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EARLY RESEARCH ON THE FIBROUS MICROSTRUCTURE OF MEAT

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Abstract

The older scientific literature on the histology of skeletal muscle describes the transverse fragmentation of myofibrils to form sarcomere disks and the attachment of Z lines to the sarcolemma in contracted muscle. These phenomena may now be explained by recent research on the cytoskeletal framework of muscle fibers, particularly desmin and vinculin. The formation of sarcomere disks might be developed as a simple method to study cytoskeletal integrity in meat. In early research, contraction bands and the disappearance of transverse striations during contraction caused considerable confusion with ordinary light microscopy. These problems were largely resolved by the use of polarized light, and polarized light microscopy may be a useful technique for the study of contraction bands and the measurement of sarcomere length in meat.

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Introduction

The study of food microstructure has brought together scientists from diverse backgrounds who share the belief that microstructural analysis is a useful approach for the elucidation of the rheological properties of natural and synthetic foods. While the major interest is naturally in new techniques and discoveries, an occasional backward glance can also be rewarding since early histologists made many observations that relate to current topics of interest. This is particularly true in the study of meat microstructure, since many of the commercially important properties of meat relate to the larger microstructural units of muscle such as muscle fasciculi and their connective tissue framework. The brief historical survey reported here was undertaken to search for early ideas and observations that might be of interest in current research. Much of the older literature is somewhat difficult to read because different names are used for microstructural components. A translation table is given to facilitate the use of older references on muscle microstructure.

Fascicular Microstructure

When simple optical lenses were used to examine the microstructure of muscle it was found that the smallest fibrous units that are visible without magnification are composed of bundles of muscle fibers (Figure 1). These bundles were called either fasciculi (plural of the Latin word for a small bundle) or lacertae (plural of the Latin word for arm-muscle), although only the former word has survived in general use. The transverse striations of skeletal muscles, or striae as they were often called, were first seen without histological staining when some type of restrictive illumination was employed. Muscle tissues were mostly examined as unstained teased preparations. Transverse sections were obtained by free-hand sectioning of dried muscle followed by rehydration (Carpenter, 1868). Three different situations

were detected whereby a fiber could terminate intrafascicularly, that is, somewhere along the length of a fasciculus: (1) a blunt or rounded ending that was anchored in the endomysium (Figure 2), (2) a tapered ending anchored in the endomysium, and (3) a fiber that terminated on a small diameter microtendon within the fasciculus. The tension that can be generated per unit cross sectional area of muscle is increased if there are hidden fibers terminating intrafascicularly (Huxley, 1957).

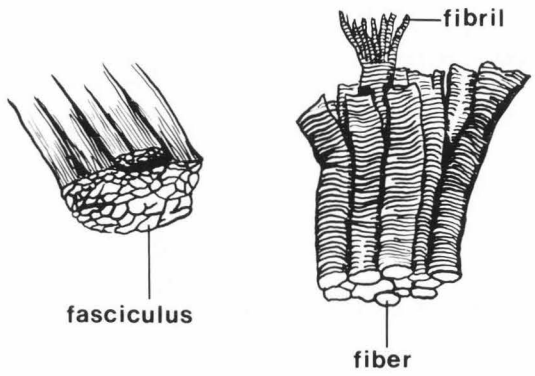

Figure 1. Three levels of muscle structure that are clearly visible by light microscopy - fasciculi, fibers and fibrils (from Quain, 1856).

The fibers of nearly all striated skeletal muscles are unbranched, but early histologists soon found a few exceptions, as in the muscle fibers that are attached in the skin or in the surface layer of the tongue (Figure 2). Some histologists now believe that striated skeletal muscle fibers may split longitudinally in diseased (Isaacs et al., 1973) or regenerating muscles (Schmalbruch, 1976).

Microstructure of Myofibrils

Sometime before his letter to the Royal Society of London published in 1674, Antony van Leeuwenhoek had discovered that most of the interior of striated skeletal muscle fibers is filled with what we now call myofibrils (Needham, 1971). Leeuwenhoek was adept at using some form of dark-field illumination with his single-lens microscope (Casida, 1976), but he never divulged its secret and more than a century passed before the development of achromatic objectives for compound microscopes enabled any further progress on the microstructure of muscle. A most intriguing debate then unfolded.

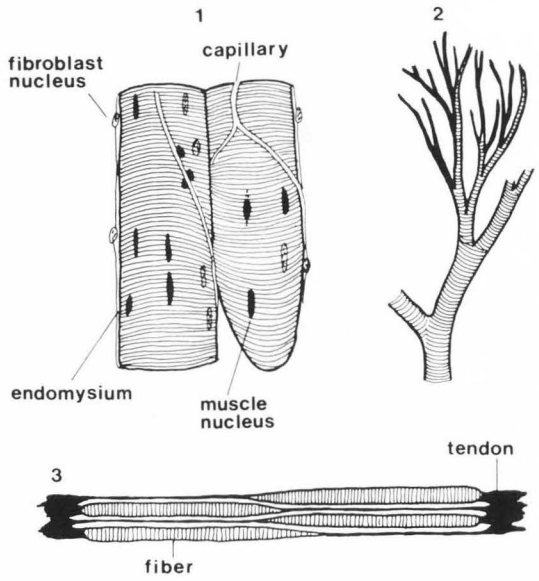

Figure 2. The ends of muscle fibers: (1) a normal conical end; (2) a relatively rare branched end; and (3), a diagram to illustrate the effects of intrafascicularly terminating muscle fibers on the contraction tension per unit area of muscle (from Schiefferdecker, 1891; Schäfer and Thane, 1898; Huxley, 1957).

Dilute acetic acid was used to make muscle nuclei visible, but it also produced some other effects. Instead of fragmenting longitudinally to reveal myofibrils, muscle fibers sometimes started to fragment transversely so that laterally adjacent sarcomeres maintained their adhesion and separated into Bowman's discs (Figure 3). A similar effect was obtained by treating muscle fibers with ammonium carbonate, in which case the discs were formed by a fracture through the light striation (Clarkson, 1896). This led to quite a serious debate as to which of the two structures, fibrils or discs, was the more fundamental unit within the fiber. Perhaps the lateral integrity of Bowman's discs might be worth a reinvestigation from the point of view of meat tenderness.

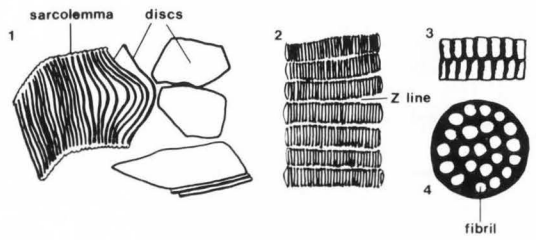

Figure 3. The splitting of muscle fibers into transverse Bowman's discs in (1) a muscle fiber from the tail muscle of a lizard, and (2) a muscle fiber from the leg of a beetle; and (3) profile and (4) transverse sectional views of a small disc (from Benda and Guenther, 1895; Schäfer and Thane, 1898).

On the winning side of the debate were those who believed that myofibrils were the fundamental unit of muscle, but the technique that was used to produce clearly visible striated myofibrils was as artificial as the acetic acid treatment used to produce discs. Muscle fibers were first hardened for a long period in absolute alcohol and were then teased apart with dissecting needles. What we now call myofibrils were then often called muscle columns or the equivalent term in Greek, sarcostyles. One observation in particular gave priority to the myofibrillar theory over the disc theory. In some living muscle fibers, it was possible to observe separate myofibrils but never separate discs. Fibrils were more easily seen in red muscle than in white muscle. At the macroscopic level it was well known (Quain, 1856) that the fatigue-resistant muscles responsible for the maintenance of posture or repetitive movements generally had a darker red coloration than did the muscles used for energetic voluntary or propulsive movements. This coloration persisted even in completely exsanguinated muscles and was evidently due to a pigment that was intrinsic to the muscle fibers - a pigment that we now know as myoglobin. It was known that most of the muscle fibers in dark red muscles had distinct longitudinal striations, but that their transverse striations were less distinct. These are characteristics that we now attribute, respectively, to a greater volume of sarcoplasm loaded with mitochondria between the myofibrils and to differences in sarcomere construction. Whatever the underlying cause, however, observations on red muscles won the day for myofibrils rather than discs being the fundamental unit of muscle contraction.

Table 1. List of older names for parts of the sarcomere

A BAND
Anisotropic band
Q band, Querscheibe (transverse band)
Brücke's lines
Sarcous element of Bowman
Metabolic Schicht (layer) of Ebner
Interseptal zone of T.H. Huxley.
Principal disc of Engelmann

I BAND
Isotropic band
J band
Intermediate disc of Krause
Hyaline substance of Schäfer
Septal zone of T.H. Huxley

Z LINE
Zwischenscheibe (intermediate disc)
Krause's membrane
Dobie's line (Dobie's granules)
Grundmembran (foundation membrane)
Telophragma (end partition)
Disque Mince (thin disc) of Renaut
Amici's Streifen (stripe)
Ende Scheibe (end disc) of Merkel
Plasmophore (form-carrier) of Holmgren
Septal line of T.H. Huxley

N BAND
Nebenscheibe (neben = next to) of Merkel
Accessory disc of Engelmann

H ZONE
Qh (heller = clearer)
Median disc of Hensen or Hensen's line

M BAND
Mittelsheibe (middle band)
M line
Median membrane of Hensen
Mesophragma (middle partition) of Heidenhain

Others
E disc (Z line refraction near N band)
Terminal disc of Merkel

Flögel's granules
(at edge of stretched A band)

CS(contractionsscheibe, contraction band)

Microstructure of Sarcomeres

A translation of older terms for parts of the sarcomere is given in Table 1. The term sarcomere (muscle part) has a

long history. When myofibrils were first isolated and examined with a compound light microscope, the myofibril appeared to be composed of a bead-like string of minute globular particles (Figure 4).

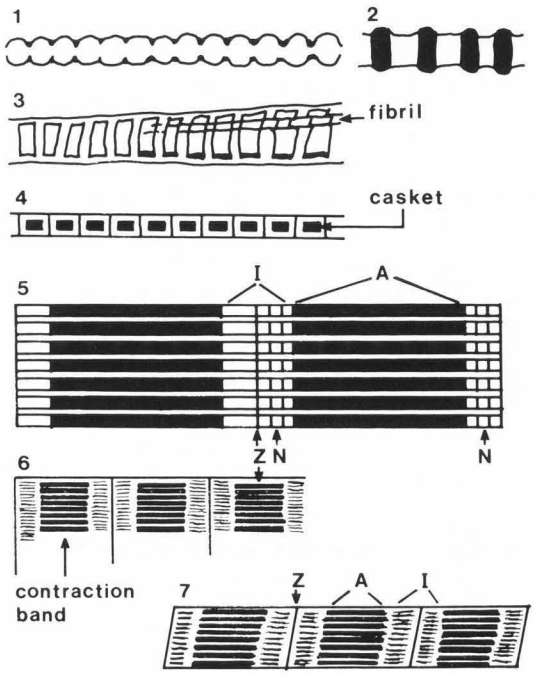

Figure 4. Early ideas on the microstructure of the sarcomere: (1) the fibril as a bead-like string of particles, (2) each bead resolved as an A band, (3 and 4) the A bands as muscle caskets, (5) the sarcomere model derived from hematoxylin-stained sections, and some clairvoyant views of (6) contracted and (7) relaxed sarcomeres (from Rosenthal, 1881; Huxley, 1881; Quain, 1856; Lewis and Stohr, 1913; Schiefferdecker, 1891).

Bowman (1840) called each particle a sarcous element, but they later became known as muscle caskets or cases. The reason for this is that when isolated myofibrils are examined with transmitted light in a fluid with a lower refractive index than that of the myofibril, a bright line moves inwards parallel to each edge of the myofibril. As a general principle in optical microscopy, the bright line or Becke line moves into the component with the higher refractive index. Thus, the dark A bands are separated from each other by light I bands, and the I bands merge with the bright line along the inner edges of the myofibril (Figure 4). The A bands then appear as dark rectangles (caskets) within the myofibril. Even at this early point, it was known (Quain, 1856) that a thin line or partition (Z line) ran across the middle of the bright band (I band) between each casket (A band). The unit from one Z line to the next Z line became known as the sarcomere. When muscle fibers were artificially fragmented into transverse Bowman's discs, the point of separation occurred along the Z line at each end of the sarcomere (Schäfer and Thane, 1898).

Although it is often assumed that the optical and mechanical deficiencies of early microscopes were the main problem in obtaining a clear view of the microstructure of muscle, this is probably not the case. Bracegirdle (1978) photographed a modern section of muscle with an 1826 microscope and obtained a reasonable representation of nuclei and striations. In the converse case, an 1849 preparation of muscle photographed with a modern microscope, both the string of beads effect and the casket effect were seen in isolated myofibrils. Thus, further advances in the elucidation of the microstructure of the sarcomere were mainly a consequence of improvements in the techniques of preparing muscle tissue for microscopic examination, and improvements in microscope design were supplementary. Although Leeuwenhoek had used saffron to stain some of his muscle fiber preparations (Bracegirdle, 1978), it was not until the latter half of the nineteenth century that differential staining underwent rapid development as a consequence of steady progress in industrial organic chemistry and the manufacture of new dyes.

In addition to the study of isolated myofibrils, it now became possible to examine longitudinal sections of sarcomeres. In the hands of Heidenhain (1913), the natural dye hematoxylin derived from logwood was developed into a technique that has not yet been surpassed for the staining of transverse striations in paraffin-embedded muscle. With progress on the use of a mordant to bind the dye specifically to certain structures, with elucidation of the chemical changes involved in "ripening" the staining mixture, and with the development of destaining techniques to enhance the degree of differential staining, the iron hematoxylin method enabled the development of a model of sarcomere structure that was to last until the advent of electron microscopy.

The most conspicuous fault of the nineteenth century model of sarcomere structure was that it missed the essential key to the mechanics of contraction - that A and I bands contain overlapping

filaments that are pulled past each other when the muscle contracts. Although an individual filament is below the resolution of ordinary light microscopy, there were a few investigators who had a subliminal grasp that a filamentous substructure might exist below the limits of resolution. Both the A and the I bands of individual myofibrils sometimes appear with an extremely fine longitudinal grain. In the words of Schäfer and Thane (1898), about the A band, "With high powers the sarcous element may be made out to be composed of a sarcous substance, which stains with haematoxylin, and is pierced by short tubular canals which extend from the clear interval as far as the middle of the disc; these canals give it a longitudinally striated appearance." A similar effect was seen in the I band: "Fine longitudinal striae which appear to be due to delicate extensions of the sarcous substance (perhaps delicate septa), may also, under favourable circumstances be seen traversing the clear intervals." This may be nothing more than an artifact due to post mortem changes in fibrillar structure, but when several of the early investigators came to engrave the illustrations for their idealized concepts of sarcomere structure, the engravers' shading naturally adopted a filament-like pattern (Figure 4).

Early Ideas on Contraction

Early investigators who believed in the existence of muscle caskets proposed some very unsatisfactory theories about muscle caskets causing contraction by attracting each other like small magnets. Even at the time that they were proposed, such ideas did not receive serious attention because it was thought that the muscle caskets disappeared in contracted muscle (Schäfer and Thane, 1898). In the contracted state, myofibrils often had a homogeneous appearance without striations. This was called the transitional stage or uebergangsstadium.

When illuminated with transmitted polarized light, muscle fibers are double refracting or anisotropic (Figure 5). When the analyzer was rotated until the field of view was as dark as possible, it was observed that the bands that appeared dark with ordinary light were now bright because of their double refraction of the polarized light (A bands). The pale I bands seen with normal transmitted light appeared very dim or dark with polarized light. An important point for meat scientists interested in measuring sarcomere lengths is that, with polarized light, the A band stays as the brightest band even when the fibril is fully contracted (although the apparent length of the A band may be reduced). With normal light, on the other hand, the A bands may be hard to identify in contracted muscle. Brücke gave the name disdiaclasts to the small double refracting particles that he thought might be responsible for the anisotropous bands of the myofibril (Huxley, 1980).

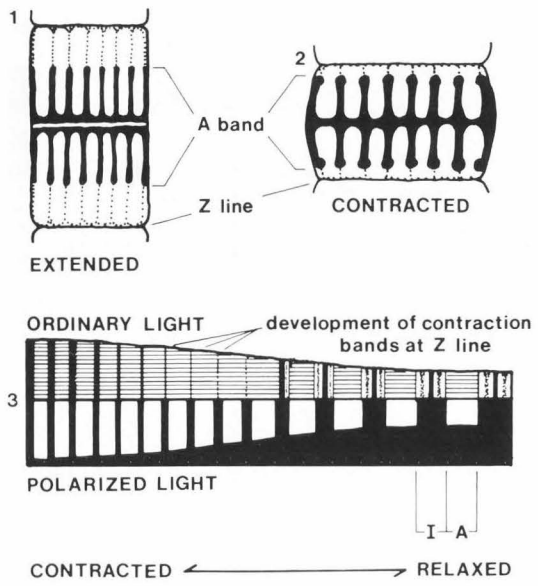

Figure 5. Early ideas on muscle contraction: (1) slightly extended and (2) contracted sarcomeres according to the theory that the I band substance was sucked into longitudinal pores in the A band; and (3) the correlation of the appearance of sarcomeres with normal and polarized light illumination as a contraction wave passes from left to right (from Schäfer and Thane, 1898; Engelmann, 1878).

The special appearance of myofibrils with polarized light (Figure 5) set in motion a wave of speculation concerning the underlying mechanisms of muscle contraction. With hindsight, we can now identify three of the more important deductions that were made.

(1) The occurrence of double refraction or birefringence suggested that there existed a precisely ordered molecular system below the limits of optical resolution.

(2) The use of polarized light showed that the A bands were still present during the transitional stage of contraction so that stretched, relaxed and contracted myofibrils all had the same basic microstructure.
(3) Studies with polarized light showed that the length of the A band remained more or less constant during muscle contraction, until the A band became concealed at each end by the increasing thickness of contraction bands.

The use of polarized light almost gave rise to the answer to how muscles contract. Krause (1869) suggested that rod-like disdiaclasts might extend the complete width of the A band, just like the thick filaments of myosin in the sliding filament theory (Huxley, 1980). Despite the clairvoyance of a few investigators, however, the most popular theory of muscle contraction remained the idea that there were longitudinal pores or channels in the A band into which the fluid substance of the I band was sucked. This idea was an attempt to base both the theory of muscle contraction and the theory of amoeboid movement on the same common basis. The ubiquitous presence of actin and myosin in motile cells (Durham, 1974) now shows that the basic intention was sound, even though all the details were wrong.

Between the Fibrils

In early studies on the sarcoplasm by light microscopy, it is difficult to believe that anything was really visible apart from a matrix of optical effects caused by the presence of the myofibrils. However, as shown in Figure 6, Dobie (1849) pulled individual myofibrils apart and found that as they came apart, "at the point where two myofibrillae are separated from each other, extended for a greater or less distance between them, there often exists a beautiful homogeneous membrane." Schäfer and Thane (1898) found that there were longitudinal lines of intercolumnar sarcoplasm that passed continuously along the length of the fiber with a bead-like swelling on each side of the Z line. In some types of muscle fibers, most of the the mitochondria are neatly arranged level with the Z line. Mitochondria often swell to many times their original size as a result of changes in the osmotic pressure around them. Perhaps mitochondria were responsible for the beads along the intercolumnar sarcoplasm.

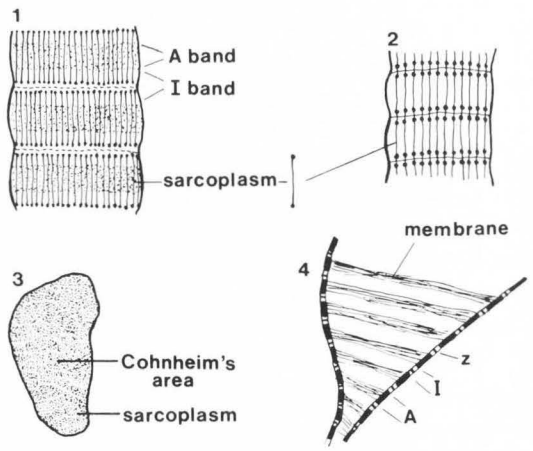

Figure 6. Sarcoplasm: (1) and (2), longitudinal elements of sarcoplasm which were conventionally drawn as a line with a bead at each end; (3), the appearance of Cohnheim's areas or fields when the groups of fibrils are separated by sarcoplasm; and (4) a membrane found between the fibrils by Dobie in 1849 (from Schäfer and Thane, 1898; Böhm et al., 1904; Dobie, 1849).

Further information on the microstructure of the sarcoplasm was obtained by gold staining. Muscle fibers were fixed with acid and then soaked in gold chloride. After a subsequent 24 hour reduction in formic acid, the sarcoplasm became stained a conspicuous dark violet color while the myofibrils were unstained. Heitzmann and Carnoy proposed that the myofibrils (now invisible) were merely artefacts due to post mortem coagulation and that muscle contraction was really caused by the reticulum of sarcoplasm between the myofibrils (Ramon y Cajal, 1937). Although this theory was rejected by muscle researchers who knew that myofibrils could often be seen in living muscle and that myofibrils were capable of contraction when taken out of the fiber, it did gain some support for a while. What gold staining has started to expose, however, might have been the sarcoplasmic reticulum as we know it today. However, there are other systems apart from membranous transverse tubules and the sarcoplasmic reticulum that run between the myofibrils.

When Bowman (1840) was demonstrator in anatomy at King's College, London, he undertook a lengthy examination of muscle fiber structure. His description of the

membrane system around the muscle fiber was particularly noteworthy and we still call it by the name he proposed - the sarcolemma. Bowman proposed that the sarcolemma might "consist of a very close and intricate interweaving of threads, far too minute for separate recognition." This would still be a reasonable description of the Type III collagen or reticular fibers that we can now see on the muscle fiber surface by scanning electron microscopy. Bowman found that the inner surface of the sarcolemma (equivalent to the plasma membrane of the muscle fiber) often showed irregularities "as if some attachment of the fibrillae to it had existed." Apart from mechanical isolation of the sarcolemma by microdissection, Bowman developed a technique of treating muscle fibers with acids or alkalis (phosphoric, tartaric and citric acids, and potash) which caused the myofibrils to swell and ooze out of the cut ends of muscle fibers. Although the myofibrils were grossly distended by this treatment and they lost their transverse striations, they preserved the "adhesion by which they are mutually held together." The transverse discs into which the muscle fiber contents could sometimes be separated by cleavage at the Z line were occasionally found to maintain their adhesion to the inner surface of the sarcolemma at the rim of the disc (i.e., in the Z line region). In contracted muscle, the sarcolemma often bulged outwards except at the points where it was bound at the midlength of the I band (Figure 7). This was confirmed in a number of studies over the next 100 years.

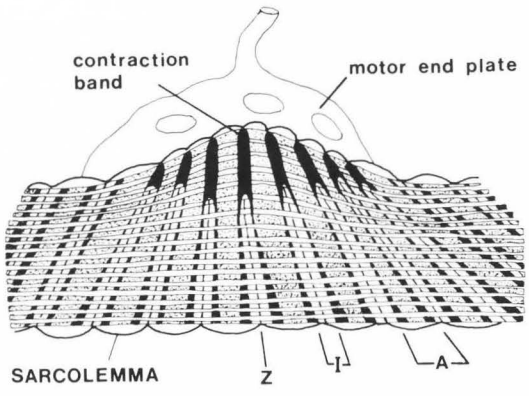

Figure 7. Formation of contraction bands beneath a neuromuscular junction showing also how the Z line adheres to the inner surface of the sarcolemma (from Cowdry, 1934).

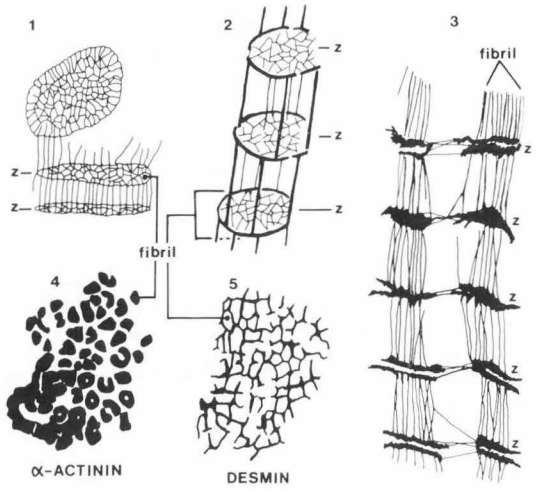

Figure 8. Cytoskeletal elements within the sarcoplasm: (1), a plan view and a lateral view of a gold chloride preparation from the 1880's showing the transverse mesh associated with the Z line and a longitudinal mesh extending between the Z lines; (2), the elements around an individual fibril seen by scanning electron microscopy after extraction of thick and thin filaments; (3), a similar view by scanning electron microscopy of the elements associated with two adjacent fibrils - note that the transverse mesh is repeated on each face of the Z line and that the transverse meshes are linked between fibrils; (4), plan view of alpha actinin at the Z line; and (5), plan view of desmin at the Z line (from Melland, 1885; Wang and Ramirez-Mitchell, 1983; Granger and Lazarides, 1978).

The explanation of Bowman's observations is quite recent (Stanley, 1983). What had to be developed first was the concept of the cytoskeleton (Figure 8). Studies by scanning electron microscopy confirm that there is an extensive meshwork of cytoskeletal filaments within the muscle fiber (Wang and Ramirez-Mitchell, 1983). The thin filaments of the sarcomere are probably anchored at the Z line by alpha actinin. The technique to show this involves the separation of the fiber into sarcomere discs. Unlike Bowman's discs which incorporated a complete sarcomere by cleavage at the Z line, the transverse discs in this modern method are prepared by solubilizing the actin and myosin from glycerinated muscle with

0.6 M potassium iodide. Maceration of the sample causes the framework of the myofibril to separate into discs by cleavage through the A band region. The discs can then be examined in a plan view. The distribution of fluorescent antibodies for alpha actinin is restricted to within myofibrils at the level of the Z line. Between these contact points appears a network pattern where antibodies for desmin are bound (Granger and Lazarides, 1978). Thus, at the level of the Z line, the desmin extends tranversely across the muscle fiber and binds the myofibrils together laterally.

Desmin was named from the Greek word for a link or bond, desmos. In order to account for the early observations on the attachment between the Z lines and the sarcolemma, something must connect the transverse desmin meshwork to the plasma membrane. As well as the meshwork that extends transversely across the fiber at the level of the Z line, there are also a few strands that connect transversely at the level of the M line. A particularly interesting feature of the transverse desmin meshwork level with the Z line is that it sometimes appears to be doubled with a layer on each side of the Z line. This feature explains how Bowman's discs could retain a connection to the sarcolemma on both rims of each disc.

Cohnheim's areas (Figure 6) are groups of myofibrils that are clumped together in transverse sections prepared for light microscopy. In recent years, this phenomenon has been dismissed as an artefact. However, artefact or not, the mechanical explanation of Cohnheim's areas might involve the desmin meshwork. Perhaps Cohnheim's areas indicate the existence of a stronger meshwork linking myofibrils that have been derived by longitudinal splitting from a common precursor.

Vinculin is involved in the attachment of actin filaments to the inner surface of the plasma membrane (Geiger, 1979; Wilkins and Lin, 1982). In skeletal muscle, vinculin forms clustered patches on the inner surface of the plasma membrane in a pattern that matches the I bands of the underlying sarcomeres (Pardo et al., 1983). Thus, vinculin is arranged in costameres or in rib-like bands around the muscle fiber. Each costamere has the appearance of a double line astride the position of the Z line, just as the desmin is doubled at this location. The costameres are matched to the state of the sarcomere - they are elongated when sarcomeres are stretched and they are shortened when sarcomeres are contracted. With transmission electron microscopy, it has been shown that the transverse filaments of the cytoskeleton sometimes connect with dense plaques on the inner surface of the sarcolemma, and that the transverse filaments level with the M line can also pull down on the sarcolemma during contraction (Pierobon-Bormioli, 1981).

Conclusion

The study of the microstructure and texture of food myosystems has been dominated by research on connective tissues and myofibrils. As shown in this brief sketch of early research, recent work on the cytoskeletal framework of muscle fibers confirms what a few Victorian histologists had already guessed: that the transverse fibrous elements within muscle fibers may be as strong as the longitudinal elements.

The myofibrillar composition of meat is basically similar to that of fish, yet these two types of food myosystems are often radically different in texture. Although differences in connective tissue content and muscle fiber arrangement certainly exist, it is possible that the basic textural difference between meat and fish has some relationship to the cytoskeleton. It is generally agreed that conditioned beef fails in tensile strength near the Z line, but this gives rise to myofibrillar fragments several sarcomeres long, not to Bowman's discs. Perhaps the characteristic texture of fish and crustacean myosystems is caused by their transverse fragmentation into Bowman's discs, whereas meat tends to form longitudinal fibrillar fragments.

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Discussion with Reviewers

C.A. Voyle: In view of the observations of Verrati (Mem. Reale Ist. Lombardo, 19, (1902) 87-133, reprinted in J. Biophys. Biochem. Cytol. (1961) 10,4, suppl., 3-59) on muscle stained by the Golgi method, do you think that the "longitudinal lines of intercolumnar sarcoplasm - with a bead-like swelling on each side of the z-line" as described by Schafer and Thane (1898) could represent the longitudinal elements of sarcoplasmic reticulum with the associated terminal cisternae?

Author: This is certainly a possibility. One of the main problems in attempting to understand the early research on meat microstructure is that it is difficult to

identify with certainty the actual nature of intermyofibrillar structures seen by early microscopists. Since Veratti's work is now widely recognized as a classic example of a "lost discovery", I concentrated on fibrous systems within the muscle fiber, as indicated in the title. Although the sarcoplasmic reticulum is obviously important in the early stages of post mortem metabolism when phenomena such as cold shortening are occurring, it is information on the cytoskeleton that is currently of particular interest with regard to meat tenderness. I am afraid that, in much of the early research, membrane systems and the cytoskeleton might have been indistinguishable, just as it is possible that silver-stained parts of the cytoskeleton might sometimes have looked like parts of the sarcoplasmic reticulum or transverse tubular system. Another problem in this area is that intermyofibrillar glycogen may also react to silver staining (Swatland, 1982, Mikroskopie, 39, 317-322) and neat intermyofibrillar networks of silver-stained glycogen have sometimes been confused with sarcoplasmic reticulum in preparations for light microscopy.

R.J. Carroll: How can one determine the collagen type by scanning electron microscopy? What is the basis for calling these fibers type III? Could they be something other than collagen?

Author: By referring to these fibers as Type III collagen or reticular fibers, I was simply referring to any type of connective tissue fiber on the muscle fiber surface.