Contributions of Collagen to the Properties of Comminuted and Restructured Meat Products

Richard C. Whiting*

Introduction

Collagen plays a major role in the texture of meat and meat products. In intact muscle, it provides the background toughness and the texture of the fiber bundles. In meat products, its effect depends on the degree of comminution and extent of gelatinization during cooking. It also contributes to the nutritional value, flavor and succulence of meats. Extracted collagen forms or controls the textures of gelatin desserts and frozen dairy products. Collagen from various sources, such as bone protein, is beginning to be used as an ingredient to improve water and fat retention in meat products or to create new products (Webster et al., 1982; Jobling, 1984). This paper focuses on how collagen influences various textural characteristics of fresh, comminuted and restructured meats.

Collagen in Muscle

Collagen usually comprises 1% to 2% wet weight of bovine skeletal muscles but it can comprise 4% to 6% wet weight (or more) in high connective tissue muscles (Casey et al., 1985; Wiley et al., 1979; Lawrie, 1985; Bailey, 1984). Bovine epimysial tissue contains 24% collagen (wet weight) and gristle 27% collagen (E.D. Strange, unpublished data).

The types of collagen in muscle and their chemical structures have been described in detail (Aberle and Mills, 1983; Bailey, 1984, 1987, 1989; Jones, 1984; Pearson et al., 1987). Epimysium contains primarily type I collagen, the perimysium types III and I, and the endomysium type IV with types III and V. At least seven other genetically distinct collagen types have been identified in various tissues. Their locations, native functions and properties are less well understood.

Older animals have increasing numbers of insoluble collagen cross-links from oxidative deamination and reduction of specific lysine and hydroxylysine residues in the short nonhelical regions (Bailey, 1984). Insoluble cross-links reduce collagen solubility, raise conformational transition temperatures and increase meat toughness. Species, age and dietary and husbandry factors affect the quantity and extent of cross-linking (McCormick, 1989).

How collagen changes during post-mortem aging remains unclear (Gillett, 1987a; Etherington, 1987; Stanton and Light, 1988). Some research demonstrated an increase in collagen solubilization upon aging. The collagen fibers appear to swell and gelatinize at lower temperatures when subsequently heated. The practical significance of these changes is unclear.

Effect of Heating

Changes that myofibrillar proteins and collagen undergo during heating have a major impact on the textural characteristics of the meat or meat product. The rate of temperature increase, duration of heating, moisture content and highest temperature reached during the processing affect the transformations of collagen. These changes in myofibrillar and collagen proteins during cooking are summarized as follows (Bailey, 1984; Gillet, 1987a,b): 40°C-minimum toughness of myofibrillar proteins and maximum toughness from connective tissues; 50°C-contractile and sarcoplasmic protein denature, coagulate, shrink transversely, express intracellular water, become insoluble and increase their contribution to the total shear force; 60°C-collagen shrinks, shear force begins to decrease; 70°C-myofibrillar proteins shrink longitudinally and collagen begins to lose its helicity and to solubilize; 74°C-maximum hardening of the myofibrillar proteins; 75° to 80°C-the collagen melts, eventually losing all structure and strength, a process called gelatinization. The rate of gelatinization increases with temperature, occurring very rapidly at 125°C. A collagen sol does not gel until the temperature decreases to 23° to 33°C. The gel completely remelts at 45° to 50°C.

Muscle texture is affected by the fiber bundles. Observations of the tearing when a piece of cooked muscle is pulled apart show small fractures in the perimysium (Purslow, 1987). The macroscopic failure of the piece of meat is a linking of these fractures. Disrupted connective tissue strands remain on the surface of the tear. Propagation of the tear is relatively easy between the fibers, but it is much more difficult to tear the myofiber. Tensile strength of the perimysial connective tissues is only 20-30 kN/m^2, while tensile strength of the myofibers is 300-400 kN/m^2. The overall meat structure, therefore, readily separates into fiber bundles.

Moller (1981) demonstrated a bimodal Warner-Bratzler shear force pattern from myofibrillar proteins and connective tissues that changes with cooking. After heating cores of beef *semitendinosus* to 60°C, the initial shear force peak from the myofibrillar proteins was 4.27 kg. The second and higher peak from the connective tissue was 5.66 kg. After heating to 80°C, the myofibrillar shear force was greater (6.52 kg) and the connective tissue peak was reduced (5.30 kg). A slower heating rate of 0.12°C/min compared to 0.60°C/min reduced the shear force of both peaks. The myofibrillar peak was

*R.C. Whiting, U.S. Department of Agriculture, Agricultural Research Service, Eastern Regional Research Center, 600 E. Mermaid Lane, Philadelphia, PA 19118

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affected by both the heating rate to 60°C and between 60° and 80°C, while the connective tissue peak was affected by the heating rate only when above 60°C.

Comminuted Products

Meat products may be classified by several criteria. For this discussion, a useful classification criterion is particle size. At one extreme are comminuted meats where chopping reduces the connective tissue to undetectable sizes. At the other extreme are restructured beef products with large pieces of meat and intact connective tissues.

Comminuted meat products frequently contain meats (shanks) and by-products (tripe, snouts, etc.) that have high levels of connective tissues. Collagen does not bind proteins well and "dilutes" the beneficial effects of the myofibrillar proteins. Although collagen is not dissolved in a meat batter, fat particles become coated by myosin and collagen proteins during comminution. The viscosity of the batter is increased by the collagen fibers. At this point, the collagen fibers may help to stabilize the batter.

Above 65°C, the collagen triple helix begins to unfold into single strands. During continued heating, the collagen unravels and begins to dissolve, releasing the fat droplets, expelling water and forming a gelatin sol. Because of solubilized collagen's high hydrophobicity, these strands can adsorb onto the liquid fat. Since the batter has already formed the myosin gel, these changes in the collagen probably disrupt the gel more than stabilize it (Gillet, 1987b). Upon cooling, the gelatin sol consolidates and solidifies into gel pockets.

Collagen content, therefore, affects a meat product's yield, texture and stability (Jones, 1984). At low levels, collagen may be advantageous in stabilizing shrinkage and improving texture. Although all studies do not agree, added collagen generally increases the firmness and perhaps juiciness of frankfurters. But higher quantities eventually cause surface gelatin, rendered fat, poor peelability, gel pockets, outer skin wrinkling, shrivelling or bowing shrinkage and a grainy, brittle or crumbly texture (Gillet, 1987b). Textural defects are particularly evident when products are cooked to temperatures above 65°C (Kramlich et al., 1973).

Wiley et al. (1979) asserted a "rule of thumb" that the quality declines when the formulation contains more than 15% high-collagen meat. Kramlich et al. (1973) contended that the amount of high-collagen meats in a formula should be less than 25% to avoid gel pockets. Muller and Wagner (1985) reported the maximum addition of rind and sinew should be 5%. Jones (1984) described a study where five levels of tripe (0% to 40%) were added to bologna to give collagen contents from 1.0% to 3.3%. The higher tripe levels resulted in increased gel-water and fat losses. Smokehouse yields were reduced only in bologna having 40% tripe. Instron compression testing of these bolognas showed reduced hardness and chewiness values with increasing amounts of tripe. In contrast, no adverse changes in emulsion stability, cook yield and expressible juice upon cooking were found in bolognas made with 20% of the lean meat replaced by wet fibrous hide collagen compared to the all-meat controls (Rao and Henrickson, 1983). In general, it appears that the critical collagen level is about 3% of the formulation; higher amounts risk failure.

Many factors contribute to the limitations on the maximum amount of collagen a product can contain without an unacceptable quality decline. These include ionic strength in the batter, pH, fat level, comminution method, vacuum cutting and processing conditions (cooking rate and humidity) (Jones, 1984). The detrimental effect of excess collagen may be partially offset by raising the pH, increasing the ionic strength or adding phosphate (Sadowska et al., 1983). The pH and ionic strength do not directly affect the collagen (Puolanne and Ruusunen, 1981), but may improve the batter-forming ability of the myofibrillar protein.

A finer chop makes the product more sensitive to the disruptive influence of collagen. Rao et al. (1982) found that a finely-chopped bologna could be extended with 12.8% fibrous hide collagen. Coarsely-chopped bologna could contain 30% fibrous hide collagen.

Keeping chopping temperatures low and avoiding excessive chopping help retain quality (Abrosiadis and Wirth, 1984). The lean meat portion of the batter should not be chopped to temperatures above 2°C or 5°C if phosphates are used. Final chopping temperatures should not exceed 12° to 15°C or 16° to 18°C, respectively.

Cooking with too rapid a temperature rise or too high a relative humidity (RH) causes instability. Gillett (1987b) reported that beef frankfurters that have a tendency to grease-out should not be cooked with more than 30% to 35% RH. Pork can tolerate 40% and poultry 50% to 60% RH. High-collagen liver sausages should not be cooked at temperatures above 74°C or to internal temperatures above 65.5°C (Gillett, 1987b). Exceeding these temperatures increases the time that the collagen is above the transition temperature for unfolding. If the liver sausage emulsion is initially very cold, the outer portions will be over-cooked before the center reaches the final temperature. This causes formation of a gelatin ring in the outer portions of the product (Gillett, 1987b). Another type of failure occurs in large-diameter sausages when rapidly heating a very cold batter. The myosin gel sets up in the outer portions of the product before the collagen swells inside, splitting the product (Gillett, 1987b).

Addition of collagen can be used to reduce the rubberiness of low-fat frankfurters (Jones, 1984). Another beneficial use is to reduce the drip in cold cuts where the absence of reheating avoids the melting and separating of gelatin (Puolanne and Ruusunen, 1981). Increasing the collagen content lightens the product's redness by diluting the muscle's myoglobin (Rao and Henrickson, 1983; Jones, 1984; Chavez et al., 1986).

Precooked collagen from skin or other sources can greatly increase the viscosity of the meat homogenates. Heating hide collagen for 20 min at 60°C and then adding it at 2.5% to meat homogenates improves the rheological properties (Sadowska et al., 1980). The authors pointed out that gelatin and denatured collagen are strong water binders. They speculated there may also be collagen-myofibrillar interactions. As mentioned earlier, native collagen in a meat batter forms gelatin too late in the processing to be integrated into the gel structure. Instead, the collagen disrupts the homogeneity of the gel by forming gelatin islets. With pre-cooking, the collagen is solubilized at the beginning of the chop and contrib-

utes functionality to the batter. However, Muller and Wagner (1985) and Sadowska (1987) reported pre-cooking collagen had a detrimental effect on sensory quality. This technique needs further research to determine how to improve the solubilization process and utilize it beneficially. Edible bone collagen is a similar ingredient. It stiffens the texture of mechanically-recovered meats and reduces fat and water losses in a variety of meat products (Jobling, 1984).

Ground Meat

Hamburger is an intermediate particle-size product. Chavez et al., (1986) extended hamburgers with up to 20% bovine hide collagen. Added collagen improved bound moisture and sensory juiciness but decreased flavor, texture and overall acceptability.

Desinewing effectively removes a major portion of the epimysium and perimysium connective tissue from meats destined for comminuted or ground meat products (Gillett, 1987a). Cross et al. (1978) used the desinewing adaptation of the Beehive mechanical deboning machine to remove about half of the collagen. Patties from desinewed meats were usually rated as tenderer and lower in connective tissue. The degree of doneness, cooking losses and juiciness were not affected. The greatest improvement in sensory characteristics was with cuts from older carcasses and with smaller holes in the deboning machine. However, Wells et al. (1980) compared desinewed with ground mature bull and cow meats. They found that grinding was more beneficial than desinewing for improving palatability and reducing shear forces, even though desinewing removed 28% to 41% of the collagen.

Salami made from meat with approximately half the connective tissue removed by the Beehive deboner did not break down, separate or form jelly pockets as did the salami from the non-desinewed meat (Gillett et al., 1976). The salami from desinewed meat had better tenderness and texture. Addition of 0.75% polyphosphate and reduction of water content prevented gel pocket formation in the non-desinewed products.

Restructured Beef Products

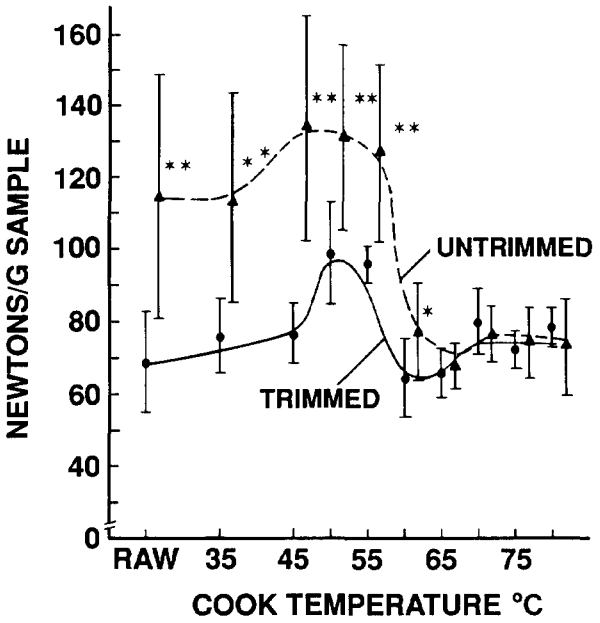
Restructured beef products are more "steak-like" when made with large pieces of meat (sectioned-and-formed or chunked-and-formed products). Flaked-and-formed restructured beef products made from meat cut with the smaller-sized Commitrol heads do not have objectionable connective tissue, but they do not have a "steak-like" texture (Huffman and Cordray, 1982). Field et al. (1969) found that the epimysium was a problem in flaked meat cut over 1 mm thick. However, some connective tissue improves the bite in many meat products. Berry (1987) concluded that smaller filaments add beneficial aspects to bind and texture.

Lower-priced beef cuts have extensive gristly and epimysial connective tissue sheets that remain substantially intact in sectioned-and-formed restructured products. Consumers object to the residue of connective tissue that remains in the mouth after mastication (Secrist, 1987). The distribution of connective tissue is very heterogeneous in this product. Perception of connective tissue is not necessarily directly related to the total amount of connective tissue. The heterogeneous distribution also causes considerable difficulties for analytical, instrumental and sensory panel analyses.

Some commercial success is being realized with restructured beef products intended for moist-heat cooking where the collagen is solubilized (Swiss steaks, pot-roasts and stews). Restructured poultry and pork products are commercially successful, in part, because their connective tissue is not as objectionable.

Strange and Whiting (1988) showed that connective tissue was quantified better by shear force measurements on uncooked than on cooked restructured steaks. They heated restructured beef steaks made from trimmed or untrimmed meat in a water bath for 1 hour at varying temperatures and measured the shear force values, using the multiple-blade Lee-Kramer cell (Fig. 1). Unheated steaks made from

Figure 1



Relationship between Kramer multiblade shear forces and cooking temperature of restructured meat products made from trimmed and untrimmed beef clods. Bars of graph are standard deviations; ** $p<0.01$ and * $p<0.05$ for Student's of shear force values between trimmed and untrimmed samples cooked at temperature shown. From Strange and Whiting, 1988.

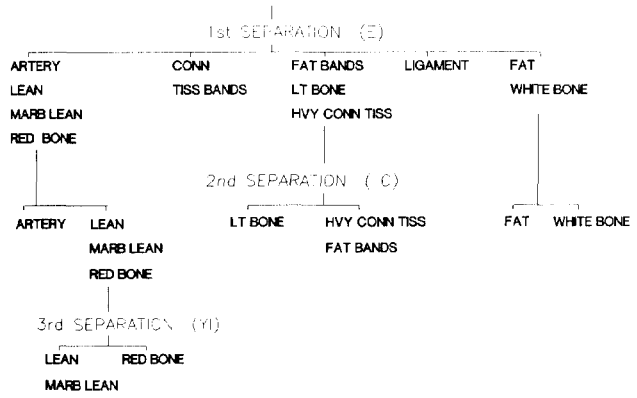
untrimmed muscles had greater shear force values than those made from trimmed muscles because of the high amounts of epimysium. The increase in myofibrillar toughening upon heating was clearly evident. Solubilization of collagen at 60°C was clearly evident in both products. The observed temperature of solubilization was lower than that usually reported, probably because of the relatively long, moist cooking. After removal of the collagen toughness (>70°C), both steaks had the same shear force. Sensory panels on similar restructured steaks cooked to 70°C on a Farberware grill showed that the panelists could distinguish the connective tissues even though the shear test on cooked

meat could not (Strange and Whiting, 1989). These shear force patterns are consistent with the results of Moller (1981) and demonstrate the need to select carefully the proper cooking regime and analytical method for the research being done.

Hand trimming the connective tissue from beef shoulder clods to make restructured steaks with 2.28% (untrimmed control), 1.95%, or 1.46% collagen significantly lowers the shear values and raises panel scores compared to product made from the untrimmed meat (Recio et al., 1986). They felt that the intermediate level of trimming would be practically and economically feasible. Berry et al. (1986) made steaks from USDA Choice chucks trimmed to yield 0.9%, 1.3% and 1.7% collagen. The shear force values were 13.7, 16.0 and 21.7 kg, respectively. Texture panelists found more gristle and webbed tissue in the latter steaks. These were also more distorted and fibrous after cooking with greater hardness and cohesiveness. Berry et al. (1988) conducted a consumer test on restructured steaks containing 0.9%, 1.3% and 1.6% total collagen. A marked increase in the number of responses of high and excess gristle occurred with the highest level of collagen. Strange and Whiting (1989) hand trimmed the tendon, epimysium and gristle from chuck muscles and then added known amounts back into the products (Table 1). With steaks having a range of collagen from 0.7% to 3.6%, significant correlations were found between collagen levels and shear force values ($r = 0.9$) or sensory evaluations of residual connective tissue ($r = -0.8$). An increase of 1.0% collagen increased the shear force 13 N/g (from 62 N/g in trimmed controls) and decreased the sensory connective tissue score by 1.1 units on a 10-centimeter unstructured scale. Tendon was objectionable at lower levels. They also made steaks from slices sorted to contain gristle, epimysium or neither connective tissue (Whiting and Strange, 1989). A similar relationship between collagen content and shear force values or sensory connective tissue was observed. In general, the practical limits of connective tissues in a restructured beef product made with relatively large pieces of meat are about 1.5% to 2.0% collagen.

Mechanical sorting and separation of connective tissue is becoming possible with advances in color measurements (Swatland, 1989), computer imaging and robotics. Newman (1984) and Hildebrandt and Hirst (1985) determined meat composition using television images. Two-tone (B/W) analysis is capable of differentiating collagen, elastin and bone. Konstance et al. (1988) used tristimulus color values and color difference indices and were able to distinguish connective tissues, bones, fat and ligaments from lean meats (Fig. 2). The first separation into five groups was by color difference. This was calculated as the square root of the sum of the squared differences from a white standard of the tristimulus color values (L, a and b). The second criterion was the chromaticity, defined as the sum of the squared differences in a and b values. The third separation criterion to differentiate lean meat and red bone (marrow) was the yellowness index, using CIE values. Near infrared (Williams and Norris, 1987) and fluorescence (Huss et al., 1985; Swatland, 1987) analyses also have potential for differentiating tissue types.

Figure 2



Scheme of separation of beef chuck components for a restructuring process using visible light.

Heiland et al. (1989) proposed to trim slices of beef chuck with a high-pressure water jet. Chucks would be sawn into slices up to 19 mm (3/4 in) thick. Each slice would pass under

Table 1. Sensory Scores and Kramer Shear Forces for Restructured Beef Products Made with Added Connective Tissue.

Connective Tissue	*Level Added*	*Connective Tissue Score*[1]	*Kramer Shear Force*[2] *Raw*	*Cooked*
Control		6.42	62.0	78.8
Epimysium	5%	6.47	80.3*	78.8
	10%	4.96*	91.2*	74.8
Gristle	5%	5.76	81.8*	76.6
	10%	4.94*	98.1*	72.5
Tendon	2.5%	4.52*	71.4*	83.9
	5%	3.48*	83.6*	87.1*

[1] Mean values of a ten-centimeter unstructured scale, 10 minimal, 0 excessive. Mean Sensory Score differences were evaluated using the Bonferroni least significant difference (LSD) method.

[2] Kramer shear force values in N/g. Means evaluated using Student t test.

* Significantly different from control ($p<0.05$).

the color television-computer analyzer where the location of bone, heavy connective tissue and fat would be determined. Then the water jet mounted on a computer-controlled robot arm would cut up the chuck. A water jet with a 0.15 mm orifice diameter, 55,000 psi water pressure (1.2 Gpm) can cleanly cut a 3/4-inch thick beef chuck steak at a rate of 9.5 meters per min. Red meat, fat and connective tissue are completely severed while bone is only slightly scored. They feel an automated processing system composed of saws, scanners, computer, water jets and separators of cut-up pieces can be economically feasible.

Blade tenderizers are used on meat before their incorporation into restructured product with limited success (Flores et al., 1986; Berry, 1987; Gillett, 1987a; Rolan et al., 1988). The blade punctures the interior portions of the muscle and the peri/endomysial connective tissue as well as epimysium. The epimysium and gristle are not degraded or removed. Success for this method appears to depend on the muscle used and process details.

Enzymes are used commercially to tenderize meats. The plant and fungal proteases (papain, bromalin, and ficin) are more active on myofibrillar and sarcoplasmic proteins than on the heavy connective tissue. Consequently, they are not suitable for tenderizing restructured beef products (Rolan et al., 1988). Pepsin will hydrolyze collagen fibers but is active at a low pH (Bailey and Etherington, 1987). Bacterial collagenases from *Clostridium histolyticum* have the promise of specificity and attack muscle collagen (Cronlund and Woychik, 1987; Bernal and Stanley, 1987) but are not successful in a meat product (Foegeding and Larick, 1986). Miller et al. (1989) added 0.01% Vibrio collagenase into restructured beef steaks, held them for 24 hours at either 2° or 11°C, and analyzed them raw and after heating for 1 hour at 40°C. The collagenase increased collagen solubility in both raw and heated steaks. Shear force values for heated control, extensively-trimmed control and enzyme-treated steaks were 126, 75 and 108 N/g, respectively.

Microorganisms that produce collagenases are pathogens or related to pathogens. This makes demonstrating their safety, obtaining regulatory approval and sensory testing difficult.

Acids can solubilize collagen. Wenham and Locker (1976) marinated beef in 1.5% acetic acid. This treatment effectively tenderized the *sternomandibularis* muscles (7.3% collagen) but had minimal effect on *longissimus dorsi* muscle (2.3% collagen). The acid treatment caused connective tissue swelling and water retention. Marinating (Kijowksi and Mast, 1986) and tumbling (Kotula and Heath, 1986) chicken meat in acetic or lactic acid tenderized the meat. Whiting et al. (1987) seamed beef chuck muscles and immersed them in 0.5 M lactic acid for 30 min at 23°C. The epimysium became swollen and translucent. After rinsing, the muscles were diced and made into restructured steaks. This treatment significantly decreased the amount of collagen and reduced the shear force values compared to steaks made from untrimmed meat (Table 2). Steaks from extensively hand-trimmed (no epimysium) muscles were also evaluated for comparison. The lactic acid slightly lowered the pH and adversely affected water holding, binding and color of uncooked steaks. The process can probably be improved by rinsing with a buffered solution and using binding systems other than salt and phosphate. Color would not be a problem with precooked, breaded or institutional products.

Summary

The endomysial and perimysial connective tissues play a major role in the texture of intact steaks. A certain amount of this collagen, primarily perimysium and endomysium, may make a positive contribution to the texture of intact and restructured meats. In comminuted products, the connective tissue is disrupted and not directly detectable by the palate. Modest amounts improve the firmness and elasticity. However, excessive amounts lead to batter instability and loss of fat and water. Epimysium and gristle are major problems in restructured beef products. Currently, no economical means of removing this connective tissue has passed from the research laboratory onto the production floor.

Table 2. Properties of Restructured Steaks after Treating the Epimysium with Lactic Acid.

	Untrimmed Control	*Trimmed Control*	*Lactic Acid*
pH	5.80[a]	5.78[a]	5.52[b]
Hydroxyproline (mg/g)	1.80[b]	0.78[a]	0.99[a]
Shear Force (N/g)	96[c]	61[a]	80[b]
Cook Loss (%)	27.5[a]	29.3[a]	34.4[b]

Values in a row with the same superscript are not significantly different by Duncan's multiple range test ($p>0.05$).

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Discussion

R. Field: I would like to compliment all three speakers on the job that they did today. I for one will be referring to these three papers for several years to come. I think they were excellent. I believe I will start with a comment and have you respond. Dr. Duance, your theory as to why collagen affects meat tenderness, I thought was interesting. What you told us was that mature collagen, perhaps partly because of the tensile strength that is developed during heating, shrinks around the muscle fibers. As the muscle fiber shrinks, moisture is squeezed out of them and the meat is tougher. I think that is probably true for meat that is dry-heat cooked, to, say 65° to 70°C internal temperature. I'm not sure it has anything to do with variation in tenderness of meat that is cooked below the shrinking point of collagen; and I'm not sure that it has anything to do with tenderness in moist-heat cooked meat that usually is cooked in the presence of plenty of moisture and also is cooked to a higher temperature so that the collagen has completely gelatinized, or at least much of it has. I wonder if you agree with what I'm saying, that your theory for differences in tenderness as a result of mature collagen is perhaps limited to dry-heat cooked meat that is cooked to 65° to 70°C?

V. Duance: I'm not sure it's that clear here becauase if it's wet cooked, collagen still shrinks and has the ability to shrink the actual meat even though the water content, as moisture, is actually surrounding the meat.

Field: I know that it shrinks, but the point is that it continues to gel. Gelation occurs and you have almost a complete gelation of the collagen.

Duance: Well, with heating to much higher temperatures, as somebody mentioned pressure cooking, collagen does probably gelatinize, but the effect of total amount of collagen, due to peptide bond cleavage and, therefore, the influence of any toughening is negated at that point. The myofibrillar components of muscle also constitute texture, so I'm not saying that collagen is the only component of texture. You have to take that into consideration.

R. McCormick: Dr. Duance, you presented a lot of evidence that crosslinking proceeds in an orderly process from immature, reducible crosslinks to mature, nonreducible crosslinks. What is the trigger? Clearly, under a given set of circumstances, you can follow that crosslinking process quite closely. But, what is the signal that allows reducible crosslinks, immature crosslinks, to mature? And, how much variability is there in that process?

Duance: Well, we really don't know. It's a spontaneous reaction. Pyridinoline, for instance, is a crosslink that is apparently present in very young tissues, so that's a fairly rapid process and the transitions for hydroxy-lysinonorleucine to pyridinoline appears to be relatively quick. The time span for the production of the other crosslinks may be much slower. What is predicted for the rate of the formation of these crosslinks, we don't know. It probably is the closeness or toughening of the collagen molecule in the tissue.

Now in an environmental process, we've heard that it's not very different to tell these apart for the most part, it will depend on the environment and the connective tissue. Things like the glucose may be able to crosslink, if the collagen is there for a very long time. It turns over slowly, as we know, and this is a time-dependent process which may, in humans, take decades to occur. We know in diabetes, for instance, that it occurs much more rapidly because of the higher glucose levels in serum. But this is something which occurs over decades. The formation of things like compound M and histidino-hydroxy-lysinonorleucine may be much more quick, over a matter of years, but it's still relatively slow.

E. Aberle: Richard, you commented that the collagen is glycosylated and implied that that had some effect on the ability to crosslink. I wonder if you or Dr. Duance would want to comment further on the effects of glycosylation of the collagen, on its ability to crosslink and on its stability?

Duance: Yes. I actually have not come across that piece of information before. I am a little surprised at it in that one of the very first steps in collagen once it passes over the cell is the removal of the procollagen peptide, the alignment of the collagen into fibrillar form, and then it enzymatically crosslinks with the lysyl oxidase pathway. That's a fairly quick process. I'm then surprised that the rate that we know glycation occurs of most proteins would seriously inhibit that process. The rate of glycation is relatively slow compared with the enzymic pathway and I'm surprised that it has a great deal of effect.

McCormick: To clarify that, there have been a number of interesting observations made concerning dietary carbohydrate and collagen crosslinking. Phil McClain reported some years ago that rats fed a diet high in reducing sugar such as glucose or fructose had muscle collagen that did not crosslink as much or as completely as animals fed a starch diet.

In recent years, there has been a great deal of interest in copper deficiency and one of the criteria for producing a copper-deficient animal is feeding a high glucose or fructose rather than starch diet. One hypothesis is that dietary fructose or glucose interferes with the availability of copper which

decreases lysyl oxidase activity and produces decreased crosslinking.

A second theory is that high levels of glucose interfere with collagen fibril formation, decrease lysyl oxidase activity and would produce less crosslinked collagen. Now whether this results in greater glycation or glycosylation of lysyl or hydroxylysyl residues, I can't say. They are hypotheses for the observation that diets high in reducing sugar result in less crosslinked collagen.

M. Judge: Rich, I wonder if you would perhaps elaborate on your comments about the relative importance of energy in the diet and other factors that, such as those that influence hypertrophy, have on crosslinking and other things, and perhaps put it in the context of a young bull on a high-energy diet that is growing rapidly for two reasons. Can we separate those effects on quality and maturation of collagen?

McCormick: That's a good question that I've given a lot of thought to. And, as I look through the research that's recorded, much of which is yours, I've come to the conclusion that, on the basis of solubility, or insolubility of collagen alone, we have a hard time deciding whether the end result of what we have (more soluble collagen vs. more insoluble collagen) is actually going to result in a more desirable or more tender product.

One part of the equation is separation of the collagen pool into soluble and insoluble fractions. The second part of the equation is determining what the nature and the extent of crosslinking in the insoluble pool is. A good example is a bull versus a steer, both of which have been growing rapidly, muscle fibers hypertrophying for some length of time. Androgens have a strong anabolic effect on collagen synthesis, so for the bull with increased growth there will be increased collagen production. Even though the soluble-collagen pool size may be larger in the bull, we're going to have one (the bull) that is decidedly tougher than the other.

The differences in collagen characteristics are in large part due to the nature of collagen in the insoluble pool, how it is crosslinked. In terms of what hypertrophy is doing, it's interesting that intramuscular collagen synthesis may be keeping up with the production of myofibrillar protein when an animal is growing rapidly, suggesting that there may not be such a dilution effect, that we may have as much collagen there as myofibrillar protein. The fact that more collagen is synthesized suggests that more of it may crosslink and crosslink to completion than in a less rapidly growing animal. Histological evidence indicates also that in hypertrophy, the endomysium becomes larger and more pronounced relative to what's happening in normal or non-hypertrophying muscle.