

THE NATURE OF TENDERNESS

by

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The sum total of our knowledge of meat quality has been acquired in three stages. The first, the empirical phase—the try-it-and-see or learn-by-experience approach—has doubtless been in operation for thousands of years. It is sobering to us as late twentieth-century meat scientists to realize just how much practical information was gained in this long pre-scientific period. Even such a primitive people as the Carib Indians, the original cannibals, had acquired a great knowledge by trial-and-error observation long before the time of Columbus. We are told by Rouse (1963) that the Carib kept their captives without food for five days and, immediately after slaughter, removed the meat from the hot bodies and washed it. Most of the meat was cooked and eaten immediately with (according to Rouse) “many signs of enjoyment . . . the women receiving the arms and legs and the men the rest of the body.” The remainder was smoked for later feasts. The parallels between the Carib’s empirical methods and modern packing practice are obvious: the pre-slaughter withholding of food (though five days seems rather excessive), the beneficial effects of early-post-mortem washing, the hot-boning operation, and the preservative action of smoke. Since the women received meat from the extremities, we must assume the existence of a grading system designed to assure the dominant sex of the more delectable cuts. And we can interpret the immediate roasting of the meat as a recognition that early-post-mortem heat treatment is an excellent safeguard against spoilage. With a little imagination, we might suppose that the advantages of pre-rigor cooking were really discovered many centuries ago in the islands of the Caribbean. Had their homeland been in a more frigid climate—who knows?—these remarkable people might even have observed cold shortening and found the means to overcome the problems we now associate with it.

The addition of a scientific approach to the empirical method, in the late nineteenth century, marked the start of the second phase of knowledge acquisition. It was characterized by a conscious effort to learn more about the tissue and the factors affecting

it. In the area of tenderness, for instance, it was no longer sufficient merely to recognize that some cuts were consistently more tender than others; it became desirable to attempt an explanation for the observed differences in terms of specific meat components. Perhaps the very first meat-tenderness investigator of this era was K. B. Lehmann of Würzburg, who showed just 80 years ago that toughness is related to connective-tissue content. This second period was responsible for a cataloging and partial elucidation of the factors associated with tenderness variation—age, breed, sex, fat content, etc.—and some idea of the complexity of meat became apparent. But the period introduced little of practical consequence or direct packing-house practicability which would not have been learned through simple empirical reasoning. Perhaps its greatest contribution was to supply scientific justification for the use of previously established practices: to demonstrate that the practical man was right all along, though frequently for the wrong reason (E. H. Callow, personal communication).

The start of the third phase of knowledge accumulation occurred about 30 years ago. It was signalled by a remarkable paper by Bate-Smith (1948b) in the first volume of “Advances in Food Research.” The author referred to “the bewildering growth of fundamental knowledge of muscle,” and to the absence of “any striking application of the principles of modern biochemistry to the technology of handling meat animals and meat.” He then proceeded to blend two previously unmixed disciplines: bio-chemistry (in the form of Szent-Györgyi’s theory of muscular contraction and Needham’s views of glycolysis) and technology (in the form of meat processing and practical determinants of meat quality). In retrospect, this paper, to me, marks the beginning of a quite revolutionary line of new thinking, for in effect it says for the first time: Meat and muscle are not two different materials, to be studied by mutually exclusive groups of investigators; meat *is* muscle, and the more we learn about one, the more we know about the other. It is this concept which characterizes the third, and current, phase of meat investigation—the desire not only to correlate cause with effect, but also to understand (and perhaps to control) the dynamic aspects of this remarkably complex substance. This concen-

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tration on change—change of bond strength, of composition, of length, of ATP, of filament overlap, of pH—is the reason for the rapid progress made in recent years, and the basis for the present tenderness symposium.

Connective Tissue

Because of its long association with meat tenderness, connective tissue merits first consideration as a cause of toughness. Its principal component, collagen, has been the subject of many reviews, and your attention is drawn particularly to the Proceedings of last year's conference for further information.

The contraction of muscle is initiated and maintained by the contractile proteins. Movement would be confined to the muscle fiber, however, if it were not for the connective tissue, which acts as a coordinator of the forces separately generated in individual fibers and as a transmitter of the resultant action. It has many other vital functions to perform in the living musculature: it supports, it separates, it protects, it provides a bed for vascular and neural tissue, and it prevents over-extension of the muscle and consequent damage to the delicate contractile structure. To undertake these manifold duties, connective tissue is located in several different sites in muscle, each structural unit of which is surrounded by a sheath or sleeve: the fiber by the endomysium, the fiber bundle by the perimysium and the entire muscle by the epimysium. Because of the great differences in muscle size, shape, complexity and function, a wide range of connective-tissue contents exists among the muscles of a carcass.

Of the numerous components of connective tissue, collagen is of most relevance to meat toughness. The basic structural unit of this protein is tropocollagen, a long, thin molecule of MW 300,000 and 280 nm in length. It has been likened (Piez, 1966) to a hose of diameter one inch and length 17 feet to give an idea of its high axial ratio. Perhaps a rope of the same dimensions would be a better analogy, as it is composed of three peptide chains wound around each other and bound together by intramolecular links to form a very compact and strong molecule.

A unit of such small size, of course, would present absolutely no problem of toughness, no matter how strongly its constituent peptides were cemented together. Unfortunately the same small unit would also be totally useless in the living animal. Nature has overcome this problem by precisely aligning the tropocollagen molecules in a so-called "quarter-stagger" overlapping formation, and then bonding them

together at frequent intervals to prevent sliding under tension. The combination of longitudinal overlap and latitudinal crosslinkage is responsible for the strength and relative inelasticity of collagen in life. It is this same combination which is responsible for the sometimes appreciable effect of collagen on meat tenderness.

The relationship between collagen and toughness is complex, and only recently has the situation been clarified. The early observation of Lehmann (1897) that connective tissue determined tenderness received support from many investigators during the next 60 years, but several studies indicated little or no correlation. It was observed, for instance, that veal contains more connective tissue than beef despite its obviously greater tenderness (Bate-Smith, 1948a; Wilsson *et al.*, 1954). More recently it was shown (Bendall and Voyle, 1967) that longissimus muscles of young calves contain three times the collagen percentage found in those of older cattle. Such results are clearly not in harmony with a simple and direct influence of collagen on tenderness.

Part of the reason for the major discrepancies was revealed in the early 'sixties, when it was shown that the collagen of young animals responds quite differently to various treatments from that of their more mature brethren. The percentage of intramuscular collagen solubilized by collagenase, for example, or by near-boiling water, is much greater in veal than in the meat of older animals (Goll *et al.*, 1964 a, b, c). Similarly, the forms of collagen which are soluble in neutral salt solutions or dilute acids fall to quite low levels between birth and 1-2 years of age (Carmichael and Lawrie, 1967).

A firm chemical and structural foundation for these observations has become available in very recent years. It now seems clear that many of the covalent intermolecular bonds in collagen—those linking the tropocollagen molecules together—are relatively labile in the young animal, being readily fractured by pH change, heat, or denaturing agents. With increasing age, however, they change to a much more thermostable form (Bailey, 1972), the more easily reducible crosslinks declining in amount until they are virtually absent at maturity (Bailey and Shimokomaki, 1971; Shimokomaki *et al.*, 1972). This age-dependent decrease in the proportion of labile to stable bonds is of obvious interest and concern to the meat researcher, for it directly determines the increasing resistance of the collagen fiber to physical breakdown during cooking. Thus the "quality" of the collagen, the ease with which the structure can be ruptured into smaller units, is of greater significance than the quantity.

Perhaps the best measure of the collagen contribution to toughness would be the product of its amount and its relative stability.

What practical means to achieve meat tenderness are suggested by this new knowledge? The only frank reply is that it is still too early to say. No immediate answer to collagen toughness is within our reach. But the results of these studies have certainly opened new avenues for exploration and (maybe) exploitation. Not just whole molecules or macromolecules, but specific chemical linkages, can now be pinpointed for special attention. And, not just their chemical or physical destruction, but their biological formation, can be examined for practical possibilities. Modification of collagen production in life, or manipulation of its structure after death, may be attainable goals, in terms not of reduction in its quantity but of change in its "quality." Controlled nutritional deficiencies or specific feed additives might be developed to delay the transition of the "youthful" and easily broken crosslinks to the more stable form. The pre-slaughter injection technique might eventually be used to circulate an enzyme specific for cleavage of the mature linkage, rather than the broad-spectrum proteolytic enzymes used currently in a sort of shotgun routine. The future for collagen control in meat, although not assured, is bright.

The Contractile Proteins

If collagen were the only cause of toughness, we could conclude that its quantity is the sole determinant of tenderness differences among the muscles of a carcass, while its quality is solely responsible for tenderness differences among corresponding muscles of varying age. We would expect, therefore, that a muscle of low collagen content from a young animal would be very acceptably tender. There are times, however, when this correlation falls down completely. The most celebrated failure of the relationship was detected in 1961, when the loins of New Zealand lambs, exported in frozen form to England, were often found to be remarkably tough, sometimes indeed to the point of inedibility. Now, the longissimus muscle does not have an unduly high collagen content, and lambs of 6-10 months of age are clearly to be regarded as youthful. The major quality defect thus presented both a great concern to the meat industry of the country and a real challenge to meat science. Many relevant papers and several specific reviews (Marsh, 1972, 1974; Locker *et al.*, 1975) have been published since the first detection of the phenomenon, and it will suffice in this report to summa-

rize the cardinal findings on which our current knowledge of it is based.

The work of the Cambridge (England) school of some 25-30 years ago (Bate-Smith and Bendall, 1947, 1949; Bendall, 1951; reviewed and updated by Bendall, 1973) established the principal changes undergone by muscle during its first few post-mortem hours. During the initial delay period, the tissue's extensibility and content of adenosine triphosphate (ATP) remain high, but creatine phosphate declines quite rapidly. Fairly abruptly, the rapid phase of rigor onset then commences; ATP decreases because there is no longer any creatine phosphate to assist its resynthesis, and its fall is paralleled by a declining extensibility. Throughout both the delay and rapid phases, lactic acid is accumulating as a result of anaerobic glycolysis, so the pH falls steadily. But eventually rigor mortis is fully established—the tissue is virtually depleted of ATP, it is rigid and almost inextensible, and acid production has ceased through either exhaustion of the glycogen reservoir or pH inactivation of the enzyme system. Although these observations were made some years before the appearance of Hanson and Huxley's (1955) theory of muscular contraction, they are completely compatible with the sliding-filament hypothesis, and the onset of inextensibility is now considered to be due directly to cross-linkage formation between the thick and thin filaments of the sarcomere.

The Cambridge workers found that post-mortem shortening occurred only during the rapid phase, and (at least in muscles of normal glycogen content) was quite small unless the temperature was raised toward 37°C. If they had extended their observations from rabbit psoas muscle to a distinctly red tissue—any bovine or ovine muscle, for instance, or even the red soleus or semitendinosus of the rabbit—they might well have added the discovery of cold shortening to their achievements. Instead, this phenomenon remained unknown and unsuspected for more than a further decade. It was Locker and Hagyard (1963) who first reported that bovine muscles, excised in a pre-rigor condition and exposed to temperatures near 0°C, shortened by 50% or more, the length change taking place almost entirely during the pre-rigor or delay phase. Four years earlier, Locker (1959) had noted that "various muscles of the ox enter rigor mortis in differing states of contraction . . . related to the strains present in the muscles of the hung carcass." In the following year (Locker, 1960), still some time before the first detection of cold shortening, he had shown that "relaxed muscles are tenderer than partly contracted muscles," suggesting to him

that "it should be possible to improve the quality of the longissimus by hanging the carcass in such a way that this muscle is stretched or prevented from shortening." These three remarkable papers, spanning a period of only four years, opened up a whole new area of meat science and muscle biology and offered an entirely fresh approach to tenderness technology.

Many studies since that time have revealed the great magnitude of the toughening caused by cold-provoked shortening in beef and lamb. If the length change is small, toughness is not greatly increased, but, with increasing shortening during the pre-rigor phase, toughness rises steeply until the tissue may be 4-5 times as tough as its unshortened control (Marsh and Leet, 1966). With still further shortening, major internal rupturing of the structure takes place because some of the sarcomeres supercontract (occasionally by 80% of initial length), this producing complete fractures in nearby areas (Marsh *et al.*, 1974). The rupturing certainly causes very appreciable tenderizing, but is of only academic interest for a number of reasons: it can only be produced in small samples capable of very rapid cooling, it causes massive drip loss (Marsh and Thompson, 1958), and the end product is so distorted as to be entirely unacceptable to the consumer.

With this knowledge, we can now see why New Zealand lamb underwent such a quality decline in a relatively short period of time. Because of greatly increased lamb production, the meat industry had installed blast freezers to speed operations. In many packing houses, the new equipment was installed in areas previously occupied by cooling floors, where the newly slaughtered carcasses had formerly resided for some hours at ambient temperature. Henceforth, therefore, hot carcasses were being exposed to a very cold air-blast within an hour or two post-mortem, their muscles still being in a strictly pre-rigor condition and thus capable of considerable cold shortening before freezing intervened (Marsh *et al.*, 1968; McCrae *et al.*, 1971). It is important to stress that the installation of blast freezers was not directly responsible for the problem, rather, it was the elimination of the delay period before freezer entry which caused the damage. Once the possibility of post-mortem shortening had been overcome, blast freezing produced at least as tender a product as the former slow freezers had achieved.

Since these cold-shortening studies were made either on lamb carcasses or on excised bovine muscles, it is reasonable to ask if the same thing would necessarily occur with the much larger (and therefore far slower cooling) beef side. Experiments in many lab-

oratories during recent years have shown that, although the effect is smaller, it still exists to a quite marked degree in beef, and must be regarded as a serious practical problem in that species. Furthermore, a worsening of the situation may be anticipated as faster and earlier post-mortem chilling methods are introduced in the interests of improved hygiene (Ingram, 1972), reduced weight loss (Taylor, 1971), or accelerated processing. An educated awareness of cold-shortening and its consequences, on the other hand, may well prevent any further deterioration or even reverse it. Indeed, an enlightened view may ultimately affect much more than packing-house practice. There is evidence (Meyer *et al.*, 1977) that the beneficial effects of marbling and fat cover on quality may be little more than a reflection of the slower cooling rate (and hence reduced cold-shortening) of the larger and fatter carcass, as suggested some time ago (Marsh, 1974). Confirmation and extension of this concept could well affect feedlot practices and perhaps (eventually) beef grading, for eating quality would then depend much less on fat cover and content and correspondingly much more on early post-mortem carcass treatment.

What is the mechanism by which cold-shortening causes such a great toughening? The shortening is necessarily the result of interaction between thick and thin filaments, so it is the contractile proteins, principally actin and myosin, which must be primarily responsible for the tenderness decline. An attempt was made three years ago (Marsh and Carse, 1974) to explain the length/tenderness relationship in terms of the overlap of thick and thin filaments within the sarcomere. The theory accounted rather well for the whole quite complex shape of the experimental data, from high stretch to high shortening, but its validity is now doubtful. It assumed a constant "background" toughness due to connective tissue within any one muscle (regardless of the extent of its pre-rigor length change), but later studies elsewhere have suggested that this may not be so (Rowe, 1974). Furthermore, there may be an interaction between the contractile filaments and the connective tissue (Dransfield and Rhodes, 1976). In addition, the recent confirmation by Locker and Leet (1975, 1976a, b) and Locker *et al.* (1976) of the presence of so-called "gap" filaments in muscle has complicated the comparatively simple picture of a few years ago. It appears, indeed, that the New Zealand group believes this new component to be a major contributor to the tensile properties of meat (Locker *et al.*, 1977). Perhaps wisely, their hypothesis is confined to general descriptive terminology, avoiding the hazards of a mathematical treatment.

The most fundamental questions we can ask about cold-shortening concern the mechanism of the effect. Why does cold exposure provoke a contraction-like response and why does it appear to be confined to distinctly red muscles? Recent evidence (Buege and Marsh, 1975) suggests that the calcium ions which trigger the shortening are released from the mitochondria responding to normal post-mortem anoxia, and not from the sarcoplasmic reticulum reacting to cold. Red muscles are known to contain both more mitochondria and a less well developed sarcoplasmic reticulum (SR) than white muscles, thus in the red tissue more mitochondrial calcium will be released when anaerobic conditions prevail, and less SR will be available to gather it up. We believe that the SR of red muscle is just about able to cope with the released mitochondrial calcium ions at room temperature, but at lower temperatures the liberated calcium ions overload the SR's sequestering ability, and shortening follows. In white muscle, the reverse holds true, fewer mitochondria release less calcium, and a more extensive SR has no difficulty at all in absorbing it even at low temperatures. No practical way of preventing the length change can be envisioned at present as a result of this hypothesis (which, incidentally, still awaits confirmation). Progress in other areas of biological research has been so rapid in recent years, however, that we cannot discount the possibility of early-post-mortem mitochondrial calcium-ion control by appropriate treatment.

It has been my intention to discuss only the nature of meat tenderness, and not the means by which tenderness can be achieved or controlled. That is the duty of the following speakers. Nevertheless, it is my hope that you will not consider this presentation in isolation from the others, or merely as a rather academic discourse remote from the real world. My success depends upon your willingness to relate the chemistry of collagen to the tenderness of meat, the muscle-shortening cause to the meat-toughening consequence, and the fundamental approach to the practical goal.

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