor | Extremely poor | |
| | | | | plus | | minus | | | | | |
| Aroma | | | | | | | | | | | |
| Flavor Fat | | | | | | | | | | | |
| Lean | | | | | | | | | | | |
| | Extremely tender | Very tender | Tender | Medium | | Fair | | Tough | Very tough | Extremely tough | |
| | | | | plus | | minus | | | | | |
| Tenderness | | | | | | | | | | | |
| | Extremely juicy | Very juicy | Juicy | Medium | | Fair | | Dry | Very dry | Extremely dry | |
| | | | | plus | | minus | | | | | |
| Juiciness | | | | | | | | | | | |

Descriptive Terms

- Aroma
1. Mild ----
 2. Sharp ----
 3. Strong ----
 4. Faint ----
 5. Foreign ----
 6. _____
 7. _____
 8. _____

- Flavor
1. Flat ----
 2. Mild ----
 3. Mellowed ----
 4. Rich ----
 5. Strong ----
 6. Old ----
 7. Bitter ----
 8. Acid ----
 9. Salty ----
 10. Sweet ----

- Color of Lean
1. Light brown ----
 2. Dark brown ----
 3. Red and brown ----
 4. Gray ----
 5. Irridescent ----

- Texture
1. Stringy ----
 2. Dense, compact ----
 3. _____
 4. _____
 5. _____

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Preference _____
(among samples judged at one time)

Scorer _____

Table 23. Individual Shear Force Readings by Position (in pounds)

| Sample Number | Central Reading | Second from Center | External Reading |
|---------------|-----------------|--------------------|------------------|
| 1 | 55.0 | 70.0 | 77.5 |
| 2 | 50.7 | 60.5 | 62.0 |
| 3 | 21.6 | 22.1 | 19.3 |
| 4 | 16.1 | 13.1 | 13.4 |
| 5 | 26.8 | 24.2 | 26.5 |
| 6 | 26.5 | 25.6 | 33.5 |
| 7 | 42.5 | 45.5 | 48.5 |
| 8 | 43.0 | 41.5 | 49.8 |
| 9 | 24.4 | 24.9 | 24.6 |
| 10 | 15.3 | 15.2 | 17.7 |
| 11 | 26.1 | 21.8 | 27.0 |
| 12 | 20.2 | 18.3 | 17.7 |
| 13 | 33.7 | 38.8 | 41.2 |
| 14 | 17.8 | 13.8 | 16.8 |
| 15 | 15.1 | 12.4 | 11.8 |
| 16 | 21.7 | 22.7 | 25.2 |
| 17 | 29.3 | 28.2 | 26.0 |
| 18 | 19.0 | 24.7 | 24.6 |
| 19 | 21.2 | 15.4 | 17.9 |
| 20 | 24.0 | 25.2 | 30.5 |
| 21 | 20.2 | 17.7 | 18.7 |
| 22 | 15.6 | 19.8 | 20.4 |
| 23 | 22.4 | 20.7 | 19.7 |
| 24 | 17.8 | 17.5 | 12.9 |
| 25 | 19.2 | 18.9 | 18.7 |
| 26 | 17.3 | 15.1 | 12.9 |
| 27 | 16.7 | 15.4 | 22.2 |
| 28 | 13.7 | 15.5 | 14.4 |
| 29 | 14.1 | 15.7 | 12.7 |
| 30 | 16.9 | 14.0 | 15.1 |
| 31 | 24.9 | 32.0 | 32.2 |
| 32 | 16.9 | 15.6 | 13.0 |
| 33 | 29.6 | 29.8 | 22.2 |
| 34 | 11.1 | 9.6 | 10.4 |
| 35 | 17.4 | 19.9 | 21.8 |
| 36 | 15.8 | 24.8 | 29.1 |
| Average | 23.3 | 24.1 | 25.2 |

Analysis of Variance: Position, $F = 197.95^{**}$;
 Sample, $F = 264.7^{***}$.