Effects of Early-Postmortem Muscle pH and Temperature on Meat Tenderness

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For a number of reasons, the pH and temperature changes taking place in early-postmortem muscle are of interest and importance to the meat investigator. First, they are prominent, simple to measure, and (up to a point) reasonably well characterized. Second, their rate and extent are often relatively easy to modify and control, at least experimentally — for instance, by electrical stimulation (ES) or preslaughter drug treatment to alter the pH/time pattern appreciably, and by adjustment of live-animal feeding plane or of postmortem cooling severity to change the temperature/time relationship significantly. Third, relatively minor changes in either or both of these parameters are able to exert readily detectable effects on many quality attributes, including color, water binding, flavor, ease of curing, and storage life. Fourth, quality problems of major concern — PSE meat in pork, and shortening-induced toughness in beef and lamb — are the direct consequences of the interplay between pH fall and temperature decline in the early-postmortem period, the former resulting from an abnormally rapid pH descent while the temperature is still high, and the latter from a rapid temperature descent while the pH is still high (or, more precisely, while the tissue is still in a prerigor state).

Although we are still far from a full understanding of these pH- and temperature-mediated effects, nevertheless much of their mystery has been removed as a result of comprehensive and thorough research studies undertaken in the past few years. For some of them, indeed, these investigations have led directly to the development of highly successful control measures; perhaps the best example is the use of ES to eliminate the problem of shortening-induced toughness in lamb and beef.

It is not my intention, however, to discuss the state of our present knowledge of the pH/temperature/quality relationship. Rather, it is to focus attention on the state of our present *ignorance* of one small but highly relevant aspect of it. My target for destruction is the almost universally held belief that meat aging (in the sense of beneficial tenderizing) commences only with rigor completion and the attainment of a low tissue pH: a myth on which so many conclusions have been based, for which so little evidence exists, and by which we have for so long been deluded. This untenable dogma has been repeated so often that we have come to accept it as an axiom of meat science, a self-evident truth that is in need of no proof at all.

Consider, for instance, these direct quotations from papers and reviews of the past eight years:

> "Aging, a postrigor process, has a true tenderizing effect" (Locker et al., 1975).
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> ". . . the onset of aging occurs only after the full achievement of rigor mortis" (Davey and Gilbert, 1976).
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> "The onset of aging seems to coincide with the achievement of rigor" (Gilbert et al., 1977).
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> "It appears that the onset of tenderizing begins at about the time muscles enter rigor" (Davey and Chrystall, 1980).
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> "Conditioning starts when the muscle goes into rigor" (Penny, 1980).
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> "Because rigor is achieved much earlier than normal, aging can commence at the higher muscle temperatures" (Chrystall and Devine, 1980).
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> "The events of rapid pH drop and rapid rigor formation of electrially stimulated muscle . . . create the proper conditions for the tenderness changes to occur" (Dutson et al., 1980).
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> ". . . the pH decline is necessary to produce the tenderness attributed to electrical stimulation" (Dutson et al., 1982).

I find no valid evidence to support or justify these very positive statements. There is no doubt that uncritical and simplistic interpretation of ES effects has been largely responsible for propagating the false concept. One reason for the tenderizing action of stimulation is often stated to be the rapid attainment of a low pH while muscle temperature is still high, a combination of parameters supposedly releasing and/or activating the proteolytic enzymes of the lysosome. But the power of this argument is nullified from the start by the confounding effects of cold-shortening prevention and fiber fracture, either or both of which can exert a strong tenderizing action on the tissue. It is simply not logical to conclude, just because the net effect of three concurrent processes is beneficial, that each one of them is necessarily contributing to the observed improvement.

A similar flaw in reasoning is seen in a recent study (Dutson et al., 1982), in which ES produced no improvement at all in tenderness — indeed, it caused a small though insignificant toughening — when it was applied to stressed-heifer carcasses of high ultimate pH. From the absence of both tenderness improvement and appreciable pH fall, the authors concluded that tenderizing requires a significant and rapid pH decline. But in muscles of ultimate pH 6.6, cross-

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bridge formation commences very early, an effect that has two consequences. (1) It eliminates or greatly reduces the extent of cold shortening, so ES cannot exert its first tenderizing action of preventing a massive length change: there is simply no length change to prevent. (2) It eliminates or greatly reduces the extent of stimulation-provoked contraction, so ES cannot exert its second tenderizing action of rupturing the tissue through super contraction; there is simply no super contraction to perform the rupturing. It is unnecessary, therefore, to invoke the lack of pH fall to account for the lack of tenderizing; the absence of both cold shortening (to toughen the control) and of tissue fracture (to tenderize the stimulated sides) can explain it entirely. Indeed, if high pH is to be held responsible for anything in that study, it surely should be for the very *high* tenderness ratings of the control-side muscles — 6.3 on an eight-point scale — rather than for the failure of the tissue to tenderize even further.

The case against high-pH aging is thus both weak and vulnerable. By contrast, there is a great deal of evidence from several directions to support the opposite view: That high-pH tenderizing can and does occur, and to a very significant extent. Consider, first, meat which (because of a low glycogen level at death) resides for a longer-than-normal time in a high pH range. We are accustomed to thinking of this material of high ultimate pH primarily in terms of its dark-cutting or DFD appearance. But there seems no good reason why it should not also be regarded as a tissue in which the altered pH/temperature relationship may affect the aging process appreciably; if aging is indeed enzyme-dependent, these two parameters and their interaction might be expected to influence its rate and extent substantially. Many investigations have been undertaken to examine this pH/tenderness connection, the studies extending to several species: mutton (Bouton et al., 1972), venison (MacDougall et al., 1979), rabbit (Miles and Lawrie, 1970), and beef (Bouton et al., 1957, 1973; Penny et al., 1963). Without exception, large tenderness differences *in favor of the high-pH tissue* were detected. A very recent study by Fjelkner-Modig and Rudérus (1983) is particularly informative; unaged longissimus muscle of young bulls had a mean sensory tenderness rating of 4.6 on a 0-9 scale when the ultimate pH was above 6.2, but of only 1.5 in those of ultimate pH below 5.8. To be sure, color and flavor differences in the reverse direction were also found; but we are here concerned with pH in relation to tenderness, and not with practical recommendations to the meat industry. The evidence is overwhelmingly in favor of the concept that tenderness proceeds much faster when the pH is higher than normal.

Second: Khan and Lentz (1973) demonstrated a very significant positive correlation between early-postmortem muscle pH and tenderness in beef. In this work, of course, the authors were dealing with a pH that was still falling, and not with a high ultimate pH as in the studies described earlier. But the result appears to be the same: If the early-postmortem high-pH state is prolonged, whether permanently (by glycogen depletion) or temporarily (by inherently slow glycolysis), the meat will be more tender than when the zone of high pH is traversed rapidly. If significant cold shortening had occurred in the Khan-Lentz investigation, the result would undoubtedly have been reversed, for the muscles of higher pH (and thus of greater cold-shortening potential) would then have been provoked to shorten and toughen more than those which were glycolyzing faster. Cold shortening is now recognized, however, as being far less of a problem in well-finished carcasses than was once believed, and in these Choice steers that were not exposed to abnormally severe cooling conditions, little or no shortening would be expected. Furthermore, since ES was not used, no supercontraction-induced tissue rupture could have occurred. In the absence of both cold shortening and fiber fracture, the observed tenderizing effects can be explained only as a result of the relatively prolonged residence time of the proteins in a high-pH or prerigor environment.

Third: Pearson et al. (1973) showed that glycolysis in the muscles of sheep, treated antemortem with high levels of reserpine, was extremely slow; mean longissimus pH at 24 hours postmortem was 6.33 and still falling. Cold shortening was not inhibited by the drug, and indeed was greater than in the muscles of control animals. Yet the meat was exceptionally tender, requiring little more than half the shearing force that was needed by the control material from untreated animals. The authors offered an explanation based on the increased water-binding capacity of high-pH meat, but this is clearly invalid: at least one of the muscles (the biceps femoris) that displayed the greatly enhanced tenderness had a mean ultimate pH not differing significantly from the untreated controls, and there is no evidence to indicate that the others failed to reach a normal ultimate pH eventually. With the benefit of more recently acquired knowledge, we may now offer a much more plausible explanation: That extensive proteolysis took place while the pH was high and falling only very slowly, the tissue becoming very tender despite its maintained ability to cold shorten.

Fourth: We obtained fairly strong evidence, a few years ago, that very early-postmortem muscle temperature was a major determinant of beef tenderness; in fact, a correlation approaching 0.8 was found between loin tenderness and the temperature earlier attained by the longissimus muscle at 2-4 hours postmortem (Lochner et al, 1980). In a later study (Marsh et al., 1981), we exposed one side of each carcass to 37°C from dressing completion until 3 hours postmortem, after which normal chilling was applied; the opposite side received regular chilling. The "hotbox" treatment resulted in a sensory-tenderness improvement of 1 to 2 units on a 1 to 8 scale (an observation, incidentally, that leads us to believe that much of the higher eating quality of fatter carcasses is due to the much slower cooling brought about by the fatty insulation). Now, although this large enhancement of tenderness was induced by temperature elevation, we are convinced that its rate and extent were very dependent on the high pH and or the prerigor state of the musculature during the hotbox treatment. The rate of tenderizing here attained is far higher than that occurring during normal chiller aging (when both temperature and pH are low), and several times greater than that found by Wilson et al. (1960), who exposed steaks for 24 hours to a temperature of 43°C *after* rigor onset and the attainment of a low pH. It is the *combination* of a relatively high pH and a high temperature, rather than either of them separately, that is responsible for the rapid tenderizing in the early-postmortem hotbox treatment.

Fifth: In an extension of this study, we (Marsh, Lochner and Kragness: paper in preparation) have demonstrated the pH influence more clearly. Using hotbox conditions on some beef sides and relatively mild chilling conditions (to avoid cold-shortening complications) on others, we found, as expected, a quite high correlation between tenderness and 3-hour muscle temperature, but a negligible relationship between tenderness and 3-hour muscle pH. When the points making up this pH/tenderness relationship were stratified according to 3-hour temperature, however, a very different picture emerged; within each of the three temperature ranges, greater tenderness was strongly associated with *higher* 3-hour pH values. If much faster cooling rates had been used, of course, the muscles of high 3-hour pH would have been tougher than the others because of their greater cold shortening; but with this possibility intentionally prevented by milder cooling, the true effect of early-postmortem pH could be observed, unconfused by length change.

Sixth: To examine this pH influence in greater depth, we (Marsh et al., 1981; Takahashi, Lochner and Marsh, paper in preparation) used a modified ES procedure to adjust muscle pH without causing any fiber rupture. Cooper and Eccles (1930) showed that low-frequency current causes muscles to contract vigorously and reversibly, whereas higher frequencies produce massive damaging contractures because each new stimulus arrives before the muscles have fully relaxed from the previous one. We applied the hotbox treatment to both sides of each carcass to prevent cold shortening, and 2-Hz ES to only one side of each to accelerate pH decline without tissue rupture. In every paired-side comparison where the control-side (unstimulated) pH at 3 hours was above 6.1, the stimulated side was tougher, on average by more than one unit. Since two of the three postulated tenderizing mechanisms of ES (shortening prevention and fiber rupture) had been eliminated by use of hotbox and low-frequency current, we are forced to conclude that the third one proposed (rapid acidification) actually works in the *contrary* direction, significantly toughening the tissue relative to that in which glycolysis proceeds only slowly.

These recent observations, together with new interpretations of earlier studies, leave no room either for doubting the importance of early-postmortem pH and temperature to meat eating quality, or for perpetuating the theory that aging commences only with rigor onset and ultimate pH attainment. For these conclusions to have any real significance, however, it is necessary to discard the notion that early-postmortem glycolytic rate depends solely on temperature; if that were true, muscle pH at any one temperature and early-postmortem time would be able to vary among carcasses over a quite narrow range (and then only because of relatively small differences in initial pH). This does not imply that early work on rabbit (Bate-Smith and Bendall, 1949), beef (Marsh, 1954) and lamb (Marsh and Thompson, 1958) was wrong. Rather, it indicates that glycolytic rate as measured in an excised muscle (in the older studies) is not the same as that observed in a muscle that remains attached to the carcass or side; the former responds only to temperature while the latter has other (and still unknown) controllers. A moderate degree of variability in rate of pH fall among corresponding muscles of different carcasses has been reported by Tarrant and Mothersill (1977) and Bendall (1978). In our own studies of this pH behavior (Marsh et al., 1981), we have observed a remarkably wide range of 3-hour longissimus pH values, spanning almost 1.5 units despite the maintenance of a steady 37°C temperature (by use of the hotbox) to eliminate any confounding temperature influence. Thus although bovine muscle almost always reaches an ultimate pH in the region of 5.5, the rate at which it proceeds toward that value is extremely variable — almost as variable, in fact, as can be found among pig carcasses.

Now we must consider the possible relevance to meat quality of these recent findings. I believe there are several consequences, some helping to explain past anomalies and others indicating new directions that may be worthy of further exploration.

1. Beef tenderness is a highly variable attribute, even when all known or suspected influences are well controlled: the age, breed, sex, fatness, preslaughter treatment and slaughter of the animal, and the dressing, cooling, storage and cooking of the carcass and meat. The present evidence introduces a new factor, previously unrecognized because of our relative ignorance of the early-postmortem state, yet of potentially considerable significance in relation to tenderness. It might prove impossible, of course, to control this rate of pH fall or to manipulate it to our advantage; or it might be undesirable to do so if the treatment resulted in greater cold shortening through slower rigor onset. But it would certainly be very gratifying to be able to *explain* the often observed wide ranges of tenderness in terms of measurable parameters, instead of vaguely shrugging them off as yet another consequence of animal variability.

2. The belief that aging commences only with rigor completion and low-pH attainment has been a major discouragement to study the properties and possible tenderizing role of the neutral protease(s) in meat. The present demonstration that tenderizing mechanisms may be operating strongly in prerigor, high-pH muscle offers a powerful incentive to pursue CAF (and perhaps other enzymes of near-neutrality optimal activity) with renewed vigor. Except for the absence of oxygen, conditions in early-postmortem muscle are very similar to those of the tissue just before slaughter, and we have long recognized the continual turnover of muscle proteins (involving breakdown as well as resynthesis) in living muscle: why, then, do we insist that this protease-activated breakdown ceases abruptly at the moment of animal death?

3. The extent of pH fall has been used frequently as an indicator of ES effectiveness and (by inference) of the extent of tenderizing. There can be no objection to this practice if appreciable cold shortening would otherwise occur, for shortening-induced toughening is so severe that its elimination must retain first priority. But in this country, with its generally rather slow chillers and its usually quite well-insulated carcasses, cold shortening is rarely of a sufficient magnitude to cause significant toughening. Any tenderizing that results from ES in these circumstances must thus be a function of the *difference* between (rather than the sum of) a beneficial tissue-rupturing effect and a detrimental fast-glycolysis effect, so pH fall will provide only a very poor measure of tenderizing efficiency. If, in addition, we were to introduce low-voltage stimulation (in response, say, to a

revised code of electrical practice), then the sole remaining ES effect on tenderness would be to cause a significant toughening despite rapid pH fall; indeed, quality *deterioration* would then vary with glyolytic rate. While this scenario is fictional at the present time, it provides an example of the need for continual vigilance when procedures are changed without consideration of understanding of the underlying mechanisms.

4. The concepts developed in this paper permit a single explanation to be offered for several observations presently requiring separate interpretations. I have referred already to the stressed-heifer study of Dutson et al. (1982); the inability of ES to provoke either tenderizing or pH fall was taken as evidence that, under other conditions eliciting both effects, one of them must depend on the other, specifically tenderizing on pH decline. Mention was also made above of the reserpine-treated sheep whose meat was remarkably tender despite high and even enhanced cold-shortening ability (Pearson et al., 1973); for want of a better explanation, the authors invoked the known high water-binding capacity of high-pH meat (even though the ultimate pH of the tissue was not elevated). A third example, not previously discussed, is the recent work of Chrystall et al. (1982), whose study closely resembled that of Dutson et al. (1982). The authors examined the effects of ES on the tenderness of meat from exercise-stressed lambs; 12 animals were chased vigorously just prior to slaughter, but only six of the carcasses were stimulated. The rather unexpected result of the investigation was this: For each of the six different muscles studied, the mean shearing-force requirement was higher for those receiving ES treatment than for the unstimulated controls. For two of the six muscles, the differences were significant ($P<.05$). The observation was accounted for in terms of a presumed failure of the musculature to relax when the stimulating current was finally switched off; the partial contraction that supposedly resulted from the aborted relaxation was held responsible for the observed toughness.

Now, all three of these studies (and, indeed, others also) are readily explained by a single mechanism if the evidence for high-pH tenderizing is accepted. In each of them, *the greater tenderness was found where the higher pH persisted for the longer time.* (It is stressed once again that this broad statement does not apply in general to conditions that encourage appreciable cold shortening, unless [as in the Pearson study] the tissue remained at a high pH for an extremely long period.) The means by which this lengthened high-pH state is brought about is of no consequence; it may be through an act of commission as in the reserpine treatment of sheep (Pearson et al., 1973), or of omission, for instance, by *not* stimulating carcasses of exercise-stressed heifers (Dutson et al., 1982) or lambs (Chrystall et al., 1982), or by *not* applying 2-Hz current to normal unstressed beef carcasses (Marsh et al., 1981). The quite simple concept of high-pH tenderizing brings all of these widely varying (and as widely interpreted) observations under a single explanatory umbrella.

I conclude with a relevant and perhaps instructive quotation from the writings of William of Occam (or Ockham) who lived in England almost 700 years ago. William's statement, usually referred to as Occam's Razor, is quoted in various ways; a common one is "Entities should not be multiplied beyond necessity." According to Bertrand Russell (1948), the original version stated "It is vain to do with more what can be done with fewer." Russell's commentary on this dictum is itself worth quoting: "If everything in some science can be interpreted without assuming this or that hypothetical entity, there is no ground for assuming it." And then he (Russell) added a personal note: "I have myself found this a most fruitful principle in logical analysis."

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Biochemistry and Biophysics Discussion

G. W. Davis, Texas Tech University: Would you please discuss how your views of gap filaments differ from those of R. H. Locker?

Robson: First of all, I think Dr. Locker had something very important and certainly has made a very significant contribution. There is no doubt that his model is incorrect. But early models are always incorrect. I think in his model, for instance, a gap filament actually runs as a core of thick filaments in adjacent sarcomeres and that clearly is incorrect. There is no core protein, at least I don't think so, in the middle of the thick filament. But, nevertheless, there are some types of fine filaments. And they do, in fact, seem to be decorated by the titin or connectin antibody. He has something and I think in the end it will turn out to be very important. Perhaps Dr. Davey will comment, but I think it's a very important contribution that Dr. Locker has made and we'll simply have to modify the way titin looks. It probably runs basically from the N_2-line on one side in the I-band to the N_2-line on the other side, and it does not go through the core; it wraps around the outside of the thick filaments.

C. E. Allen, University of Minnesota: Abe, in terms of what you have just reviewed, how would you interpret the action of nonproteolytic meat marinades that we know have an action in terms of tenderness of collagen? I'm thinking about water uptake vs. breaking linkages.

Aberle: Marinades contain a lot of different salts, organic acids — various things which could affect the hydration integrity of that collagen helix. The important thing that has come to me in doing this review is that, if we consider the collagen structure in the absence of water, we're making a great mistake. Water is an integral part of it and it contributes to the structure of that helix and its stability, no doubt about it.

J. M. Regenstein, Cornell University: Dr. Marsh, must the mechanism in high pH be proteolysis? Do you have direct evidence or is it possible that the mechanism of high pH might be different?

Marsh: I have no evidence that it's necessarily proteolytic and I think, at one stage, I did say the mechanism is enzyme catalyzed. We've always assumed that it's proteolysis though I did notice a recent Japanese paper which claimed that calcium was having some direct effect on the contractile protein, I think, and the suggestion that it might be a toughening mechanism. So, yes, I have to agree with you, Joe; in our lab we have no certain evidence that proteolysis is involved.

T. R. Dutson, Texas A&M University: I'd like to address this to Dr. Marsh, or anyone else who'd like to comment on it. It appears from his presentation that we're in a state that Prigogine, the Nobel Prize winning physicist, said has a dissipating structure. A dissipating structure being one that we're at a point where we have enough perforations in the system that it must be changed. And as Dr. Marsh mentioned, we may need to change some of our thinking; but I think we need to be rather careful in doing this and Dr. Marsh has brought out some interesting data, some data that I have also looked at. And an interesting concept which I agree with wholeheartedly — that the changes or the events that take place in the first 1 or 2 or possibly 3 hr postmortem — are probably some of the most effective in causing changes in tenderness which are measured some time later. I mentioned that in my 1977 paper and Dr. Marsh has quoted some of that data. One of the differences I see, and that he also pointed out, is in many cases you look at pH differences under those treatments and you find that is fairly well associated and Dr. Marsh pointed out it's just an association. But there are two, possibly three, things that I think he didn't bring out. One is particularly the dark cutting study that we did. He mentioned that during that period there would be no massive contraction and, therefore, no contracture bands. We many times in stimulated muscle find very few or no observable contracture bands in some of the muscle. That may be because we haven't found them, but they are not near as numerous as we find in other cases. But the tenderization seems to be the same. And in that study there could have been because we monitored the contraction of both sides, and the contraction appears to be the same type as in animals which had not been stimulated and did not produce dark cutters. The second thing that may need to be considered is that of cold shortening. He mentioned that the differences in shortening may be causing the differences in the tenderness that occur between high pH and low pH which has normally been observed. But in many cases of observing electrically stimulated samples, we find no difference in shortening in many of the cases we have looked at and even in observing some of Bendall's statement. So, if there is no difference in shortening and no difference in contracture bands, then what is the cause of the difference relative to the pH in these samples? If the pH is lower, there is no difference in shortening and there is no apparent difference in contracture. So I think some of these things still need to be considered and, as Prigogine said, when you're in dissipating structure, you have many things perturbing this and you have to consider all of the elements coming from all sides. I really appreciate Dr. Marsh's looking at this. In the last couple of papers that I've written, I've stated that Dr. Marsh's 1981 paper raises some questions that will probably get us much closer to the understanding of the mechanisms of tenderness

in postmortem muscle.

Marsh: Thanks for the complimentary remarks, Thayne. I must say in our experience we have had limited experience with microscopy of this tissue; but in our experience, when we have looked at electrically stimulated beef, that is, with regular current (50 or 60 cycles in this country), then we do in fact always find a degree of fracturing. When we look at the 2 cycles per second stimulated material we invariably find no fracturing. Now that is only our experience and I do agree with you that other people have found much less extensive fracturing. But I would point out in that connection that in my 1981 RMC paper I did a few calculations and it turns out that, with a sarcomere of 2 in length, if only one of those sarcomeres had a transverse split in it, only 1 in every 500, then we would still have a break in that tissue at 1 mm intervals. The degree of fracturing or the degree of rupture required to do this can be almost infinitesimally small. I'm not suggesting certainly in our work it is much more than 1 in 500. But, at least theoretically, one break in every 500 units, one damaged unit in every 500 sarcomeres, would be enough to make that meat remarkably tender. We don't have to have every unit in every link in a chain a weak one. The chain is going to break with only a single weak link in it, and I'm suggesting that there could be as many as 499 strong links in that chain and one weak one. Does that answer your point, Thayne?

Dutson: Well, somewhat, but we have considered your comment in that paper about the number of breaks that need to be placed, and as we've looked at the fragmentation or the fracture bands or disruptures in those samples, we have looked at many, many more than this 500 and find none of these observable events. And, you know, it goes up to more than 100 fold. So I think in those cases we don't find any of that, but we still see the difference in tenderness at those pH's. In those same experiments, we find no difference in cold shortening. So I think there is still a bit of controversy here that we may need to consider all of this. Like Prigogine said about dissipated structure, "when it comes to a level of nondissipation it will be a higher level of organization."

Marsh: One point, I think, that I did not bring out. I took the familiar line that there are only three possible mechanisms that have been suggested for electrical stimulation's effectiveness, and in one case I knocked out two of them and said "if this is correct the third one must be operative but it's in the wrong direction." There is a flaw in my argument there, of course. Who knows, there may be a 4th or 5th or 6th possible mechanism for electrical stimulation and that's probably the point you're aiming at, Thayne. I would stress, however, that, as I said at the beginning, I had about six lines of evidence and individually they may not be very strong. Put them all together and I think we've got a pretty good case against this myth that a low pH rigor state is necessary. I do certainly agree with you, Thayne, maybe there's a 4th or 5th or 6th mechanism that we don't even guess at the moment.

A. M. Pearson, Michigan State University: Would you speculate on the enzymes that may be involved in the tenderization?

Marsh: No, as a matter of fact, I had a question to ask Rich Robson. That is a bit outside my territory though obviously I'm moving in that direction. And I would like to pass your question on to Rich Robson. In particular, apart from the calcium activated factor (CAF), do you see any other possibilities of near neutral pH optimum for proteases, Rich?

Robson: No, not really. CAF can explain almost everything as Bill Dayton would . . . (laughter). I think the only problem you have at that pH would be that the calcium levels required might not be quite high enough. But Bill then solved that question when he came up with the low-calcium activated form of CAF.

W. R. Dayton, University of Minnesota: I pretty much agree with Rich. The localization studies we and some others have done on the calcium activated protease (along the same lines as what Rich has done with nebulin and titin) put the protease at the Z-disk, which is the most susceptible and identifiable structure degraded by the protease. I think these studies, coupled with the presence of low calcium CAF, would provide nice circumstantial evidence, at least, that the protease may in fact play a role in breaking up the structure of the myofibril along the lines of what Dr. Marsh indicated. It doesn't have to happen in too many places in order to have a very profound effect. And it is certainly at the right place to do that.

G. R. Schmidt, Colorado State University: Dr. Marsh, do you feel you could screen 1,000 carcasses and select and measure them and they would be highly more tender than the bottom 10%?

Marsh: I think that would give some indication. At one stage, Jim Lochner and I had the idea that we could have a combination temperature and pH probe and insert it into the loin at 3 hr and give a much better measure of eating quality than the present quality grading system. But we were thinking of a several quality probe. One thing that we had to give up on that idea was within a single longissimus muscle there can be at the one 3-hr time (constant temperature) a tremendous variability in pH. Thayne, I think, has had the same experience here. With a difference of 2 cm in the same sectioning plane on a single longissimus at 3 hr postmortem (constant temperature), the pH might differ by .5 of a unit or more. So that would not be such a good feature from that point of view. However, I do think that 3-hr pH will definitely give some indication when coupled with 3-hr temperature of eating quality, certainly of tenderness.

Davey: If you take prerigor beef, thick muscle and you put a strip into isotonic sucrose, leave it at 15°C, this situation will cause it to shorten by anything up to 50%. So you could then allow it to set in rigor mortis in the sucrose solution and attempt to get a correlation between tenderness and shortening. And indeed there is one. It is a straight line relationship; there is no increase in tenderness over the whole range of shortening. A little bit of histological examination shows that the fibers are convoluted and crimped to a massive degree and only the external responsive fibers to the sucrose have actively shortened. So it seems just possible that some of this so-called tenderizing at high temperatures may relate to some physical phenomenon of this sort. And I further ask the question, if you raise the temperature to about 43° to 44°C where heat shortening is going to be much more active and intense and perhaps spread itself more universally throughout the fibers, do you get the same situation arising?

Marsh: I'm very well aware of your early work on lamb where you did show a distinct toughening effect of heat rigor.

We have encountered that just occasionally when we've gone too high with our hot box temperature. When we've put it up to 43° to 44°C the meat was reasonably tender, but it wasn't as tender as we believe it would have been had we been back at 37°C. On the other hand, quoting Wilson et al. (1960), where they took steaks already in rigor, they were able to hold some of their meat. In fact, I think they said their best tenderizing conditions were 24 hr at 43°C, heavily laced with every antibiotic in the radiation method available then, of course. But in that case, their meat was in rigor, there was no possibility of the heat shortening. The best I can say is that we have got some indication of it happening if we accidentally went too high, but 37°C for a maximum of 3 hr postmortem did not seem to produce that problem.

Davey: I think it has to be remembered that most proteolytic enzymes of the usual sorts have a high temperature tolerance and, in fact, the aging enzymes seem to be most active around 60°C. Therefore, one would expect perhaps with the high temperature of 43°C, one would have even enhanced the result. I do mention something else in relation to proteolysis, which is interesting, in work that is going on in New Zealand at the present time. We are blessed with a lot of geothermal activity in New Zealand and the local university has been isolating various bacteria and other organisms from these steam borers and has been isolating the proteolytic enzymes from them, and they have a temperature optimum, if you'd like, to about 95°C. This is a remarkable thing and we are in the process of collaborating with them to see whether these proteolytic enzymes can be injected into meat to be used as a tenderizing process.

Dutson: Relative to proteolytic enzymes, I'd like to ask Rich or Bruce or anyone who might know, do you think maybe some of the ATP dependent proteases could be involved in this early postmortem tenderization? This was an observation made by Lawrence Yates in our laboratory some time ago that, in this early postmortem period, it seems that is where the largest change takes place. When Lawrence incubated muscle at the same temperature and the same period of time at early postmortem, he got changes. If he did it later postmortem after rigor, he got much less change taking place. Do you think that it could be the ATP dependent proteases that are actually causing some of these changes while there is ATP there?

Robson: Do you really have any evidence that there are any ATP dependent proteases inside the muscle cell? If you don't, then we will have to rule out this question. I really don't think there is any good evidence of any significant amount of ATP dependent proteases inside the muscle cell.

Dutson: I would have to go back to Goldberg's data, he thought there was sufficient quantity within the muscle cell.

Robson: He was at Iowa State for a seminar recently and I've seen this and I really don't think that he has shown clearly that he has the ATP dependent proteases inside the muscle cell. Now maybe Bill Dayton might want to comment.

Dayton: I would agree with you very strongly, he has possibly shown there might be something in muscle tissue, but we all know from bitter experience that doesn't necessarily mean the muscle cells.

Dutson: Relative to the Z-line and the breaking of the Z-line, that being one of the places that CAF is most active, in most of the high temperature work that I have done, any time you have a high temperature/high pH system, you seem to affect the Z-line structure more than you do in a high temperature/low pH system; but if you take those myofibrils from the pH incubated meat and subject them to fragmentation (fragmentation by the method that is used at Iowa State or by our method) you get more fragmentation of those myofibrils at the low pH/high temperature than you do at the high pH/high temperature.

Robson: I guess I would say, many times when we look at Z-line structure, we have a tendency, I think, to simply look and see if we see a dense line. And we interpret that to mean that it is intact when in fact it may not be. Wang at the University of Texas has shown very interestingly that one of the things that happens when you have a breakdown of titin and nebulin is that those proteins tend to translocate. It is almost like they snap over to the Z-line. So the Z-line structure itself may be very fragile, but under a variety of instances that structure that you're looking at is not for instance just -actinin. It's now -actinin and a kind of coagulant of titin and nebulin that has moved over there. I'm not sure that I'm really answering your question, but I do think that one has to be very careful when you look at Z-line structures in any kind of postmortem muscle and suggest that it is intact or it is not.

Davis: Dr. Huiatt, do you have anything to add to this discussion?

T. W. Huiatt, Iowa State University: One thing on the ATP proteases subject. I also understand from Goldberg's work that the major substrates for these are denatured proteins or proteins that have somehow come off the ribosome with inappropriate sequences and that the normal proteins are not the primary substrates for these enzymes. So I think what you want to look at is the proteases that are involved, as Bruce Marsh has said, in the normal turnover of muscle protein should still be functioning at this time, and it is not really clear that the ATP dependent proteases are the ones that are doing this. And a comment on the second question. I think Dr. Davey's work has suggested that not only breakage at the Z-line, but also destruction of linkages between adjacent Z-lines to allow slippage of the myofibrils past each other, also are important in tenderization. So breakage at the Z-line and fragmentation of the myofibrils may not be the whole answer, some of the desmin crosslinks may also be important.

Dutson: Also, in Locker's paper of last year's RMC, he sort of interpreted some of Davey's data to mean that there really was much more tenderization when you have the Z-line structure intact. He assumed from his comparison of different pieces of data that Z-line structure really had no relationship to tenderness.

M. C. Hunt, Kansas State University: I would like to ask Dr. Aberle if he might speculate on any early postmortem changes that might occur in stromal proteins.

Aberle: From the very limited evidence that we have on thermal shrinkage temperatures, I would say that the changes in the collagen are toward lesser stability from zero time after death through 12 hr and that occurs very quickly. Whether that has any real relationship to early postmortem tenderization, I really don't know. It may, because it is a decrease in stability.