MYOFIBRILS AND MEAT QUALITY

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Myofibrils occupy about 83% of the volume of muscle fibres in living muscle and provide the majority of the structural protein. They are therefore a prime candidate to play a role in two key aspects of the eating quality of meat, water-holding and toughness. We shall see, however, that these attributes are determined by the interaction of muscle fibres with connective tissue and, although we shall concentrate on the contribution made by myofibrils, connective tissue cannot be left out of the discussion. We shall approach these topics primarily from a structural viewpoint. All levels of structure, from single protein molecules to macroscopic features like the perimysial network that can be seen by eye, contribute to our understanding of these topics.

WATER HOLDING

Lean meat contains about 75% water. The content of water is important economically because meat is sold by weight. Both the content of water and its distribution within the muscle are important for determining the properties of muscle and its quality; for example, it is possible to have two pieces of meat with the same content of water, but one soft and exudative and the other firm and dry.

Meat can lose water not only by evaporation from its surfaces but also by drip oozing from the cut ends or by expulsion of fluid during cooking. Conversely, during processing, when meat is treated with salt or with acid marinades, water is taken up either from the external bathing medium or, if the meat has been injected, from internal pockets formed at the injection sites.

Although much has been written on the subject of water-holding (see Hamm 1960; 1986), we have found it illuminating to study water-holding from a structural viewpoint (Offer and Knight 1988 a,b). In this section we shall discuss the mechanisms by which water is lost from, or gained by, muscle and how these affect the distribution of water between compartments.

Most of the water in living muscle is in the myofibrils in the spaces between thick and thin filaments (fig.1). The general hypothesis which we are testing is that gains or losses of water originate in changes in the volume of myofibrils. In the raw state we suppose that these occur by a lateral expansion or shrinking of the filament lattice (fig. 1).

Drip Loss

We have tested the hypothesis that drip losses originate from shrinkage of the myofibrillar filament lattice by determining the centre-to-centre separation of filaments in muscle post-mortem. Although in beef *rectus abdominis* muscle there is initially little change in spacing with time, at the onset of rigor there is a 4.4% decrease in spacing, corresponding to a 9% volume decrease (fig.2).

The shrinkage is partly due to the fall in pH of the meat post-mortem. The pH fall reduces the negative charge on the thick and thin filaments and hence reduces the repulsive forces between them causing the filament lattice to shrink to a new equilibrium where the reduced electrostatic pressure is balanced by the reduced restraint from transverse structural elements (Matsuda and Podolsky 1986). The other effect is that the attachment of myosin heads to actin at rigor, even at constant pH, diminishes the filament lattice spacing, probably because the myosin heads attach with the least strain only at a preferred filament spacing (Matsubara et al. 1984). On this basis we would expect that in DFD meat with a high final pH the lattice would not shrink so much and we can thus see why drip loss is diminished in the DFD state.

Where does the fluid expelled from the myofibrils accumulate? Transverse sections of beef *sternomandibularis* muscle fixed at various times post-mortem show that soon after death there are no large channels in the meat; the fibres fill the endomysial network, and the fibre bundles fill the perimysial network (fig.3b), but by about 4-6 h post-mortem, sufficient myofibrillar shrinkage has occurred for the fibre bundles to shrink away from each other leaving fluid-filled gaps around the perimysial network (fig.3c). At rigor, the shrinkage is sufficient for the fibres to separate from one another leaving fluid-filled gaps also between the fibres and the endomysial network (Hegarty and Heffron 1974; Offer et al 1987; fig.3d).

The remaining question is how this extra-cellular fluid reaches the surface as drip. The lower side of a piece of meat drips more than the upper side, so we think the main force driving out drip is gravity (Offer 1984). However, the upper surface produces some drip and we think it likely that hydrostatic pressure produced in the meat by the myofibrillar shrinkage pressure also plays a role. Because in the micrographs the channels between fibre bundles appear wider than those between fibres, we have supposed that drip flow is predominantly between fibre bundles. Recently we have tested this notion by applying under a small hydrostatic pressure a solution of fluorescein-tagged serum albumin and following its appearance at the far side of the meat. The first fluorescence appears in the coarser features of the

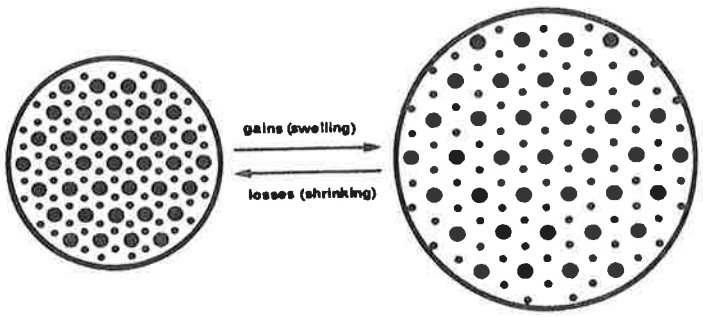

Fig. 1. Hypothesis explaining the origin of changes in water-holding. On the left hand side is shown schematically a transverse section of a myofibril. On the right hand side the same myofibril is shown with an expanded filament lattice.

perimysial network (Offer, Starr, Almond and Knight unpublished results). We conclude that drip flow is predominantly along the channels formed between fibre bundles.

In the PSE state drip flow is increased. Preliminary X-ray diffraction experiments show that the shrinkage of the myofibrils is about twice as much in the PSE state as in normal muscle (Knight, unpublished results; fig.2), so the extra amount of drip is easily accounted for. As yet we have not studied the extra-cellular spaces in PSE muscle, but we suppose that this extra shrinkage widens the channels along which drip flows. What causes the extra shrinkage of myofibrils in PSE? It is well established that PSE muscle results when the pH of the meat is low while the carcass is still warm (Wismer-Pedersen 1959). This combination causes the denaturation of several proteins. Although the denaturation of sarcoplasmic proteins may contribute to the increase in light scattering power, it would be difficult to explain changes in the water-holding other than by a change in a myofibrillar protein and Penny (1967a) has shown that myosin is de natured under the conditions producing PSE. We have recently investigated by electron microscopy of negatively stained myosin molecules what changes in shape of the myosin molecule occur when myosin is kept at pH 6.0 and 35°C, conditions which PSE muscle has experienced and under which myosin loses half its ATPase activity in about 8 min (Penny 1967b) . We find that after 5 or 10 minutes the average length of the myosin heads decreases significantly from 19 to 17 nm (Sharp and Offer, unpublished results). Assuming that in rigor muscle the long axis of the heads lies in a plane containing the long axes of the thick and thin filaments, and lies at an angle of 50° to the muscle fibre axis, a reduction of 2 nm in the myosin head length would reduce the surface-to-surface distance between thick and thin filaments by 2 sin 50° nm = 1.5 nm. Taking the centre-to-centre distance between thick and thin filaments to be about 24 nm in normal rigor muscle, the reduction in myosin head length of 2 nm would therefore cause an additional 6% reduction in the filament lattice spacing or a 12% reduction in myofibrillar volume (fig.2). Thus the seemingly minute change in the shape of the myosin head as a result of denaturation is sufficient to account for the enhanced exudation in the PSE state, a graphic illustration of the importance of understanding molecular events to explain phenomena in meat science.

Water-Uptake

We have examined the response of myofibrils to salt solutions by irrigating myofibrils on a microscope slide (Offer and Trinick 1983; Offer et al. 1987). When myofibrils are treated with a series of NaCl solutions of increasing concentration and buffered to pH 5.5, little happens until a NaCl concentration of 0.6 M is reached. At this concentration the myosin from the centre of the A-band (approximately the H-zone) is extracted and the myofibril swells laterally. The swelling increases at 1 M NaCl. In 1 M NaCl the average increase in cross-sectional area of rabbit myofibrils is 92% ± 52% (SD) (Offer et al. 1987). At much higher NaCl concentrations no extraction of myosin and no swelling occurs (Knight and Parsons, unpublished results), as might be expected from the results of swelling in meat pieces (Callow 1932).

The inclusion of 10 mM pyrophosphate, the simplest polyphosphate, causes substantial changes in the pattern of extraction. There is little change when myofibrils are irrigated with 0.3 M NaCl plus 10 mM pyrophosphate, but when the NaCl concentration is increased to 0.4 M, extraction begins from the ends of the A-band and continues until all, or nearly all, of the A-band has dissolved. In the presence of pyrophosphate some lateral swelling occurs, but this is smaller on average than with NaCl alone. For rabbit myofibrils the average increase in cross-sectional area in 1 M NaCl, 10 mM pyrophosphate was only 39% ± 45% (SD).

How can the salt-induced swelling be explained? An important clue is that myosin solubilisation and swelling often accompany one another. NaCl at 0.4 to 1 M concentration is known to be a structure-breaker and one of its main effects on the myofibril is to cause depolymerisation of the thick filament to myosin molecules. In the absence of pyrophosphate, the myosin molecules formed will tend to remain attached to the actin filaments in the overlap region (fig.4b). Now we know that in the myosin molecule the tails are very flexibly attached to the myosin heads, so once the thick filament shaft is depolymerised, there will be a tendency for the myosin molecules to explore space (fig.4b). In collaboration with Barry Millman and Bernie Nickel we have proposed that in the unswollen lattice the ability of the tails to rotate is greatly restricted by the presence of neighbouring decorated thin filaments. Swelling of the lattice would allow greater freedom and this provides substantial entropic pressure causing the lattice to swell (fig.4c). We have provided some support for this hypothesis by irrigating myofibrils, depleted of myosin by previous salt-extraction in the presence of pyrophosphate, with a myosin solution. The myosin binds to the thin filaments and the myofibrils swell (Offer et al. 1987).

With this hypothesis we can explain why salt-induced swelling in the presence of pyrophosphate is small for

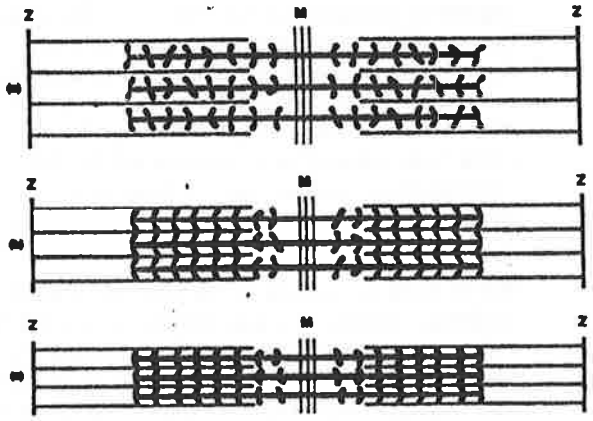

Fig. 2. Lateral shrinkage of a myofibril post-mortem. (a) Living muscle. The myosin heads are detached and the filament spacing relatively wide. (b) Normal rigor muscle. The pH has fallen, the myosin heads have attached to the thin filaments and the filaments are closer together. (c) PSE muscle. Shortening of the myosin heads causes the filaments to be still closer.

myofibrils and much larger for meat pieces. In our irrigation experiments, myofibrils are exposed to a large excess of salt solution. Pyrophosphate promotes the dissociation of actomyosin, so in its presence the myosin molecules formed by depolymerisation of the thick filament will tend to be extracted and be unavailable to drive the entropic swelling mechanism. When meat pieces are treated with a relatively small volume of salt and polyphosphate, a smaller fraction of the myosin will be extracted and a larger swelling would be expected.

Endomysial Restraint

We can now ask how the swelling behaviour of muscle fibres is modified in meat by the presence of connective tissue. The salt-induced swelling of dissected single muscle fibres is very variable from fibre to fibre (Offer et al. 1987). This variability arises because some fibres when dissected lack an endomysial sheath, whereas others are enclosed by one. The presence or absence of an endomysial sheath was determined by dissolving out myofibrillar proteins with the detergent SDS. A sheathed fibre can be recognised because after this treatment an empty sheath can be observed. It turns out that fibres lacking an endomysial sheath swell substantially in salt, but those possessing one swell to a much smaller extent. This clearly confirms the conclusion of Wilding et al. (1986) that the endomysium acts as an important mechanical restraint on swelling. Fibres lacking an endomysial sheath swell greatly at first in salt but subsequently shrink below the starting volume (Offer et al. 1987). Sheathed fibres swell much less but the swelling remains constant with time. This may be explained by the loss of myosin from the naked fibres.

The marked effect of time post-mortem on the swelling of rabbit fibres observed by Wilding et al. (1986) results from the relative ease, after a short conditioning period, with which the endomysium can be stripped off the fibre, presumably as a result of proteolysis. Changes in the swelling of fibres with time of conditioning can be explained in terms of the proportion of fibres that are sheathed, and no effect of time on the salt-induced swelling of either naked or sheathed fibres was apparent (Knight and Elsey, unpublished experiments). These results suggest that the variability in the suitability of meat for processing depends on the ease with which on comminution the muscle fibres are stripped from their endomysia. The implication is that, for high water uptake and myosin extraction, a time of processing should be chosen when comminution causes the endomysium in a high proportion of the fibres to be removed or ruptured.

Cooking Losses

When meat is cooked, a variable amount of lateral shrinkage occurs at temperatures between 40°C and 60°C; substantial longitudinal shrinkage occurs at temperatures above 60°C (Bouton et al. 1976; Locker and Daines 1976). Together these may result in up to about a 40% volume decrease on cooking. We will now discuss the structural changes responsible.

Light microscopy of transverse sections of cooked meat show that, on cooking at temperatures up to 90°C, the muscle fibres shrink within their endomysial sheaths leaving larger fluid-filled gaps than are present at rigor (Schmidt and Parrish 1971; Offer et al. 1984) (fig.3). Evidently the fibres shrink of their own accord and are not compressed. Isolated single muscle fibres shrink laterally beginning at about 40°C and the maximum rate occurs at 60°C (Bendall and Restall 1983). Lateral shrinkage also occurs on heating myofibrils, making it clear that fibre shrinkage is an active, not a passive, process (Offer et al. 1984). Such shrinkage is probably similar in nature to that occurring in PSE but requires a higher temperature because of the greater protection to denaturation of myosin given by actin compared with ATP. It should be emphasised that the lateral shrinkage of muscle fibres would not in itself cause any loss of water from the meat and would not therefore cause any change in size of the meat. The expelled water would initially merely accumulate in the spaces between fibres. Over a short time-period gravity may drain some fluid to the surface as it does in PSE, but larger fluid losses from these gaps probably requires the tension generated by the thermal shrinkage of connective tissue.

Beginning at about 50°C, the collagen of the endomysial sheaths begins to denature. Isolated sheaths shrink both longitudinally and laterally (Light and Bailey 1983; Purslow and Champion unpublished results), but in meat, since the sheaths touch the muscle fibres at intervals along the fibre axis, longitudinal shrinkage of the sheaths would initially be resisted. Lateral shrinkage of the endomysial sheaths would exert pressure on the fluid between the fibre and the sheath, driving it out to the

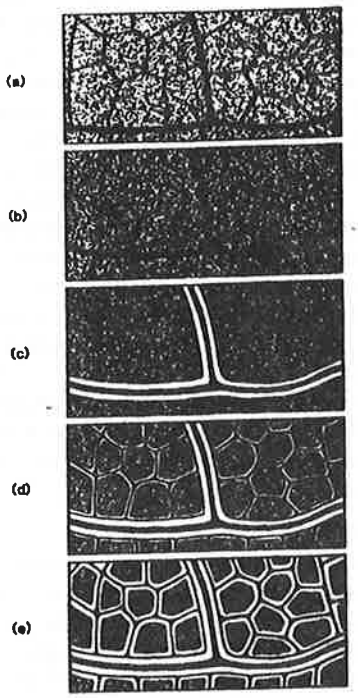

Fig. 3. Stages in the swelling or shrinkage of muscle. Each diagram depicts a transverse section including parts of three fibre bundles separated by perimysium (thick lines). The fibres (stippled) are separated by the endomysial network (thin lines). Starting with living muscle (b), shrinkage of the fibres occurs post-mortem (c and d) giving rigor muscle (d) with gaps between fibre bundles and between fibres. In PSE and cooked muscle (e) the gaps are larger. Starting with rigor muscle (d), salt-swelling of the fibres would initially cause disappearance of the gaps (c and b) and finally swelling of the muscle as a whole (a).

surface, the pressure increasing with temperature. If lateral shrinkage of the endomysial sheaths occurs, the muscle fibres will get closer together and the meat as a whole will shrink laterally. But the amount of shrinkage of the sheaths and therefore the amount of water lost from the meat in the process would depend on the viscous resistance and therefore the size of the meat; this may explain the large variability in lateral shrinkage observed. Beginning at about 64°C, when the collagen of the perimysium begins to denature (Mohr and Bendall 1969), this flow of fluid along channels to the surface will be augmented by the perimysial network exerting pressure on the fluid between fibre bundles.

Although lateral shrinkage of muscle fibres and connective tissue occur independently, their longitudinal shrinkage is functionally coupled because of the residual links between them. Muscle strips begin to shrink in length at 64°C, the degree of shrinkage increasing with temperature (Bouton et al. 1976; Bendall and Restall 1983). Muscle fibres and myofibrils also shrink longitudinally (Aronson 1966; Hostetler and Landmann 1968; Offer et al. 1984).

We can therefore speculate that, beginning at 50°C and increasing dramatically at 64°C, the connective tissue will exert a longitudinal compressive force on the muscle fibres. When some critical temperature is reached where the fibres also tend to shrink longitudinally, both connective tissue and fibres shrink co-operatively. Unfortunately very little is known about this step. We do not know, for example, what change in cross-sectional area of the muscle fibres and the endomysial sheaths accompanies this longitudinal shrinkage. If the endomysial sheaths remain of constant cross-sectional area, inevitably the longitudinal shrinkage will be accompanied by a corresponding decrease in volume and fluid loss. We can regard this process as the muscle fibres and connective tissue retracting away from the water. The proportions of water lost from the fibres and from the fluid-filled gaps in this process remain to be determined.

TOUGHNESS

In contrast to the very clear role that myofibrils have in determining the water-holding properties of meat, their role in determining its toughness is not yet clear. When meat is chewed, it is pulled apart in the mouth. The difficulty with which this is achieved is perceived as the sensation of toughness. Although a variety of mechanical tests have been applied to meat, we shall concentrate on the results of tensile tests because they are easiest to interpret (Bouton et al. 1975). We shall lay particular emphasis on breaking strength since this correlates reasonably well with the sensory perception of toughness (Stanley et al., 1972). We shall discuss what happens to the structure of muscle when strips are pulled apart in a direction parallel to the fibre axis. The question we shall particularly address is whether the muscle fibres or the connective tissue dominate the breaking strength in the relaxed, rigor and cooked states.

Relaxed Muscle

Strips of muscle excised soon after death can be extended very readily to nearly twice their rest length with a low load (about 0.1 kg/cm^2). If the load is removed, the strips return to their original length (Davey and Dickson 1970). Further extension is resisted very strongly by the perimysial connective tissue (Davey and Dickson 1970; Locker and Leet 1975; Purslow 1988).

At rest length the collagen fibres in the criss-cross lattice of the perimysium are crimped and make an angle θ of about 55° to the muscle fibre axis. Extension or shortening of the muscle causes a change in θ and stretches out the crimp (Rowe 1974; Purslow 1988). This sets up a restoring force which is small for moderate changes in the sarcomere length. However, at about twice rest length the collagen fibres lose their crimp and the network resists extension very strongly (Purslow 1988).

In isolated muscle fibres or fibre bundles the perimysium is no longer present and they can be extended to four times rest length (Locker and Leet 1975; 1976a). In relaxed muscle the cross-bridges are not attached and therefore sliding of thick and thin filaments can take place readily. Continuity between successive Z-discs is provided by the gap filaments which form an integral part of the thick filament, emerge from its tips and continue to the Z-discs (see reviews by Locker 1984; Squire et al. 1987; Offer 1987). Gap filaments are probably made of the protein titin (connectin) which extends all the way from the M-line to the Z-disc.

Thus when relaxed muscle is extended, extension occurs by sliding of non-interacting thick and thin filaments and the elongation of gap filaments outside the A-band. Much of the resistance to stretch at moderate extensions is provided by the gap filaments, with the sarcolemma and endomysium making a greater contribution at higher extensions for single fibres (Podolsky 1964). When muscle strips are extended to a similar degree (about

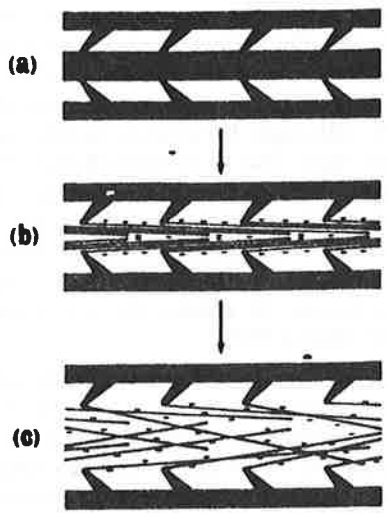

Fig. 4. Mechanism of swelling of myofibrils in salt. (a) Longitudinal section through muscle showing two thin filaments and one thick filament with myosin heads linking them. (b) In salt the thick filament shaft depolymerises but before swelling the motion of the myosin tails is restricted. (c) After swelling the myosin tails are able to move through a larger angle.

twice rest length), the high resistance to stretch of the perimysium comes into play.

Rigor Muscle

At the onset of rigor, there is a very marked increase in stiffness of muscle (Bate-Smith 1939). Beef *sternomandibularis* muscle in rigor shows a high resistance to extension by light loads (Davey and Dickson 1970), but if the load is increased, a critical point is reached (~1 kg/cm^2 at an extension of about 18%) where the muscle flows without further increase of load to a length of about 1.8 times rest length (Locker and Daines 1975b; Locker and Leet 1976b; Locker and Carse 1976). Further loading lengthens the muscle to about twice rest length at which point the perimysial connective tissue resists further extension. When the load is removed, the muscle returns only a small way towards its original length, so evidently the structural changes produced are irreversible. The breaking strength of unaged beef *sternomandibularis* muscle is about 2.9 kg/ cm^2 (Locker et al. 1983) but the breaking strength varies widely from muscle to muscle and with the sex of the animal (Locker and Wild 1982a).

The stiffness of rigor muscle diminishes with ageing and strips of aged rigor muscle can be stretched to about twice rest length by a load (0.1 kg/cm^2) about as small as that required by relaxed muscle (Davey and Dickson 1970). This is because the yield point of beef *sternomandibularis* muscle is drastically reduced by ageing (Locker and Wild 1982a). As with the unaged rigor muscle, the aged muscle shortens only slightly when the load is removed and extension beyond twice rest length requires a high load (Davey and Dickson 1970) . Ageing post-rigor causes no reduction in the breaking strength of rabbit muscle that had entered rigor unrestrained and only a small reduction in muscle that had been restrained on the carcass (Stanley et al. 1971).

With regard to the plastic extension of unaged rigor muscle under high loads, there are strong indications that extension occurs by an increase of sarcomere length brought about by an increase in length of the I-bands with little or no damage to the ultrastructure (Locker and Leet 1976b, Hegarty et al. 1973, Locker and Wild 1982a), although breakages in the thick filaments have also been seen (Rowe 1982). The most likely explanation is that the thick and thin filaments can creep by the preferential detachment of highly strained cross-bridges followed by their rapid reattachment in relatively unstrained positions (Kuhn 1978).

We can now consider the mechanism by which *aged* rigor muscle can be easily extended. In beef *sternomandibularis* muscle aged at 15°C no breaks are observed by light microscopy prior to extension, but after extension to twice the initial length, many breaks were seen in the fibres with substantial gaps separating the fractured ends (Davey and Dickson 1970; Locker and Wild 1982a). The fractured fibres were still, however, enveloped in their endomysial sheaths. In these aged fibres the sarcomere length did not increase on stretching, but it is not clear whether the number and size of the gaps was sufficient to explain the extension, or whether some other mechanism (eg slipping of fibres or myofibrils) also occurred.

The fractures formed when aged muscle is extended occur mainly at the junction of the thin filaments and the Z-disc, but occasionally at the junction between the A-band and I-band (Davey and Dickson 1970; Locker and Wild 1982a). It is often supposed that weakening of the myofibril must occur specifically at the sites where fracture is observed, but it is then difficult to see why fracture can occur at these two different sites. At the junction of each thin filament with the Z-disc and, to a lesser extent, where the thin filament enters the A-band, there is a rapid change in the amount of load-bearing material. Such a 'step' in the load-bearing structure will cause concentrations of stress at the step (eg Timoshenko and Goodier 1954), (fig. 5). These concentrations of stress, over and above the remotely applied stress on the ends of the muscle fibres, make fracture most likely to occur at these sites even if the thin filaments were uniformly weakened by proteolysis.

Cooked Muscle

Studies on the extension of relaxed and rigor muscle set the scene for what is the most important state for the study of eating quality, the cooked state. Cooking produces a marked change in the mechanical properties of meat. The plateau on the load-extension curve shown by rigor muscle essentially disappears and the yield point becomes far less obvious, but is sometimes visible as an inflection on the rising load-extension curve (Locker and Daines 1975b; Bouton et al. 1975; Locker and Carse 1976; Locker et al. 1983). Moreover, when the load is removed, the muscle shortens nearly back to its original length.

Cooking at 60°C slightly lowers the initial stiffness below that of uncooked rigor muscle, but cooking at 70-90°C raises both the initial and final stiffness to about twice that of uncooked muscle (Locker et al. 1983 but see Locker and Carse 1976). Furthermore, the breaking strength of unaged beef *sternomandibularis* muscle cooked to

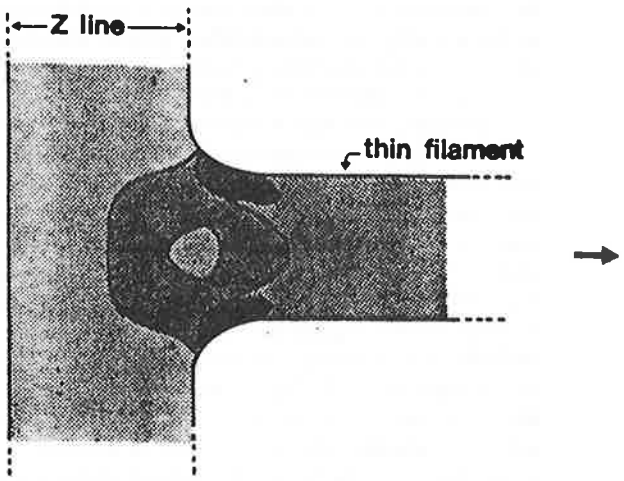

Fig. 5. Schematic diagram of stress concentrations at the Z-disc/thin filament junction. Increased density of shading indicates a higher stress concentration. Greatest stress concentration occurs at the corner of the step.

50-80°C (4 to 5 kg/cm^2) is considerably greater than that of uncooked muscles and that for cooked cold-shortened muscle is similar or only a little higher (Bouton et al. 1975; Locker et al. 1983). With more extreme cooking (3 h at 100°C) of normal-length muscle, where collagen would be expected to be largely solubilised, the breaking strength fell to 1.8 kg/cm^2 (Locker and Carse 1976; Locker et al. 1983).

We can now consider the structural changes that occur in meat on cooking. The collagen fibres in meat are attached to the muscle fibres and their shrinkage in length on heating is partially restrained. The criss-cross lattice of collagen fibres in the perimysium survives cooking at 80°C, although the fibres are now decrimped (Locker and Daines 1975a). Purslow and Lewis (unpublished experiment) dissected out strips of perimysium after cooking meat at various temperatures. As expected, in the raw state perimysial strips had a low stiffness at extensions up to about 90% but a much higher stiffness at higher extensions. The strips of cooked perimysia were markedly stiffer at extensions up to 90% and the stress-strain plots were more nearly linear. The breaking strength of the cooked perimysia (about 3.7 MPa) was about 60% higher than in the uncooked state. In part the increased stiffness is likely to arise from the decrimping of the collagen fibres and in part due to restrictions in the way 8 can alter with stretch.

In contrast to the decreased extensibility of whole muscle and perimysia after cooking, single muscle fibres are more extensible after cooking (Wang et al. 1956, Hostetler and Cover 1961). Unfortunately, the stress-strain curves and breaking strength of cooked single muscle fibres have not been determined.

With regard to changes in muscle fibres when meat is cooked, the thick filaments beg in to fuse and the thin filaments disintegrate at about 60°C, but A- and I-bands are still present (Schmidt and Parrish 1971). Locker and his co-workers have provided evidence that the gap filaments survive cooking (Locker et al. 1977; Locker and Wild 1982b) even though as a result of proteolysis there may be many nicks in the titin (King and Kurth 1980, King et al. 1981). Unfortunately, we have very little understanding of the structure of the cooked myofibril and it is possible that load-bearing structures formed from the thick and thin filaments are present which are too disorganised or have too fine a structure to be detected by microscopy. Myosin and actomyosin form gels on heating above 40°C (Samejima et al. 1969) both at high ionic strength and low ionic strength where in the uncooked state myosin would be present as molecules or filaments respectively. The cohesive strength of a 105 mg/ml actomyosin gel is about 0.3 kg/cm^2 (Nakayama and Sato 1970), so the higher concentration of actin and myosin in the cooked A-band might alone provide a strong structure. It is not at all clear what happens to actin and the other proteins of the thin filament on cooking. So while it is plausible that the only load-breaking structures in the cooked myofibril are gap filaments periodically reinforced by the myosin gel in the A-band, this assumption needs to be critically tested.

When unaged beef *sternomandibularis* muscle is cooked and stretched by up to 30%, the A-band appears unchanged and extension occurs almost entirely in the I-band with the filaments becoming clearer (Locker and Wild 1982b). At 50% extension, the A-bands are markedly stretched and are then seen to contain an array of fine filaments in a freckled matrix, presumed to be gap filaments in a fragmented coagulum of actomyosin. At a stretch of 77% the sarcomere lengths extend by a similar proportion and the A-bands are 72% longer, the I-bands 98% longer and the Z-discs 67% longer (Locker et al. 1977). When muscle that went into rigor at twice excised length prior to cooking is extended to breaking point, fracture occurs between the A- and I-bands. It was concluded that gap filaments were broken (Locker et al. 1977). Thus in such extended material the gap filaments may provide the breaking strength but it cannot be concluded that the same would be true for cooked muscle of normal length.

If the muscle is aged for 1 day at 15°C, cooked and then stretched by 50%, the I-band, but not the A-band, extends and broken filaments are seen in the I-band, presumably due to proteolytic attack. In some cases short filaments emerge from the edge of the A-band. It thus appears that in cooked aged meat the I-bands have been sufficiently weakened that extension takes place there at tensions too low to extend the A-band.

We can now discuss the relative contributions that muscle fibres and connective tissue make to the fracture properties of cooked muscle. When a transverse slice of cooked muscle is pulled apart in a direction perpendicular to the fibre axis, cavities initially open between fibre bundles caused by the breakdown of the junction between the perimysium and the endomysial network on one side of it (Carroll et al. 1978, Purslow 1985, 1987). As the meat is pulled further apart, some of the cavities join up along a path which follows the boundaries between fibre bundles. Further extension is resisted by strands of the perimysial network originating from nodes of the perimysial network which bridge the gap (Purslow 1985). At still higher extensions, the highly-elongated perimysial strands break and complete fracture occurs.

The breaking strength of cooked muscle in longitudinal tensile tests is five to ten times higher than in the lateral tensile tests (Purslow 1985). The first event is local separation (debonding) of neighbouring fibre bundles (Purslow 1985). As the load is increased, the fibre bundles are the first elements to rupture, presumably when they are extended beyond a critical point (Carroll et al. 1978; Purslow 1985). The gaps between the fractured ends are bridged by perimysial strands which become more aligned with increased extension and finally break. Thus both in the lateral and longitudinal tests the perimysium is the last structure to break and therefore appears to provide the breaking strength in unaged cooked meat.

Three lines of evidence support this notion. If the perimysium contributed all the breaking strength of cooked muscle, the expected breaking strength of cooked muscle would be about 0.05 x 3.7 MPa = 0.19 MPa

assuming the perimysium occupies 5% of the cross-sectional area of muscle. This compares with an observed breaking strength of cooked muscle of 0.4 MPa. Another piece of evidence is the qualitative similarity between muscle and perimysia in their increase in stiffness on cooking and the shape of the load-extension curve. Finally Locker and Carse (1976) estimated the contribution made by connective tissue to the mechanical properties of muscle by extracting myofibrillar proteins with alkali. They found that the stiffness at low loads of their alkali-extracted strips increased markedly on cooking. The extracted strips after cooking had a breaking strength of about 3 kg/cm^2 compared with 4.9 kg/cm^2 for the unextracted strips. This implies that the connective tissue provides at least 60% of the strength of cooked muscle and this must be regarded as an underestimate since we do not know the extent to which collagen is damaged by the alkali treatment.

Despite this strong evidence that the perimysial connective tissue determines the breaking strength of unaged cooked muscle, it would be wrong to extrapolate this conclusion to other states. The most clear-cut evidence for the importance of muscle fibres comes from studies on conditioning. There is overwhelming evidence that during ageing several myofibrillar proteins are proteolytically cleaved (see Penny 1980) and, as explained above, in aged rigor muscle there is evidence that the myofibril is mechanically weakened. In contrast, the connective tissue is altered relatively subtly during ageing (Kopp and Valin 1981). Whatever subtle biochemical degradation of the perimysium occurs on ageing, this apparently has little consequence for its strength; the breaking strength of cooked muscle in lateral tensile tests is quite unaffected by ageing (Bouton and Harris 1972; Purslow and Lewis unpublished experiments), yet in longitudinal tensile tests the breaking strength falls by about one third after ageing. This is at first difficult to understand because the perimysial connective tissue, at least in unaged cooked muscle, is the last structure to break and should therefore determine the strength, and it would be difficult to suppose that the perimysial strands were weakened by ageing in the longitudinal tests but entirely unaffected in the lateral tests.

The most reasonable way of reconciling these facts is to suppose that, although the perimysial connective tissue alone is responsible for the breaking strength of cooked *aged* muscle, the fracture behaviour alters as result of conditioning and the muscle fibres as well as the connective tissue contribute to the strength of cooked *aged* muscle (Purslow, unpublished theory). In general terms, differences in the degree of interaction between the fibre bundles and perimysium in aged and unaged muscle would affect the strength of the whole composite. For example, conditioning might weaken the muscle fibre sufficiently that on application of stress, fracture of the fibre bundles occurred rather than their separation. The stress concentration at the site of fracture of the fibre bundle could then be transmitted to the perimysium and the next fibre bundle, and so on across the muscle causing fibre bundles and perimysium to fail simultaneously at a lower load than in unaged muscle (Purslow, unpublished theory).

Although the toughness of cold-shortened meat is often cited as evidence that muscle fibres contribute to toughness, it seems possible that some, at least, of the toughening could be due to changes in the breaking strength of the perimysial connective tissue due to reorientation of collagen fibres with muscle shortening (Rowe 1977). The relative contributions of connective tissue and muscle fibres to cold-shortening toughening remain to be determined.

It seems to us that our basic understanding of the mechanical properties of meat has now advanced to such an extent that in the next two or three years we should be in a position to explain in structural terms the toughness or tenderness brought by a wide variety of treatments.

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