Mechanisms and Strategies for Improving Meat Tenderness

B.B. Marsh*
T.P. Ringkob
R.L. Russell
D.R. Swartz
L.A. Pagel

Those who cannot remember the past are condemned to repeat it.

– G. Santayana

It is 25 years since Locker and Hagyard (1963) reported their discovery of cold shortening, and 40 years since Bate-Smith (1948) published his classical paper on rigor mortis and beef aging. It is 60 years since Warner (1928) presented his results on a mechanical test for meat tenderness. And it is just a few months over 80 years since Lehmann (1907) reported his study of toughness in relation to connective tissue and fiber diameter. These annversaries are more than mere historical milestones; they are disturbing reminders of the pathetically small progress we have made toward tenderness improvement and the routine attainment of tender meat.

There are, to be sure, several valid reasons for our continuing lack of success. One that can be clearly recognized in retrospect is the series of changes in meat-industry practice that have had a highly detrimental effect on eating quality. The gradual reduction of postmortem delay between slaughter and chilling, the application of faster and faster chilling rates, and the continuing trend toward leaner beef have all been introduced for very good reasons, and could not have been avoided; but they have also had significant negative effects on tenderness. Perhaps we should be prepared for yet another self-inflicted wound, for early evidence (to be discussed below) suggests that the use of repartitioning agents may result in appreciable toughening.

A second reason for our failure is our long-term reliance on the empirical approach to the toughness problem. There is nothing inherently wrong with trial-and-error methodology, which has produced wondrous advances in many fields; but when, year after year, it proves of no avail (as it has done in the tenderness area), we must recognize that empiricism has reached its limit. Our future success or failure will be determined by the extent to which the muscle biologist, the meat scientist and the industry technologist are willing to come together, sharing and overlapping their separate areas of basic knowledge, development skills and practical expertise.

There is a third reason for our continuing inability to control tenderness: a lack of appreciation of the highly dynamic nature of pre-rigor muscle. The brief period between life and death of the musculature presents us with a unique and still much under-utilized opportunity to influence the tissue's ultimate structure and properties, for muscle at this time is a highly sensitive and vulnerable material. We are already acquainted (albeit rather superficially) with two early-postmortem phenomena, cold shortening and rigor mortis, and with the use of electrical stimulation (ES) to suppress the first of these by accelerating the second. Yet a vast amount remains to be learned of this phase, in relation to both basic knowledge and practical application. What do we really know, for instance, of the role of heat shortening as a toughening agent under mild chilling conditions? To what extent can observations on pre-rigor excised muscles be extrapolated to carcasses? Does tenderness continue to improve with progressively longer stimulation? Why does accelerated glycolysis produce such variable and even conflicting results in different situations? The answers to these and other questions are important both because they will contribute to our knowledge of toughness and tenderness and because they will almost certainly contribute to the practical attainment of tenderness and higher eating quality.

There are two early-postmortem parameters that are at least partly controllable by the plant operator: the rate of cooling (through manipulation of air temperature and velocity) and the rate of rigor onset (primarily through carcass stimulation). An ability to modify these rates, however, is of little value if we do not know the extent to which each should be altered for greatest benefit, or the degree to which changes in one of them will reinforce or cancel the beneficial effect of the other. The tenderness literature is of only limited assistance, for it is full of contradictions; it is not difficult to locate references to both the tenderizing **and** the toughening effects of high early-postmortem temperatures, for instance, or to the increased tenderness **and** the increased toughness due to accelerated pH decline. We are thus in a very frustrating position; we know that these two rates – cooling and pH fall – affect tenderness, and we know how to modify them, but we do not know the extent to which they should be modified to optimize eating quality.

*B.B. Marsh, R.L. Russell, D.R. Swartz and L.A. Pagel, Muscle Biology Laboratory, University of Wisconsin, 1805 Linden Drive, Madison, WI 53706

T.P. Ringkob, Animal Science Department, University of Nevada, Reno, NV 89557

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Tenderness and Glycolytic Rate

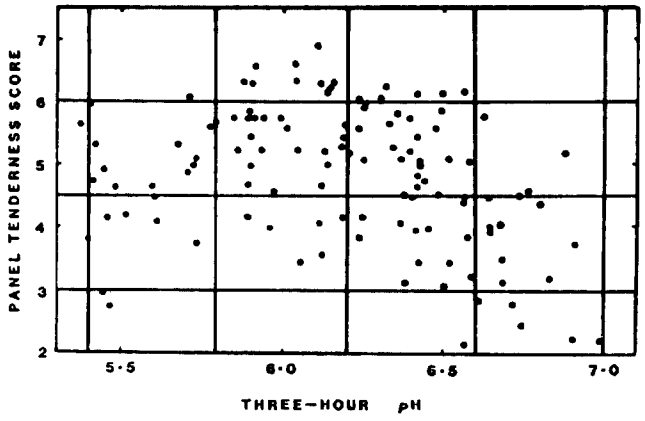
Partly because of this major gap in knowledge, we recently undertook a study (Marsh et al., 1987) in which both glycolytic and cooling rates were intentionally varied over very wide ranges, and both were carefully monitored so that their individual and combined effects on tenderness could be assessed. The investigation yielded consistent but rather unexpected results, and we have been forced to question some of the widely-held current views on tenderness and its attainment. A brief summary of these results is necessary before we discuss the implications of the study.

The experiment used 60 steers, all from the same source and of similar carcass characteristics. Four early-postmortem chilling rates were compounded with three ES modes to give 12 treatment combinations, each of which was applied to single sides of five carcasses; the contralateral sides were not stimulated, but were subjected to the same cooling conditions as their ES-treated pairs. These differential treatments produced very broad spectra of cooling and glycolytic rates; mid-loin temperatures at three hours postmortem (T_3) ranged from 19° to 37°C, and *longissimus* pH values at three hours (pH_3) from 5.4 to 7.0. Of several measurements made during cooling, the one of greatest significance was this latter, pH_3, a rate parameter depending primarily on the degree of prior stimulation but also strongly influenced by cooling severity.

The panel tenderness evaluations (1-8 scale) of unaged loin steaks from all 120 sides are shown in Fig. 1, plotted against pH_3 values. Rapidly cooled control sides predominated, of course, in the high pH_3 region (slow glycolysis), whereas the sides receiving prolonged stimulation and delayed cooling had low pH_3 values, to the left; other combinations of the two treatments were responsible for most of the points in the mid-region. It was not surprising to observe an association of toughness with slow glycolysis and a decrease in toughness with somewhat faster glycolytic rate, to a pH_3 of about 6; this behavior would be expected from current knowledge of cold shortening and its attendant toughening effect. What **was** surprising was the declining tenderness with still faster glycolysis, that is, in those muscles that had reached (or were approaching) their ultimate pH values by three hours postmortem. It is true that a great tenderness scatter partly obscured this result. Within any narrow pH_3 zone, there was a spread of about three panel units out of a total range of about five units; this we tentatively attribute to varying proteolytic activity among the 60 carcasses. But the overall trend was unmistakable: Tenderness was maximal when early-postmortem glycolysis proceeded at an **intermediate** rate, and declined if the rate was either slower or faster. The table in Fig. 1 summarizes the graphed data, indicating that both ends of the tenderness scale were improved by the attainment of a pH_3 of about 6; in this intermediate region, there were more muscles in the most tender category (scoring about 6) and fewer – in fact, none at all – in the really tough category that scored below 3.

The pattern is seen more clearly if the pH_3 values are grouped into ranges of 0.1 unit, even though with this fine degree of subdivision some groups (shown by open circles) contained fewer than four samples (Fig. 2). Warner-Bratzler shear values (Fig. 3) fully supported the panel results, a minimum in force requirement occurring in steaks that had

Figure 1

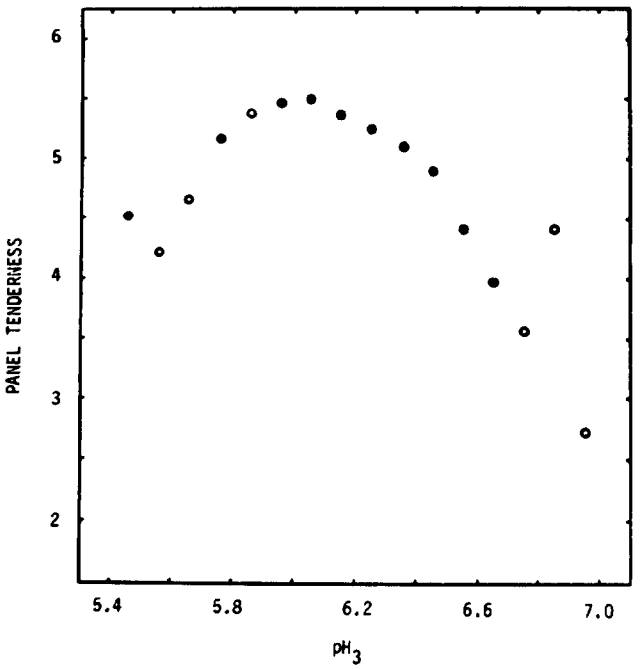


TENDERNESS DISTRIBUTION (%) BY pH_3 RANGE

6-7	5	28	14	0
4.5-6	60	56	52	22
3-4.5	25	16	32	50
2-3	10	0	2	28

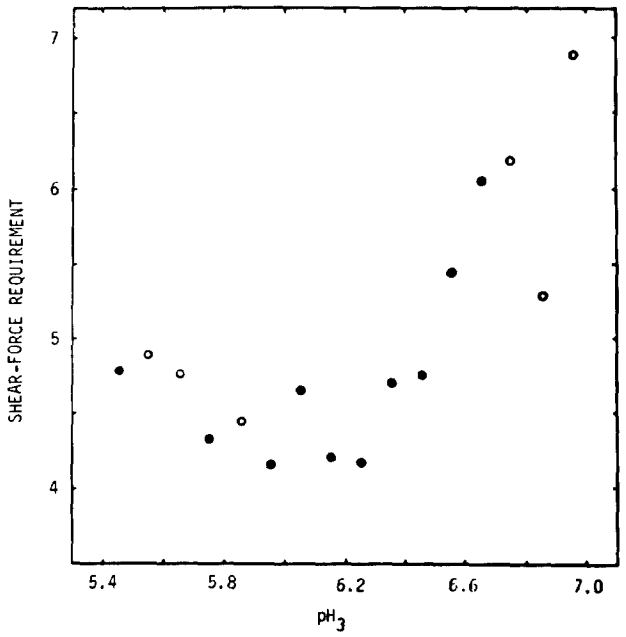
Panel tenderness in relation to glycolytic rate. Unaged steaks; tenderness scale 1 – extremely tough, 8 – extremely tender.

Figure 2



Panel tenderness in relation to glycolytic rate; data grouped into 0.1 intervals of pH_3. Unaged steaks; closed symbols 6-16 steaks per point, open symbols 1-4 steaks per point.

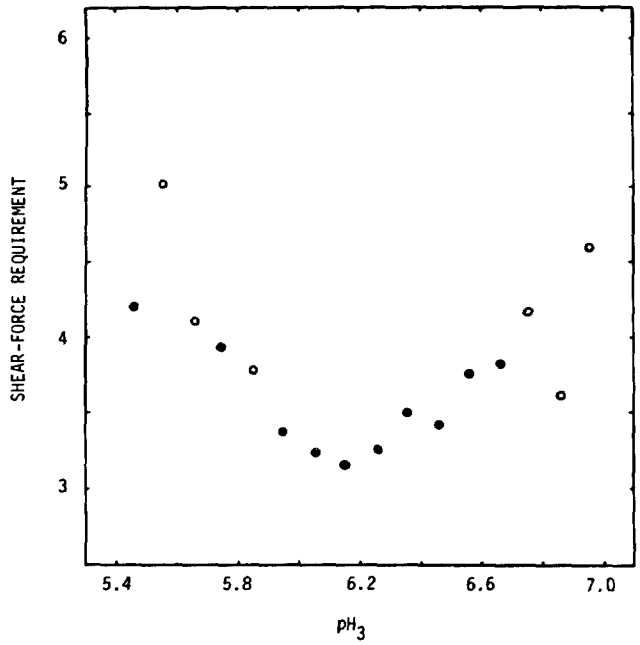
Figure 3



Shear-force requirement in relation to glycolytic rate. Unaged steaks; cores of 1.25 cm diameter. Data grouping and symbols as in Fig. 2.

pH_3 values of about 5.8 to 6.2. Even after two weeks' aging at 2°C, the roughly quadratic relationship persisted (Fig. 4), and in fact became rather more pronounced, for steaks of very low pH_3 tenderized to a smaller extent than those of intermediate values near 6. This difference in aging effectiveness cannot be ascribed to variations in ultimate pH, since all but two of the steaks had reached normal low ultimate values

Figure 4



Shear-force requirement in relation to glycolytic rate. Aged steaks (14 days at 2°C); cores of 1.25 cm diameter. Data grouping and symbols as in Fig. 2.

(5.30 to 5.56) before 48 hours postmortem, when the 14-day aging period commenced. It is also to be noted that the totally unaged steaks of pH_3 about 6 were as tender on average as the fully aged steaks that had attained very low pH_3 values.

Most of the points in the low-pH_3 (fast glycolyzing) region represented sides that had received an atypically slow or delayed cooling, so the possibility arises that the left limb of the relationship might be due to this abnormal chilling treatment. Essentially the same feature – a toughening with high glycolytic rate – has since been found, however, in separate studies in Wisconsin and Nevada, both of them employing only normal chilling conditions. The toughening cannot be ascribed, therefore, to the exceptionally slow cooling imposed on some of the sides of the original study.

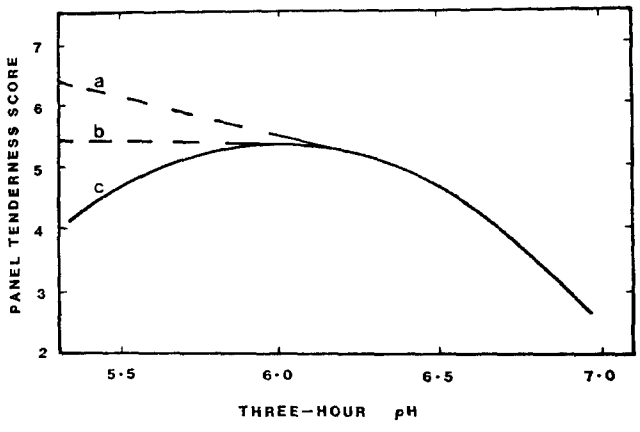
All tenderness evaluations – panel unaged, and Warner-Bratzler unaged and aged – thus lead to the same conclusion: Tenderness is optimized by the attainment of an intermediate rate of early-postmortem glycolysis, regardless of the procedures (ES and/or cooling rate) by which this rate is induced.

Implications of the Rate/Tenderness Relationship

This finding is really a rather simple one. Expressed inversely, it merely states that tenderness is reduced by both very low and very high rates of early-postmortem glycolysis, and the first of these conclusions – that slow glycolysis leads to toughness – is already well-known from cold-shortening studies. The sole novel feature is thus the toughening effect of very rapid pH decline (Fig. 5c). Yet despite its simplicity, the result is a surprising one, for it contradicts the very well entrenched (though unsubstantiated) view that, if fast glycolysis is good, then faster glycolysis will be at least as good (Fig. 5b) and probably better (Fig. 5a).

For several reasons, we believe this finding may also be a rather important one, able to explain a number of puzzling past observations and to open a new avenue for investigation.

Figure 5



Tenderness/pH_3 relationships: (a) and (b) hypothetical (see text for description), (c) observed.

First. A fairly rigorous test of any new concept is an ability to explain previously inexplicable or irreconcilable results (of which, sadly, there is no scarcity in the tenderness field). The paradoxical toughening effect of ES, when coupled with delayed or very slow cooling, (Takahashi et al., 1984; Unruh et al., 1984, 1986; Pommier et al., 1987) is one such example of a still unexplained set of observations; another is the sometimes toughening, sometimes tenderizing effects of two or three hours' maintenance of muscle temperature at 35° to 40°C (Lochner et al., 1980; Lee and Ashmore, 1985; Koh et al., 1987). If we accept the popular view that faster glycolysis promotes tenderness throughout the whole achievable range of glycolytic rates, then we must believe that tenderness will continue to improve (or, at the very least, will not decline) as ES becomes more severe and/or chilling becomes more lenient, for both of these treatments accelerate pH decline. It is thus impossible to explain the toughening effects observed at the high rates of glycolysis taking place in these two sets of observations. If, on the other hand, we accept the new evidence that peak tenderness coincides with an intermediate glycolytic rate, the paradox disappears; the slow or delayed chilling of lean carcasses can itself produce rates of pH fall close to that required for maximum tenderness, and any further rate increase (whether caused by vigorous ES treatment or by greater carcass fatness) will result in very low pH_3 values that we can now associate with decreasing tenderness.

This peak-tenderness hypothesis can account also for the highly variable effectiveness of stimulation-induced tenderizing reported by different investigators. Depending on the stimulation parameters, the severity of chilling, and the size and fatness of the carcasses, glycolysis may by chance attain the most tenderness-effective rate. It is as likely (and perhaps **more** likely), however, to fall short of this desirable rate, or to drastically overshoot it, the extent of tenderizing then being disappointingly small, negligible or even negative. It is not the effect of ES alone, but the combined effect of ES **and** muscle temperature, that determines the rate of pH fall, and thus the extent of tenderizing. For this reason, pH_3 appears to be a valuable parameter; it is a catch-all resultant of all factors affecting glycolytic rate, primarily stimulation and temperature, and as such it obviates the need for separate evaluations of these variables.

It is possible that the new hypothesis may also explain the decreased tenderness of meat from animals treated with β-agonists (Jones et al., 1985; Hamby et al., 1986; Hanrahan et al., 1987; Smith, 1987), an effect that apparently cannot be accounted for by reduced fat and consequent cold shortening (Hamby et al., 1986). A significant increase in peripheral glycolysis has been reported by Eisemann et al. (1988) in clenbuterol-treated steers; if this much-increased rate carried over (as is likely) into the early-postmortem phase, it would probably exceed that which we can now equate with maximal tenderness.

Second. This work forces us to re-examine current theories of the nature of tenderness and tenderization. There is no obvious conflict between the present results and many earlier observations that stressed the importance of suppressing cold shortening; the devastating effect of this phenomenon far outweighs all other influences on tenderness. But the prevention of cold shortening is merely a forestalling of excessive toughening, and not really a process of tenderizing; on its own, it serves only to move the tissue's texture into a "neutral" zone that is neither markedly tough nor noticeably tender, and certainly does not ensure a desirably tender end-product (Marsh, 1981). True tenderness, by contrast, depends not only on eliminating the negative effects of shortening, but also on accentuating the positive influence of other beneficial postmortem processes.

The nature of these "other processes" remains unclear, but we can now be virtually certain that lysosomal-enzyme release and/or activation is **not** one of them. Our results show unambiguously that the conditions promoting increased catheptic activity – fast pH fall in a still-warm carcass – are precisely those that cause the toughening phase associated with low pH_3 values. The lysosomal-enzyme theory has been in vogue for some years despite a lack of direct evidence for it and a number of objections against it (Dayton et al., 1981; Allen, 1988), and it has led to the unfortunate belief that the tenderizing effectiveness of ES can be gauged by the size or the rate of pH decline that stimulation causes. The use of pH fall as a measure of ES efficacy must now be restricted to the shortening-prevention phase – the right limb – of the tenderness/glycolytic-rate relationship; if it is used to encourage still faster glycolysis beyond the desirable intermediate rate, it will result in a significant tenderness decline.

Our rejection of catheptic action as an important tenderizing agent does not necessarily apply to the longer-term aging phase, for it is conceivable (though unproven) that lysosomal enzymes may play a role during the tissue's prolonged chiller residence. Nor does it imply that CAF or some other neutral protease is necessarily involved in tenderization, even though the observed decline in tenderness with very rapid pH fall is in accord with CAF's known behavior (Dayton et al., 1976). Certainly another early-postmortem influence, additional to that of cold shortening, must be invoked to account for the quadratic relationship between glycolytic rate and tenderness, but we are not yet entirely convinced that it is neutral protease activity.

Third. There are practical as well as theoretical implications in these observations. In the United States, the tenderizing effectiveness of ES has been rather disappointing, and the process would probably be used only rarely if it were not for its several cosmetic effects and its facilitation of early grading (Savell et al., 1980; Terrell et al., 1982). The results described in this paper provide a reason for this often-indifferent performance of ES, and suggest a way – the attainment of an intermediate rate of glycolysis – by which this performance can be improved and perhaps optimized. It is conceivable that pH_3 or some related variable may eventually find use within industry as a monitoring tool, or as a guide to eating-quality evaluation. Much remains to be done, however, before our present limited knowledge can be utilized in practice; the relationship between glycolytic rate and tenderness must be confirmed, for instance, and its applicability to carcasses of widely differing characteristics must be examined. Perhaps the greatest problem will be the actual measurement of glycolytic rate, for it is obviously unrealistic to propose the routine excision of muscle samples for iodoacetate homogenization (as was done in our study). Yet the alternative - in situ pH determination by probe electrode – is a highly unreliable procedure that yields very misleading

results in actively glycolyzing muscle, as Bate-Smith (1939) demonstrated almost 50 years ago.

Even if it never attains direct use, however, the pH_3 concept is relevant to the practical attainment of tenderness. It demonstrates that glycolytic rate is a function of both ES and cooling rate, and not of stimulation alone; thus carcasses subjected to less severe chilling conditions will require correspondingly less ES treatment. It explains why the stimulation of fatter (and thus slower-cooling) carcasses not only fails to incease tenderness, but may actually toughen them, for their non-stimulated glycolytic rate is probably close already to the optimum. And it indicates that an intermediate glycolytic rate is as effective in tenderizing as two weeks' aging of meat that has undergone either a lower or a higher rate: surely an incentive for further interest and study, even if not for direct and early application.

Strategies for Improving Tenderness

The results of this study have been reported at some length for several reasons: they are certainly new, probably interesting, and possibly important. But they have been presented mainly to stress the great significance of the pre-rigor state of muscle and to encourage its fuller investigation. We are already aware of many of the chemical changes taking place in the tissue at this time, and of the effects that these changes can exert on the physical properties of the product: its color, water binding and tenderness, for example. But we are certainly not aware of **all** the changes or **all** the effects. Indeed, with our present very limited knowledge we are not even able to anticipate the consequences of quite simple changes in early-postmortem processing; who could have predicted, for instance, that a combination of slower chilling and electrical stimulation (each of them separately promoting tenderness) would cause a significant toughening?

The degree to which we can control tenderness thus depends very largely on the extent to which we can understand early-postmortem muscle. It is fortunate that the usually separate interests of the meat technologist and the muscle biologist come together at this time, while the tissue is in transition from a contractile machine to a food; pre-rigor muscle is thus a connecting bridge and a communication channel between the two research areas.

The need for this collaboration is not confined, however, to the early-postmortem phase; there are other aspects, not directly related to rigor, that merit as high a research priority. Collagen's contribution to toughness has been recognized since the turn of the century (Lehmann, 1907), and a fuller knowledge of its age-dependent structure and degradation is vital to a complete control of the tenderizing process, particularly in older animals. The role of titin as a cause of toughness is far from clear. Proteolytic enzymes abound in muscle; which of them play a part in tenderization, and what conditions encourage their activity? Each of these topics is the subject of intense research in fields unrelated to meat quality, yet each exerts (or is likely to exert) a powerful influence on tenderness, so a closer relationship between basic and applied investigators would be highly beneficial.

The need is thus not just for the acquisition of isolated data; it is for their integration into a coherent structure and their extrapolation toward both basic understanding and practical application. This concept was clearly recognized 80 years ago by the mathematician J.H. Poincaré: "Science is built up with facts, as a house is with stones. But a collection of facts is no more a science than a heap of stones is a house."

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Discussion
Meat Tenderness

J. Regenstein: Bruce, did you try a quadratic fit or some higher fit which may give you more curvature? On computers these days, would some of those fits actually give you a slightly better fit of your data?

B. Marsh: I think that was tried, Joe. Possibly Tom Ringkob could help me out there. I believe they did try up to about the fifth power and this is the better one. This came out as good as any.

Regenstein: Mohammad, on the experiment where you had the three different muscles with the initial Warner Bratzler shear values being very different, the final being all the same; I don't follow the logic of why those three muscles have different levels of calcium-activated protease, or CDP as you call it. It is not clear to me that it fits the explanation you gave, because they all come down to the same level. So the *psoas* would have no activity that is actually related to it. The others have more room to go. It seems to be that there is a bottom, so that the logic of that is what I was a little unhappy with.

M. Koohmaraie: You can't tell your whole life story in 40 minutes. If you noticed, I said at least "theoretically" when I was talking about that. Now there are three different muscles that have three different fiber type compositions. They have differences as far as function is concerned, and there are a lot of things different other than CDP alone. One of the major explanations that I have to come up with, since *psoas major* has only 50% of the activity in the *longissimus* (LD), is how come it doesn't go down? OK? The *psoas major* contains a large percentage of red fibers. The work at Wisconsin from about 20 to 30 years ago showed the zinc content of the red fibers is about 3 to 4-fold higher than that in white fibers. We are working on this problem right now. We have 30 documents which show that zinc concentration of a sub-physiological level would completely inhibit the CDP activity. So simply that's why I said "theoretically." Interpretation of the data by itself is not the whole story. There is a lot to analyze. The zinc content in *psoas major* is expected to be much higher than in the LD and that could be an explanation why you don't get any tenderization with *psoas major*. We are addressing that right now.

Marsh: I would just like to add a point there. I think there are two points that do need to be watched in this sort of comparison, Mohammad. First of all, the *psoas* is tender, exceptionally tender to begin with, largely because it is a stretched muscle on the carcass. The sarcomere lengths are very long, far longer than 2 microns. The other point is that when muscles have tenderized down to a certain value, no matter which muscles we are looking at, they are just not going to tenderize any further, regardless of what enzyme you have present. We find with our beef, which is all *longissimus* muscle, if we have them at the start without aging with 8 Warner Bratzler units, they might come down to 3 units within 3 weeks. Others that started with 4 units were reasonably tender, but also would come down to 3. I think there is a base line at which we hit this sort of background toughness, if you like, which is not going to be affected. So I do feel you should be cautious in transliterating this work among different muscles. The *psoas* is already about as tender as it was going to be and I don't think that a large or small amount of CAP would really make any difference to further tenderization.

Koohmaraie: I kind of disagree and kind of agree, but I have to say that I disagree with you. We find shear data in the literature which goes down to 2. Why doesn't the *psoas major* go to 2? Myofibrillar fragmentation, as far as I am concerned, is the best estimate of tenderness. Shear force value of cooked steaks and so forth has some problems. If you measure myofibrillar fragmentation index, it predicts that *psoas major* is a tough muscle. It does not fragment at all. Why?

Marsh: If that's the case, I suggest that myofibrillar fragmentation index is a very poor determinant of tenderness.

R. Merkel: Has anybody done it (MFI) with rabbits? It doesn't work.