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Collagen and elastin content of four beef muscles aged varying periods of time

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NOTE TO USERS

COLLAGEN AND ELASTIN CONTENT OF FOUR BEEF MUSCLES AGED VARYING PERIODS OF TIME

by

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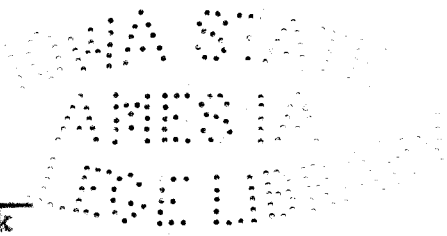

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INTRODUCTION

The biochemistry of muscle has become a most extensive field of knowledge since the first announcement of a chemical attack which was the description of myosin or muscle clot in distinction from muscle plasma by Kühne in 1864. While much has been learned relative to the proteins present, little is known of the biochemical alterations which occur in this fraction during the storage of muscles so commonly utilized with the edible meats.

Therefore further characterization of the changes as they occur with time should more adequately describe muscle protein and at the same time assist in an understanding of the salient factors of storage and quality maintenance under heat treatment, as well as other types of processing.

Without question the changes may be visualized as autolytic changes in the same category with other post mortem events. Certainly the reactions are primarily enzymatic reactions proceeding at very slow rates because of low temperature and without benefit of the physiological controls imposed by the living tissue. In addition aerobic oxidation affects those areas which are exposed, as is very evident in the browning which takes place as methemoglobin is produced at the surfaces. On these exposed areas, changes due to the growth of bacteria and molds take place after some lapse of

time, and dehydration brings about concentration of inorganic salts as well as protein denaturation. These changes penetrate into the tissue in more or less degree according to the temperature and humidity of the storage room, the condition of the original tissue, and the measure of sanitary conditions observed throughout the process.

It has long been observed that changes occur in beef muscles during storage at the usual temperature (35°F.) for periods up to thirty or forty days which result in an increase in tenderness and a change in flavor. Further, it has been assumed that the tenderization was due to an alteration in the connective tissue brought about by enzymatic or other chemical factors operating during the storage period. Since collagen is the more abundant and more labile of the two components of connective tissue, it has seemed reasonable to explain the changes as partial conversion of the collagen to gelatin. This study was undertaken to test that postulation by following the collagen and elastin content of beef muscles during the usual storage period by chemical methods. Histological, mechanical, and organoleptic tests on the same muscles were performed in another study.

REVIEW OF LITERATURE

Progress in Knowledge Regarding the Chemistry of Skeletal Muscle

It has been more than a century since Bracconet announced in 1820 the isolation of sucre de gelatine, and almost exactly that long since Tilanus' first analysis of elastin in 1844. Kühne published in 1864 the results of his studies on muscle plasma, the clot of which he called myosin, his isolation of histidine, and his work with enzymes on muscle decomposition. Mülder who is responsible for giving the name "protein" to this class of nitrogenous substances was a contemporary of Kühne's. About the same time von Fürth was developing the technique of fractional coagulation by heat and making wide use of the technique of salting out muscle proteins with various concentrations of ammonium sulfate. Mülder, Liebig, and Berzelius were particularly interested in the chemistry of gelatin, which proved to be a versatile substance, convenient to work with, and very important to the tanning industry. These chemists handed their torch to Siegfried, who is responsible for the widely-accepted reticulin hypothesis in connection with tendon structure, and later Hofmeister and Halliburton assumed

leadership in this area of investigation.

Probably because of the relative ease of obtaining the material free from muscle most of these chemists made extensive use of tendons and ligaments for the study of connective tissues, and in their writings gave minute directions for the preparation of collagen from tendons, and elastin from ligamentum nuchae. Along with the preparation which often took weeks of time and long, tedious extractions with water, crude enzymes, and dilute reagents of the kind available, they have recorded the results of countless experiments to explain the chemical properties of the proteins in which they were interested.

As soon as a few of the constituents of muscle were recognized, men trained in greatly divergent fields seized upon the newly acquired knowledge to help them solve problems in their own areas of science. In Europe the chemists Abderhalden and Hoppe-Seyler continued the study of the connective tissues, refining the methods of extraction, repeating and carrying further the analyses as better equipment and reagents and more knowledge became available.

In England Dorothy Jordan Lloyd (1, 2, 3, 4, 5) and Marriot (6), partly with the assistance of the leather trade, have been studying for many years the physical and chemical properties as well as the structure of gelatin. Just recently in Scotland Ames (7) has been supplementing their work on the properties of collagen and elastin.

In the United States Chittenden and associates (8) at Yale and Gies and coworkers (9, 10, 11, 12, 13) at Columbia attempted to clarify some of the complexities of collagen and gelatin chemistry during the first decade of this century. More recently Bergmann's interest in amino acids led him to the study of muscle proteins. Niemann and Chibnall were also active in this area. Bogue (14) studied gelatin extensively from the standpoint of the tanning industry, using skins as his source of collagen. Theis and others (15, 16, 17, 18, 19, 20, 21, 22) published within recent years some well controlled studies on the structure and reactions of the collagen and gelatin molecules as revealed by their reactions with formaldehyde, quinone, and other reagents.

In addition to these biochemists, Mirsky (23) investigated the soluble proteins of muscle and especially the phenomenon of denaturation. Neurath and others (24) also studied this phenomenon which is closely allied to the fields of physiology and pathology. In these fields many workers are studying not only the functioning of normal muscles, but of muscular atrophy and dystrophy as well.

Two of the most fascinating branches of the study of the chemistry of muscle are very recent developments. One of these has sprung from a continuation of the application of physical chemistry to the study of the muscle proteins which Gortner initiated and Cohn and Edsall have continued. It has

resulted in the deliniation of the structures of myosin and collagen and the development of hypotheses to explain their functioning by means of X-ray diffraction studies and the electron-microscope. W. T. Astbury (25, 26) in England and R. S. Bear (27) at Massachusetts Institute of Technology are two of the most active workers in this area. They have been able by correlating X-ray patterns with the composition of these and other proteins, so far as it is known, to make postulations not only as to size and configuration of the myosin and collagen molecules, but also to the arrangement of amino acids in the molecules. Schmitt, Hall and Jakus (28) have been studying the same problem with the electron microscope. Carey (29, 30) has been trying to apply the principles of wave mechanics to the observations made histologically of muscles in rigor in an effort to explain the phenomenon.

The other area of muscle chemistry which is of recent development is that of the application of enzymatic techniques. Northrop (31) as early as 1921 studied the comparative hydrolysis of gelatin by pepsin, trypsin, acid, and alkali. Since that time Meyerhof, Lippmann, Ochoa, Cori, and Szent-Gyorgyi have been active in the study of the enzyme reactions of muscle. Meyerhof and Szent-Gyorgyi (32, 33) have perhaps been the most interested in reconstructing a picture of the contractile tissue, myosin, and the method of its action. The latter's postulation of a myosin-actin-

adenosinetriphosphate complex, very sensitive to different temperatures and to certain inorganic ions, is too new for wide acceptance as yet.

There are on the Continent a number of centers in central Europe, especially Russia, where chemists, for example A. Kuntzel (34) and Fokina (35), are working on the composition and functioning of muscular tissue. More results from their laboratories will probably be available in the near future.

Chemical Studies on Meat

No mention has so far been made of a group of workers in the chemistry and physics of muscle proteins working with a somewhat different point of view. These investigators, like the physical chemists and physiologists, took the information gained by Kühne, Liebig, Mülder, and Hofmeister and in the later part of the nineteenth century began to apply it to experiments on animal tissue destined for use as food. These workers were interested for the most part in explaining the effects of storage under varying conditions and of heating on animal tissues. Often the results were measured by a combination of organoleptic tests, mechanical devices, histological procedures, and chemical determinations. The main objective was the determination of the factors responsible for quality in meat, and how they might be

influenced to improve that quality.

The first laboratory to attack the problem was that of K. B. Lehmann and associates (36) at the University in Würzburg. He reported in 1907 the results of ten years' work on two muscles of beef: a tender one, the psoas, and a tough one, which Lehmann called the flank skin muscle. He devised a dexometer, a machine which imitated the action of human biting as nearly as possible and determined that the toughness ratio of psoas to flank was 1/2.4. Histological studies showed the fibers of grown cattle to be two to two and one half times as thick as those of calves. He used Schepilewsky's method (37) for determining collagen in which he extracted a 25-gram sample with water, then with 5 per cent sodium hydroxide at room temperature, and later with hot 0.5 per cent sodium hydroxide. He determined the nitrogen in the filtrate, which he assumed was the result of a conversion of the collagen to gelatin. He used the Hofmeister factor, 5.6, to convert the gelatin-nitrogen figure to collagen. The psoas had 0.3 to 0.5 per cent connective tissue by this method and the flank 0.8 to 1.4 per cent. Lehmann stored these two muscles for a period of eight days and found a tenderizing effect with aging, more evident in the case of the flank muscle. This change in tenderness was not so striking in the case of meat boiled five minutes, thirty minutes, or sixty minutes, but the same trend was evident. In another experiment he found that freezing and thawing lessen the toughness

of muscle, ripened muscle showing the tenderizing to a greater extent. In studies on cooking meat, Lehmann also found that although in the raw state flank muscle was 2.63 times as tough as psoas, after one to one and one half hours' boiling they were equal in tenderness, and after three hours the flank was more tender than the fillet. The psoas might be tougher after short cooking than it was in the raw state. His explanation of this fact was that the coagulation of "muscle albumins" made the muscle more difficult to cut, as did the loss of muscle elasticity. If the tenderizing of connective tissue did not compensate for this, the meat was tougher cooked than raw. The work in Lehmann's laboratory was handicapped by lack of specialized equipment such as the modern laboratory has, but his experiments were admirably conceived and carried out, considering that he was a pioneer in this type of work. His appraisal of his work was very sensible, and it pays him tribute to say that though similar experiments have since been done with much refinement of technique, very few of his conclusions have been brought into question.

Simultaneously with the work of Lehmann, Grindley and his associates (38) at the University of Illinois were carrying out and publishing the results of a series of studies entitled, "The Chemistry of Flesh." In general their plan included numerous determinations upon a few materials. The determinations they made are of little help at the present

time because the distinction between coagulable matter and non-coagulable matter, ash, fat, and phosphorus, are no longer of very much significance. The work is significant, however, as being the first to apply chemical methods alone and on a large scale to the solution of the problem of producing quality in meat.

In 1917 a United States Department of Agriculture bulletin by Hoagland, McBride and Powick (39) traced the progress of acidity, proteose-nitrogen, non-coagulable nitrogen, amino-nitrogen, ammoniacal- nitrogen, soluble organic phosphorus, and the acidity of the kidney and external fats of seven quarters of beef stored just above freezing for fourteen to one hundred and seventy-seven days. They found the same changes in the beef as had been found in beef autolyzed under aseptic conditions for seven to one hundred days, which excluded bacteria as the chief cause of changes during aging. The increase in tenderness due to storage was complete in two to four weeks. The coagulable-nitrogen decreased and non-coagulable-nitrogen increased continuously. The amino-nitrogen and ammoniacal-nitrogen increased but in the latter case, the rate decreased as it accumulated. The total soluble phosphorus was irregular but the proportion of the phosphorus which was inorganic increased. The classification of nitrogen fractions into coagulable and non-coagulable is again not very helpful in comparing the results

with work of the present time, but the problem was clear-cut, and the conclusions well drawn, although in many cases results were too few in number and too irregular to permit the making of positive statements.

In the middle 1930's a number of publications relating chemical characteristics to quality in beef appeared. Noble, Halliday, and Klaas (40) in connection with studies on tenderness and juiciness of beef which they determined mechanically, stated that the more juice in the beef, the richer it was in solids, total-nitrogen, and in one case, coagulable-nitrogen. L. C. Baker (41) listed the constituents of meat acting as indices of change and included the following: (1) lactic acid content, (2) pH, (3) moisture, proteins and extractives, (4) hemoglobin and color, (5) ammoniacal-nitrogen, (6) free fatty acid. He noted that "little fundamental work has been done on the proteins of meat since the time of Halliburton and von Fürth."

From Kansas State College, Mackintosh, Hall, and Vail (42) published in 1936 some observations pertaining to the tenderness of meat which included the statement that the higher the collagen-nitrogen, the less tender the meat, the higher the shear value, and the lower the palatability scores. Brady (43) on the basis of thirteen animals he tested and Hammond (44, 45) stated that tenderness is a function of texture, which, in turn, depends upon the size of the

fiber bundle of the muscle, implying that collagen is not a major factor. The larger the bundle the "finer" the texture. It is readily apparent, too, that the larger the bundle, the less connective tissue there would be in a given area of muscle, and for this reason it would be more tender. Steiner (46) in studying post mortem changes in beef at different temperatures concluded that the beef decreases in toughness on aging because the muscle fibers become inelastic and brittle.

McCarthy and King (47) in studying chemical changes in meat stored for forty-eight hours at 60°F. found a more rapid rise in sulfhydryl content and in soluble-nitrogen compounds than in the usual thirty-day ripening period at 35°F. There was a comparable rate of disappearance of ascorbic acid and a more rapid rise in hematin-type pigments in the press juice.

Helen Johnson (48) studied the water content of frozen poultry after six to nine months' storage. There was no correlation between bound water or water content and "juiciness." The amount of bound water as determined by vapor pressure increased with storage and with cooking. More water was held as bound water by those samples stored at -12.2°C. than by those stored at -23.3°C.

Bendall (49) investigated the effect of cooking on the creatine, creatinine, phosphorus, nitrogen, and pH values of raw, lean beef. He found a rise in the ratio of free to

total creatine, and a rise in pH due to hydrolyzed linkages in the protein molecule. There was little effect on the phosphorus or non-protein nitrogen.

Ramsbottom, Strandine, and Koonz (50) compared the tenderness of twenty-five beef muscles, both raw and cooked. They noted that the cooked muscles decreased in tenderness due to coagulation and denaturation of muscle proteins and the shrinking and hardening of muscle fibers. The tissue was cooked in lard at 250°F. to an internal temperature of 170°F. The authors compared the shear readings of raw and cooked collagenous tissue, the readings being 120 and 21.5 respectively. Elastic connective tissue readings decreased from 81.1 to 42.3. This is interesting inasmuch as it is unlikely that much if any hydrolysis of collagen takes place in the brief cooking period, and it would appear to be impossible for any breakdown in the elastin to have occurred, other than coagulation.

Deatherage and Harsham (51) report that tenderness is not a smoothly increasing function of the length of the storage period in carcasses held at 33° to 35°F. At seventeen days there was a break in many cases, the tenderness value being lower at twenty days, but improving somewhat by the thirty-first day.

In general, the studies on tenderness and cooking which have involved mechanical measurements and organoleptic and

histological procedures have been reviewed in the thesis of Dorothy Harrison (52), in which the histological, organoleptic, and mechanical tests performed in connection with this thesis problem are reported.

Investigations on Storage at Temperatures Below 0°C.

In the early 1920's Fearon and Foster (53) studied some of the autolytic changes in meat frozen and thawed. They concluded that beef cannot be frozen and thawed again without marked changes taking place in the appearance, palatability, and general physical state of the meat. The first chemical results they published were in a report to the Fourth International Congress of Refrigeration in Paris, 1924. In unfrozen beef Foster (54) found the soluble-nitrogen content on autolysis increased from 10.5 per cent to 13 per cent of the total-nitrogen in ten days and then remained constant. In beef frozen at -8°C., the soluble-nitrogen changed from 10.5 to 16 per cent of the total-nitrogen in two days. Fearon and Foster allowed a 20-per cent mixture of liver scraped from the connective tissue and mixed with toluene and distilled water to autolyze for nine days. The filtrate obtained at the end of this period was treated with trichloracetic acid or metaphosphoric acid to precipitate the coagulable-protein. The filtrate obtained was designated by

these chemists as the soluble-nitrogen component of their preparation. These results would seem to indicate a greater tissue hydrolysis at lower temperatures.

Reay (55) in a report of the Food Investigation Board, 1930, says the important change during storage is a colloidal one. The muscle globulin loses part of its power to hold water, probably because it undergoes some form of denaturation by the action of the concentrated salt solution in the partly frozen muscle. If so, the forces attracting water to the proteins in muscle are exceedingly weak since there is no physico-chemical evidence that there is any appreciable "chemically" bound water in muscle.

Tressler, Birdseye and Murray (56) report that freezing at -20°F. effects a marked tendering, which is complete in five weeks.

D. B. Finn (57) in discussing the denaturation of the proteins in muscle juice by freezing at -2° to -3°C. says that denaturation reaches a maximum in twenty-five to forty days and amounts to about 20 per cent of the total-coagulable-nitrogen. The critical pH is 6, below which there is a rapid increase. From 25 to 30 per cent of the total-protein is denatured at -2° to -3°C. in forty days. One fraction of the proteins is more easily denatured than are the others, probably part of von Fürth's myogen fraction. This can be explained by the variation in pH and changes in salt

concentration. An interesting suggestion is made in Finn's comment that "if there is an intimate relationship between 'drip' and denaturation it would appear that the former could be reduced by keeping the pH in the frozen state at or above pH 6." A possible method would be to perfuse the muscle with phosphate buffer.

S. S. Drozdov and N. S. Drozdov (58) report, in studies on beef frozen at -23°F., a shift in pH to the acid side, an increase in lactic acid, glucose, and acid-soluble and inorganic phosphorus. There was some increase in protein-nitrogen, but no changes in residual- and amino-nitrogen values.

In his report as the chief of the Bureau of Animal Industry (1939) J. R. Mohler (59) reports that freezing at 20°F., -10°F., and -40°F. increased the tenderness as compared with storage at 34°F.

Beard and Nelson (60) stored beef frozen at various temperatures for periods up to 365 days. They found that the rate of shrinkage increased up to ninety days and decreased thereafter. The pH decreased from 6.2 to 5.7 at ninety days and then increased to 6.6 at the end of 120 days. The tenderness increased up to 120 days.

Hankins and Hiner (61, 62) found tenderizing greatest in meat frozen at -23.3°F. They concluded the tenderizing effect was due to the mechanical action of freezing and to enzyme

action. In commenting on the histological characteristics of beef in relation to temperature of freezing they reported that in beef frozen at 18°F. there were large interfibrillar ice areas, but none inside the cells. As the freezing temperature was lowered, however, intrafibrillar freezing occurred and damage to the fiber was progressively more serious. As the freezing temperatures were lowered, precipitated proteins and nuclear fragments outside the cell walls became more extensive. Meat that had been aged for five days before freezing increased consistently in tenderness. Drip losses during subsequent thawing decreased as the freezing temperatures were lowered. Increased intrafibrillar freezing and rupturing of fiber walls permitted the proteins to reabsorb a large proportion of the water originally frozen in the meat, it was believed. As the studies were carried out within twenty-four to thirty-six hours, more work was needed involving longer storage and larger samples.

The Characteristics of Skeletal Muscle

Skeletal muscle is a tissue made up of long, narrow multi-nucleated fibrils enclosed by a thin elastic membrane called the sarcolemma. The fibrils lie in the same direction and are bound together into fibers which are held together in bundles by a loose connective tissue framework

called the endomysium and surrounded by a sheath of connective tissue called the perimysium. The bundles or fasciculi are enclosed in a thicker layer of connective tissue called the epimysium and as such make up the entire muscle.

Within the muscle fibers are the sarcoplasm and the myofibrils. The myofibrils, when viewed under the microscope, give the appearance of longitudinal striations. The myofibrils are composed of alternating dark and light portions. The dark portions of all myofibrils in a given fiber lie adjacent to each other and form the dark or anisotropic cross fiber striations. Likewise, the light portions of the myofibrils form the isotropic cross striations. In the lighter bands the myosin is more highly folded or is disposed at angles so that the bands cause cross striations to appear, usually surpassing the longitudinal ones in distinctness.

There is still some confusion among authorities in regard to the number of the proteins of muscle cells and the terminology to be employed. E. C. Bate Smith (63, 64) recognizes the following soluble proteins: myosin, which makes up 63 per cent of the muscle protein; globulin X, 9 per cent; and myogen and myoalbumin, which together make up 11 per cent. The myofibrils are thought to be composed of myosin, which is the contractile part of the tissue. A plausible explanation of this phenomenon has been worked out by

Astbury (26) and others on the basis of X-ray diffraction studies of myosin. The analysis of the diffraction patterns of myosin, which is a fibrous protein with a very long back-bone chain, show that this chain has the ability to fold, thus becoming shorter or contracting. The shortening or contractility is confined to the anisotropic bands and supposedly affects the ionization and hence the length of the myosin chains by (1) gross change of pH, (2) a localized change in the neighborhood of certain groups, and (3) esterification of some side-chain groups which previously contributed to the total charge. Calcium and magnesium are localized in this refracting band, but the adenine nucleotides are in isotropic bands in relaxed muscle, diffusing to anisotropic bands in fatigue. The folded chain is known as α-myosin and when extended so that the polypeptide backbone is almost straight it is β-myosin. It is thought that linkages between atoms or groups of atoms in the side chains give α-myosin what stability it has, and also endow it with an inherent reversible long-range elasticity.

The insoluble proteins in muscle are the albuminoids, collagen and elastin, about which more will be written later, since they constitute the main subject of this study.

In addition to proteins and water, skeletal muscle contains nitrogenous extractives, among which are amino acids, creatine, creatine-phosphate, urea, adenine, xanthine,

carnosine, anserine, hypoxanthine, and non-nitrogenous extractives such as lactic acid, pyruvic acid, glycogen, and the like. Inorganic salts are present in complex equilibrium as well as the complicated enzyme systems needed for the building of muscle tissue, its maintenance, and its functioning.

Post mortem changes in muscle

When muscle tissue is excised the catalytic (enzymatic) processes which function continually during life do not cease but continue as autolytic recreations affected by entirely different conditions. For instance there is a decreasing supply of substrates which are no longer replaced, end products which accumulate, and the withdrawal of physiological controls which are effective in living organisms. Different conditions of temperature, hydrogen ion concentration, and other environmental factors pertain, so that autolysis is greatly altered. For example the bicarbonate ion is destroyed, esterified phosphate is hydrolyzed to orthophosphate, and the proteins which contributed half the buffer strength in vivo now contribute only one third. Orthophosphate, carnosine and anserine become the most important compounds in the buffering system. The first noteworthy effect is the appearance of a rigidity which occurs essentially simultaneously with a marked decrease in glycogen,

the appearance of lactic acid, and the production of heat, (5 calories per gram of muscle). This phenomenon, rigor mortis, has been studied in many laboratories, notably those of Lehmann (36), Hoagland (39), E. C. Bate Smith (65) in Cambridge and Hoet and Marks (67) in Hampstead, England. The rigor is not caused by the lactic acid produced nor the accompanying lowering of the hydrogen ion concentration, as Hoet has shown by producing rigor in hypoglycemic animals. The lactic acid does, however, aid in determining the amount of shortening and the tension developed by the muscle. Hoet thinks the failure of the mechanism which effects the change from phosphoric acid and glycogen to hexosephosphate is necessary for the appearance of rigor. In case of the absence of glycogen in the muscles, rigor is weak and evanescent, but early. In normal muscles rigor is due to failure of the synthetic mechanism for want of oxygen, and is strong and persistent. E. C. Bate Smith (65) has shown that in rigor there is no evidence of actual insolubility of the soluble muscle proteins nor of any denaturation. The connective tissues do not contribute to the stiffening of the muscle in rigor. Mirsky (23), however, disagrees with Bate Smith and reports that as much as 30 per cent of the muscle protein becomes insoluble in rigor.

The significant changes in rigor are as follows:

1. Increasing acidity. The pH of intercellular fluids is normally 7.3 to 7.5, according to Fenn and Maurer

(68), and of the interior of fibers 6.9 or lower. In post mortem changes a pH of from 5.6 to 6.0 is common, and pH 5.3 is possible.

2. Lower glycogen content.

3. Decrease in elasticity.

4. Lowered resistance to electric current. Callow (69, 70, 71) says the lower electrical resistance is accompanied by a change from a closed to an open structure, in which the membranes around the fibers are broken. Massage brings about a very rapid drop in electrical resistance. Rapid cooling of the carcass delays the drop in resistance and prevents the development of an open structure. If, however, a muscle with a pH below 5.8 cools slowly, it does develop Callow's so-called open structure.

5. Microscopic appearance of the muscle fibers. P. C. Paul (72) has discussed the appearance of rigor nodes in histological studies of beef muscles during rigor and its resolution, as well as during subsequent storage. The rigor nodes consist of widened areas in the fiber where the cross striations are packed densely, sometimes in and sometimes out of alignment, alternating along the fiber with narrow areas with wider, less distinct cross striations. Sometimes the contraction evident in the node has pulled the neighboring fibers into a wavy or twisted configuration which is a passive rigor distortion.

From Szent-Gyorgyi's laboratory (32) has recently come an ingenious explanation of muscular contraction. Chemists there have separated the original myosin of Danilewsky and Halliburton into two proteins, one of which, actin, can exist in an active fibrous form and in an inactive, globular form. Fibrous or F-actin combines with a complex of myosin and adenosine triphosphate when excitation occurs. The more stable contracted state which is poor in energy is assumed spontaneously. Then the splitting of adenosine triphosphate sets in supplying the energy for relaxation. Rigor mortis is explained by the conception that in such an unexcitable system there is left a slightly salt-precipitated and thus slightly contracted adenosine triphosphate-free actomyosin, which upon introduction of adenosine triphosphate undergoes the resolution of rigor.

If the histological characteristics, i.e., the longitudinal or cross striations are obliterated and the interior of the fiber appears granular, this condition is evidence of disintegration. The sarcolemma may or may not be broken and the granular material escaping into the intercellular spaces.

Dorothy Harrison in the thesis referred to above (52) studied longitudinal sections of the muscles used in this study and graded the muscles as to proportion of collagen and elastin observed. With one day's storage the fibers of psoas major of animal I were thrown into deep macro waves

with occasional nodes, but those of animal IV were straight to slightly wavy. The cross striations were distinct and widely spaced but the longitudinal striae were barely discernible. Only small amounts of collagen and fat could be observed between the fibers in animal I, but there was a moderate increase in collagen, and a large increase in the fat deposits in animal IV. In the latter animal the fibers and striations appeared more gnarled.

In the longissimus dorsi of both animals the longitudinal striations were quite prominent but the cross striations were visible only under high power. Kinks, Z-Z contractions, twists, and waves which are present in passive contraction were common, but not rigor nodes. Fat globules, collagen, and tangles of elastic tissue fibers might be distinguished.

In the semitendinosus muscle dense strips of elastic tissue could be discerned in almost every section made. In general the fibers were fairly straight, but in animal IV they were thrown into shallow waves. The cross striations were fine and close together, and the longitudinal markings were clearly visible.

Sections from the biceps femoris were characterized by few straight fibers and an abundance of Z-Z and waved contractions with distinct cross striations. Nodes were observed and strips of dense collaginous tissue could be distinguished.

The histological picture at the end of two and five days had changed little except that in a few cases disintegration had started. In general, as aging progressed beyond two days there was a tendency for the fibers to become straighter, with fewer waves, Z-Z contractions, twists, and kinks. There were two types of fiber changes which indicated disintegration: one was an increasing fragility in the muscle fiber, like worn textile fibers; the other was a disintegration of the protoplasm over an area in the fiber extending over a few or many cross striations. In these areas the sarcolemma might be intact or broken, but all striations disappeared and the material appeared granular. The strips of disintegration became longer and more numerous with aging. Some muscles showed the maximum amount of disintegrated tissue after ten days of storage, but in others the process was progressive for twenty or thirty days. Thus as the meat became aged, it became more tender, and the fibers straighter and more fragile.

In the cooked muscles the connective tissue appeared granular and in muscles in which there were large amounts of collagen a film of granular tissue often covered the entire section. Usually cooked fibers were straighter than fibers in the raw sections.

The histological rating of the proportion of connective tissue in the muscles studied is given in table 1.

Table 1.

Histological Rating of the Relative Amount of Connective Tissue in Certain Muscles from Two Animals

Muscle / Animal number	Collagenous conn. tissue		Elastic conn. tissue		Total	Ave.
	Raw	Cooked	Raw	Cooked		
Psoas major						
I	3*	3	3	3	12	3
IV	5	5	3	3	16	4
Longissimus dorsi (ribs)						
I	5	5	3	3	16	4
IV	7	7	5	5	24	6
Longissimus dorsi (loin)						
I	5	5	3	3	16	4
IV	7	7	5	5	24	6
Semitendinosus						
I	5	5	7	7	24	6
IV	7	7	7	7	28	7
Biceps femoris						
I	5	5	3	3	16	4
IV	7	7	5	5	24	6

*1 = none, 3 = small, 5 = medium, 7 = large amounts of collagen and elastin.

Abstracted from Harrison, Dorothy (52).

The ratings show a larger proportion of collagenous tissue than elastic tissue in all muscles except the semitendinosus. The psoas major rated lowest of the muscles. These histological ratings were in accord with the values obtained by

shearing sections of the muscles with the Warner-Bratzler shearing apparatus. Miss Harrison concludes this section,

> Thus the proportion of connective tissue, particularly elastin, has an effect on the amount of force required to shear a piece of meat of a given size, or in other words on the tenderness of meat.

Denaturation and coagulation

Certain agents cause the proteins of muscle to become denatured or coagulated. These include the physical agents heat, pressure, freezing, irradiation, sound waves, and surface forces; the chemical agents hydrogen ion, hydroxyl ion, organic solvents; and the organic solutes urea, guanidine salts, acetamide, and formamide, as well as enzymes. According to Neurath, Greenstein and Putnam (24), "the concept of denaturation as a reversible reaction does not warrant general application." The following may be considered as evidence of denaturation:

1. Decrease in solubility,
2. Loss of biological activity,
3. Loss of crystallizing ability,
4. Increased reactivity of constituent groups, e.g., disulfide, sulfhydryl, and phenolic,
5. Changes in molecular shape,
6. Susceptibility to enzymatic hydrolysis.

Mirsky (23) differentiates between the denaturation

which occurs in dehydration, in rigor mortis, and probably in muscular contraction, and, on the other hand, that produced by heat and acid. In the former type there is some change in internal configuration, but it is far less drastic than in the latter type. This is shown by the fact that bonds are broken to free sulfhydryl groups which can be titrated in the case of denaturation by heat or acid, but none can be detected in denaturation by dehydration. Reconstituted proteins, such as enzymes, retain their functional ability in the case of dehydration, also.

A more drastic or long-continued treatment with these agents results in permanent loss of solubility, biological activity, and other similar properties and is termed coagulation.

The Characteristics of Collagen and Gelatin

Collagen and elastin are the two constituents of the connective tissue framework which encloses the muscle fibers. The collagen fibers run in straight or wavy courses in all directions throughout the muscle, and are concentrated at the terminal parts of the muscle in the tendons and fascia which attach the muscle to bones, other muscles, or organs. These fibers are not elastic, but their wavy nature permits considerable stretching of those particular areas. Each fiber is colorless and birefringent and is composed of fibrils

which account for the longitudinal striations. The tissue is characterized by a sparsity of cells and much intercellular material, and also by the ability to proliferate freely, whereas muscle fibers do not increase in number. Interfibrillar fat enmeshed in collaginous tissue is partly responsible for its being termed white connective tissue in contrast to elastin, the yellow connective tissue.

The collagen fibers consist of fibrillae 0.5 mm. in diameter which have a micellar structure formed by chain molecules and have as an envelope a network of loose, unoriented single fibrillae. Siegfried, Lloyd (5) and others disagree with this explanation of Kuntzel's (34), insisting that the collagen fibers are surrounded by a film of protein called reticulin. The fibrils never divide but bundles may branch dichotomously. Compared to elastin fibers, collagen fibers are not elastic, but Lloyd and Marriot (4) have shown that strips 0.1 mm. in diameter show reversible extension to the extent of 1 $\pm$ 0.4 per cent for every gram of load, up to 7 or 10 grams, at which they usually break. This is equivalent to a load of from 1,400 to 2,000 pounds per square inch.

The fibrils swell under the influence of acetic acid, sodium hydroxide, and potassium hydroxide, and are dissolved by boiling dilute acid or base. Collagen is insoluble in water and salt solutions, hot or cold. It is hydrolyzed to

amino acids by boiling concentrated acids and alkalis, but steam or boiling dilute acid converts collagen into gelatin. Collagen is least liable to hydrolysis at pH 5 to 6, which is slightly above its isoelectric point of pH 4.80 to 4.85. At pH 7 there is no acid or base fixation and at this pH in eight hours at 70°C., 17 per cent of the total-nitrogen has gone into solution. Above pH 5.4, ammonia is evolved, increasing rapidly with increased pH. Fokina (35) pointed out that the internal micellar structure of collagen changes slightly in the range of pH 7.0 to 3.5, but from 3.5 to 1.0 the structure is completely changed. Hofmeister (73) found that after thirty hours' boiling collagen no longer gelatinizes, but forms two peptones. One of these is insoluble in alcohol but precipitated by platinic chloride; the other is soluble in alcohol and also precipitable by platinic chloride. Halliburton (74) reported that ether and alcohol extraction render collagen more difficult to convert into gelatin. There is little of interest in the concentration of electrolytes in collagen, other than that collagen contains chloride, but the muscle cells themselves do not.

Collagen is not a simple anhydride of gelatin but a polarization complex produced by chemical condensation, according to Bogue (14). Collagen contains 48.85 per cent carbon, gelatin 48.28 per cent; collagen 8.01 per cent hydrogen, gelatin 7.84 per cent; collagen 18.02 per cent

nitrogen, gelatin 17.56 per cent; and the heat of combination of collagen is 5387 calories, while that of gelatin is 5350. The carboxyl equivalent of gelatin is 0.9 mM per gram, while that of collagen is only 0.35 mM per gram.

Dorothy Jordan Lloyd (1, 2, 3, 4, 5, 6) and her co-workers have studied the swelling and hydration of collagen and gelatin with great thoroughness. Collagen fibers swell less than gelatin and do not change their volume over a wide range about the isoelectric point. The extent to which proteins are hydrated depends upon their R-groups, the longer R-groups and the more oxygen and nitrogen atoms and double bonds, the more water can be held. Water is held by (a) an osmotic effect; (b) hydration of the proteins, the so-called bound water; and (c) a physical separation of the component parts of the fiber bundle. Sodium salts of protein and their chlorides hold more water than the original protein, and the same thing holds true as the collagen fibers become disorganized. Hydration is checked, however, by coordination between the imino group and the ketonic oxygen atom. Salts promote swelling near the isoelectric point, but they do not weaken a direct carbonyl-imino linkage between the backbones of adjacent molecules.

Lloyd (5) and others also studied the chemical reactions of collagen and said that moderately concentrated strong alkalis could break all types of linkages between protein

molecules; for instance, she found the following:

$\frac{N}{2000}$ NaOH(pH 10.8)	some swelling of collagen (rat-tail.)
$\frac{N}{1000}$ NaOH(pH 11.1)	reticular tissue ruptured but swelling is complete in twenty-four hours.
$\frac{N}{500}$ NaOH(pH 11.4)	reticular tissue weakened and swelling is progressive and leads ultimately to solution.

Ames (7) studied the loss of nitrogen when collagen is converted to gelatin. He discovered that the loss of nitrogen in limed material takes place during the soaking period, and he identified three types of gelatin with iso-electric points at pH 4.75, 5.6 and 8, according to whether they were derived by alkaline, hot water, or acid extraction. He said, in part, "Apparently unless acid is used collagen will not break down into gelatin without loss of nitrogen. In case of water hydrolysis alone, the nitrogen evolved is one fifth that lost during a soak in alkali." He quoted Emmet and Gies (12) who were of the opinion that the nitrogen lost probably comes from the amides of aspartic and glutamic acids. He recalled that the collagen molecule has twenty-two residues of dicarboxylic acids, nineteen of them as acid amides. He quoted Theis and Jacoby (18) who found a loss of histidine and lysine with alkaline soaking and reduced formaldehyde combining power. This leads them to believe that amino groups may be lost. The best evidence, however,

is that the nitrogen lost by collagen in alkali comes from amide groups which form cross linkages between polypeptide chains. Heat causes groups of chains to separate and rearrangement to take place. When acid is used, the amide cross linkages are immediately broken. Collagen treated with calcium chloride contracts (18) and takes on the characteristics of heat-denatured collagen, except that the isoelectric point is lowered instead of raised.

Theis (18, 19, 20, 21, 22) and others have made an exhaustive study of the reactions of collagen with formaldehyde and quinone. Formaldehyde forms methylene bridges between imino groups or amino groups in juxtaposition, or it forms methylol groups. Its reaction with quinone increases the structural strength of the collagen due to bonding between polypeptide chains. The reactive centers of quinone combine with amine groups of the protein which are in juxtaposition.

Within the last decade Bear (27), Astbury (25, 26), Schmitt (28), and their collaborators have been able to find out much about the collagen molecule by means of X-ray diffraction studies and the electron microscope. There is no doubt but that it is a fibrous protein of very high molecular weight with a preponderance of amino acids having short-chain R-groups. About one third the residues consist of glycine and alanine, and almost another third of proline and hydroxyproline. Astbury (26) thinks the basic sequence

is proline - glycine - some other amino acid, except that in every eighteenth sequence proline is missing and some other takes its place. Because of the imino ring of proline, the backbone chain is shorter than the beta configuration of the other fibrous proteins, but it is practically inextensible. Because of the proline rings it is of cis-configuration. Astbury summarized from his own work and that of many contemporaries the following list of amino acids in gelatin, which must approximate those in collagen.

Table 2

Amino Acid Content of Gelatin.

Acid	Per cent	Acid	Per cent
glycine	25.5 or 26.5	serine hydroxylysine	3.3
alanine	8.7	threonine	1.4
leucines	7.1 or 8.5	tyrosine	0
phenylalanine	1.2 or 2.6	tryptophane	0
proline	19.7 or 17.5	cystine	0.2
hydroxyproline	14.1, 14.4 or 14.7	methionine	1.0
arginine	9.1 or 8.7	aspartic acid	3.4
lysine	5.9	glutamic acid	5.8
histidine	0.9	amide nitrogen	0.33
hydroxylysine	0.94	total nitrogen	18.3
molecular weight - about 24,000			
average residue length - 2.86Å			
number of residues - 216			

There are, however, many high spacings in the true collagen X-ray pattern that have not been observed in the gelatin photographs.

Pauling conceived of the collagen molecule as having the chain folded accordion-fashion and held in these folds by hydrogen bonds. Theis (15), using his diagram, explained the contraction of collagen on heating thus:

> Since rise in temperature increases the vibrational energy of the molecule it would be natural then for the collagen fibers upon heating to contract, because of the reduction of cohesion due to the ionic attraction of the zwitterions and to the short link.

He defined the shrinkage temperature of a protein as the point at which the increasing disruptive tendencies exceed the diminishing cohesive forces. This is a measure of the structural stability of collagen expressed in temperature units.

Titration within the pH range two to thirteen affects the salt linkages. The effect on the hydrogen bond is noticeable at pH 1 and pH 13.

Collagen is digested by trypsin in alkaline solution and by pepsin in weak acid solution, while elastin is digested only very slowly by trypsin. This has been used as a means of separating the two by some modern workers.

Collagen, as has been said, is hydrolyzed by steam, hot water, or dilute acids to gelatin. It is insoluble in cold water, alcohol, ether, chloroform, carbon disulfide, benzene,

or absolute alcohol. It is precipitated by tannic acid, picric acid, the usual alkaloidal reagents, platinic chloride, and basic lead acetate. Potassium dichromate and formaldehyde form well defined compounds with gelatin. Its most characteristic properties are the ability to take up a large quantity of water, and, if not too dilute, to form a gel.

Because collagen is so difficult to study most of the knowledge we have about both collagen and gelatin has come as a result of studying the latter. Gelatin, it is said, is collagen with its molecules disoriented. Most of the chemistry of gelatin has been treated for this reason in the previous paragraphs on collagen. The isoelectric point is pH 4.6. It is rapidly digested by trypsin and is hydrolyzed by hot acids and alkalis. The products of hydrolysis have been studied very little so far, but some workers have identified some products of definite composition. The course of the early stages of hydrolysis with alkali, trypsin, or pepsin is similar, but is quite different with acid.

Alkaline solutions of gelatin reduce cupric oxide. Bogue said that gelatin breaks down above pH 8.

Lloyd, Marriot and Pleass (3) have suggested that in the swelling of gelatin in salt solutions, Fajan's principle may be applied: salts in which the ions are of equal size and valence are associated with less water than salts in which the ions are unequal in size and valence.

Sponsler, Bath and Ellis (75) by means of X-ray diffraction studies have determined that in the gelatin molecule the length of an amino acid residue along the backbone chain is 2.8Å. The width of the side chains is 11Å and the thickness is 4.3Å. Repetition distance along the backbone does not change with swelling as the water content increases from 0.2 per cent to 90 per cent, which is evidence that the polypeptide chain does not change. In general, the width of the side chains increases with increasing amounts of water. With 0.2 per cent, 15 per cent, and 33 per cent of water the numbers of water molecules bound to each molecule of gelatin are 4, 260, and 750 respectively.

The Characteristics of Elastin

The elastic fibers run singly without forming bundles, often dividing dichotomously and anastomose to form a network. They are highly refractive and extremely elastic, and when massed together appear yellow. They are very resistant to heat, weak acids, and weak alkalis. They are digested by pepsin in acid solution and slowly by trypsin in neutral solution. The isoelectric point is pH 4.18.

In attempting to obtain a picture of the composition of elastin Stein and Miller (76) were able to assert that in hydrolyzing elastin 29.4 per cent (roughly one third) of the constituent amino acid residues were glycine, 15.2 per cent

proline, 1.0 per cent arginine, 0.23 per cent cystine, 0.38 per cent lysine. The average molecular weight of the amino acids liberated was 104. The minimum molecular weight (576 amino acid residues) was 49,500. There was some question about the valine content of elastin, the best estimate being about one ninth of the amino acids present. To some workers the most outstanding difference between the composition of collagen and elastin has been the lower content of glycine and higher content of the leucines present in elastin.

Elastin is characterized by extreme stability to most chemical reagents. It is hydrolyzed by hot concentrated acids and alkalis, but can be put into solution only by 90 per cent formic acid at 95°-100°C. for twenty-four hours.

Table 3

Amino Acid Content of Elastin (28).

Acid	Per cent	Acid	Per cent
Glycine	29.4	Methionine	0.38
Alanine	0	Tyrosine	1.6
Valine	13.5	Tryptophane	0
Aspartic acid	0	Proline	15.2
Arginine	1.0	Hydroxyproline	2.0
Lysine	0	Leucine fraction	30.0
Histidine	0	Phenylalanine	3.34
Cystine	0.23	Amide-nitrogen	0.04

The Methods Previously Used for the Determination of Collagen and Elastin in Skeletal Muscle

Schepilewsky's method

Great strides were taken in clarifying the chemistry of connective tissue between the first publication of a method for the determination of collagen by Schepilewsky (37) and the description of Mitchell and Hamilton's method which appeared in 1926. In Schepilewsky's method, which Lehmann (36) used with modifications, the connective tissue was removed from the more soluble muscle proteins by trituration in a mortar with water, and subsequent sieving. Then the connective tissue residue was extracted with 5 per cent sodium hydroxide for fifteen hours in the cold. The collagen was extracted from the elastin with hot 0.5 per cent sodium hydroxide, and nitrogen determinations were made on the filtrate. Lehmann extracted the tissue for twenty-four instead of fifteen hours at room temperature, then filtered the mixture through a Buchner funnel covered with cotton. The residue was thoroughly washed and the cotton with the connective tissue was cooked in 0.5 per cent sodium hydroxide and filtered. The filtrate contained all the collagen and the elastin remained with the cotton on the filter. Nitrogen determinations were made on the filtrate and collagen calculated by using the Hofmeister factor, 5.6. Samples weighing 25 grams

were used. The results obtained were as follows:

Animal		Connective tissue (%) psoas	Connective tissue (%) flank skin muscle
I	7-year-old cow	A. 0.493 B. 0.533	A. 0.961 (shank) B. 0.796
II	3-year-old ox	A. 0.188 B. 0.188	A. 1.473 B. 1.243
III	11-year-old cow	A. 0.423 B. 0.312	A. 1.411 B. 1.482
IV	2-1/2 to 3-year-old cattle	A. 0.323 B. 0.323	A. 0.774 B. 0.756

Lehmann was careful in the original table to describe the extent of trimming of perimysium and fascia done before removing the samples - a point of utmost importance in interpreting results. The range of values for psoas was 0.3 to 0.5 per cent and for flank skin muscle 0.8 to 1.5 per cent or almost a ratio of one to three.

Lehmann did not state how long his cotton and connective tissue was cooked with 0.5 per cent sodium hydroxide, but considering the fact that Lowry, Gilligan and Katersky (80) found it necessary to autoclave samples at fifty pounds for four hours, it is probable that Lehmann's extraction was inadequate. It is also probable that intracellular proteins were extracted in the boiling 0.5 per cent sodium hydroxide and determined as collagen. The checks obtained in duplicate determinations impress one as remarkable indeed for such a limited number of samples of such a variable tissue as muscle.

Mitchell and Hamilton's method

Mitchell, Hamilton and various associates (77, 78, 79) developed a method for the determination of collagen and elastin, using the method of Schepilewsky as the basis. Published first in 1926, this method has been modified by the authors several times between 1926 and 1937. As it was last published the method consisted of grinding 100-gram samples of meat in a one-quart ball mill with 300 cubic centimeters of water and two pounds of 3/4-inch porcelain balls for ninety minutes at 100 R.P.M. The contents were poured on a 100-mesh sieve and washed with about a gallon of water, the connective tissue being worked with the fingers until it felt firm and rubbery. The residue was autoclaved at eighteen pounds' pressure for one-half hour with 400 cubic centimeters of water. The mixture was filtered through a linen filter and washed eight times by decantation, after boiling one minute with 100 cubic centimeters of water each time. Collagen-nitrogen was determined on the filtrate. The residue was washed from the sieve into a beaker with 200 cubic centimeters of cold trypsin solution and incubated sixteen hours at 38°-40°C. The digest was filtered, washed, and the nitrogen determined on the residue and from this the elastin in the sample was calculated.

Mitchell, Hamilton and others (78) commented on the difficulty of sampling, even with 100-gram samples. The fat

deposits in the muscle gave them difficulty in handling the samples, but they found that the use of warm or hot water for washing was helpful in overcoming this difficulty. It will be noted that they avoided the use of alkali, relying upon thorough maceration and repeated washing to extract the soluble proteins of muscle which would interfere with the collagen determination. The reason given by the authors was the possibility of extraction of collagen by the alkali and its consequent loss in the discarded filtrate. They pointed out that collagen is soluble in 1 N. sodium hydroxide. Intracellular proteins not removed in the exhaustive washing were digested out with trypsin to leave the elastin, on which nitrogen determinations were made.

On the basis of the work of Lowry, Gilligan and Katersky (80) there is some question as to whether the autoclaving was sufficient to change the collagen to gelatin. From the standpoint of the technician this method is unnecessarily tedious and time-consuming. The method has the merit, however, of avoiding any drastic chemical treatment which might be injurious to the connective tissues.

Mitchell gave the following values for collagen-nitrogen as per cent of total-nitrogen in the psoas muscle: 9.9, 8.7, 9.6, 10.6, 9.6, 7.6, 7.6, 7.2 with an average of 8.85. For longissimus dorsi the values were 8.8, 8.4, 7.2, 9.6, 8.9, 8.7, 7.2, 6.8 with an average of 8.2. For round, 8.4, 8.0, 11.2, 11.0, 10.8, 12.6, 12.4, 10.3 with an average of 10.6.

Braun's method

Braun (81), whose interests were physiological and histological rather than chemical, fitted submucosa from sheep's intestine over a glass tube and boiled it in a 40-per cent urea solution for one to two hours, washed it, and stained it. Then it was placed between two slides, one of which had a hole cut in it. The slide arrangement was boiled in aqueous 40-per cent urea solution until elastin fibers alone appeared. This is a very ingenious arrangement for the qualitative removal of soluble muscle proteins and collagen, which is soluble in boiling 40-per cent urea upon prolonged extraction.

Hoppe-Seyler and Lang's method

In 1933 Hoppe-Seyler and K. Lang (82) published a method for the determination of collagen in liver tissue. It was based upon the tryptic digestion of liver brei over a period of three weeks, after which the mixture was centrifuged, washed, extracted with alcohol and ether, and dried to constant weight. The method, in addition to being lengthy, has the disadvantage of the possibility of loss of collagen during the long digestion period with trypsin.

The same year G. Farkas (83) published comments on several modifications of a method used by Bechmann, E. Schmidt, H. Wagner and Scholer, König and Scholl, Barran, and Striegel

employing picric acid or ammonium molybdate to precipitate the gelatin. These reagents are not specific for gelatin, however, and results obtained by these methods would be subject to serious question.

E. C. Bate Smith's method

E. C. Bate Smith (63, 64) outlined a scheme for the approximate determination of the proteins of muscle in 1934, in which the soluble proteins were extracted with 7 per cent lithium chloride instead of sodium hydroxide. This extracts 98 per cent of the soluble protein if nine extractions are made and 95 per cent if six are made. This method also substitutes exhaustive extraction with 0.01 N. hydrochloric acid for the tryptic digestion used by many laboratories to remove the residual intracellular fraction. The method in outline is this: a 5-gram sample and 10 grams washed sand are weighed into a 40-milliliter centrifuge tube and extracted with 35 milliliters cold 7-per cent lithium chloride at 0°C. with the tube shaken in a horizontal position for thirty minutes. The mixture is centrifuged, the supernatant poured off, and the process repeated five more times. Then the residue is extracted six times with 0.01 N. hydrochloric acid in the cold and washed with water. It is then autoclaved for two hours at two atmospheres' pressure (121°C.). The mixture is centrifuged, the supernatant liquid poured off,

the residue is washed, and the washings added to the filtrate. Nitrogen determinations on the filtrate give collagen-nitrogen and on the residue, elastin-nitrogen.

Bate Smith recommends this method in that it avoids errors from two sources to which other methods are susceptible: (1) if a considerable amount of intracellular residue is present this fraction breaks down to a small extent during autoclaving, and the amount so broken down is determined as collagen; (2) a certain small amount escapes tryptic digestion and is estimated as elastin. The errors in these two cases may amount in extreme cases to as much as 25 per cent of the collagen and 50 per cent of the elastin. Using his original method which employed tryptic digestion instead of hydrochloric acid extraction to remove residual intracellular proteins, Bate Smith found the collagen-nitrogen to be 10 per cent of the total-coagulable-nitrogen in beef topside (round). By the modified method, in fresh rabbit muscle 13-24 per cent of the total-protein was collagen and 1 per cent of the total-protein was elastin.

The method has been worked out to enable the technician to determine the soluble as well as the insoluble proteins quantitatively, which tends to make the procedure cumbersome and lengthy. Other than this and the fact that there is no provision for removal of fat and phospholipids, there seems to be no objection to the method. On the contrary, Bate Smith has worked out the source and magnitude of likely errors so

painstakingly that it gives the student confidence in his recommendations.

Spencer, Morgulis and Wilder's method

Spencer, Morgulis, and Wilder (84) worked out a micro-method for determining collagen, in which they used ground acetone-dried tissue, autoclaved a 100-200 milligram sample with 4 milliliters water for two hours at 15-20 pounds and precipitated the gelatin with tannic acid. The gelatin precipitate was digested with sulfuric acid and the nitrogen determined by direct nesslerization.

The method is based on the assumptions that tannic acid precipitates small amounts of gelatin quantitatively, that gelatin is not affected by autoclaving alone or with muscle powder, and that non-protein-nitrogen substances do not interfere.

The method has the merit of being simple, but is questionable from two angles: the adequacy of the time of autoclaving, for dried material especially, and the specificity of the tannic acid for gelatin alone. If any products of hydrolysis of gelatin result from the autoclaving, they would fail to appear in the tannic acid precipitate, while in those methods which extract the soluble-nitrogen and fat before autoclaving, then determine the nitrogen in the filtrate, not only gelatin but any of its breakdown products influence the value obtained.

Krylova's method

Krylova (85) separated out the plasma proteins, then extracted the collagen by autoclaving for two hours under 1.5 atmospheres' pressure. After concentrating the filtrate he added alcohol, then copper sulfate and determined the nitrogen in the precipitate by the usual Kjeldahl method.

The specificity of the precipitating agent and the adequacy of the autoclaving time would be open to question in this method.

Bell, Morgan and Dorman's modification of Mitchell and Hamilton's method

Bell, Morgan and Dorman (86) adapted Mitchell and Hamilton's method to cooked samples. These workers ground the entire amount of material available and used 50-gram samples for determinations. They ground the meat in a ball mill for ninety minutes, washed it exhaustively, and autoclaved it at 15 to 18 pounds' pressure for two hours. The residue was washed thoroughly with hot water, and nitrogen determinations run on aliquots of the filtrate and washings. No elastin determinations were made.

This method differs from Mitchell and Hamilton's principally in the pre-treatment of the sample, and the recommendation that a test for tryptophane be made in case of a milky filtrate.

Lowry, Gilligan and Katersky's method

Lowry, Gilligan, and Katersky (80) developed a method for the determination of collagen and elastin in normal and abnormal human heart muscle. They extracted two- to four-gram samples (A) which were minced in a mortar with sand and allowed to stand with 40 milliliters 0.1 N. sodium hydroxide at room temperature overnight. The mixture was centrifuged, the supernatant liquid poured off, and another extraction made with 0.1 N. sodium hydroxide for two hours. Vigorous stirring at the beginning of the extraction and before centrifuging was necessary. The reaction of the residue was adjusted to pH 7 by adding 40 milliliters of distilled water, one drop 0.1 per cent phenol red, and adjusting the color to a faint pink with 0.1 N. hydrochloric acid. After centrifuging, the residue was extracted with 40 milliliters of a 1:3 mixture of ether and 95-per cent alcohol, then with ether, centrifuging and pouring off the supernatant each time. The centrifuge tube was brought to constant weight at 100°C. (B). Twenty milliliters of water was added and the mixture was autoclaved at fifty pounds' pressure for four hours. The liquid was poured off and the tube again dried to constant weight (C). The per cent of collagen in the sample was calculated by subtracting the weight after autoclaving from that before autoclaving, dividing by the weight of the sample, and multiplying by 100, or $\frac{B - C}{A} \times 100 = \%$ collagen.

For the determination of elastin the dried residue was extracted with 40 milliliters 0.1 N. sodium hydroxide in a boiling water bath for thirty minutes. After centrifuging, 40 milliliters of wash water were added, and the mixture centrifuged again. After pouring off the supernatant liquid, the tube was dried to constant weight (D). The per cent elastin in the sample was calculated by subtracting the final weight from the weight of the tube after autoclaving, dividing the product by the weight of the sample, and multiplying by 100; $\frac{C - D}{A} \times 100 = \%$ elastin.

Lowry, Gilligan, and Katersky have carefully considered in their paper most of the objections which might be raised to their method. They have assured themselves that it determines diverse collagens and have used both 0.1 N. sodium hydroxide and 0.01 N. sodium hydroxide for extraction with equivalent results. The unusually long period of autoclaving was decided upon because it was found that although tendon collagen was hydrolyzed to gelatin in two hours at fifteen pounds' pressure, some tissues gave higher and more consistent results with longer autoclaving or higher temperatures. These workers point out that a small amount of protein which is neither collagen nor elastin escapes extraction with cold alkali, and that it may be made soluble on autoclaving and thus may be included in the collagen fraction. The hydrolyzed protein gave only faint qualitative tests for tyrosine and reduced sulfur, however.

Lowry reported the results of his determinations of collagen and elastin in many organs and tissues, but of only one muscle of a rat, the adductor of the thigh. The maximum and minimum values for collagen in 21 of these muscles analyzed were 2.00 and 0.65 per cent of the wet tissue, with an average of 1.00 per cent. This was equivalent to 4.3 per cent of the dry weight of the tissue. The maximum and minimum elastin values for ten of the muscles were 0.37 and 0.07 per cent of the wet tissue with an average of 0.27 per cent, equivalent to 1.1 per cent of the dry weight of tissue.

Yoder's method

Lester Yoder (87) in Dr. Thomas' laboratory at Iowa State College has recently developed a method for determination of collagen and elastin based partly on the work of Mitchell and others, but employing alkaline extraction of the soluble proteins. Ten-gram samples of finely ground muscle were weighed into 50-milliliter centrifuge tubes and were extracted three times with warm water, centrifuging and discarding the supernatant liquid and fat each time. The residue was then mixed with 30 milliliters of water, and 0.1 N. sodium hydroxide was added to make 50 milliliters. The mixture was centrifuged to opalescence and the residue was mixed with 50 milliliters 0.1 N. sodium hydroxide and centrifuged. The supernatant liquid was poured off and the residue was centrifuged with repeated additions of water until the wash water no longer gave a coloration with phenolphthalein. The residue was then

covered with 30 milliliters hot water, the tubes capped with tin foil and autoclaved for one hour at 120°C. The tubes were filled with boiling water, centrifuged, and the supernatant solution of gelatin together with two subsequent washings with boiling water were subjected to nitrogen determination. Nitrogen determinations on the residue gave elastin-nitrogen values.

Yoder determined the total-nitrogen content of the muscle by a Kjeldahl determination on two-gram samples of the original minced tissue. Results obtained were as follows:

	Collagen-N per cent of total-N		Elastin-N per cent of total-N	
	5-gram sample	10-gram sample	5-gram sample	10-gram sample
Round steak	3.88	3.78	2.16	1.90
Porterhouse steak	5.95	5.70	2.04	1.91
Sirloin steak	3.58	3.94	1.61	1.26
Longissimus dorsi	7.00	6.16	1.10	1.01

The short period of autoclaving in this study is of questionable adequacy. Yoder himself comments on the fact that his elastin values include tissue which is not elastin but resembles it in solubility. He recognizes that further extraction to remove intracellular proteins not taken out by the alkali or water would have been desirable.

EXPERIMENTAL PROCEDURE

Preparation of the Samples

This study was undertaken as part of a larger project which included histological, mechanical, and organoleptic tests on the muscles of four animals of three different grades. The detailed description of the selection, preparation, and storage of the beef muscles, as well as the results of that part of the study will be found in the thesis entitled, "Histological, Physical, and Organoleptic Changes in Three Grades of Beef During Aging," by Dorothy Harrison (52). Two animals were used in the study of the collagen and elastin content. Animal I was a yearling steer whose carcass merited the classification, good, according to the United States grading standards, which classify meat into seven grades. Good is the third classification down the list and constitutes about twenty per cent of beef carcasses found in local markets. Animal IV was an eight-year-old dairy cow whose carcass was classified as cutter, the sixth classification, hence, very poor. The muscles of the cow were smaller than those of the other animal, and the fat and connective tissue were a bright yellow.

It was desirable to have muscles varying in tenderness and large enough to furnish material for all the tests on

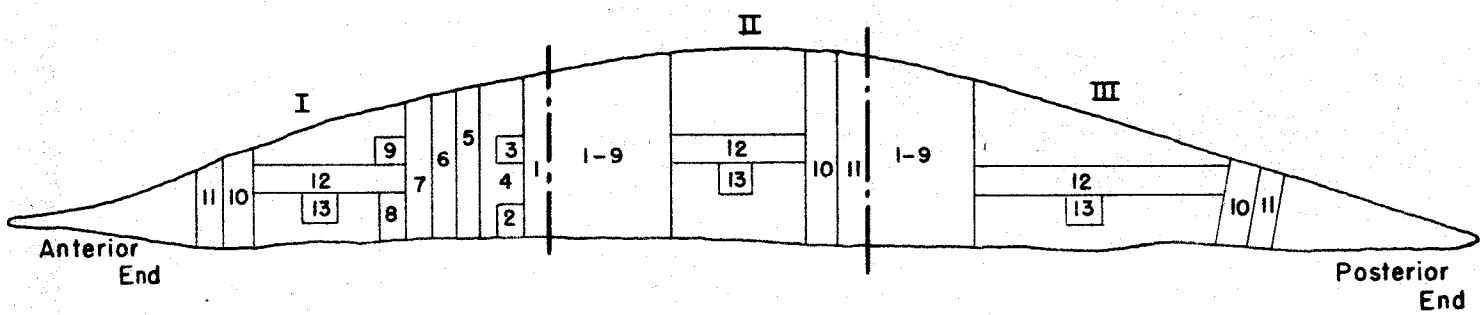
muscles stored for six different storage periods. The psoas major, the longissimus dorsi, the semitendinosus, and the biceps femoris were the muscles chosen for study. Ramsbottom and others (50) have shown that these muscles differ in tenderness. The psoas major or tenderloin is a long narrow muscle lying along the ventral surface of the backbone on either side. The longissimus dorsi or loin lies in much the same position relative to the backbone but on the dorsal side. It is several times as large as the psoas major. They make up the "eye" and the tenderloin portion of the familiar porterhouse steak. The semitendinosus and the biceps femoris are both in the round, where muscles are tougher because they are used to a much greater extent. The semitendinosus is a relatively small eclair-shaped muscle lying slightly above and posterior to the biceps femoris. This latter one is shaped roughly like a thick equilateral triangle and is several times as large as the semitendinosus. It lies below and inside the semitendinosus, and extends farther down toward the shank. A detailed description of these muscles is given in the text by Sisson and Grossman (88).

Pairs of muscles were divided into six roasts each, with the exception of the longissimus dorsi. This muscle was divided between the twelfth and thirteenth ribs into the rib portion and the loin portion. Each portion was cut into three roasts, six designated longissimus dorsi-rib, and six

longissimus dorsi-loin. This portioning may be seen in figures 1 to 4 which show diagrams of the muscles and the positions from which samples for chemical analysis were cut. The roasts varied in weight from 0.7 pounds to 4.5 pounds. Roasts from the biceps femoris were the largest with the others in the following order: longissimus dorsi > semitendinosus > psoas major. When the storage period for a particular set of muscles was complete they were brought from the meat laboratory cooler to the foods laboratory, where samples for all the various determinations were removed and the remainder of the roast was cooked. After cooking, the samples were removed promptly for the chemical and histological tests as well as for the organoleptic tests. Sampling the cooked meat presented especial difficulties because in cutting thin slices from hot meat the loss of juice was unavoidable, nor was there any way to estimate the amount lost from a particular volume or weight of the meat.

The animals were slaughtered in the Iowa State College Animal Husbandry abattoir, dressed, cut into right and left sides and hung in the cooler at 34° to 36°F. The following day the carcasses were cut between the fourth and fifth ribs, and the muscles used in the study were removed, trimmed, and cut into roasts. The roasts were labelled, placed unwrapped on enamel trays with about one-inch spaces between them, and were then stored on the open shelves

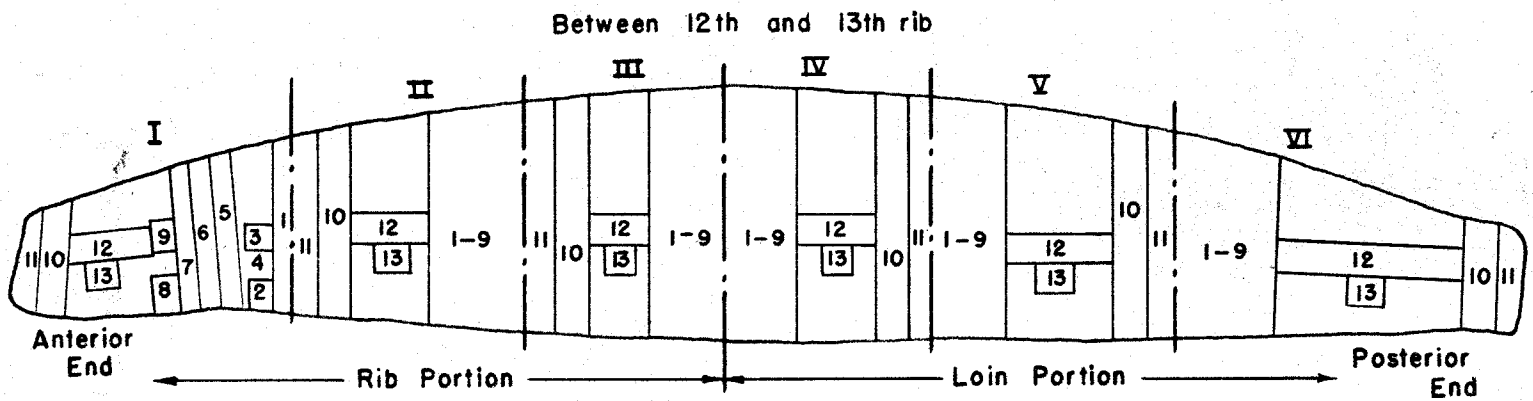
Division of the Psoas Major.



I	Roast from anterior end
II	Roast from middle portion
III	Roast from posterior end
1	Uncooked chemical sample
2	Uncooked histological cross section
3	Uncooked histological longitudinal section
4	Outside slice after cooking -- discarded
5 & 6	Slices for palatability tests
7	Cooked chemical sample
8	Cooked histological cross section
9	Cooked histological longitudinal section
10	Cooked sample for pH
11	Uncooked sample for pH
12	Shear sample
13	Press fluid sample

Fig. 1. The Psoas Major Muscle.

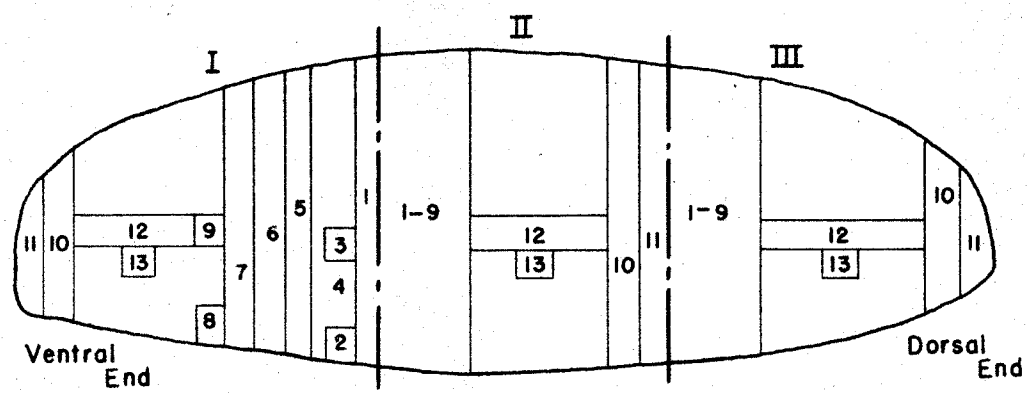
Division of the Longissimus Dorsi.



I	Roast
II	Roast from middle rib portion
III	Roast from portion adjoining loin
IV	Roast from portion adjoining ribs
V	Roast from middle loin portion
VI	Roast from posterior end
1	Uncooked chemical sample
2	Uncooked histological cross section
3	Uncooked histological longitudinal section
4	Outside slice after cooking--discarded
5 & 6	Slices for palatability tests
7	Cooked chemical sample
8	Cooked histological cross section
9	Cooked histological longitudinal section
10	Cooked sample for pH
11	Uncooked sample for pH
12	Shear sample
13	Press fluid sample

Fig. 2. The Longissimus Dorsi Muscle

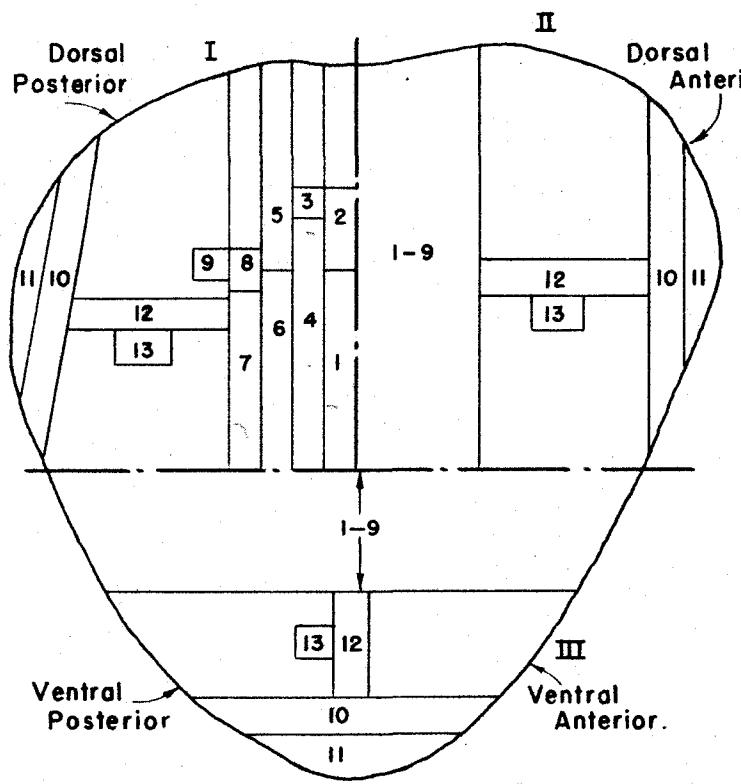
Division of the Semitendinosus



I	Roast from ventral end
II	Roast from middle portion
III	Roast from dorsal end
1	Uncooked chemical sample
2	Uncooked histological cross section
3	Uncooked histological longitudinal section
4	Outside slice after cooking--discarded
5 & 6	Slices for palatability tests
7	Cooked chemical sample
8	Cooked histological cross section
9	Cooked histological longitudinal section
10	Cooked sample for pH
11	Uncooked sample for pH
12	Shear sample
13	Press fluid sample

Fig. 3. The Semitendinosus Muscle.

The Division of the Biceps Femoris.



I	Roast from dorsal posterior
II	Roast from dorsal anterior
III	Roast from ventral posterior and ventral anterior
1	Uncooked chemical sample
2	Uncooked histological cross section
3	Uncooked histological longitudinal section
4	Outside slice after cooking--discarded
5 & 6	Slices for palatability tests
7	Cooked chemical sample
8	Cooked histological cross section
9	Cooked histological longitudinal section
10	Cooked sample for pH
11	Uncooked sample for pH
12	Shear sample
13	Press fluid sample

Fig. 4. The Biceps Femoris Muscle.

in the cooler at 34° to 36°F. for the desired aging periods. Paul (72) found that roasts wrapped in cellophane and stored at 34° to 36°F. for eighteen days became slimy. In order to avoid this condition, these roasts which were to be kept longer, were stored without wrapping although surface dehydration was expected.

The roasts were aged 1, 2, 5, 10, 20, and 30 days before sampling and testing. At the end of the proper storage period each roast was weighed, raw samples removed, the cooking carried out, cooked samples removed, and tests made as planned. The statistical pattern for the aging periods consisted of a 5x6 table for each animal in which the storage periods were determined from a table of random numbers. The patterns for animals I and IV are given in tables 4 and 5.

The samples for chemical analysis, both uncooked and cooked, were deposited in 2 to 8 oz. cork-stoppered or screw-capped sample bottles, and stored at -30°F. until extraction of the collagen and elastin. The samples weighed approximately twenty to forty grams, and, especially in the case of the samples from animal IV, were free from gross fat deposits and thick layers of the epimysium, which is the collagenous outer-covering of the muscle.

Table 4

Statistical Pattern for Aging Periods in Days for Roasts from Animal I

Muscle	A*		B*		C*	
	Left	Right	Left	Right	Left	Right
Psoas major	2	5	1	10	20	30
Longissimus dorsi (ribs)	1	30	5	10	2	20
Longissimus dorsi (loin)	10	20	2	30	1	5
Semitendinosus	2	10	5	30	1	20
Biceps femoris	5	20	1	2	10	30

*A, B, and C for the psoas major and longissimus dorsi muscles refer to roasts from the anterior end, middle portion, and posterior end of the muscles, respectively. For the semitendinosus these numbers refer to the ventral end, middle portion, and dorsal end of the muscle, and for the biceps femoris to the ventral anterior and posterior (lower part), the dorsal posterior, and the dorsal anterior.

Table 5

Statistical Pattern for Aging Periods in Days
for Roasts from Animal IV

Muscle	A*		B*		C*	
	Left	Right	Left	Right	Left	Right
Psoas major	1	20	5	30	2	10
Longissimus dorsi (ribs)	1	5	2	30	10	20
Longissimus dorsi (loin)	10	30	5	20	1	2
Semitendinosus	2	5	20	30	1	10
Biceps femoris	1	30	5	10	2	20

*See table 4 on page 60.

Basis of the Methods Chosen

The method for extracting the collagen and elastin from the muscle tissue was chosen on the assumption that both collagen and elastin are resistant to attack by alkali of approximately 0.1 N. concentration at room temperature. It is also assumed that collagen is changed quantitatively to gelatin by prolonged autoclaving in water at neutral pH, but elastin is unaffected either by the autoclaving or by subsequent treatment with hot 0.1 N. alkali. Any changes in the muscle while in storage at -30°F. pending analysis were considered of minor importance and necessarily disregarded.

In such a case, the water- and dilute-alkali-soluble constituents may be removed in two extractions with 0.1 N. sodium hydroxide, followed by neutralization. The fats and phospholipids are extracted with alcohol and ether. The residue is then autoclaved and the gelatin poured off in the supernatant liquid after centrifuging. Residual intracellular protein is removed from the crude elastin by extraction with 0.1 N. alkali at 100°C. and the residue considered pure elastin.

Moisture Determination

Duplicate samples varying from 1 to 7 grams were dried to constant weight in open aluminum drying dishes in a

constant temperature drying oven at 100° to 116°C., according to the usual procedure. The samples from animal I were also used for total-nitrogen determinations, and so varied from 1 to 2 grams in weight. Those from animal IV varied from 3 to 7 grams according to the abundance or scarcity of available material.

Total-nitrogen Determination

The samples which had been dried to constant weight for determination of moisture content were transferred to micro-Kjeldahl flasks. Five milliliters of nitrogen-low sulfuric acid were added, and the digestion carried out in the usual fashion, using as catalyst a small amount of a 1:1 mixture of copper sulfate and potassium sulfate. When the digest was free of charred material, 30 per cent hydrogen peroxide was introduced, a few drops at a time, up to a maximum of ten drops. With further boiling the digest became clear and was diluted to 50 milliliters. One milliliter was distilled into 4 per cent boric acid and titrated with 0.01 N. hydrochloric acid according to the usual micro-Kjeldahl procedure. This permitted the calculation of nitrogen in the original sample.

This determination gave considerable difficulty because the large amount of sample took from six to twelve hours for digestion. Serious bumping occurred due to the large amount of wash water in the flask, as well as the large amount of

organic material. This method was used for animal I only.

Another difficulty was the necessity of keeping samples for brief periods while they were in use, in the freezing compartment of the laboratory refrigerator. At such times, especially in the case of the cork-stoppered samples, a gross redistribution of moisture took place, making the removal of a 1- or 2-gram representative sample difficult.

Collagen Determination by the Gravimetric Method

This method is an adaptation of the method used by Lowry, Gilligan, and Katersky (80) in determining the amount of collagen and elastin in human heart muscle.

Samples of two to four grams were weighed by difference (A) into 50-ml. round-bottom centrifuge tubes. Determinations were run in triplicate. About 200 milligrams of washed fine-mesh sand was weighed into the tube. The tissue and sand were transferred to a small mortar and ground with a pestle to as smooth a paste as possible. The ground tissue was returned to the centrifuge tube, the mortar was rinsed with five milliliters distilled water, which was then thoroughly stirred into the tissue. Thirty-five milliliters of 0.1 N. sodium hydroxide were used in two portions to rinse the mortar, and then mixed thoroughly with the muscle paste. This treatment with alkali was for the purpose of removing the non-collagen and non-elastin substances including the other

nitrogen compounds. Its successful use emphasized the difference in the relative solubility of the connective tissue components as contrasted with other components of the muscle tissue. The mixture was allowed to stand at room temperature overnight, after which it was stirred and centrifuged. All centrifugation was at the approximate rate of 2,200 R.P.M. The time varied with the viscosity of the suspension. The supernatent liquid was poured off and 40 milliliters 0.1 N. sodium hydroxide stirred in. This extraction was allowed to continue for two hours, when the mixture was again centrifuged and the supernatant liquid added to the previous portion. Forty milliliters of distilled water and one drop of 0.1-per cent phenol red were added and the mixture was titrated to a faint pink with 0.1 N. hydrochloric acid (pH 7). It was necessary to stir vigorously and allow time for the diffusion of the alkali from the suspended particles. The samples were centrifuged again, and the residue extracted with 40 milliliters of a 3:1 mixture of 95-per cent alcohol and anhydrous ether. This was allowed to stand ten minutes, then centrifuged and the connective tissue extracted with 40 milliliters anhydrous ether. After centrifugation, the ether was discarded, the centrifuge tube carefully wiped off with distilled water, and dried at 100° to 116°C. to constant weight. (B)

Twenty milliliters of distilled water was added to each tube, a loose tin foil cap was bent over each, and they were

autoclaved for six hours at twenty pounds' pressure. The liquid which contained the gelatin was centrifuged off, being reserved for further analysis, and the remaining connective tissue washed with 20 milliliters of distilled water. This wash water, after centrifugation, was added to the 20-milliliter gelatin-containing portion. The tube and its contents were again dried to constant weight (C), the difference between weights (B) and (C) constituting the weight of the collagen in the sample. Per cent collagen in the sample was calculated in this manner:

$$\frac{B - C}{A} \times 100 = \% \text{ collagen.}$$

Collagen Determination by the Nitrogen Method

The portion separated by centrifuging after autoclaving and the subsequent washing (20 milliliters each) were made up to a volume of 50 milliliters. Nitrogen determinations were made on 5 milliliter aliquots of this by the standard micro-Kjeldahl procedure. Solutions were kept at refrigeration temperatures until used. The collagen content of the samples was calculated by multiplying the nitrogen content by the factor 5.38, according to the collagen-analysis by Bergmann quoted in Block and Bolling's "Amino Acid Composition of Proteins and Foods," page 268.

Elastin Determination by the Gravimetric Method

The following procedure, a modification of the method of Lowry, Gilligan, and Katersky (80), was used for animal I only.

To the centrifuge tubes containing the residue after collagen extraction, 40 milliliters 0.1 N. sodium hydroxide was added, and they were placed in a boiling water bath for thirty minutes to extract any soluble intrafibrillar-nitrogen remaining. After centrifugation the alkali was poured off, and 40 milliliters distilled water added for washing. This was centrifuged and the tube with the elastin and sand was dried as before to constant weight. (D) The elastin content of the samples was calculated in this way:

$$\frac{C - D}{A} \times 100 = \% \text{ elastin.}$$

Elastin Determination by the Nitrogen Method

In order to transfer the elastin from 50-milliliter centrifuge tubes to micro-Kjeldahl flasks for nitrogen determination it was necessary to get it into solution. This was accomplished by refluxing the samples for twenty-four hours in a water bath with three milliliters of formic acid. Twenty-five-milliliter Erlenmeyer flasks inverted over the mouths of the centrifuge tubes served as air condensers.

It was necessary to replace at intervals the formic acid which evaporated.

The digest was transferred with repeated washings with small aliquots of distilled water to micro-Kjeldahl flasks, and the nitrogen determination made by the usual micro-Kjeldahl procedure. It proved to be desirable to use formic acid for the first two washings, instead of distilled water, to ensure the removal of all formic acid-soluble material from the sand and the walls of the tube. It was also expedient to evaporate the water from the sample in the micro-Kjeldahl flask over an open flame with constant agitation to avoid bumping and to shorten the period of digestion.

Soluble-nitrogen Determinations

All the portions of supernatant liquid used for extraction except the ether portion--six in all, making a total of 240 milliliters--were pooled to make up the soluble-nitrogen fraction of the muscle samples. It consisted of supernatant liquids poured off after centrifuging as follows: two 40-milliliter portions of 0.1 N. sodium hydroxide used for extraction in the cold, one 40-milliliter portion 0.1 N. sodium hydroxide used for extraction at 100°C. for thirty minutes, one 40-milliliter portion of a 3:1 alcohol-ether mixture, two 40-milliliter portions of distilled water, and enough 0.1 N. hydrochloric acid to adjust the pH to 7.

This mixture contained all the muscle material except that determined as collagen or elastin. The volume was adjusted to 250 milliliters and nitrogen was determined by the micro-Kjeldahl method on 3 milliliter aliquots.

Special Difficulties and Considerations

There were some problems for which no completely satisfactory solutions were found.

1. It was necessary to store the collagen solutions, pending nitrogen determination. Even in the refrigerator some mold growth appeared which interfered with pipetting.

The soluble-nitrogen portions were stored at room temperature. Since they were alkaline in reaction (approximately 0.05 N.) there is some question about the loss of nitrogen from terminal amino groups.

2. The centrifuge tubes were labelled with wax marking pencil. Before each weighing this mark was removed by rubbing lightly with a slightly dampened cloth. In adding sand and attaining constant weight three separate times, this rubbing was repeated on each tube as many as twelve or more times. Under such conditions it was almost impossible to avoid putting a charge on the tube which perhaps influenced the precision of weighing to some extent.

3. Since single muscles differ greatly throughout their structure, it was found extremely difficult to obtain

three 2- to 4-gram portions from one piece of muscle, even lying adjacent to each other, which gave identical or almost identical values for collagen and elastin, and were representative of the particular sample of muscle being analyzed.

Results

This study consisted of collagen and elastin determinations on four different muscles of two animals, a yearling steer and an eight-year-old dairy cow. The muscles chosen were divided into six roasts, each of which was permitted to "age" or remain in storage at 34° to 36°F. a different length of time. The statistics department devised a plan by which the period of time each roast was to remain in storage was determined, in order to make statistical analysis of the results of the study possible. Roasts were sampled and tests were run at the following storage intervals: 1, 2, 5, 10, 20, and 30 days. The objective of the study was to find out whether or not the collagen or elastin changed as the storage period was lengthened, and, if so, in what manner and by what amount.

The results are presented in tabular form in the following pages. With the exception of dry weight determinations which represent the average of two samples, the figures were obtained by averaging the results of three determinations.

The methods used in calculating results

In tables 6 to 15 and 26 to 35 inclusive, data on the dry weight and the collagen content of the selected muscles of animals I and IV will be found expressed in several ways. The collagen content is figured in terms of collagen-nitrogen as per cent of sample; collagen-nitrogen as per cent of dry sample; collagen-nitrogen as per cent of total-nitrogen; and as collagen per cent. The last value, collagen per cent, was obtained by the gravimetric procedure previously described and was also calculated from the nitrogen content of the same sample. Results given by both methods are included for comparison.

The analytical methods have been described in previous paragraphs. Results of the collagen-nitrogen determination by the nitrogen method were corrected for variable moisture content by multiplying this value by the factor $\frac{100}{\text{per cent dry weight of the sample}}$. Since the lipid content of samples also varied, it was important to correct for this factor also. This was accomplished by expressing the collagen-nitrogen as per cent of total-nitrogen. It was possible to determine the total-nitrogen in two ways: in the one, a nitrogen determination was made on a separate portion of tissue from the same sample, in this case, on the portion which had been dried for the dry weight determinations; in the other, the nitrogen contents of the three portions into

which the tissue was separated, elastin, collagen, and soluble portions, were added. Because this latter method gave a value for the same portion of tissue as the collagen and elastin values, it was considered preferable and so was used throughout this study. A table (table 50) containing the total-nitrogen values of all the samples of the four muscles of both animals has been included in this section and will be commented upon.

In order to translate collagen-nitrogen into collagen, the former value was multiplied by the factor 5.38. This factor was computed from the nitrogen content of collagen, 18.6 per cent, as quoted in Block and Bolling's "Amino Acid Composition of Proteins and Foods," page 268 of the 1945 edition, from Bergmann and Stein (89). This collagen per cent value can be compared with the collagen value obtained directly by the gravimetric method on the same samples. Similarly the factor for converting elastin-nitrogen into elastin, 5.85, was calculated from the nitrogen content of elastin (17.1 per cent) taken from the same reference quoted from Stein and Miller (76).

Gravimetric determinations of collagen and elastin were not made on the muscles of animal IV because the determination of the nitrogen content of the extracted tissue and calculation of collagen and elastin were considered of superior dependability.

The collagen content of uncooked samples of muscles of animal I

In tables 6 to 10 inclusive it will be noted that the dry weight varies from 25 to 35 per cent and that in no case does the dry weight increase consistently from the first to the thirtieth day of storage. In the longissimus dorsi-ribs, however, and in the semitendinosus there are only minor deviations. The amount of surface of the roast exposed during storage, the length of the storage period, as well as

Table 6

Collagen Content of Uncooked Samples of the Psoas Major, Animal I, Aged Varying Periods of Time

Sample no.	I 2u	I 1u	I 4u	I 5u	I 6u	I 3u
Storage-days	1	2	5	10	20	30
Dry wt. %	28.37	32.65	32.18	28.70	30.53	28.30
Collagen-N % of sample	0.0264	0.0574	0.0402	0.0530	0.0635	0.0760
Collagen-N % dry sample	0.0931	0.1758	0.1249	0.1847	0.2080	0.2685
Collagen-N* % total-N	0.865	1.85	1.18	1.50	1.81	1.96
Collagen ** col-N x 5.38	0.5005	0.9451	0.6715	0.9929	1.1182	1.4435
Collagen*** grav. method (dry basis)	0.9655	1.3033	1.2886	1.8291	1.9172	1.7442

Averages: * - 1.53
** - 0.9456
*** - 1.5080

Table 7

Collagen Content of Uncooked Samples of the Longissimus Dorsi-ribs, Animal I, Aged Varying Periods of Time

Sample no.	IIR 7u	IIR 12u	IIR 11u	IIR 8u	IIR 9u	IIR 10u
Storage-days	1	2	5	10	20	30
Dry wt. %	24.88	26.10	28.71	28.92	26.27	29.77
Collagen-N % of sample	0.0534	0.0620	0.0644	0.1240	0.1800	0.0915
Collagen-N % dry sample	0.2146	0.2375	0.2243	0.4288	0.6853	0.3071
Collagen-N* % total-N	1.66	1.72	1.76	3.59	4.65	2.13
Collagen ** col-N x 5.38	1.1537	1.2769	1.2058	2.3052	3.6841	1.6510
Collagen*** grav. method (dry basis)	1.4529	1.8056	1.5621	3.3971	4.4081	2.0213

Averages: * - 2.59
** - 1.8795
*** - 2.4412

Table 8

Collagen Content of Uncooked Samples of the Longissimus Dorsi-loin, Animal I, Aged Varying Periods of Time

Sample no.	IIL 18u	IIL 17u	IIL 15u	IIL 16u	II 13u	IIL 14u
Storage-days	1	2	5	10	20	30
Dry wt. %	26.04	25.97	28.06	26.06	26.88	28.41
Collagen-N % of sample	0.3226	0.0573	0.1019	0.0757	0.1068	0.1427
Collagen-N % dry sample	1.2391	0.2207	0.3632	0.2905	0.3973	0.5023
Collagen-N* % total-N	9.28 (omit)	1.70	----	----	2.69	3.80
Collagen ** col-N x 5.38	6.6614	1.1865	1.9526	1.5617	2.1359	2.7004
Collagen*** grav. method (dry basis)	0.7647	1.5962	2.5255	1.6016	2.9113	3.0965

Averages: * - 2.73
** - 1.9074
*** - 2.0826

Table 9

Collagen Content of Uncooked Samples of the Semitendinosus, Animal I, Aged Varying Periods of Time.

Sample no.	III 21u	III 19u	III 20u	III 22u	III 24u	III 23u
Storage-days	1	2	5	10	20	30
Dry wt. %	29.65	27.03	27.72	28.86	34.65	29.92
Collagen-N % of sample	0.1341	0.1275	0.1720	0.1740	0.1957	0.1780
Collagen-N % dry sample	0.4525	0.4718	0.6206	0.6029	0.5648	0.5949
Collagen-N* % total-N	4.12	3.77	----	4.65	4.77	4.66
Collagen ** col-N x 5.38	2.4326	2.5364	3.3363	3.2412	3.0364	3.1982
Collagen*** grav. method (dry basis)	2.9185	3.2586	3.4900	5.0409	4.1986	3.7427

Averages: * - 4.39
** - 2.9635
*** - 3.7749

Table 10

Collagen Content of Uncooked Samples of the Biceps Femoris, Animal I, Aged Varying Periods of Time.

Sample no.	IV 29u	IV 26u	IV 25u	IV 27u	IV 28u	IV 30u
Storage-days	1	2	5	10	20	30
Dry wt. %	25.37	26.69	28.61	27.19	28.23	28.06
Collagen-N % of sample	0.0495	0.0575	0.0970	0.0620	0.1394	0.1039
Collagen-N % dry sample	0.1951	0.2155	0.3390	0.2280	0.4938	0.3703
Collagen-N* % total-N	1.43	1.69	2.49	1.68	3.41	2.66
Collagen ** col-N x 5.38	1.0489	1.1585	1.8225	1.2257	2.6547	1.9907
Collagen*** grav. method (dry basis)	1.4388	1.5704	2.9942	2.0060	3.1917	2.3964

Averages: * - 2.23
** - 1.6502
*** - 2.2663

the efficacy of the sample-bottle closure during the period of frozen storage were evidently the major factors which caused variations in dry weight. The amount of fatty tissue in the sample also affects the dry weight determination. At the end of the storage period (thirty days) the dry weight varied from 28.30 per cent for the psoas major to 29.92 per cent for the semitendinosus.

The collagen-nitrogen content, like the dry weight, fails to show any consistent trend in any of the muscles. Individual muscles differ in collagen content expressed in collagen-nitrogen as per cent of total-nitrogen, as may be seen by comparing the averages of the six different-storage-period figures (assuming for this comparison that length of storage does not affect the collagen)--1.53 for the psoas major (the tenderest), 2.59 and 2.73 for the rib and the loin portions of the longissimus dorsi, 4.39 for the semitendinosus, and 2.23 for the biceps femoris. These values are in accord with commonly accepted ratings of comparative tenderness, and with the results obtained by Ramsbottom and others (50).

In all instances in these five tables the gravimetric method for the determination of collagen gives higher results than the nitrogen method. The averages of the six different-storage-period values for each muscle vary from 9 to 59 per cent higher than those computed from the nitrogen content.

Since the latter method is less subject to accidents and errors in the laboratory, it appears to be preferable.

The collagen content of cooked samples of muscles of animal I

In tables 11 to 15 inclusive, data obtained from cooked samples of these same muscles are presented. It is apparent that the dry weight was much higher because of the loss of water in cooking and the loss of liquid in slicing the hot roasts. The dry weight in all cases is above 30 per cent of the weight of the sample, and in two instances above 40 per cent, with values distributed irregularly. The cooking was described in the thesis of Dorothy Harrison (52).

Table 11

Collagen Content of Cooked Samples of the Psoas Major, Animal I, Aged Varying Periods of Time.

Sample no. / Storage-days	I 2c / 1	I 1c / 2	I 4c / 5	I 5c / 10	I 6c / 20	I 3c / 30
Dry wt. %	32.97	36.11	36.76	37.39	34.23	32.69
Collagen-N % of sample	0.1094	0.0837	0.0234	0.0526	0.0666	0.0651
Collagen-N % dry sample	0.3318	0.2318	0.0636	0.1407	0.1945	0.1991
Collagen-N* % total-N	3.10	1.97	0.496	1.24	1.57	1.41
Collagen ** col-N x 5.38	1.78	1.26	0.342	0.756	1.05	1.07
Collagen*** grav. method (dry basis)	0.334	1.70	0.621	0.663	0.832	1.06

Averages: * - 1.631
** - 1.043
*** - 0.868

Table 12

Collagen Content of Cooked Samples of the Longissimus Dorsi-ribs, Animal I, Aged Varying Periods of Time.

Sample no.	IIR 7c	IIR 12c	IIR 11c	IIR 8c	IIR 9c	IIR 10c
Storage-days	1	2	5	10	20	30
Dry wt. %	36.03	33.23	36.87	32.69	34.70	34.97
Collagen-N % of sample	0.1198	0.0830	0.0530	0.0895	0.0603	0.1127
Collagen-N % dry sample	0.3324	0.2497	0.1437	0.2738	0.1737	0.3223
Collagen-N* % total-N	3.02	1.99	1.29	2.13	1.33	2.48
Collagen ** col-N x 5.38	1.7878	1.3424	0.7725	1.4719	0.9338	1.7327
Collagen*** grav. method (dry basis)	2.1273	2.0503	0.9397	1.1343	0.8562	1.8819

Averages: * - 2.04
** - 1.3402
*** - 1.4983

Table 13

Collagen Content of Cooked Samples of the Longissimus Dorsi-loin, Animal I, Aged Varying Periods of Time

Sample no.	IIL 18c	IIL 17c	IIL 15c	IIL 16c	IIL 13c	IIL 14c
Storage-days	1	2	5	10	20	30
Dry wt. %	40.47	35.06	36.18	36.67	36.62	36.01
Collagen-N % of sample	0.1615	0.1457	0.0973	0.0602	0.1079	0.0494
Collagen-N % dry sample	0.3991	0.4155	0.2689	0.1642	0.2947	0.1371
Collagen-N* % total-N	3.57	3.49	2.49	1.56	2.34	1.09
Collagen ** col-N x 5.38	2.1456	2.2337	1.4456	0.8827	1.5843	0.7370
Collagen*** grav. method (dry basis)	2.5031	2.8007	1.9036	1.0112	1.6165	0.8334

Averages: * - 2.42
** - 1.5048
*** - 1.7781

Table 14

Collagen Content of Cooked Samples of the Semitendinosus, Animal I, Aged Varying Periods of Time

Sample no.	III 21c	III 19c	III 20c	III 22c	III 24c	III 23c
Storage-days	1	2	5	10	20	30
Dry wt. %	37.22	28.90	31.99	36.06	33.48	34.73
Collagen-N % of sample	0.1272	0.2268	0.1323	0.1305	0.1473	0.1911
Collagen-N % dry sample	0.3453	0.7847	0.4136	0.3619	0.4400	0.5502
Collagen-N* % total-N	2.63	5.39	2.82	3.08	3.23	4.19
Collagen ** col-N x 5.38	1.8563	4.2185	2.2235	1.9456	2.3654	2.9579
Collagen*** grav. method (dry basis)	2.1896	5.4744	2.3382	2.8529	2.7020	3.3486

Averages: * - 3.56
** - 2.5945
*** - 3.1510

Table 15

Collagen Content of Cooked Samples of the Biceps Femoris, Animal I, Aged Varying Periods of Time

Sample no.	IV 29c	IV 26c	IV 25c	IV 27c	IV 28c	IV 30c
Storage-days	1	2	5	10	20	30
Dry wt. %	31.86	38.84	32.08	39.66	42.61	34.75
Collagen-N % of sample	0.0708	0.0949	0.0881	0.0850	0.1078	0.1673
Collagen-N % dry sample	0.2277	0.2915	0.2746	0.2143	0.2530	0.4815
Collagen-N* % total-N	1.63	1.95	1.96	1.98	2.10	3.69
Collagen ** col-N x 5.38	1.2241	1.5671	1.4762	1.1521	1.3601	2.5885
Collagen*** grav. method (dry basis)	1.6671	2.0986	1.4930	1.0886	1.5948	2.8058

Averages: * - 2.22
** - 1.5614
*** - 1.7913

It consisted of cooking the roasts on a rack in a deep kettle in which they were covered with bland lard at 96° to 98°C. and cooked to an internal temperature of 70°C.

The results for collagen-nitrogen as per cent of dry weight and as per cent of total-nitrogen were calculated as previously described. Again the values are very irregular. For example in the psoas major the collagen-nitrogen expressed as per cent of total-nitrogen was 3.10 on the first day of storage, but 0.496 on the fifth day, a difference of 500 per cent. The length of storage, it appears from these data, seems to play a minor role, if any, in the magnitude of the collagen content. A more reasonable explanation for the wide differences in value is the location in the muscle from which the sample was cut, and the presence or absence of dense layers of endomysium or perimysium. Ramsbottom and co-workers (50) found that samples taken from the anterior end of longissimus dorsi were less tender than those cut from the middle or posterior portions. However, samples from the anterior portion of the longissimus dorsi of animal I did not contain more collagen than those from the middle portion, according to these results.

In all muscles except the collagen-low psoas major, the gravimetric method gave higher values than the nitrogen method, but the difference is not so great as in the uncooked samples, ranging from 17 per cent lower to 21 per cent higher.

The magnitudes of values for collagen content are somewhat lower in the cooked than in the uncooked samples, which may be explained to some extent at least by the partial breakdown of collagen to gelatin with the application of moist heat.

Comparison of results obtained in this study with results reported by other recent investigators

Mitchell (78) reports the value of collagen as per cent of total-nitrogen in the psoas major as 8.85 per cent compared with 1.53 per cent in this study. The discrepancy probably resides in large measure in the amount of trimming which was done to remove the epimysium from the outside of the muscle. These workers report that 8.3 per cent of the total-nitrogen is collagen-nitrogen in the longissimus dorsi, whereas 10.6 per cent of the total-nitrogen is collagen-nitrogen in the round. In the present study the corresponding amounts are 2.59 per cent for the longissimus dorsi and 4.39 per cent for the semitendinosus, one of the muscles of the round.

Spencer, Morgulis, and Wilder (84) by means of precipitation of the collagen with tannic acid, subsequent digestion, and direct nesslerization, obtained values for collagen in tenderloin which averaged 12.6 per cent of the total-nitrogen. According to Lowry (80), substances other than collagen are precipitated by the tannic acid and determined as collagen, making the results excessively high.

Bell, Morgan, and Dorman (86), using a modification of Mitchell and Hamilton's method, determined the collagen in beef, both uncooked and cooked, and found 5 to 8 per cent of the total-nitrogen to be collagen-nitrogen in uncooked meat and 3 to 6 per cent in cooked meat, without considering the collagen content of the residue removed in the original washings with water.

Bate Smith (64) reported the collagen in rabbit muscle by extraction with lithium chloride and dilute hydrochloric acid to be 13 per cent of the total-protein in fresh rabbit muscle and 24 per cent in frozen rabbit muscle, but did not discuss values in beef muscle.

Lowry (80) reported an average collagen content of 4.3 expressed as per cent of dry tissue in the adductor muscle from the thigh of a rat. This was determined by the gravimetric method used in this study.

Yoder (87) obtained an average collagen-nitrogen of 6.58 expressed as per cent of total-nitrogen for the longissimus dorsi and of 3.83 for round steak.

It is difficult to compare the results of collagen determinations by different workers because of the use of such a variety of tissues and the various bases upon which results are expressed. Compared with Mitchell and others' results, the values for collagen-nitrogen as per cent of total-nitrogen in beef muscles in this study is low. Lowry

and others' determination of collagen as per cent of dry weight in rat muscle is in agreement with the results of this study. Yoder's results are somewhat high for the longissimus dorsi, but are of the same order for round.

The elastin content of uncooked samples of muscles of animal I

The next set of tables, 16 to 20, contain the data obtained on the elastin content of the four beef muscles. It is evident that there is no change in the elastin content with storage and there is a marked difference in the elastin content of different muscles. The psoas major has an average

Table 16

Elastin Content of Uncooked Samples of the Psoas Major, Animal I, Aged Varying Periods of Time.

Sample no.	I 2u	I 1u	I 4u	I 5u	I 6u	I 3u
Storage-days	1	2	5	10	20	30
Dry wt. %	28.37	32.65	32.18	28.70	30.53	28.30
Elastin-N % of sample	0.0027	0.0122	0.0092	0.0092	0.0093	0.0174
Elastin-N % dry sample	0.0095	0.0374	0.0286	0.0321	0.0305	0.0615
Elastin-N* % total-N	0.0885	0.394	0.271	0.260	0.265	0.449
Elastin ** elas-N x 5.85	0.0556	0.219	0.167	0.188	0.178	0.360
Elastin*** grav. method (dry basis)	0.431	0.250	0.169	0.280	0.500	0.500

Averages * - 0.288
** - 0.1946
*** - 0.355

Table 17

Elastin Content of Uncooked Samples of the Longissimus Dorsi-ribs, Animal I, Aged Varying Periods of Time.

Sample no.	IIR 7u	IIR 12u	IIR 11u	IIR 8u	IIR 9u	IIR 10u
Storage-days	1	2	5	10	20	30
Dry wt. %	24.88	26.10	28.71	28.92	26.27	29.77
Elastin-N % of sample	0.0092	0.0107	0.0381	0.0098	0.0211	0.0204
Elastin-N % dry sample	0.0370	0.0410	0.1327	0.0339	0.0803	0.0685
Elastin-N* % total-N	0.285	0.326	0.104	0.284	0.545	0.475
Elastin ** elas-N x 5.85	0.2164	0.2398	0.7760	0.1982	0.4696	0.4006
Elastin*** grav. method (dry basis)	0.3834	0.7923	0.9592	0.1307	0.9308	0.6658

Averages: * - 0.337
** - 0.3834
*** - 0.6437

Table 18

Elastin Content of Uncooked Samples of the Longissimus Dorsi-loin, Animal I, Aged Varying Periods of Time.

Sample no.	IIL 18u	IIL 17u	IIL 15u	IIL 16u	IIL 13u	IIL 14u
Storage-days	1	2	5	10	20	30
Dry wt. %	26.04	25.97	28.06	26.06	26.88	28.41
Elastin-N % of sample	0.0145	0.0121	----	----	0.0265	0.0227
Elastin-N % dry sample	0.0557	0.0466	----	----	0.0986	0.0799
Elastin-N* % total-N	0.418	0.359	----	----	0.667	0.604
Elastin ** elas-N x 5.85	0.3257	0.2725	----	----	0.5766	0.4673
Elastin*** grav. method (dry basis)	0.5596	0.4205	1.0293	0.4954	0.8589	0.6829

Averages: * - 0.512
** - 0.4105
*** - 0.6744

Table 19

Elastin Content of Uncooked Samples of the Semitendinosus, Animal I, Aged Varying Periods of Time.

Sample no.	III 21u	III 19u	III 20u	III 22u	III 24u	III 23u
Storage-days	1	2	5	10	20	30
Dry wt. %	29.65	27.03	27.72	28.86	34.65	29.92
Elastin-N % of sample	0.1259	0.1834	----	0.1726	0.1585	0.1602
Elastin-N % dry sample	0.4248	0.6786	----	0.5981	0.4574	0.5354
Elastin-N* % total-N	3.88	5.38	----	4.62	3.87	4.19
Elastin ** elas-N x 5.85	2.4842	3.9685	----	3.4977	2.6749	3.1310
Elastin*** grav. method (dry basis)	3.4145	4.4100	3.4907	4.0090	3.3391	3.9299

Averages: * - 4.39
** - 3.1513
*** - 3.8205

Table 20

Elastin Content of Uncooked Samples of the Biceps Femoris, Animal I, Aged Varying Periods of Time.

Sample no.	IV 29u	IV 26u	IV 25u	IV 27u	IV 28u	IV 30u
Storage-days	1	2	5	10	20	30
Dry wt. %	25.37	26.69	28.61	27.19	28.23	28.06
Elastin-N % of sample	0.0142	0.0116	0.0278	0.0130	0.0342	0.1718
Elastin-N % dry sample	0.0560	0.0435	0.0972	0.0478	0.1211	0.6123
Elastin-N* % total-N	0.411	0.341	0.713	0.352	0.839	4.400 (omit)
Elastin ** elas-N x 5.85	0.3275	0.2544	0.5684	0.2795	0.7082	3.581
Elastin*** grav. method (dry basis)	0.7573	0.6138	0.6609	0.8158	1.1299	1.7154

Averages: * - 0.531
** - 0.4276
*** - 0.7955

elastin-nitrogen content in per cent of total-nitrogen of 0.288, the longissimus dorsi 0.337 and 0.512, the biceps femoris 0.531, and a considerably higher value for the semitendinosus, 4.39.

Just as the gravimetric method gave higher results in the determination of collagen, it gave values in elastin 21 to 82 per cent above those obtained by determining the nitrogen content of the elastin fraction in all except the biceps femoris.

The results in terms of elastin by the nitrogen and gravimetric methods for the four uncooked muscles of animal I are: psoas major, 0.1946, 0.355; longissimus dorsi-ribs, 0.3834, 0.6437; longissimus dorsi-loin, 0.4105, 0.6744; semitendinosus, 3.1513, 3.8205; and biceps femoris, 0.9532, 0.9489, if the sample IV30u is included. If sample IV30u is not included, the results are 0.4276 and 0.7955. In these figures, like those for the other muscles, the value by the nitrogen method is considerably below the value obtained by the gravimetric method.

The elastin content of cooked samples of muscles of animal I

Tables 21 to 25 present the elastin-nitrogen and elastin data from the cooked samples of these same muscles. It might be noted in the beginning that the cooked sample from the roast described in the preceding section, number IV 30u, as having an excessive elastin value by the nitrogen method,

and a high one by the gravimetric method, has in the cooked sample by both methods a moderate value, which seems to indicate the uncooked sample was atypical. No trend can be distinguished in the elastin values with the progress of length of storage time, as was to be expected. The average elastin-nitrogen values are slightly higher for the psoas major than for the uncooked samples of the same muscle, but the elastin values are almost identical. In the longissimus dorsi-ribs, the nitrogen expressed as per cent of total-nitrogen is somewhat higher (about 30 per cent) but the elastin calculated on the basis of nitrogen content is almost identical. The elastin by gravimetric determination is somewhat higher, as usual.

In the longissimus dorsi-loin the agreement in elastin-nitrogen as per cent of total-nitrogen is very close between the cooked and the uncooked samples, but there is a difference of 0.09 per cent in the calculated elastin content. In the semitendinosus the agreement is close between the cooked and uncooked samples, whether expressed as elastin-nitrogen as per cent of total-nitrogen or as elastin. The biceps femoris values for uncooked samples are unduly influenced by the thirty-day storage sample. If this value is discarded, the uncooked determinations are only slightly higher than the cooked ones, with the exception of elastin by the gravimetric method which is almost twice as high as the value calculated from the nitrogen content.

Table 21

Elastin Content of Cooked Samples of the Psoas Major, Animal I, Aged Varying Periods of Time.

Sample no.	I 2c	I 1c	I 4c	I 5c	I 6c	I 3c
Storage-days	1	2	5	10	20	30
Dry wt. %	32.97	36.11	36.76	37.39	34.23	32.69
Elastin-N % of sample	0.0329	0.0358	0.0102	0.0159	0.0173	0.0127
Elastin-N % dry sample	0.0998	0.0991	0.0277	0.0425	0.0505	0.0388
Elastin-N* % total-N	0.934	0.842	0.216	0.375	0.407	0.275
Elastin ** elas-N x 5.85	0.5836	0.5795	0.1620	0.2485	0.2953	0.2269
Elastin*** grav. method (dry basis)	0.2639	0.6773	----	0.2086	0.1513	----

Averages: * - 0.508
** - 0.3493
*** - 0.3253

Table 22

Elastin Content of Cooked Samples of the Longissimus Dorsi-ribs, Animal I, Aged Varying Periods of Time.

Sample no.	IIR 7c	IIR 12c	IIR 11c	IIR 8c	IIR 9c	IIR 10c
Storage-days	1	2	5	10	20	30
Dry wt. %	36.03	33.23	36.87	32.69	34.70	34.97
Elastin-N % of sample	0.0325	0.0255	0.0140	0.0140	0.0130	0.0280
Elastin-N % dry sample	0.0902	0.0767	0.0380	0.0428	0.0375	0.0801
Elastin-N* % total-N	0.817	0.612	0.340	0.332	0.288	0.614
Elastin ** elas-N x 5.85	0.5275	0.4485	0.2222	0.2503	0.2193	0.4684
Elastin*** grav. method (dry basis)	9.6886 (omit)	0.4366	0.9055	0.4374	0.0432	0.3652

Averages: * - 0.501
** - 0.3560
*** - 0.4376

Table 23

Elastin Content of Cooked Samples of the Longissimus Dorsi-loin, Animal I, Aged Varying Periods of Time.

Sample no.	IIL 18c	IIL 17c	IIL 15c	IIL 16c	IIL 13c	IIL 14c
Storage-days	1	2	5	10	20	30
Dry wt. %	40.47	35.06	36.18	36.67	36.62	36.01
Elastin-N % of sample	0.0220	0.0355	0.0263	0.0124	0.0123	0.0117
Elastin-N % dry sample	0.0543	0.1012	0.0727	0.0338	0.0336	0.0325
Elastin-N* % total-N	0.485	0.849	0.674	0.321	0.267	0.259
Elastin ** elas-N x 5.85	0.3175	0.5918	0.4251	0.1977	0.1965	0.1901
Elastin*** grav. method (dry basis)	0.0694	1.4565	0.2336	0.3900	0.1458	----

Averages: * - 0.476
** - 0.3193
*** - 0.4591

Table 24

Elastin Content of Cooked Samples of the Semitendinosus, Animal I, Aged Varying Periods of Time.

Sample no.	III 21c	III 19c	III 20c	III 22c	III 24c	III 23c
Storage-days	1	2	5	10	20	30
Dry wt. %	37.22	28.90	31.99	36.06	33.48	34.73
Elastin-N % of sample	0.1520	0.1937	0.1938	0.1841	0.1482	0.2508
Elastin-N % dry sample	0.4084	0.6702	0.6058	0.5105	0.4427	0.7221
Elastin-N* % total-N	3.15	4.61	4.14	4.32	3.25	5.50
Elastin ** elas-N x 5.85	2.3883	3.9193	3.5427	2.9854	2.5889	4.2228
Elastin*** grav. method (dry basis)	1.9943	4.0755	3.6727	2.8787	2.7134	4.0467

Averages: * - 4.16
** - 3.2746
*** - 3.2302

Table 25

Elastin Content of Cooked Samples of the Biceps Femoris, Animal I, Aged Varying Periods of Time.

Sample no.	IV 29c	IV 26c	IV 25c	IV 27c	IV 28c	IV 30c
Storage-days	1	2	5	10	20	30
Dry wt. %	31.86	38.84	32.08	39.66	42.61	34.75
Elastin-N % of sample	0.0130	0.0389	0.0208	0.0120	0.0366	0.0155
Elastin-N % dry sample	0.0408	0.1002	0.0648	0.0303	0.0859	0.0446
Elastin-N* % total-N	0.299	0.799	0.464	0.279	0.711	0.342
Elastin ** elas-N x 5.85	0.2386	0.5860	0.3790	0.1772	0.5023	0.2608
Elastin*** grav. method (dry basis)	----	0.7195	0.5492	0.1944	0.4534	0.2521

Averages: * - 0.482
** - 0.3573
*** - 0.3614

Comparison of results obtained in this study with results reported by other recent investigators

The results of this study cannot be readily compared with those of Mitchell and others (78). The data of this study are reported for specific muscles, whereas the results obtained by Mitchell and others are reported for commercial cuts of meat. Some of these cuts, as the round, contain several muscles. The elastin-nitrogen values obtained in this study are somewhat higher than the 0.02 to 0.07 per cent of total-nitrogen for the psoas major and the 0.05 to 0.11 per cent reported for porterhouse steak by Mitchell and others

(78). In the round, Mitchell found 0.15 to 0.48 per cent and in the navel 0.74 to 1.42 per cent of the total-nitrogen. In the rib-eye (longissimus dorsi) he obtained 0.01 to 0.24 per cent of elastin-nitrogen. The values reported in this study vary from 0.104 to 0.545 with an average of 0.337 for this muscle.

Bate Smith (64) gave the value 1 per cent of the total-nitrogen to the elastin content of all his rabbit muscles. The psoas major he reported as having a total connective tissue content of 1 per cent and other muscles 3 to 4 per cent, presumably on a per cent of sample basis.

Lowry, Gilligan, and Katersky (80) reported only one kind of skeletal muscle analyzed by their method, the adductor muscle of the thigh of the rat. The average value they obtained was 1.1 per cent of the dry weight of the tissue, which is somewhat higher than the 0.42 to 0.68 per cent obtained for beef semitendinosus in this study by a similar extraction procedure. The two tissues as well as the method of sampling were so different in the two cases, that closer agreement than this could hardly be expected.

The collagen content of uncooked samples of muscles of animal IV

The data obtained for muscles of animal IV are given in the following tables: 26 to 30, 31 to 35, 36 to 40, and 41 to 45, and are similar to data for animal I given in preceding tables.

Table 26

Collagen Content of Uncooked Samples of the Psoas Major, Animal IV, Aged Varying Periods of Time.

Sample no.	I 94u	I 96u	I 95u	I 93u	I 91u	I 92u
Storage-days	1	2	5	10	20	30
Dry wt. %	28.01	32.29	32.80	32.86	31.80	37.08
Collagen-N % of sample	0.0502	0.0461	0.0527	0.0536	0.0959	0.0630
Collagen-N % dry sample	0.1792	0.1428	0.1607	0.1631	0.3016	0.1699
Collagen-N* % total-N	1.5925	1.6245	1.6420	1.7143	2.6585	2.0123
Collagen ** col-N x 5.38	0.9641	0.7683	0.8646	0.8775	1.6226	0.9141

Averages: * - 1.8740
** - 1.0019

Table 27

Collagen Content of Uncooked Samples of the Longissimus Dorsi-rib, Animal IV, Aged Varying Periods of Time.

Sample no.	IIR 100u	IIR 101u	IIR 97u	IIR 102u	IIR 99u	IIR 98u
Storage-days	1	2	5	10	20	30
Dry wt. %	28.05	28.04	29.71	26.97	28.46	32.89
Collagen-N % of sample	0.0882	0.1613	0.2354	0.2279	0.1415	0.1576
Collagen-N % dry sample	0.3144	0.5752	0.7923	0.8450	0.4972	0.4792
Collagen-N* % total-N	2.6964	4.5930	6.6703	6.1867	4.0721	3.9266
Collagen** col-N x 5.38	1.6915	3.0946	4.2626	4.5461	2.6749	2.5781

Averages: * - 4.6909
** - 3.2413

Table 28

Collagen Content of Uncooked Samples of the Longissimus Dorsi-loin, Animal IV, Aged Varying Periods of Time.

Sample no.	IIL 105u	IIL 108u	IIL 107u	IIL 106u	IIL 104u	IIL 103u
Storage-days	1	2	5	10	20	30
Dry wt. %	26.87	25.35	28.14	31.76	28.74	28.35
Collagen-N % of sample	0.2108	0.1477	0.1354	0.1592	0.1283	0.1977
Collagen-N % dry sample	0.7845	0.5826	0.4812	0.5013	0.4464	0.6973
Collagen-N* % total-N	6.4325	4.3379	3.9356	4.4289	3.4945	5.3112
Collagen ** col-N x 5.38	4.2206	3.1344	2.5889	2.6970	2.4016	3.7515

Averages: * - 4.6568
** - 3.1323

Table 29

Collagen Content of Uncooked Samples of the Semitendinosus, Animal IV, Aged Varying Periods of Time.

Sample no.	III 111u	III 112u	III 109u	III 114u	III 113u	III 110u
Storage-days	1	2	5	10	20	30
Dry wt. %	24.76	24.99	26.51	30.34	29.38	33.44
Collagen-N % of sample	0.1613	0.1460	0.1490	0.2010	0.3910	0.2808
Collagen-N % dry sample	0.6515	0.5842	0.5621	0.6625	1.3308	0.8397
Collagen-N* % total-N	4.6396	4.4416	4.2717	5.5928	9.7762	6.6692
Collagen** col-N x 5.38	3.5051	3.1430	3.0241	3.5643	7.1597	4.5176

Averages: * - 5.8985
** - 4.1523

Table 30

Collagen Content of Uncooked Samples of the Biceps Femoris, Animal IV, Aged Varying Periods of Time.

Sample no.	IV 115u	IV 117u	IV 116u	IV 119u	IV 120u	IV 118u
Storage-days	1	2	5	10	20	30
Dry wt. %	25.88	27.56	27.43	30.61	27.44	26.33
Collagen-N % of sample	0.1374	0.1580	0.1503	0.1770	0.1128	0.2983
Collagen-N % dry sample	0.5309	0.5733	0.5479	0.5782	0.4111	1.1329
Collagen-N* % total-N	3.9501	4.5191	4.0963	4.5084	2.9676	7.5552
Collagen** col-N x 5.38	2.8562	3.0844	2.9477	3.1107	2.2117	6.0950

Averages: * - 4.5995
** - 3.3843

Animal IV was a dairy cow of cutter grade, according to United States standards, having smaller muscles, intensely yellow fat, and a large amount of connective tissue enveloping the muscles excised for analysis. An effort was made to cut away and discard this layer, but the task was found to be very difficult.

It will be noted that the dry weight of the uncooked samples of animal IV almost parallel those of animal I. There was somewhat more dehydration at the end of thirty days in the psoas major and the semitendinosus, but these muscles were small and so each roast had a large surface area exposed in proportion to volume.

Again the collagen-nitrogen values show no relation to

the duration of storage. Individual determinations of collagen-nitrogen as per cent of total-nitrogen show a wide variation in collagen content, ranging in the psoas major from 1.59 to 2.66 per cent and in the biceps femoris from 2.97 to 7.56 per cent. The values for the rib and loin sections of the longissimus dorsi are almost identical. As in all cases, the semitendinosus has the highest collagen content, with the biceps femoris and the longissimus dorsi about equal, and the psoas major having the least.

The collagen content of cooked samples of muscles of animal IV

The collagen-nitrogen values for the cooked samples have about the same range of dry weight as those of animal I, from 32.21 to 49.06 per cent with the extremes in the smallest muscles.

Table 31

Collagen Content of the Cooked Samples of the Psoas Major, Animal IV, Aged Varying Periods of Time.

Sample no.	I 94c	I 96c	I 95c	I 93c	I 91c	I 92c
Storage-days	1	2	5	10	20	30
Dry wt. %	42.47	36.31	34.66	49.06	32.21	37.18
Collagen-N % of sample	0.0410	0.0509	0.0462	0.0456	0.0597	0.0456
Collagen-N % dry sample	0.0965	0.1402	0.1333	0.0929	0.1853	0.1226
Collagen-N* % total-N	0.9941	1.2368	1.1568	1.3250	1.4593	1.1193
Collagen ** col-N x 5.38	0.5192	0.7543	0.7172	0.4998	0.9969	0.6596

Averages: * - 1.2152
** - 0.6912

Table 32

Collagen Content of Cooked Samples of the Longissimus Dorsi-rib, Animal IV, Aged Varying Periods of Time.

Sample no.	IIR 100c	IIR 101c	IIR 97c	IIR 102c	IIR 99c	IIR 98c
Storage-days	1	2	5	10	20	30
Dry wt. %	39.96	42.13	39.96	35.46	34.56	36.27
Collagen-N % of sample	0.0920	0.1308	0.1122	0.2632	0.1693	0.1907
Collagen-N % dry sample	0.2302	0.3105	0.2808	0.7423	0.4899	0.5258
Collagen-N* % total-N	2.1551	3.1362	2.5394	5.7321	3.6150	4.4129
Collagen ** col-N x 5.38	1.2385	1.6705	1.5107	3.9936	2.6357	2.8288

Averages: * - 3.5985
** - 2.3130

Table 33

Collagen Content of Cooked Samples of the Longissimus Dorsi-loin, Animal IV, Aged Varying Periods of Time.

Sample no.	IIL 105c#	IIL 108c	IIL 107c	IIL 106c	IIL 104c	IIL 103c
Storage-days	1	2	5	10	20	30
Dry wt. %	26.59	38.97	36.36	35.59	35.17	36.22
Collagen-N % of sample	0.6265	0.5219	0.2620	0.5040	0.2633	0.2989
Collagen-N % dry sample	2.3561	1.3392	0.7206	1.4161	0.7486	0.8252
Collagen-N* % total-N	18.1937	11.0034	5.5723	11.0041	5.9133	6.3459
Collagen ** col-N x 5.38	12.6758	7.2049	3.8768	7.6186	4.0275	4.4396

Averages: * - 7.9678
** - 5.4335

#Values for this sample omitted from calculations.

Table 34

Collagen Content of Cooked Samples of the Semitendinosus, Animal IV, Aged Varying Periods of Time.

Sample no.	III 111c	III 112c	III 109c	III 114c	III 113c	III 110c
Storage-days	1	2	5	10	20	30
Dry wt. %	36.57	33.69	35.41	33.30	36.27	33.97
Collagen-N % of sample	0.3398	0.3891	0.4157	0.6619	0.7096	0.4111
Collagen-N % dry sample	0.9292	1.1549	1.1740	1.9877	1.9564	1.1334
Collagen-N* % total-N	6.9670	9.2733	9.3029	13.4790	13.9124	9.1757
Collagen ** col-N x 5.38	4.9991	6.2134	6.3161	10.6938	10.5254	6.0977

Averages: * - 10.3517
** - 7.4743

Table 35

Collagen Content of Cooked Samples of the Biceps Femoris, Animal IV, Aged Varying Periods of Time.

Sample no.	IV 115c	IV 117c	IV 116c	IV 119c	IV 120c	IV 118c
Storage-days	1	2	5	10	20	30
Dry wt. %	37.94	37.43	40.59	41.70	35.69	35.78
Collagen-N % of sample	0.3834	0.3664	0.2853	0.2170	0.2738	0.2458
Collagen-N % dry sample	1.0105	0.9789	0.7029	0.5204	0.7672	0.6870
Collagen-N* % total-N	7.1943	6.5505	6.2199	4.6746	5.4649	5.0248
Collagen ** col-N x 5.38	5.4365	5.2665	3.7816	2.7998	4.1275	3.6961

Averages: * - 5.8548
** - 4.1847

The values for collagen-nitrogen as per cent of total-nitrogen are interesting in the cooked samples of this animal. In the psoas major and the longissimus dorsi these values decrease about 35 per cent in the case of the psoas major and 25 per cent in the case of the longissimus dorsi-ribs. This may be accounted for in part by assuming some collagen breakdown in the cooking process, and possibly some difference in the slicing out of the samples. The three less tender muscles, however, show much more striking increases in collagen-nitrogen values. The increases in the average of the six determinations of samples stored different lengths of time were 71, 75, and 27 per cent in the longissimus dorsi-loin, the semitendinosus, and the biceps femoris, respectively. No satisfactory explanation can be made for this large increase at the present time.

Comment must be made, however, on the difficulty of extracting the soluble-nitrogen from the cooked samples with 0.1 N. sodium hydroxide. As soon as the well ground sample was mixed with the alkali, a viscous, granular-appearing permanent gel was formed, from which it was extremely difficult to separate the collagen and elastin by centrifugation. There is a slight possibility that even with five extractions and much vigorous and repeated stirring, a small amount of this apparently denatured protein remained in the centrifuge tube and was extracted in the autoclave to be determined as

collagen. The residues were white, however, or grayish-white, and from all appearances extraction was complete. However, it is difficult to see why this fact should influence the values for three muscles and not for the other two.

Values for sample IIL 105c have been omitted because the sample so labelled was uncooked and atypical.

The elastin content of uncooked samples of muscles of animal IV

The elastin-nitrogen data from uncooked samples of animal IV are presented in tables 36 to 40. It is evident no relation between elastin content and length of storage exists in these samples.

Table 36

Elastin Content of Uncooked Samples of the Psoas Major, Animal IV, Aged Varying Periods of Time.

Sample no.	I 94u	I 96u	I 95u	I 93u	I 91u	I 92u
Storage-days	1	2	5	10	20	30
Dry wt. %	28.01	32.29	32.80	32.86	31.80	37.08
Elastin-N % of sample	0.0123	0.0103	0.0130	0.0133	0.0226	0.0082
Elastin-N % dry sample	0.0439	0.0319	0.0396	0.0405	0.0711	0.0221
Elastin-N* % total-N	0.3902	0.3630	0.4050	0.4254	0.6265	0.2619
Elastin ** elas-N x 5.85	0.2568	0.1866	0.2317	0.2369	0.4159	0.1293

Averages: * - 0.4120
** - 0.2443

Table 37

Elastin Content of Uncooked Samples of the Longissimus Dorsi-rib, Animal IV, Aged Varying Periods of Time.

Sample no.	IIR 100u	IIR 101u	IIR 97u	IIR 102u	IIR 99u	IIR 98u
Storage-days	1	2	5	10	20	30
Dry wt. %	28.05	28.04	29.71	26.97	28.46	32.89
Elastin-N % of sample	0.0101	0.0086	0.0086	0.0144	0.0140	0.0111
Elastin-N % dry sample	0.0360	0.0307	0.0289	0.0534	0.0492	0.0337
Elastin-N* % total-N	0.3088	0.2449	0.2437	0.3909	0.4029	0.2766
Elastin ** elas-N x 5.85	0.2106	0.1796	0.1691	0.3124	0.2878	0.1971

Averages: * - 0.3113
** - 0.2261

Table 38

Elastin Content of Uncooked Samples of the Longissimus Dorsi-loin, Animal IV, Aged Varying Periods of Time.

Sample no.	IIL 105u	IIL 108u	IIL 107u	IIL 106u	IIL 104u	IIL 103u
Storage-days	1	2	5	10	20	30
Dry wt. %	26.87	25.35	28.14	31.76	28.74	28.35
Elastin-N % of sample	0.0129	0.0150	0.0191	0.0158	0.0186	0.0192
Elastin-N % dry sample	0.0480	0.0592	0.0679	0.0497	0.0647	0.0677
Elastin-N* % total-N	0.3936	0.4405	0.5552	0.4395	0.5066	0.5158
Elastin ** elas-N x 5.85	0.2808	0.3463	0.3972	0.2907	0.3785	0.3960

Averages: * - 0.4752
** - 0.3483

Table 39

Elastin Content of Uncooked Samples of the Semitendinosus, Animal IV, Aged Varying Periods of Time.

Sample no.	III 111u	III 112u	III 109u	III 114u	III 113u	III 110u
Storage-days	1	2	5	10	20	30
Dry wt. %	24.76	24.99	26.51	30.34	29.38	33.44
Elastin-N % of sample	0.0588	0.0759	0.0603	0.0357	0.0558	0.0756
Elastin-N % dry sample	0.2375	0.3037	0.2275	0.1177	0.1899	0.2261
Elastin-N* % total-N	1.6737	2.3090	1.7287	0.9933	1.3952	1.7957
Elastin ** elas-N x 5.85	1.3894	1.7766	1.3309	0.6885	1.1109	1.3227

Averages: * - 1.6493
** - 1.2698

Table 40

Elastin Content of Uncooked Samples of the Biceps Femoris, Animal IV, Aged Varying Periods of Time.

Sample no.	IV 115u	IV 117u	IV 116u	IV 119u	IV 120u	IV 118u
Storage-days	1	2	5	10	20	30
Dry wt. %	25.88	27.56	27.43	30.61	27.44	26.33
Elastin-N % of sample	0.0123	0.0139	0.0196	0.0174	0.0166	0.0270
Elastin-N % dry sample	0.0475	0.0504	0.0715	0.0568	0.0605	0.1025
Elastin-N* % total-N	0.3536	0.3976	0.5342	0.4432	0.4367	0.6838
Elastin ** elas-N x 5.85	0.2779	0.2948	0.4183	0.3323	0.3539	0.5996

Averages: * - 0.4749
** - 0.3795

The range of values in different determinations on the same muscle is much more limited in the case of elastin-nitrogen than in the case of collagen-nitrogen. In the psoas major the values extend from 0.26 to 0.63 and in the semitendinosus from 0.99 to 2.3 per cent of the total-nitrogen.

The elastin content of cooked samples of muscles of animal IV

In the cooked samples the results are slightly lower on the average than those of the uncooked samples. The distribution of values shows no regularity and no relation to the length of storage period. Cooked and uncooked samples from the same roast do not always correspond in the magnitude of the elastin-nitrogen content, but there is a general tendency, as shown in the tables to do so, which is evident especially in the averages.

Table 41

Elastin Content of Cooked Samples of the Psoas Major, Animal IV, Aged Varying Periods of Time.

Sample no.	I 94c	I 96c	I 95c	I 93c	I 91c	I 92c
Storage-days	1	2	5	10	20	30
Dry wt. %	42.47	36.31	34.66	49.06	32.21	37.18
Elastin-N % of sample	0.0180	0.0144	0.0083	0.0132	0.0078	0.0113
Elastin-N % dry sample	0.0424	0.0397	0.0239	0.0269	0.0242	0.0304
Elastin-N* % total-N	0.4364	0.3499	0.2078	0.3836	0.1907	0.2774
Elastin ** elas-N x 5.85	0.2480	0.2322	0.1398	0.1574	0.1416	0.1778

Averages: * - 0.3076
** - 0.1821

Table 42

Elastin Content of Cooked Samples of the Longissimus Dorsi-rib, Animal IV, Aged Varying Periods of Time.

Sample no.	IIR 100c	IIR 101c	IIR 97c	IIR 102c	IIR 99c	IIR 98c
Storage-days	1	2	5	10	20	30
Dry wt.%	39.96	42.13	39.96	35.46	34.56	36.27
Elastin-N % of sample	0.0101	0.0171	0.0148	0.0122	0.0123	0.0093
Elastin-N % dry sample	0.0253	0.0406	0.0370	0.0344	0.0356	0.0256
Elastin-N* % total-N	0.2370	0.4100	0.3350	0.2657	0.2626	0.2152
Elastin ** elas-N x 5.85	0.1480	0.2375	0.2165	0.2012	0.2083	0.1498

Averages: * - 0.2876
** - 0.1936

Table 43

Elastin Content of Cooked Samples of the Longissimus Dorsi-loin, Animal IV, Aged Varying Periods of Time.

Sample no.	IIL 105c#	IIL 108c	IIL 107c	IIL 106c	IIL 104c	IIL 103c
Storage-days	1	2	5	10	20	30
Dry wt. %	26.59	38.97	36.36	35.59	35.17	36.22
Elastin-N % of sample	0.0152	0.0153	0.0177	0.0102	0.0188	0.0073
Elastin-N % dry sample	0.0572	0.0393	0.0487	0.0287	0.0535	0.0202
Elastin-N* % total-N	0.4414	0.3226	0.3765	0.2227	0.4222	0.5499
Elastin ** elas-N x 5.85	0.3346	0.2299	0.2849	0.1679	0.3130	0.1182

Averages: * - 0.3788
** - 0.2414

#Values for this sample were omitted from all calculations.

Table 44

Elastin Content of Cooked Samples of the Semitendinosus, Animal IV, Aged Varying Periods of Time.

Sample no.	III 111c	III 112c	III 109c	III 114c	III 113c	III 110c
Storage-days	1	2	5	10	20	30
Dry wt. %	36.57	33.69	35.41	33.30	36.27	33.97
Elastin-N % of sample	0.0576	0.0801	0.0816	0.0700	0.0715	0.0527
Elastin-N % dry sample	0.1575	0.2378	0.2304	0.2102	0.1971	0.1453
Elastin-N* % total-N	1.8098	1.9090	1.8261	1.4255	1.4018	1.1763
Elastin ** elas-N x 5.85	0.9214	1.3911	1.3478	1.2297	1.1530	0.8500

Averages: * - 1.5914
** - 1.1488

Table 45

Elastin Content of Cooked Samples of the Biceps Femoris, Animal IV, Aged Varying Periods of Time.

Sample no.	IV 115c	IV 117c	IV 116c	IV 119c	IV 120c	IV 118c
Storage-days	1	2	5	10	20	30
Dry wt. %	37.94	37.43	40.59	41.70	35.69	35.78
Elastin-N % of sample	0.0100	0.0071	0.0064	0.0072	0.0153	0.0134
Elastin-N % dry sample	0.0264	0.0190	0.0158	0.0173	0.0429	0.0375
Elastin-N* % total-N	0.1876	0.1269	0.1395	0.1551	0.3054	0.2739
Elastin ** elas-N x 5.85	0.1544	0.1112	0.0924	0.1012	0.2510	0.2194

Averages: * - 0.1981
** - 0.1549

If the values at all of the storage periods are averaged the values are lower than the elastin-nitrogen content of animal I in all cases except in the psoas major. This is contrary to popular opinion, because carcasses of lower grade are usually considered to be of higher connective tissue content, including both collagen and elastin. However, Mitchell and others (78) found no correlation between grade of animal and elastin content in analyzing carcasses of various grades. In his study the longissimus dorsi of an old cow, graded common, which according to United States standards was next to the lowest grade acceptable for edible meat, contained the same amount of elastin-nitrogen figured as per cent of total-nitrogen (0.02) as that of a choice steer. A fourteen- or fifteen-year-old cow, graded canner, the lowest grade of all, gave for the elastin content of the same muscle a value of only 0.04 per cent of the total-nitrogen.

Summary of the collagen and elastin data on animal I and animal IV

In table 46 the averages of the six determinations of collagen- and elastin-nitrogen as per cent of total-nitrogen for uncooked and cooked samples of both animals are summarized. The observations made concerning the averages of individual determinations can easily be followed in these columns:

(1) There is a progressive increase in collagen- and elastin-nitrogen as per cent of total-nitrogen in the muscles as follows: the psoas major, least; the longissimus dorsi and the biceps femoris almost equal; and the semitendinosus with the highest value. This increase is verified by the statistical analysis as shown in tables 47 and 48.

(2) Animal IV (cutter grade) had a higher collagen-nitrogen content and a lower elastin-nitrogen content than animal I (good grade). That this difference in collagen is significant may be seen by examining the statistical analysis in tables 47 and 48. The significance of the difference in elastin content, however, as shown statistically, is almost all due to difference in one muscle, the semitendinosus.

(3) There was a consistent decrease in collagen and elastin in the cooked samples except for the collagen-nitrogen values of the three tougher muscles of animal IV. The statistical analysis showed that this decrease was very little greater than the experimental error, however, and is not significant at the 5 per cent level.

(4) In addition, there is the lack of any relation between the collagen or elastin content in any of the muscles of either animal and the length of storage at 34° to 36°F., which is shown consistently throughout tables in this section. In tables 47 and 48 statistical verification of this will be found.

Table 46

Average Collagen-nitrogen and Elastin-nitrogen Content of Four Beef Muscles from Animals I and IV.

Muscle	Uncooked Collagen-N per cent of total nitrogen	Cooked Collagen-N per cent of total nitrogen	Uncooked Elastin-N per cent of total nitrogen	Cooked Elastin-N per cent of total nitrogen
Animal I				
I (psoas major)	1.53	1.63	0.288	0.508
IIR (longissimus dorsi-ribs)	2.59	2.04	0.337	0.501
IIL (longissimus dorsi-loin)	2.73	2.42	0.512	0.476
III (semitendinosus)	4.39	3.56	4.39	4.16
IV (biceps femoris)	2.23	2.22	0.531	0.482
Average (four muscles listed)	2.694	2.374	1.212	1.532
Animal IV				
I (psoas major)	1.8740	1.2152	0.4120	0.3076
IIR (longissimus dorsi-ribs)	4.6909	3.5985	0.3113	0.2876
IIR (longissimus dorsi-loin)	4.6568	7.9678	0.4752	0.3788
III (semitendinosus)	5.8985	10.3517	1.6493	1.5914
IV (biceps femoris)	4.5995	5.8548	0.4749	0.1981
Average (four muscles listed)	4.3439	5.7976	0.6645	0.5527
	3.51	4.08	0.938	1.042

The data contained in table 46 are shown in graphical form in figures 5, 6, 7, and 8. The irregularity of the distribution of the values in each group is apparent in both the collagen and the elastin portions of all four graphs, which include the results from uncooked and cooked samples of both animals. The position of the horizontal line which runs through the group of dots which denotes the value at a particular storage period indicates the average content of collagen or elastin in that muscle. The relative amounts of collagen and elastin in the various muscles of both animals may be readily appraised by noting the position of this line. The large increase in collagen content along with a decrease in elastin content found in animal IV, especially in the semitendinosus, is very evident if figures 5 and 6 are compared with figures 7 and 8, and the relative positions of these horizontal lines noted.

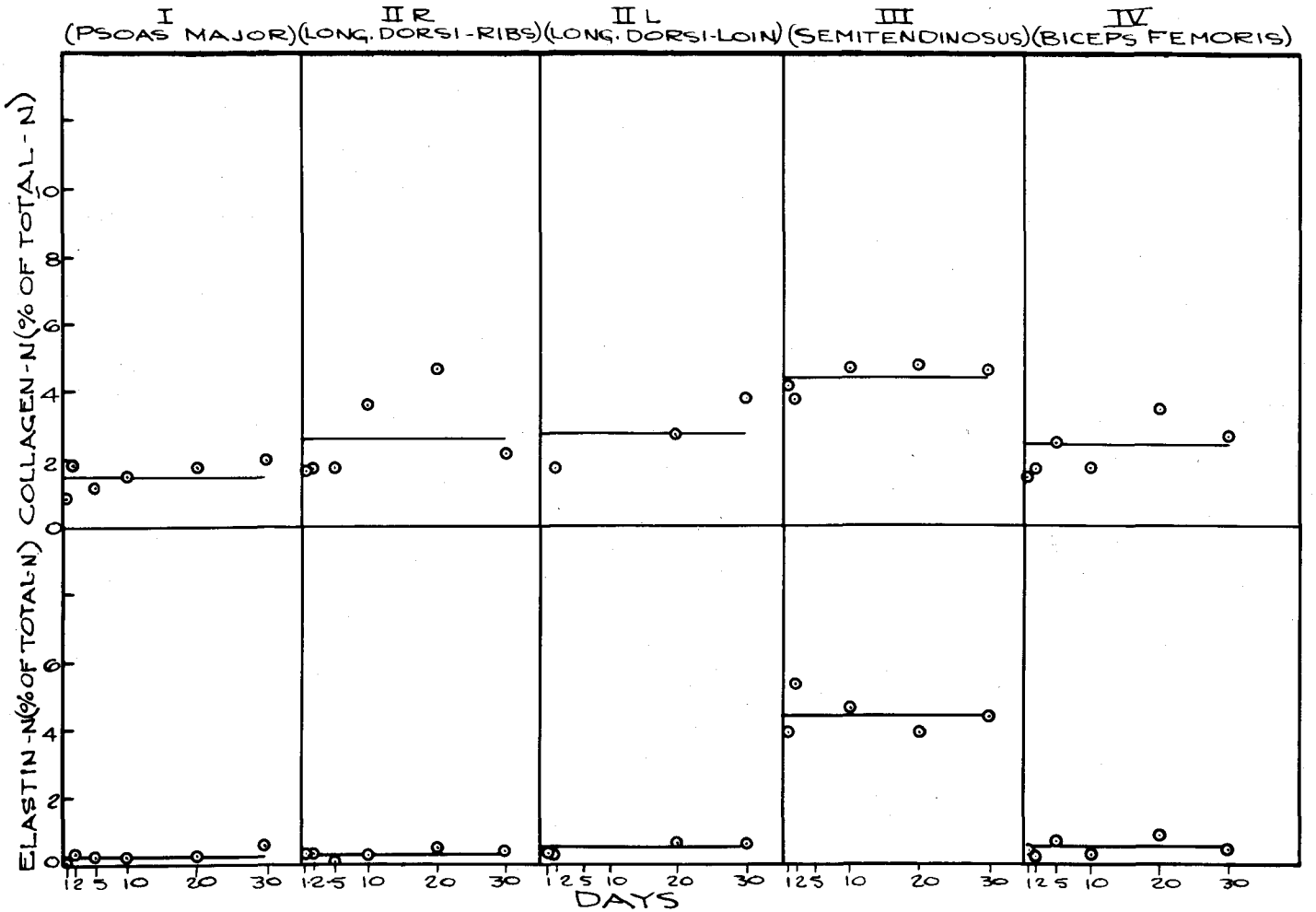


FIG. 5 COLLAGEN AND ELASTIN CONTENT OF UNCOOKED SAMPLES OF FOUR BEEF MUSCLES (ANIMAL I) *(The horizontal line represents the average of values plotted)*

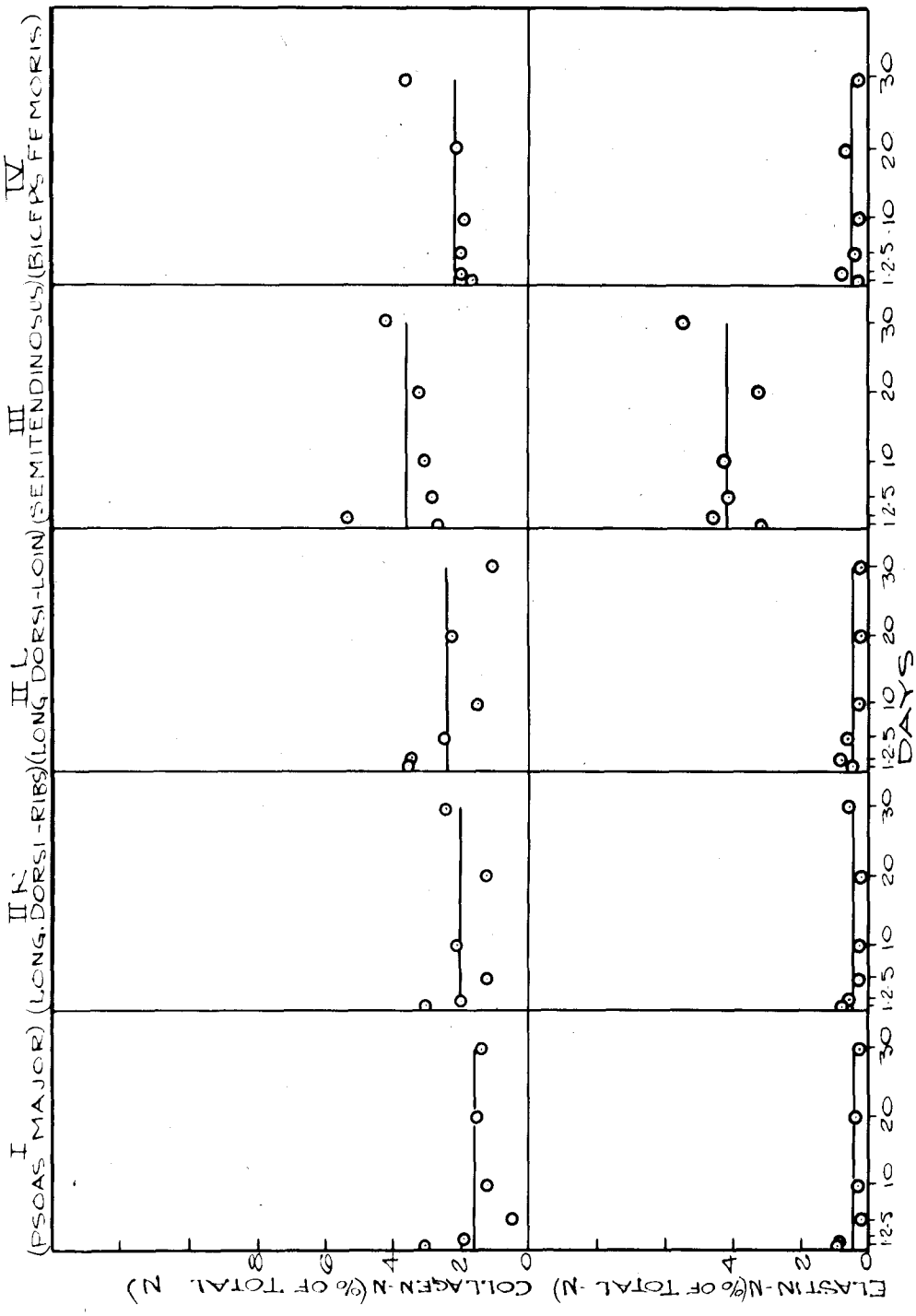


FIG. 6 COLLAGEN AND ELASTIN CONTENT OF COOKED SAMPLES OF FOUR BEEF MUSCLES (ANIMAL I) *(The horizontal line represents the average of values plotted)*

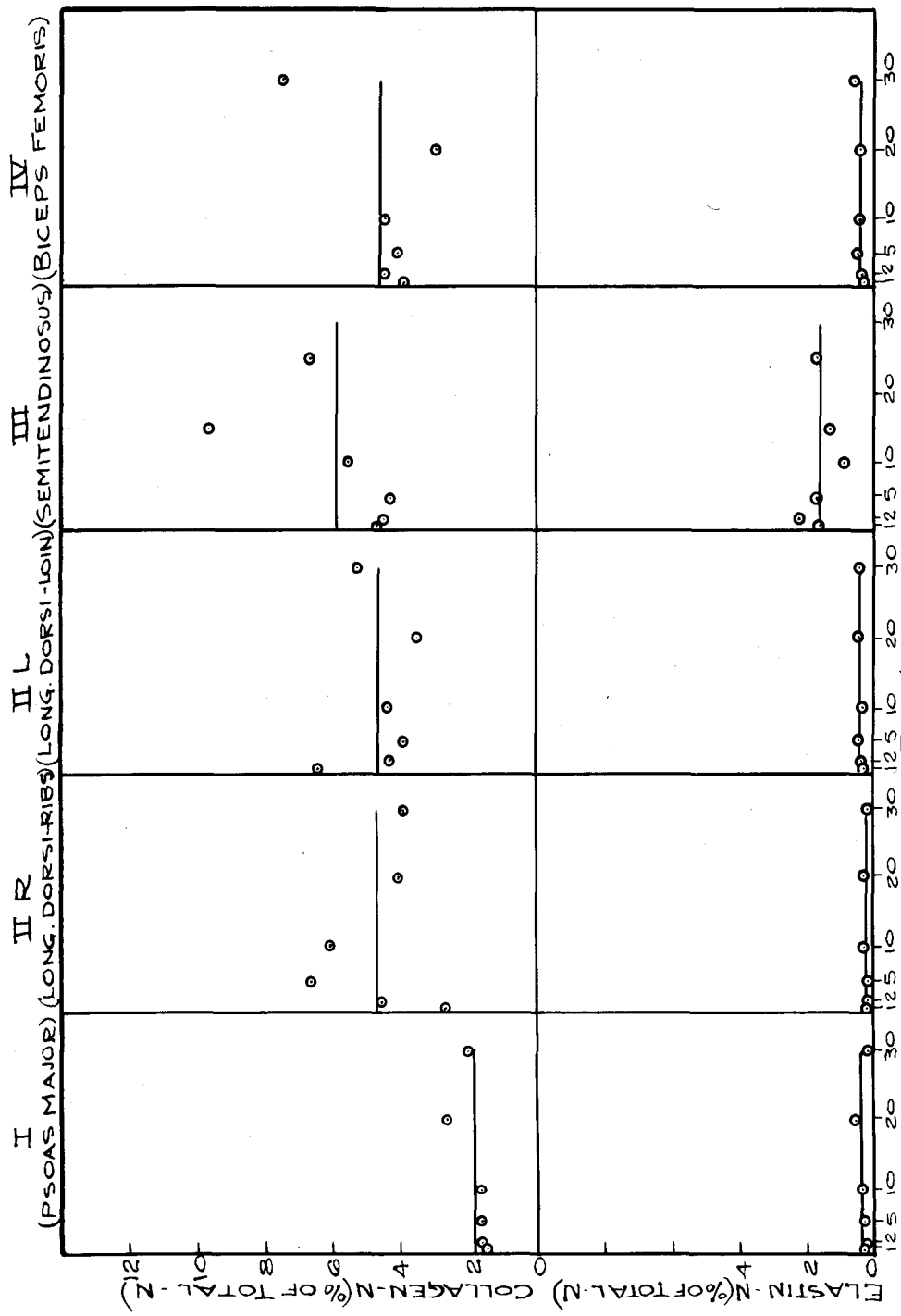


FIG. 7 COLLAGEN AND ELASTIN CONTENT OF UNCOOKED SAMPLES OF FOUR BEEF MUSCLES (ANIMAL IV) *(The horizontal line represents the average of values plotted)*

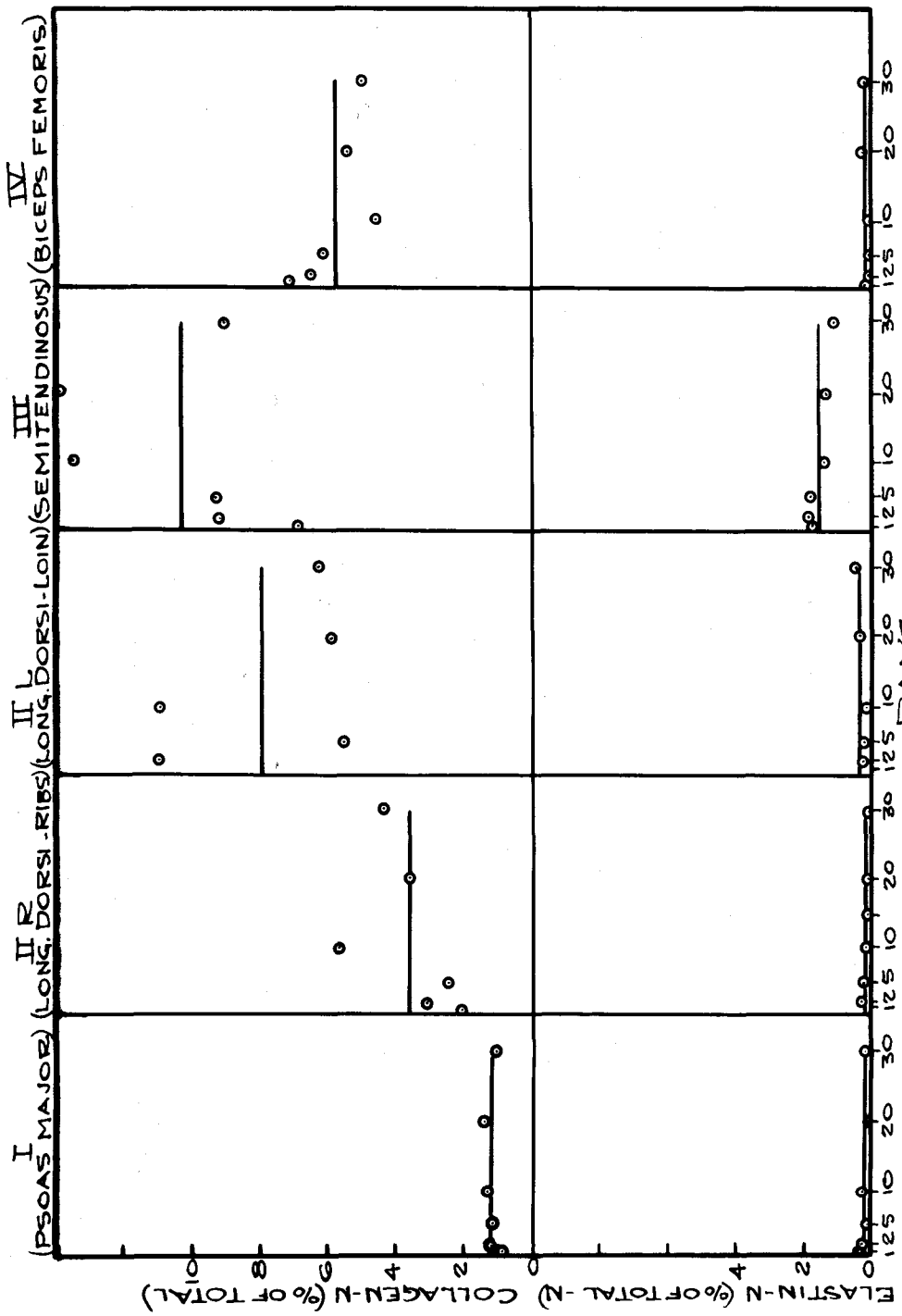


FIG. 8 COLLAGEN AND ELASTIN CONTENT OF COOKED SAMPLES OF FOUR BEEF MUSCLES (ANIMAL IV) (*The horizontal line represents the average of values plotted*)

Table 47

Analysis of Variance of Collagen Content of Four Muscles of Animal I and Animal IV.

Variation	Degrees of freedom	Sum of squares	Mean squares
Total	119	788.806405	
Animals	1	195.141	195.141**
Muscles	4	254.88	63.721**
Dates	5	12.89	2.577
Dates x muscles	20	40.56	2.028
Error (a)	29	114.76	3.957
Cooking	1	10.11	10.108
Cooking x muscles	4	31.15	7.788
Cooking x dates	5	11.24	2.249
Cooking x dates x muscles	20	22.201	1.110
Error (b)	30	96.86	3.229

** Significant at P = .01.

Table 48

Analysis of Variance of Elastin Content of Four Muscles of Animal I and Animal IV.

Variation	Degrees of freedom	Sum of squares	Mean square
Cells	59	172.62	
Animals	1	11.03	11.029**
Muscles	4	124.25	31.062**
Dates	5	.79	.158
Dates x muscles	20	2.67	.134
Error	29a	33.88	1.168

** Significant at P = .01.

Table 49

Analysis of Variance of Soluble-nitrogen Content of Four Muscles of Animal I and Animal IV.

Variation	Degrees of freedom	Sum of squares	Mean square
Total	119	1286.10	
Animals	1	126.38	126.38**
Muscles	4	653.88	163.47**
Dates	5	8.98	1.80
Dates x muscles	20	86.40	4.32
Error (a)	29	158.07	5.45
Cooking	1	12.56	12.56
Cooking x muscles	4	45.07	11.27
Cooking x dates	5	28.58	5.72
Cooking x dates x muscles	20	47.10	2.36
Error (a)	30	119.09	3.97

** Significant at P = .01.

Dorothy Harrison in her histological studies on these muscles used an arbitrary rating scale for scoring muscles, according to the amount of collagen and elastin which was evident in the stained sections she observed. Her scoring is shown in Table 1, and the basis used is listed below the table. The scores may be compared with the average collagen and elastin content of the muscles as shown in table 46. The two tables are in agreement in these things: (1) that the psoas major has the least amount of collagen and elastin, the semitendinosus the largest amount; (2) that the longissimus dorsi-ribs, the longissimus dorsi-loin, and the biceps femoris are roughly equivalent in the amounts of these components of connective tissue; (3) that animal IV has consistently a higher collagen content than animal I. The point of difference is that although animal IV gave histological evidence of having more elastin, it actually had less by chemical determination.

Total nitrogen content of four beef muscles, uncooked and cooked, of animal I and animal IV

The values obtained for total-nitrogen for all the muscles of both animals calculated by adding the nitrogen content of the elastin fraction, the collagen fraction, and the soluble-nitrogen fraction are given in table 50. These values were used in computing the collagen-nitrogen and elastin-nitrogen as per cent of total-nitrogen, as referred to previously in this section.

Table 50

Total Nitrogen Content of Four Beef Muscles, Uncooked and Cooked, of Animals I and IV, Determined by Addition of the Elastin-nitrogen, the Collagen-nitrogen, and the Soluble-nitrogen Content.

Animal and Muscle no.	Storage in days					
	1	2	5	10	20	30
	%	%	%	%	%	%
I, I-uncooked	3.0521	3.0946	3.3944	3.5342	3.5118	3.8744
IV, Iu	3.1522	2.8378	3.2095	3.1266	3.6073	3.1308
I, IIRu	3.2236	3.2867	3.6685	3.4538	3.8751	4.2899
IV, IIRu	3.2710	3.5119	3.5291	3.6837	3.4749	4.0136
I, IILu	3.4685	3.3674	----	----	3.9725	3.7597
IV, IILu	3.2771	3.4049	3.4404	3.5946	3.6715	3.7223
I, IIIu	3.249	3.399	----	3.741	4.109	3.820
IV, IIIu	3.5132	3.2871	3.4881	3.5939	3.9995	4.2104
I, IVu	3.4537	3.4061	3.8978	3.6910	4.0732	3.911
IV, IVu	3.4784	3.4963	3.6692	3.9260	3.8011	3.9483
I, I-cooked	3.5243	4.2525	4.7146	4.2345	4.2539	4.6068
IV, Ic	4.1242	4.1156	3.9939	3.4415	4.0910	4.0741
I, IIRc	3.9765	4.1635	4.1210	4.2115	4.5203	4.5600
IV, IIRc	4.2609	4.1706	4.4184	4.5917	4.6832	4.3214
I, IILc	4.536	4.1805	3.9006	3.8576	4.6113	4.5151
IV, IILc	3.4435	4.7431	4.7018	4.5801	4.4527	4.7101
I, IIIc	4.820	4.210	4.681	4.257	4.554	4.563
IV, IIIc	4.8773	4.1959	4.4685	4.9106	5.1005	4.4803
I, IVc	4.3418	4.8668	4.4839	4.301	5.1496	4.5315
IV, IVc	5.3292	5.5935	4.5869	4.6421	5.0102	4.8917

The effect of cooking on the collagen-nitrogen, elastin-nitrogen, and soluble-nitrogen content of four beef muscles of animal I and animal IV.

The effect of cooking, as described in the preceding section, on the collagen-nitrogen and elastin-nitrogen is shown in table 51.

Table 51

The Effect of Cooking on the Collagen-nitrogen, Elastin-nitrogen, and Soluble-nitrogen Content of Four Beef Muscles of Animals I and IV.

Animal and Muscle no.		Per cent of total-nitrogen					
		Collagen-N	Difference	Elastin-N	Difference	Soluble-N	Difference
I I	uncooked	1.53		0.288		98.1885	
	cooked	1.63		0.508		97.8600	
			+0.10		+0.220		-0.3285
I IIR	uncooked	2.59		0.337		96.9387	
	cooked	2.04		0.501		97.4603	
			-0.55		+0.164		+0.5216
I IIL	uncooked	2.73		0.512		96.7237	
	cooked	2.42		0.476		97.0986	
			-0.31		-0.036		+0.3749
I III	uncooked	4.39		4.39		91.2169	
	cooked	3.56		4.16		92.2806	
			-0.83		-0.023		+1.0637
I IV	uncooked	2.23		0.531		96.5976	
	cooked	2.22		0.482		97.3002	
			-0.01		-0.049		+0.7026
IV I	uncooked	1.87		0.412		97.714	
	cooked	1.22		0.308		98.4772	
			-0.65		-0.104		+0.7632
IV IIR	uncooked	4.69		0.311		94.9979	
	cooked	3.60		0.288		96.1133	
			-1.09		-0.023		+1.1154
IV IIL	uncooked	4.66		0.475		94.8680	
	cooked	7.97		0.379		91.7324	
			+3.31		-0.096		-3.1356
IV III	uncooked	5.90		1.65		92.4603	
	cooked	10.35		1.59		88.1617	
			+4.45		-0.06		-4.2986
IV IV	uncooked	4.60		4.75		94.9257	
	cooked	5.85		0.198		93.9471	
			+1.25		-0.277		-0.9786

It will be noted that in general there is a slight decrease in value. Exceptions occur in the cases of the collagen-nitrogen of the three less tender muscles in animal IV and the psoas major of animal I, where a very small increase occurred in both collagen- and elastin-nitrogen. The longissimus dorsi-ribs, animal I, showed an increase in elastin-nitrogen only. Conversely, there is to be seen an increase in the soluble-nitrogen except in the psoas major, animal I, and the longissimus dorsi-loin, the semitendinosus, and the biceps femoris of animal IV.

DISCUSSION OF RESULTS

The data just presented have shown two facts with significant implications: one is that the degradation of collagen, as far as may be determined by a chemical method, is not responsible for the tenderization of beef as aging progresses; the other is that the elastin content is not related to the age, grade, or toughness of the carcass.

It has long been assumed, as was previously pointed out, that beef becomes more tender upon storage or "aging" because the connective tissue, or more particularly collagen, was partially degraded by acid or enzyme action to gelatin or similar products as the aging progressed. The results presented in the previous section show that this degradation does not take place, and that the collagen and elastin content of the tissues are unchanged chemically. That some change has taken place is shown by the change in tenderness to shear and to organoleptic tests. The explanation of this change must be sought in physical rather than chemical alterations. It may be found that changes causing or accompanying changes in pH which occur with the resolution of rigor mortis and the progress of aging bring about swelling or hydration of the collagen molecule and thus weaken the resistance of the tissue to a shearing force, such as biting.

Not much is known about the hydration of collagen, although the relationship between gelatin and water has been widely studied. Since collagen so closely resembles gelatin, being what Bogue calls a "polarization complex produced by chemical condensation," it seems reasonable to expect chemical behavior in regard to hydration similar to that shown by gelatin. The analogy in the behavior of the two proteins might be valid in regard to the character of the reaction but not necessarily in the degree or speed with which it progresses. If it is true that the hydration of collagen resembles that of gelatin, then as the pH rises, polar groups are liberated and so are enabled to bind water molecules, thus inducing swelling and weakening in the fiber.

If the suggestion that a physical change involving greater hydration of the collagen molecule brought about by a change in pH or other environmental factors should prove to be the explanation for the tenderization brought about by aging, then control of pH or the other responsible environmental factors would be one of the keys to the production of tender beef.

Since collagenous tissue in the skin, joints, and arteries is the tissue most affected by aging and certain other degenerative processes in the living animal, an explanation of the changes in hydration to which collagen is subject, and the conditions upon which these changes depend,

would be of great value. It has long been recognized that one of the most characteristic symptoms of age is dehydration and toughening of collagenous tissue, which might well be the reverse of the "aging" process undergone by beef carcasses subsequent to the resolution of rigor mortis.

Another entirely different kind of implication of the study just reported concerns tests for tenderness in meat. Food technologists are constantly in search of more reliable or convenient tests for measuring qualities of foods they are studying. The possibility of using histological sections stained to render the collagen and elastin visible has been suggested. Dorothy Harrison used slides so stained that she was able to use an arbitrary rating scale to estimate roughly the amount of collagen and elastin present. The validity of this approximate determination of the amount of collagen and elastin in animal tissue depends upon the obtaining of a representative sample of the tissue to be assessed. This becomes more and more difficult as the size of the portion of tissue being examined becomes smaller, as in a histological section. The examination of many sections reduces this hazard, but is often prohibitive as far as time and cost is concerned. In this study, Miss Harrison's approximate ratings agreed with the chemical findings except in the elastin content of animal IV, which was rated very high in elastin according to the histological rating scale, but tested lower than animal I in elastin by chemical methods.

SUMMARY

Collagen and elastin determinations were made on samples from four muscles of two beef animals: one a steer which was graded good by United States standards, and the other an eight-year-old dairy cow graded cutter, or next to the lowest grade by these same standards. The roasts were stored 1, 2, 5, 10, 20, or 30 days at 34° to 36°F. in the college meat cooler. At the end of the storage period, samples of the roasts both cooked and uncooked were kept at -30°F. until chemical analysis was made. Because samples were necessarily small, micro-methods were employed. The method used was a modification of the Lowry, Gilligan, and Katersky method (80) so that micro-Kjeldahl determinations of nitrogen content might be used to obtain results, rather than the gravimetric procedure.

Giving due consideration to the limitations of the method, evidence for the following conclusions has been presented:

1. That the length of the storage period under conditions used in this study has little if any effect on the collagen or elastin content of muscle. Hence the explanation of the increase in tenderness of muscles during aging must be sought in factors other than the chemical degradation of collagen and elastin.

2. The muscles studied vary in both collagen and elastin content. The psoas major has the least amount of both collagen and elastin; the semitendinosus has the largest amount; and the longissimus dorsi and the biceps femoris are intermediate and roughly equivalent in the quantity of both of these proteins they contain.

3. The amount of collagen and elastin in a muscle or in an animal cannot always be correlated with the grade, according to United States standards. For example, animal IV of an inferior grade contained less elastin than animal I of good grade.

4. Increases in elastin do not always accompany increases in collagen or in apparent toughness. For example, animal IV had a high collagen content and high toughness rating, but a relatively low elastin content.

5. Cooking in fat at 96° to 98°C. to an internal temperature of 70°C. changes the collagen and elastin content of these four muscles very little, if at all.

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