A New Theory of Tenderness in Meat, Based on Gap Filaments

R. H. Locker*

Tenderness is a quality of meat highly regarded by the consumer, who may have a very clear idea of how he rates it. For the meat scientist, interested in putting a number on it, it poses problems. Tenderness, or more often toughness, is ideally measured by selected human jaws. Fortunately for experimental convenience and precision, mechanical shear values correlate fairly well with panel scores, although there is some doubt as to what shearing devices actually measure, and whether the various models now in use measure the same thing. In spite of this, shear force remains the preferred measure of toughness in research. It is a more complex parameter than tensile strength along the fibre. The relationship between the two is not entirely agreed (for a discussion see Locker et al., 1977). An interesting visual demonstration of stretching around the blunt shearing wedge of the MIRINZ tenderometer (MacFarlane and Marer, 1966) was provided by Davey and Winger (1980, their Fig. 16). Considering all the evidence, it seems reasonable to conclude that tensile strength is the dominant factor in shear force. We have given a great deal of attention to tensile properties in recent work, since these are more amenable to structural interpretation, and, used properly, can distinguish the contributions of various components. But understanding what is being measured by such parameters still has complications.

Firstly, muscle is a composite of contractile machinery enveloped in connective tissue. Knowledge of the former has advanced enormously under the stimulus of the sliding filament theory of muscular contraction, and with the advent of sophisticated techniques in molecular biology. Collagen chemistry has flourished to a comparable degree, although the organization of the protein into connective tissue, particularly at the macro-end of the scale, has been an area of neglect. This essay will concentrate on the myofibrillar component. It is of course what I know best, but it is also the eminently controllable factor, capable of being grossly modified for better or for worse by post mortem treatment. Early research on tenderness over-emphasized connective tissue, of necessity, because new doors had yet to open. Recognition of the role of state of contraction swung the emphasis probably too far in the direction of the myofibril. Interest in connective tissue revived somewhat with the important concept of the perimysium as a collagen net. Rowe (1974) found the net slack and crimped in relaxed muscle, but taut in stretched or contracted muscle. Locker and Daines (1975) independently observed the net in cooked muscle where it is tensioned by thermal shrinkage. These were in fact rediscoveries since the net had already been beautifully photographed in both crimped and taut form by Moran and Hale (1928). The superb scanning electron micrographs of Rowe (1981) offer a new and more sophisticated view of both perimysium and endomysium. It is now time for a more balanced view of the myofibrillar and connective tissue components and some attempt will be made at the end of this paper to integrate the two into a unified theory.

There have been a few attempts to explain tenderness in terms of our new grasp of fine structure in muscle, and to explain why tenderness changes so much under certain treatments such as aging or cold shortening. There is still a basic validity in these attempts, but this paper will probe deeper and interpret the myofibrillar contribution to toughness in a radical way. More attention will be paid to fine structure, as actually observed in the cooked state. The ideas that follow are novel because they are based on a third set of filaments, readily observable within the myofibril, but so far largely ignored. I refer to the "gap-filaments", first observed twenty-one years ago, reported on spasmodically since under various names, and the subject of my undivided attention since 1973.

Working between the worlds of muscle morphology and meat tenderness, I have gradually realized that gap filaments (G-filaments) are not only significant in the myofibrillar component of tenderness, but in fact its chief determinant. My present view is that the only tension-resisting unit of the myofibril to survive cooking with any integrity is the array of G-filaments, whatever the degree of contraction. The roots of these ideas have already been presented in some detail (Locker et al., 1977).

I had hoped that this new work would have stimulated activity on G-filaments amongst academic muscle biologists and meat scientists but after five years this is barely detectable, at least from my remote viewpoint. G-filaments seem to be in a state of limbo, lacking respectability in either world. For that reason I have chosen to come to this conference, and be my own advocate.

A lack of enthusiasm amongst the theorists of muscular contraction is perhaps not surprising as the filaments have no obvious role in the sliding filament theory, and indeed present complications. It is ironic that both the Huxleys, so firmly associated with the sliding filament theory, have observed and then ignored evidence for G-filaments. In their original paper, H. E. Huxley and Hanson (1954) described ghost myofibrils

*R. H. Locker, Meat Industry Research Institute of New Zealand, P.O. Box 617, Hamilton, New Zealand

Reciprocal Meat Conference Proceedings, Volume 35, 1982.

resulting from extraction of myosin. To account for their obvious structural continuity, in spite of the centre of the sarcomere appearing empty under phase contrast, they proposed S-filaments linking the ends of actin filaments. These were last heard of in Huxley and Hanson (1957), when the S-substance was estimated by interference microscopy as 3% of the myofibrillar protein. The gap filaments appear to have been first seen by A. F. Huxley and Peachey (1961) while investigating the ability of stretched fibers to contract. They presented an electron micrograph of a sarcomere stretched beyond overlap point (their plate 3.3). The legend reads "The suggestion of fine filaments connecting the ends of the two major sets of filaments may be a result of super-position in the section or may represent an additional component of the myofibril." The term "suggestion" was an understatement, and there is no other mention of this important observation anywhere in the text. It was left to Sjöstrand (1962) to describe and name the "gap filaments".

There now seems good reason to believe that the G-filaments are composed of a protein called "connectin" by Maruyama et al. (1976) and more recently "titin" by Wang et al. (1979, 1980). Contributions towards "connectin" were collected in proceedings of the Third International Conference on Fibrous Proteins held in New Zealand in 1979 (Maruyama et al., 1980; Locker and Daines, 1980; King and Kurth, 1980).

In relation to meat science, the visual evidence alone for G-filaments has become too compelling to ignore. Since the first paper appeared (Locker et al., 1977) new results have reinforced the evidence (Locker and Daines, 1980; Locker and Wild, 1982a, b, c; Locker et al. 1982). This seems an appropriate time to present a more coherent account. A more concise account will be presented elsewhere (Locker, 1982).

Organization of G-filaments Within the Sarcomere

Studies based on the electron microscopy of very highly stretched beef fibres led to a model (Fig. 1) for the arrangement of G-filaments within the sarcomere (Locker and Leet, 1976a). Sarcomeres stretched by 4-5 times from excised muscle length show G-filaments to advantage in the wide gap that opens between the A- and I-filaments. The proposed connections were based mainly on the following observations:

(a) I-filaments appear to be unconnected to gap filaments, remaining quite intact and unstretched at maximum extension.

(b) A-filaments, which appear to be continuous with the G-filaments, are usually both stretched and dislocated, having slid out of register toward one Z-line or the other with destruction of the M-line. The degree of dislocation is limited, for reasons unknown, by a residual overlap of 0.6μ which remains at the centre of the sarcomere, even when stretched to 12μ.

(c) There appear to be about half as many filaments in the gap as there are A-filaments in the normal A-band.

(d) Extraction of myosin from dislocated A-bands leaves an array of fine filaments showing the above overlap. In some fibers A-bands do not dislocate, and when these are extracted an array of fine filaments is also seen, but here they emerge from the remnants of A-filaments protected from solution within the surviving M-line structures.

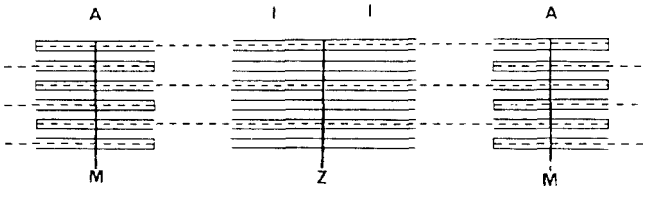

Fig. 1. Each G-filament (dotted) acts as core to two A-filaments in adjacent sarcomeres, linking these A-filaments through the Z-line, and independently of I-filaments.

The model proposes that each G-filament (dotted) forms a core to an A-filament, emerging at one end only, passing between the I-filaments, through the Z-line, between the I-filaments of the next sarcomere, and into a second A-filament, again terminating as core. Thus the G-filaments have a curious symmetry centered on the Z-line, linking half the A-filaments in any A-band to A-filaments in one adjacent sarcomere, and half to those in the sarcomere on the other side.

They are continuous through the Z-line but, it must be noted, not through the sarcomere. Adequate anchoring of ends within the A-band is therefore a pre-requisite for maintenance of structural continuity under stress. There is additional evidence for continuity through the Z-line in that G-filaments can be dragged through the Z-line under great stress, taking the N_2-line with them (Locker and Leet, 1976b).

The gap filaments are highly elastic in live muscle, and remain elastic (but less so) and strong after heat denaturation (Locker and Wild, 1982c). They are resistant to a variety of powerful protein solvents but are vulnerable to proteolytic enzymes (Locker et al., 1977).

The role of G-filaments is still unclear. It seems possible that they might function as a series elastic component in muscle. As a "core" protein they might be involved in organizing the laying down of A- and I-filaments in the differentiation of muscle. It does seem definite that G-filaments support the N_2-line (Locker and Leet, 1976b) which may act as a perforated spacer, marshalling the I-filaments from a square array at the Z-line into a hexagonal array at the edge of the A-band. This arrangement may also serve to guide I-filaments back into the A-band when they have been fully withdrawn. This occurs at maximal stretch (100-110%) in whole ox sternomandibularis (Locker and Leet, 1975), but the muscle can still contract back to excised length without damage.

Gap Filaments and Yield Point in Raw Meat

Pre-rigor muscle offers only slight resistance to extension. In the relaxed state there are no bridges between actin and myosin filaments, which can slide over each other readily. It follows that G-filaments must offer little resistance also, since a loading of only about 0.1 kg/cm^2 is needed for 80% extension in sternomandibularis (Davey and Dickson, 1970). Beyond this point the collagen net begins to offer resistance.

In rigor meat, bridges between actin and myosin impose a low extensibility, until at about 18% extension and loads of 0.9-1.6 kg/cm^2 the strip suddenly reaches a point where it continues to extend readily without further loading. This is the "yield point" of the muscle (Locker and Daines, 1975b; Locker

and Carse, 1976; Locker and Wild, 1982a, b). Histological appearance of yielded muscle varies with rigor temperature and between fibers, but in general the response is an erratic opening up of I-bands without actual breakage (Locker and Wild, 1982a). Our interpretation of micrographs is that at the yield point I-filaments snap near the Z-line (or pull out of it) while the G-filaments stretch. The strip then sags until the collagen net takes up the strain.

Yield point is observed in a variety of beef muscles, and varies with rigor temperature and state of contraction or stretch. Sometimes the state of contraction or the nature of the connective tissue causes the muscle to break before it yields (Locker and Wild, 1982a). Yield point has also been observed in rabbit psoas (Locker et al., 1982).

If the meat is aged for 1 day at 15°C before loading, the yield point falls drastically to about 0.1-0.2 kg/cm^2 (Locker and Wild, 1982a, b). The I-bands snap cleanly along the A-I junction. It seems that both I-filaments and gap filaments have been rotted by catheptic action.

The Characteristics of Cooked Meat

The real business of a tenderness theory is with cooked meat. The term "cooked" covers many different degrees of heat denaturation. I will now attempt to define the essential characteristics of cooked meat and the point at which these appear.

Davey and Gilbert (1974), using beef sternomandibularis, found a two-step rise in shear force with cooking temperature during a 1 h cook. The steep rises obtained at 40-50°C and 65-75°C were identified with the denaturation of the myofibrillar and connective tissue components respectively. However, since the steep rises in shortening and weight loss occurred only in the second phase (60-80°C), in parallel with a strong endothermic reaction, it appears that this phase is compound, reflecting marked changes not only in connective tissue but also in the myofibrillar proteins.

Locker and Carse (1976) cooked beef strips by immersion for 10 and 40 min at 50, 60, 70 and 80°C. Cooking loss and shortening were small at 50 or 60°C but between 10 and 40 min at 70°C rose steeply to about two-thirds of the values for 40 min at 80°C. A yield point was only just discernible on loading by hand after 40 min at 60°C or 10 min at 70°C, and had disappeared after 40 min at 70°C. Another characteristic of cooked meat is a sudden and clean cleavage between the blunt jaws of the MIRINZ tenderometer (Macfarlane and Marer, 1966). This property appeared as the yield point was disappearing, that is after 40 min at 60°C or 10 min at 70°C (Locker and Carse, 1976).

The load-extension curves of cooked muscle strips consist of at least two distinct linear phases, the second representing a sudden increase in extensibility, apparently due to some myofibrillar event (Locker et al., 1982). The effect is seen after cooking for 40 min at temperatures of 60-100°C. More recently we have confirmed this effect at 60°C and compared it with results at 80°C (Locker and Wild, 1983).

To summarize these general results on meat as a whole, it appears that much of the character of cooked meat is achieved within an hour at 60°C. There is a transition from the stiffness of rigor with a "yield" point to a superficially elastic state in which the muscle may be readily stretched by about 90% and can still return to near original length without obvious structural change (Locker et al., 1982). The major hydration losses and much of the rise in shear force have still to occur. I will now consider more specifically the effect of cooking on connective tissue and on the A-, I- and G-filaments.

The Fate of the Filaments

What happens to the various filaments of which connective tissue and myofibril are made, as cooking progresses? Which are the important survivors?

(a) *Connective tissue*

While collagen fibers dominate the mechanical properties of this tissue, it must be acknowledged that most of the observations described relate to the whole tissue, including the intricately structured proteoglycans which make up the "ground substance", and there is some risk in switching from the stability of the whole to that of collagen striations.

Connective tissue undergoes profound change in the 60-70°C region. Davey and Gilbert (1974) found the thermal shrinkage of perimysial connective tissue from sternomandibularis muscle (strained from a homogenate) to occur at 62-68°C. Macfarlane et al. (1981), using similar material from semimembranosus, found by differential calorimetry a thermal transition temperature of 63°C. These results are in fair agreement with histological studies. Giles (1969) found the striations of collagen fibrils were unaffected after 140 min at 60°C, but gradually disappeared at 70°C (although even at 140 min some striated fibrils remained). More recently a number of workers have applied scanning electron microscopy to cooked meat, and in these cases endomysial connective tissue was observed. Cheng and Parrish (1976) found in longissimus, brought rapidly to 60, 70 and 80°C that the endomysium was intact at 60°, but much coagulated by 70°C. Jones et al. (1977) found endomysium in semitendinosus to be unaffected after 45 min at 50°C, but congealed at 60°C. Leander et al. (1980) found in longissimus and semitendinosus, roasted to 63, 68 and 73°C, that coagulation of endomysium had begun at 63°C and was severe at 68 and 73°C.

The collagen of the perimysium is probably the most important to tenderness and it may be concluded from the above that this "melts" a little above 60°C, although it seems that endomysial collagen may succumb at a slightly lower temperature. The two collagens represent a different genetic mix: types I and III in perimysium and types I and V in endomysium (Bailey et al., 1979). Thermal shrinkage has the effect of pre-tensioning the collagen net. After 40 min at 80°C the perimysium has assumed a striking "crossed diagonal" configuration. The contraction is strong enough to throw the muscle fibers into an irregular "cooking crimp", which may be avoided by cooking under restraint (Locker and Daines, 1975a). The collagen fibers themselves, originally inextensible, have now assumed a rubber-like elasticity.

(b) *The A-filaments*

Myosin itself is readily denatured by heating. Rabbit myosin in 0.5 MKC1 at pH 6.2 flocculates in 2-3 min at 53°C (Locker, 1956). It is somewhat more stable in situ. In beef strips heated for 1 h at various temperatures, extractability of myosin fell below 20% at 53°C and had almost vanished at

60°C (Davey and Gilbert, 1974). The myosin head (S-1) is more vulnerable than the tail, aggregating even at 35°C, while myosin rod uncoils rapidly at 50°C forming a three dimensional network. After 20 min at 60°C in 0.6 MKC1, pH 6.0, only 30% of the original α-helix content remains (Samejima et al., 1981).

Histological evidence from several sources on the survival of A-filaments, is reasonably consistent. Giles (1969) found in four beef muscles that the A-filaments were somewhat changed after 20 min at 60°C and barely discernible at 100 min. This change was rapid at 70°C. Schmitt and Parrish (1971) broiled thick steaks to a given internal temperature and found the A-filaments intact at 50°C, visible in longitudinal but not transverse sections at 60°C, and not visible at all at 70°C or 80°C. In muscle cooked for 40 min at 80°C we found the coagulated A-band had a speckled appearance, which became more pronounced on stretching (Locker et al., 1977). We have also found (unpublished results) that in muscle stretched by 100% and cooked in rigor for 10 min at 50-80°C there was no change in A-filaments at 50°C but they became progressively more ghostly at 60, 70 and 80°C. Leander et al. (1977) found in muscle heated to 63, 68 and 73°C, that an overall granular appearance was evident at 63°C, although A-filaments still appeared to have some integrity. At 68°C little more than granules remained.

The fate of the A-filaments is important in ideas about tenderness, since the denatured A-band is considered to be the strong unbreakable unit of the sarcomere. We have recently penetrated the murk of the coagulum and obtained a clear answer (Locker and Wild, 1982c). Rigor muscle was cooked for 40 min at 80°C and then loaded to breaking point. It recovered and was restretched by 60% during fixation. The A-band was seen to have opened up to a parallel array of fine filaments embedded in fragments of flocculum. The same result was obtained if the muscle had first been cold shortened to the point where the I-band had disappeared. I conclude that the array consists of G-filaments, originally embedded in a tight coagulum of actomyosin, which has broken up on stretch. The concept of the A-band as a fused structure of coagulated A-filaments must be abandoned.

(c) *The I-filaments*

Giles (1969) found the I-filaments had lost fine structure after 20 min at 60°C and thereafter progressively coagulated. After 20 min at 70°C they had begun breaking at the A-1 junction. Schmitt and Parrish (1971) found the I-filaments were still well defined when muscle had reached 60°C, but had disintegrated by 70°C (in fact their photographs show a few filaments remaining). In muscle cooked for 40 min at 80°C we found the I-filaments had disappeared, leaving a much sparser array of thicker filaments which became clearer on stretching (Locker et al., 1977).

Since I-filaments disintegrate so early, it is curious that no one has asked what maintains the undoubted continuity of cooked muscle. The situation resembles the "ghost" story over again. My interpretation of the sparse array of survivors in the I-band is that they are G-filaments, unevenly coated with coagulated actin. They are about half as numerous as were the A-filaments. When stretched muscle is cooked the I-filaments do not disintegrate, but appear to coalesce to thicker filaments, apparently around a G-filament (ibid.).

(d) *The G-filaments*

The preceding sections have already claimed the G-filaments as the only survivors in the A- and I-bands, but G-filaments too must suffer denaturation at some stage of cooking. It is my belief that G-filaments denature steadily during an hour at 60°C. They then lose the extreme extensibility of their native state but retain a more moderate extensibility, which along with that of denatured collagen, confers the pseudo-elastic character of cooked meat. There is no single conclusive piece of evidence for denaturation at 60°C, but it is entirely consistent with a number of miscellaneous observations:

1. Yield point has almost vanished after 40 min at 60°C.
2. A clean bite in the tenderometer first appears at this stage.
3. The biphasic load-extension diagram, characteristic of cooked meat (Locker et al., 1982) is seen at this stage.
4. The exact shape of the shortening-toughening curve seen in meat cooked 40 min at 80°C is reproduced (at lower shear values) in meat cooked 40 min at 60°C (see later).
5. The ability of meat to be tenderized by pressure-heat treatment is lost within an hour at 60°C (see later).
6. It is possible that the maximum rate of aging in meat at 60°C is due to more ready attack on denatured G-filaments (see later).

Taken together, these observations point to the fact that G-filaments become cooked at 60°C yet remain strong while A- and I-filaments are rapidly losing any significance to strength.

This discussion leads to the conclusion that 60°C is a critical temperature in the cooking of meat. The G-filaments become cooked (and sole survivors), while by a narrow margin, the perimysium remains undenatured. In plain words, meat at this stage consists essentially of cooked myofibrils and raw connective tissue. At 70°C both components rapidly become cooked, and then on extension, pre-tensioned G-filaments and a pre-tensioned collagen net stretch in parallel, each making a significant contribution to strength.

Prolonged Cooking

We have so far been concerned with the emergence of the cooked state. What subsequent changes occur if cooking is more rigorous?

Shear force peaks after an hour at 75-80°C, when changes in hydration are completed (Davey and Gilbert, 1974). Our standard cook of 40 min at 80°C therefore represents a "fully cooked" meat.

More prolonged or extreme cooking produces a slow decline in shear force (ibid.) as connective tissue suffers hydrolysis. Cooking has been carried to extreme lengths in this laboratory. Davey et al. (1976) cooked beef strips for up to 100 h at 80°C. Shear force fell substantially between 1 and 12 h, only a little between 12 and 24 h, and then remained almost unchanged up to 100 h. Initial differences due to cold shortening or aging were preserved. On the other hand initial large differences due to age of the animal were almost eliminated by 24 h (Davey and Niederer, 1977). Similarly at 100 or 120°C shear force changed little from 3 to 4.5 h (ibid.).

My experience with sternomandibularis is that after 3-4 h at

100°C, connective tissue is destroyed to the point where adhesion between fibres is quite minimal (which unfortunately makes tensile measurements difficult). Yet after 2 h at 100°C half the tensile strength remained and after 8 h, a third (Locker and Carse, 1976). When muscles were restrained either at excised length or maximal stretch during rigor and cooking for 4 h at 100°C, the stretched samples broke on average at 2.7 kg/cm², equivalent to two-thirds of the strength of the unstretched, based on equal numbers of fibres (Locker et al., 1977). Since the stretched samples had no overlap of A- and I-filaments and connective tissue had been destroyed, only G-filaments could have maintained continuity. Micrographs showed clearly that these had indeed survived in the gap, and were the point of breakage.

I can only conclude from the above observations that in grossly over-cooked meat it is the survival of G-filaments that maintains a considerable residual strength after connective tissue has succumbed. The stability seems extreme in view of the possibility of random breakage of peptide bonds under such prolonged heating. The preservation of margins of toughness due to shortening or to aging emphasizes the involvement of G-filaments in such effects, and will be further discussed.

A Basis for Cold Toughening

The relationship between tenderness and state of contraction, and modification of the latter by early exposure to cold, is now an established area of meat science. It has already been reviewed several times (e.g. Locker et al., 1975). A new review, soon to appear, will cover in more detail the substance of this section (Locker, 1983). An explanation of the shortening-toughening relationship of Marsh and Leet (1966) offers an important test for the G-filament theory.

Marsh and Carse (1974) have already attempted to explain the rise in shear force in cooked muscle, as the degree of shortening between death and rigor increases from 0-40% of excised length. They invoked the relative positions of A- and I-filaments at different degrees of shortening, to which a specific sarcomere length was allotted, based on an excised length of 2.1 μm. Their argument implies homogeneity in sarcomere length over the range, but Voyle (1969) has shown this to be untrue for cold shortened meat. Both papers seem to imply, although it is not very clearly stated, that toughening is due to elimination of the I-band as the weak link in the sarcomere. This occurs at a sarcomere length of 1.5 μm, and further shortening causes A-filaments to be compressed against the Z-line, or to penetrate it. Marsh and Carse barely discussed cooking, but appear to believe that when such sarcomeres are cooked, they become a strong continuum of overlapping A-filaments, bound together by surviving actin-myosin links. In the light of Voyle's histological observations the steeply rising phase of the curve over 20-40% shortening seems best explained in terms of an increasing statistical incidence in the sample of sarcomeres of 1.5 μm or less. I see no need to quarrel with such an interpretation, but only with the nature of the continuum.

My model (Fig. 1) also predicts the I-band as the weak link since there are only half as many G-filaments there as in the A-band. The latter unit is stronger in cooked meat, not only by virtue of a double ration of G-filaments, but also because of reinforcement by a matrix of coagulum. This accounts for the fact that when cooked muscle is stretched by up to 30%, extension of the sarcomere is confined almost entirely to the I-band (Locker and Wild, 1982c). The coagulum seems likely to consist largely of actomyosin since the affinity of actin and myosin seems to operate during coagulation. Actin-myosin mixtures produce stronger gels on heating than myosin alone (Yasui et al., 1980). As already shown, the concept of a continuum of denatured A-filaments in short sarcomeres must be abandoned in favor of one composed of G-filaments. These should form a stronger continuum than might have been expected of A-filaments, since although in my model half depend only on overlap across the Z-line, the other half are truly continuous through the Z-line. It should be noted however that since each gap filament passes through only one Z-line, a true long-range continuum is not possible, but continuity must depend on lateral linkages within the A-band.

I have proposed elsewhere (Locker, 1983) that the less steep part of the curve (0-20% shortening) could be attributed to an increase in strength of the I-band itself as it shortens, possibly due to denaturation of G-filaments in a less stretched and therefore thicker state. The steep phase (20-40% shortening) reflects the impact of sarcomeres without I-bands.

The characteristic shape of the shortening-toughening curve is reproduced exactly, but at lower shear values, if normal thorough cooking (40 min at 80°C) is replaced by either a mild cook (40 min at 60°C) or an extreme cook (24 h at 80°C). Both facts fit my theory nicely. The 60°C treatment (Davey and Gilbert, 1975) cooks only the G-filaments but not the collagen, which remains untensioned and therefore probably does not contribute much to shear force on the MIRINZ tenderometer. In the long cook (Davey et al., 1976) G-filaments survive well, but collagen is destroyed. Thus in both cases G-filaments are fully operational, while connective tissue is rendered ineffective for quite different reasons, leading to overall reduction in shear values.

The Mechanism of Aging

The literature on aging is bulky and often conflicting, but it seems agreed that aging is predominantly an attack by indigenous proteases on the myofibrils. The drastic decline in yield point (but not breaking point) with moderate aging exemplifies this (Locker and Wild, 1982a, b). The relative roles of the various cathepsins and neutral proteinases are far from clear, but between them they seriously weaken the already weaker I-band. Yield in rigor meat is due to failure of I-filaments, while G-filaments simply stretch, but after aging both sets are so weakened that they fail together at very low loads giving a clean break across the I-band. In the cooked state only the failure of G-filaments is relevant.

The first and most dramatic demonstration of the role of G-filaments in aging came from the micrographs of Davey and Graafhuis (1976). The G-filaments in fully stretched muscle (100%) largely survived a severe aging of 3 days at 25°C, but on cooking there was total collapse leaving the "gaps" empty. In view of the earlier claim that the disappearance of the Z-line during aging accounts for the weakening of the myofibril (Davey and Gilbert, 1967) it is interesting to note that the Z-line survived this very rigorous aging apparently intact. I believe the disappearance of the Z-line (nearly always reported from studies on myofibrils), may serve as an indicator of advanced

aging, but is irrelevant to tenderization. We have observed a similar destruction of G-filaments when the same muscle, glycerinated at 100% extension, was incubated in a crude muscle protease preparation and cooked (Locker et al., 1977). The destruction of G-filaments was almost as severe in the presence of EDTA as in the presence of Ca (at pH 7), suggesting that both cathepsins and neutral proteases were effective.

The vulnerability of G-filaments to proteases has also been demonstrated by irrigating over-stretched fibres with very dilute trypsin solutions or with crude muscle protease solutions. The fibres snap within minutes (Locker et al., 1977; see also references there to earlier work). Connectin, the probable protein of the G-filaments, is the only structural protein of a longitudinal filament to be markedly degraded during aging (King and Kurth, 1980; King et al., 1981; King and Harris, 1982).

The lack of response to aging seen in meat, cold shortened to maximum toughness (Davey et al., 1967) may be reinterpreted in terms of gap filaments. The current view is that the vulnerable zones, the I-filaments or normal Z-line have been removed by shortening. In my theory, tenderizing of the myofibrillar component by aging is due to attack on the G-filaments. This occurs only where an I-band is present, because in a sarcomere which has reached a length of 1.5 μm, the G-filaments exist only as cores to thick filaments and are completely sheathed in myosin tails, that part of the myosin molecule which is α-helical in structure and therefore the most resistant to proteolytic attack. Thus a protease cannot gain access to the G-filaments.

There is one possible chink in the armour. In my model each G-filament must emerge from one A-filament to disappear into another in the next sarcomere. This "G-link" should remain fully vulnerable to proteolysis down to a sarcomere length of 1.55 μm, while from 1.55-1.45 μm it should lie entirely within the Z-line (ca. 0.1 μm). At 1.5 μm the G-link will be vanishingly small. The Z-line may offer protection, since in the intact muscle it is only removed by extreme aging. At sarcomere lengths below 1.45 μm, the G-link, if not sheared by over-run of its two A-filaments, will again be exposed, but at this stage overlap of G-filaments may provide continuity in the cooked state, as formerly proposed for A-filaments.

While the aging results appear to support an "A-core" situation for G-filaments, it must be conceded that even if located outside the A-filaments they could be protected in contracted sarcomeres by the exclusion of proteases from the restricted spaces between the inter-digitated A- and I-filaments, cluttered as they are by rigor cross-links. The indigenous prcteases tend to be medium to large proteins. The molecular weights of cathepsins B, C, D and E are respectively 52, 210, 58 and 305 kD (Greenbaum, 1971), while calcium-actuated factor appears to have a molecular weight of 110 kD (Dayton et al., 1976). However even the slightest penetration of enzyme at the edges of the A-band, where the A-filaments taper, would suffice. The disorientation of A-filaments here by crumpling in contractive bands could assist. Against an exclusion mechanism is the fact that inter-digitated I-filaments do not appear to be protected during aging, as the following experiment indicates.

We have just observed (unpublished results) that cold shortened meat ages fully in terms of yield point, that it shows a drastic decline to values comparable with those for aged unshortened muscle. However shear values on the same aged samples when cooked remain at the high levels characteristic of cold shortened meat at rigor. This is consistent with the view that in short sarcomeres, I-filaments, which determine the yield point of raw myofibrils, are vulnerable to proteolysis inside the A-bands, while G-filaments, which determine the strength of the cooked meat, are not.

In unshortened muscle we found no correlation between yield point and shear force but a good correlation when the same meat was aged (Locker and Wild, 1982a). In the latter case the yield point, although directly measuring I-filament strength, also reflects the degree of proteolytic attack on the G-filaments, which determines the cooked strength.

It may be noted that the modestly sloping part (0-20%) of the shortening-toughening curve of rigor muscle becomes quite horizontal for aged meat (Davey et al., 1967, 1976), which agrees with the idea that in rigor muscle this phase is due to the increasing strength of I-bands as they shorten. This contribution is demolished by aging, but not that of sarcomeres without I-bands.

Tenderization by Pressure-heat Treatment

Australian workers have shown in a considerable series of papers (most recently Macfarlane et al., 1981) that rigor meat may be tenderized by high pressure treatment at elevated temperatures (ca. 100 MPa at 50-60°C for up to an hour). This P-H process seems to act like an accelerated aging. It reduces shear force to similar levels, but does not further reduce it in meat well-aged prior to treatment. This led the authors to propose that the same structural element is attacked in both cases. Unlike aging, it is completely effective on cold shortened meat, which gives the method a unique status. The method does not work where the pressure is applied after incubation at 60°C for an hour or more and this is one of my reasons, offered earlier, for believing that G-filaments become denatured at this temperature. The mechanism suggested for the P-H effect was that pressure reversibly depolymerises A- and I-filaments (an already known effect of high pressure) but at the elevated temperatures used, the protein monomers denature and cannot reassemble.

The technique offers another interesting test for my theory. We have just completed a study on P-H treatment, directed at the fate of G-filaments (Locker and Wild, 1983). Yield point is much affected after only 20 min at 50°C (60 MPa), but to achieve good tenderization of unshortened or cold shortened muscle, 1 h at 60°C is needed. While some histological changes were seen after the harsher P-H treatment, using relaxed or shortened sarcomeres, with or without subsequent cooking at 80°C, none suggested loss of tensile continuity on stretching. No fracturing of G-filaments was seen. However initial extensibility and breaking loads were substantially reduced by high pressure if strips were subsequently cooked at 80° (40 min) or 100°C (3 h). The severe cooking, which destroyed collagen, accentuated the difference between pressurized samples and controls.

As previously discussed, the hour at 60°C involved in the more drastic P-H treatment means, on my theory, that the myofibrils (effectively the G-filaments) have been cooked in the process. This is not true if the treatment is carried out at 50-55°C and curiously an effect on the G-filaments was direct-

ly observable only in such "raw" samples, subjected to P-H treatment for 20 min at 55°C. When extended by 50% both pressurized strips and 55°C controls showed expansion in the I-bands with remarkable uniformity (unlike the erratic expansion of rigor muscle during yielding). The controls showed well defined and preserved G-filaments stretching across the expanded I-bands, while in pressure treated samples these had fractured near the N_2-line.

We conclude that G-filaments are readily and profoundly affected by P-H treatment and that this offers a basis for the tenderization. It is still unclear whether the mechanism is a purely physical effect or whether accelerated aging is involved. For example, the "A-cores" in cold shortened meat could be stripped of protective myosin by depolymerization under high pressure, becoming vulnerable to proteolysis. We have obtained the curious result that when pressure was released at 20 min, reduction of shear force in unshortened muscle continued unabated at 60°C during the next 40 min in two samples, more slowly in two others, and not at all in two more. In contrast, tenderization in all cold shortened samples from the same six animals stopped abruptly with release of pressure.

We have found the striations of perimysial collagen survive our harshest P-H treatment, in agreement with the earlier conclusion of the Australians that connective tissue is not affected by the treatment.

Transverse Strength and Tenderness

Tensile strength of the myofibril cannot fully explain tenderness as registered by a tenderometer and even less by the teeth. Adhesion between and within fibers affects the response of meat to the complex cutting and grinding involved in chewing. This sideways strength may be divided into three categories.

(a) *The perimysium*

The arrangement of the perimysial connective tissue varies enormously between muscles according to their function. It is a subject yet to be seriously explored. For example, the psoas in flexing the back requires layers of fibers at different depths to contract differentially, thus sliding over each other. The sparse perimysium is therefore arranged in planes parallel to the back (Locker and Daines, 1976). The poor adhesion of fiber bundles is no doubt a factor in the culinary rating of fillet. Sternomandibularis on the other hand is tightly laced in all directions by a well developed perimysium, yet even here a distinct lateral anisotropy is manifested in the muscle thickening in its thinnest dimension, but not in its widest dimension, under a variety of stimuli (ibid.). Arrangement of the perimysium may greatly influence tensile properties for example in determining whether a raw muscle yields or breaks (Locker and Wild, 1982a).

The changes in the perimysium on aging are small compared to those in the myofibril. They appear to be real but subtle (for a review see Dutson, 1974). Some effects usually attributed to collagen, such as changes in thermal solubility and shrinkage temperature (e.g. Kopp and Valin, 1981), could equally well reflect modification of the complex networks of proteoglycans known as the "ground substance".

(b) *Fiber to fiber adhesion*

Rapid advances in the understanding of cell to cell adhesion in terms of such substances as fibronectins, open new possibilities in meat research. Although the sarcolemma is a rather specialized cell boundary, fibronectins are present. Ageing appears to cause marked changes in adhesion between fibers. We once observed this in a crude but dramatic way while cutting frozen sections of formalin-fixed muscle on a traditional freezing microtome. Sections of rigor muscle could be readily picked up from the surface of water containing detergent, but sections of aged muscle disintegrated immediately on the surface to a litter of fibers.

(c) *Cross-linking of myofibrils*

The myofibrils are integrated in several ways. The sarcoplasmic reticulum which envelops them provides a degree of lateral adhesion. This should be especially true for the transverse tubules, which also link the myofibrils to the cell membrane. However, lateral shrinkage in cooking appears to cause extensive tearing of the tubules away from the membrane, which itself survives in a somewhat damaged condition (Locker and Daines, 1974).

The most important intracellular transverse links are likely to be those provided by desmin. Grainger and Lazarides (1978) showed that this protein occurs as a transverse fibrous network, forming collars around every Z-line. This explains various reports of links between adjacent Z-lines and between Z-lines and cell membrane, links which appear to survive cooking (Davey et al., 1976). Desmin is one of the few structural proteins to disappear with aging from SDS-polyacrylamide gels (Young et al., 1980). It seems likely therefore that this transverse intra-fibrillar array may be an important factor in a total mechanism for aging.

This brief discussion of transverse strength in muscle is intended to acknowledge the over-simplification of my attempts to explain tenderness in terms of G-filaments, and also to indicate that as a result of new advances in molecular biology, aspects of meat tenderness, which have remained largely inaccessible, now offer some of the most important opportunities in meat science.

Finally, I will not be too surprised if my model for the connections of G-filament requires modification, or if the tenderness theory based on it does not emerge unscathed. I am confident however that the basic notion of G-filaments as the major determinant of the myofibrillar contribution to tenderness will prove as much a survivor as the G-filaments themselves.

Summary

The G-filament theory of tenderness can explain all the known modifiers of meat tenderness.

(1) **Raw meat:** Raw muscle in rigor responds to tensile stress by I-filaments tearing out of the Z-line, while G-filaments and the collagen net stretch readily. Aging causes both I- and G-filaments to snap together.

(2) **Cooking sequence:** Cooking totally changes the roles of the filaments. At 60°C, A-filaments disintegrate, while I-filaments remain visible. The G-filaments slowly denature but remain strong and elastic. The myofibrils be-

come cooked. By 70°C I-filaments have disintegrated, but the important change is the thermal shrinkage of collagen, which pretensions and elasticizes the perimysial net. The meat as a whole is now cooked and consists essentially of elastic G-filaments (the only survivors in the myofibril) stretching in parallel with an elastic collagen film, each contributing significantly to tensile strength. G-filaments survive the most rigorous cooking, but collagen succumbs. The reverse is true for aging.

(3) **Strong A-band:** In cooked meat the A-band becomes an array of G-filaments, twice as numerous as in the I-band, and reinforced with actomyosin coagulum. The weaker I-band is therefore the site of tensile failure.

(4) **Cold toughening:** Cold shortened meat toughens on cooking because of progressive elimination of I-bands in favour of a continuum of G-filaments.

(5) **Aging:** Tenderization by aging is due to proteolytic rotting of G-filaments in the I-band. Disappearance of Z-lines is largely irrelevant. Cold shortened meat does not age, because at the contraction band stage, G-filaments occur only as A-filament cores, completely protected from proteases by resistant myosin tails.

(6) **Pressure-heat treatment** also appears to operate by weakening G-filaments.

(7) **Transverse strength:** It is acknowledged that the theory, set out in tensile terms, is over-simple in that it does not cover transverse structural elements, which are undoubtedly of significance to tenderness.

References

Bailey, A.J., D.J. Restall, T.J. Sims and V.C. Duance. 1979. Meat tenderness: immunofluorescent localization of the isomorphic forms of collagen in bovine muscles of varying texture. J. Sci. Food Agric. 30:203.

Cheng, C.S. and F.C. Parrish. 1976. Scanning electron microscopy of bovine muscle: effect of heating on ultrastructure. J. Food Sci. 41:1449.

Davey, C.L. and M.R. Dickson. 1970. Studies in meat tenderness. 8. Ultrastructural changes in meat during aging. J. Food Sci. 35:56.

Davey, C.L., and Gilbert, K.V. 1967. Structural changes in meat during aging. J. Food Technol. 2:57.

Davey, C.L. and K.V. Gilbert. 1974. Temperature-dependent cooking toughness in beef. J. Sci. Food Agric. 25:931.

Davey, C.L. and K.V. Gilbert. 1975. The tenderness of cooked and raw meat from young and old animals. J. Sci. Food Agric. 26:953.

Davey, C.L., H. Kuttel and K.V. Gilbert. 1967. Shortening as a factor in meat aging. J. Food Technol. 2:53.

Davey, C.L., and Graafhuis, A.E. 1976. Structural changes in beef muscle during aging. J. Sci. Food Agric. 27:301.

Davey, C.L. and A.F. Niederer. 1977. Cooking tenderizing in beef. Meat Sci. 1:271.

Davey, C.L., A.F. Niederer and A.E. Graafhuis. 1976. Effects of aging and cooking on tenderness of beef muscle. J. Sci. Food Agric. 27:251.

Davey, C.L. and R.J. Winger. 1980. The structure of skeletal muscle and meat toughness. In "Fibrous Proteins: Scientific, Industrial and Medical Aspects" (eds. D.A.D. Parry and L.K. Creamer). Academic Press, London. 1:97.

Dayton, W.R., D.E. Goll, M.G. Zeece, R.M. Robson and W.J. Reville. 1976. A calcium-activated protease possibly involved in myofibrillar protein turnover. Purification from porcine muscle. Biochem. 15:2150.

Dutson, T.R. 1974. Connective tissue. Proc. Meat Ind. Res. Conf. p. 99.

Giles, B.G. 1969. Changes in meat produced by cooking. Proc. 15th Europ. Meat Res. Workers Conf. 15:289.

Grainger, B.L. and E. Lazarides. 1978. The existence of an insoluble Z disc scaffold in chicken skeletal muscle. Cell 15:1253.

Greenbaum, L.M. 1971. Cathepsins and kinin-forming and -destroying enzymes. In "The Enzymes" (ed. P.D. Boyer, 3rd ed.) Academic Press, New York. 3:47.

Huxley, A.F. and L.D. Peachey. 1961. The maximum length for contraction in vertebrate striated muscle. J. Physiol. 156:150.

Huxley, H.E. and J. Hanson. 1954. Changes in the cross striations of muscle during contraction and stretch and their structural interpretation. Nature 173:973.

Huxley, H.E. and J. Hanson. 1957. Quantitative studies on the structure of cross striated myofibrils. I. Investigations by interference microscopy. Biochem. Biophys. Acta 23:229.

Jones, S.B., R.J. Carroll, and J.R. Cavanaugh. 1977. Structural changes in bovine heated muscle: A scanning electron microscope study. J. Food Sci. 42:125.

King, N.L. and L. Kurth. 1980. SDS Gel electrophoresis studies of connectin. In "Fibrous Proteins: Scientific, Industrial and Medical Aspects" (eds. D.A.D. Parry and L.K. Creamer). Academic Press, London. 2:57.

King, N.L., L. Kurth, and W.R. Shorthose. 1981. Proteolytic degradation of connectin, a high molecular weight myofibrillar protein during heating of meat. Meat Sci. 5:389.

King, N.L. and P.V. Harris. 1982. Heat induced tenderization of meat. Meat Sci. 6:137.

Kopp, J. and C. Valin. 1981. Can muscle lysosomes affect muscle collagen post mortem? Meat Sci. 5:319.

Leander, R.C., H.B. Hedrick, M.F. Brown, and J.A. White. 1977. Effect of heating on the ultrastructure of bovine muscle. Proc. 30th Ann. Reciprocal Meat Conf. p. 99.

Leander, R.C., H.B. Hedrick, M.F. Brown, and J.A. White. 1980. Comparison of structural changes in bovine longissimus and semitendinosus muscles during cooking. J. Food Sci. 45:1.

Locker, R.H. 1956. The dissociation of myosin by heat coagulation. Biochem. Biophys. Acta, 20:514.

Locker, R.H. 1982. A new basis for meat tenderness in terms of gap filaments. Proc. 28th Congr. Europ. Meat Res. Workers, Madrid. In press.

Locker, R.H. 1983. Cold induced toughness in meat. In "Advances in Meat Science". (Eds. A.M. Pearson and T.R. Dutson). Avi Publishing Co., Westport (Conn.) In press.

Locker, R.H. and W.A. Carse. 1976. Extensibility, strength and tenderness of beef cooked to various degrees. J. Sci. Food Agric. 27:891.

Locker, R.H. and G.J. Daines. 1974. Cooking loss in beef. The effect of cold shortening, searing and rate of heating; time course and histology of changes during cooking. J. Sci. Food Agric. 25:1411.

Locker, R.H. and G.J. Daines. 1975a. Effect of shortening during cooking on the tenderness and histology of beef. J. Sci. Food Agric. 26:1711.

Locker, R.H. and G.J. Daines. 1975b. Rigor mortis in beef sternomandibularis muscle at 37°C. J. Sci. Food Agric. 26:1721.

Locker, R.H. and G.J. Daines. 1976. Transverse anisotropy in beef muscle. J. Sci. Food Agric. 27:186.

Locker, R.H. and G.J. Daines. 1980. Gap filaments — the third set in the myofibril. In "Fibrous Proteins: Scientific, Industrial and Medical Aspects". (eds. D.A.D. Parry and L.K. Creamer) Academic Press, London. 2:43.

Locker, R.H., G.J. Daines, W.A. Carse and N.G. Leet. 1977. Meat tenderness and the gap filaments. Meat Sci. 1:87.

Locker, R.H., C.L. Davey, P.M. Nottingham, D.P. Haughey and N.H. Law. 1975. New concepts in meat processing. Adv. Food Res. 21:157.

Locker, R.H., and N.G. Leet. 1975. Histology of highly stretched beef muscle 1. The fine structure of grossly stretched single fibres. J. Ultrastructure Res. 52:64.

Locker, R.H. and N.G. Leet. 1976a. Histology of highly stretched beef muscle II. Further evidence on the location and nature of gap filaments. J. Ultrastructure Res. 55:157.

Locker, R.H. and N.G. Leet. 1976b. Histology of highly stretched beef muscle IV. Evidence for movement of gap filaments through the Z-line, using the N_2-line and M-line as markers. J. Ultrastructure Res. 56:31.
Locker, R.H. and D.J.C. Wild. 1982a. Yield point in raw beef muscle. The effects of aging, rigor temperature and stretch. Meat Sci. 7:45.
Locker, R.H. and D.J.C. Wild. 1982b. A machine for measuring yield point in raw meat. J. Texture Studies 13: in press.
Locker, R.H. and D.J.C. Wild. 1982c. Myofibrils of cooked meat are a continuum of gap filaments. Meat Sci. 7:61.
Locker, R.H. and D.J.C. Wild. 1983. Tenderization of beef by pressure-heat treatment in relation to gap filaments. Meat Sci. submitted for publication.
Locker, R.H., D.J.C. Wild and G.J. Daines. 1982. Tensile properties of cooked beef in relation to rigor temperature and tenderness. Meat Sci. 7: in press.
Macfarlane, J.J., I.J. McKenzie and R.H. Turner. 1981. Pressure treatment of meat. Effects on thermal transitions and shear values. Meat Sci. 5:307.
Macfarlane, P.G. and J.M. Marer. 1966. An apparatus for determining the tenderness of meat. Food Technol. 20:838.
Marsh, B.B. and W.A. Carse. 1974. Meat tenderness and the sliding filament hypothesis. J. Food Technol. 9:129.
Marsh, B.B. and N.G. Leet. 1966. Studies in meat tenderness. III. The effects of cold shortening on tenderness. J. Food Sci. 31:450.
Maruyama, K., S. Kimura, N. Toyota and K. Ohashi. 1980. Connectin, an elastic protein of muscle. In "Fibrous Proteins: Scientific, Industrial and Medical Aspects". (eds. D.A.D. Parry and L.K. Creamer). Academic Press, London. 2:33.
Maruyama, K., S. Matsubara, R. Natori, Y. Nonomura, S. Kimura, K. Ohashi, F. Murakami, S. Handa and G. Eguchi. 1976. Connectin, an elastic protein of muscle. J. Biochem. 82:317.
Moran, T. and H.P. Hale. 1928. The "bloom" of meat. Ann. Report Food Invest. Board, D.S.I.R. (H.M.S.O.) p. 10.
Rowe, R.W.D. 1974. Collagen fibre arrangement in intramuscular connective tissue. Changes associated with muscle shortening and their possible relevance to raw meat toughness measurements. J. Food Technol. 9:501.
Rowe, R.W.D. 1981. Morphology of the perimysial and endomysial connective tissue in skeletal muscle. Tissue and Cell. 13:681.
Samejima, K., M. Ishioroshi and T. Yasui. 1981. Relative roles of the head and tail portions of the molecule in heat induced gelation of myosin. J. Food Sci. 46:1412.
Schmitt, J.G. and F.C. Parrish. 1971. Molecular properties of post mortem muscle. 10. Effect of internal temperature and carcass maturity on the structure of bovine longissimus. J. Food Sci. 36:110.
Sjöstrand, F. 1962. The connections between A- and I-band filaments in striated frog muscle. J. Ultrastructure Res. 7:225.
Voyle, C.A. 1969. Some observations on the histology of cold shortened muscle. J. Food Technol. 4:275.
Wang, K., J. McClure and A. Tu. 1979. Titin: Major myofibrillar component of striated muscle. Proc. Natl. Acad. Sci. U.S.A. 76:3698.
Wang, K. and C.L. Williamson. 1980. Identification of an N_2 line protein of striated muscle. Proc. Natl. Acad. Sci. U.S.A. 77:3254.
Yasui, T., M. Ishioroshi and H. Nakano. 1980. Heat induced gelation of myosin in the presence of actin. J. Food Biochem. 4:61.
Young, O.A., A.E. Graafhuis and C.L. Davey. 1980. Post mortem changes in cytoskeletal proteins of muscle. Meat Sci. 5:41.

Fresh Meats and Microbiology Discussion

M. J. Marchello, N. Dakota: Do we really have a problem with trichinae?

A. W. Kotula: Back in the early 1900's we did, but to my knowledge now there is less than ½ of one percent incidence of any trichinae occurring in any of our pork products.

I think that the last information that I saw that Zimmerman published was 0.125 percent incidence and I will let you decide whether you have a problem or not. What we were doing in our work was to try to ensure that we did not come out with any recommended cooking procedures which would not destroy the trichinae if present and we always said "if present." Now, the press looks at it one way and there is not much we can do about that. I think it is essential, however, for scientists to be aware of our concern that, if trichinae were to be present in pork, some of the methods which we had worked with and evidently some of the methods that Bill Zimmerman has worked with would not provide adequate heat to destroy the trichinae.

D. M. Kinsman, Connecticut: Gay, do you have any information about the relative merits of prebrowning as it pertains to the time element and the energy element?

Starrak: Not as specific as I would like to have. There has been some work done with that at Texas, I believe. We did find some disadvantages to prebrowning in that it does increase both the time required and the amount of energy used. Also, many cooks don't particularly like to use the browning dishes that go in a microwave oven. They are accustomed to thinking of microwave being cool cooking and there is a danger of getting burned when you find this hot dish — and a browning dish is really very hot. If you are browning meat conventionally on top of the range, you have two cooking pans to deal with rather than one. One of the main advantages of microwave ovens is that the cleanup is much easier than in conventional ovens. When you use browning dishes and skillets you wipe out the advantage that you have with microwave cooking. Perhaps somebody else knows more about the comparative energy used, but microwave does save energy in most cases. In some cases, like beef patties, it compares about the same as cooking them on a surface unit of a gas range.

Thayne Dutson, Texas: King and Harris, and King and associates weren't able to find connectin after heat treatment of muscle. There are a couple of possibilities I see and I was wondering if you would comment on them. One is that maybe their method of measurement after denaturation didn't detect the connectin. The other possibility is that gap filaments are possibly composed of something in addition to connectin. In other words, could there be a connectin component or maybe something in addition to that?

R. H. Locker: I actually had that result in my script but I took it out, being pressed for time. It is perhaps one of the nicest effects on the face of it for my theory. When you cook meat at 80 degrees, connectin disappears. Certainly this proves that gap filaments can't be of any significance in cooked meat.

M. J. Marchello, N. Dakota: I have another question in relation to the stretching of the A band and the pulling of the myosin filaments in both directions. You did show a micrograph where at times that doesn't happen and we have noticed that as well. What do you think is the reason for those