STRUCTURAL CHANGES DURING THE POST RIGOR STORAGE OF SOME LARGE BEEF MUSCLES

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Introduction: Beef muscle passes, post-mortem, into rigor-mortis, wherein it is hard and inextensible. (Bendall, 1960; Goll, 1968). If the muscle is cooked and eaten in rigor it is tough and unpalatable. About 36 hours post-mortem the muscle softens to the touch though it does not become extensible again (Bendall 1960). Jungk et al. (1967) showed that the ability to maintain isometric tension was lost after 48 hours. This tension development is most noticeable in bovine muscle at 2°C and is therefore closely related to the cold shortening effect reported by Locker et al. (1963). After rigor-mortis has apparently been "resolved" muscle continues to increase in tenderness. (Deatherage 1963; Goll 1968). Goll et al. (1964) showed that tenderness does not increase very much after 10 days at 4°C. If meat is stored, sterile, for up to six months, there is a general breakdown in the protein and crystals of L-tyrosine have been reported to appear (Drake et al. 1957).

Undoubtedly some autolysis probably catalysed by catheptic enzymes is occurring in long term sterile storage. However, it is not clear whether the initial post-rigor softening and the tenderising of the succeeding 10 or so days is enzymically catalysed. There appears to be a very low level of catheptic activity in the immediate post-rigor period (Locker 1960b; Sharp 1963; Lutalo-Bosa and Macrae 1969). Davey & Gilbert (1966) showed that tenderising effects occurring over 30 days were not related to proteolytic activity measured as non-protein-nitrogen increase. Valin (1970) showed that only 15% of the total cathepsin-D, in muscle lysosomes, was released after 8 days storage post-mortem.

It appears that the textural changes occurring in muscle post-mortem can be grouped in several overlapping chronological phases and that they are the expression of sequential changes occurring in the muscle ultrastructure. These changes appear to be confined to the interior of the muscle fibre. There is a convincing body of evidence that ageing effects are not related to alterations in the connective tissue (Steiner, 1939; Wierbicki et al. 1955; Davey et al. 1967) but McClain et al. (1970) reported that soluble connective tissue decreased slightly post-mortem.

Changes in the fibre ultrastructure so far reported to occur during post-mortem storage include:
(1) association of actin and myosin filaments at rigor onset (Bendall 1960)
(2) fission of actin-Z-line bonds during post-rigor tenderising (Davey and Dickson, 1970, Fukazawa et al. 1969)
(3) loss of lateral alignment of adjacent myofibrils (Davey and Gilbert 1969)
(4) loss of the Z line (Davey and Dickson 1970, Henderson et al. 1970)

Since individual beef muscles in one carcass differ from each other in tenderness it is necessary to ascertain whether the changes, observed in specific muscles are universal, or whether they occur to a varying extent in different muscles. We examined samples from six of the principal meat muscles from the beef hind quarter at fixed times post-mortem in the electron and light contrast microscopes. Since the degree of contraction of muscles at rigor-mortis is known to affect tenderness (Locker, 1960;(a) Partman, 1963; Herring et al. 1965a,b; Marsh et al. 1966, Herring et al. 1967)

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we also measured sarcomere lengths on the micrograhs to determine whether the muscles showed markedly differing degrees of contraction.

Methods: Three bullocks of approximately 1000lb liveweight were used. They were slaughtered and dressed in the laboratory abattoir under good hygienic conditions. The following muscles were examined, longissimus dorsi, psoas major, biceps femoris, semitendinosus (also sampled pre-rigor) semimembranosus, glutaeus medius (also sampled pre-rigor). Samples were taken at 30 hr., 4 and 10 days post-mortem at points one third of the muscle length from one end and prepared for electron microscopy by conventional methods i.e. primary fixation in 5% Glut. (Sabatini et al. 1963), post-fixation in osmium tetroxide (Palade 1952) and embedding in Araldite (Glauert 1965). Samples taken pre-rigor were held at rest length before fixing (Price et al. 1963). Muscles were left on the carcass which was conventionally hung in the chill at 2°C until 30 hr post-mortem. They were excised carefully, placed in clean polythene bags and held at 2°C for the remaining time of aging. The temperature in the round was 2°C before the excision. Swabbing of the surface revealed about 100 organisms/cm^2 at 30 hr increasing to about 2 X 10^3 organisms/cm^2 at 10 days. Samples were taken at least 4-5cm within the muscle. No traces of microorganisms were seen in any of the preparations. Sarcomeres were measured by stage micrometer. Nodes were avoided when measurements were taken.

Results: - Light Microscopy

Samples from the semitendinosus and glutaeus medius were examined pre-rigor. All the fibres were straight and showed well defined A, I and H bands and Z lines.

At 30 hr. post-mortem the fibres of the psoas, which had sarcomeres of 2.66 to 3.54 µM, were usually straight and parallel. The fibres of the biceps femoris and semitendinosus with sarcomeres of 1.69 - 2.35 µM were wavy and crimped. The longissimus dorsi, semimembranosus and gluteus medius with sarcomeres of 1.45 - 2.20 µM showed extensive crimping. Crimped fibres were frequently seen to be slightly less contracted than adjacent straighter fibres but this was not a consistent observation. All muscles showed frequent but irregularly distributed "contraction nodes". (Paul et al. 1944). These contraction nodes are lengths of the fibre consisting of 10 - 20 shortened sarcomeres, and laterally convex between stretched lengths, also of 10 - 20 sarcomeres, laterally concave. The stretched lengths were frequently torn across.

At 4 days post-mortem all muscles showed infrequent I- band breaks - either traversing the fibre along a single I band or jaggedly stepwise across a number of consecutive I-bands.

After 10 days there were more fibre breaks in all muscles than at four days. Fibre crimping and nodes of contraction persisted and it was not possible to detect any differences in the proportion of fibre breaks between any of the muscles.

Electron Microscopy: - Pre-rigor myofibrils

Muscle sampled immediately after death had the appearance of resting muscle including wide I-bands, and prominent but narrow H zones each bisected by the M line. The Z lines showed zig-zag patterns. (Huxley 1960, Franzini-Armstrong and Porter 1964).

At 30 hr. post-mortem in all muscles, some of the Z lines showed sharp perpendicular breaks and patches of dissolution, often extending into

I band filaments. Many Z lines showed the zig-zag pattern. The A bands were intact. Some myofibrils were separated laterally from each other, but sarcoplasmic reticulum elements still connected them at the level of the Z lines. Each muscle had a predominant sarcomere length range but showed in the regions adjacent to the contraction nodes, fully extended (3.6 μM) to supercontracted (<1.45 μM) sarcomeres with the associated banding patterns. The supercontraction pattern showed at sarcomere lengths below 1.45 μM (the mean length of the A band), together with a gross disorganisation of filament alignment. The myosin filaments were crumpled against the Z line, which showed a corresponding thickening. A penetration of the Z-line by myosin filaments was seen in some myofibrils.

At 4 days post-mortem the ultrastructure of the Z line itself was not as well preserved as seen after 30 hrs. and was beginning to appear amorphous. All six muscles showed I band breaks occurring between Z-line and I filaments. Even fibres appearing intact under the light microscope showed signs of I-Z weakening.

After 10 days post-mortem ageing Z - I breaks increased and the Z line was usually diffuse, amorphous and here and there obviously disappearing completely. The sarcoplasmic reticulum connections between Z lines had mostly disappeared. All 6 muscles showed these features.

The 6 muscles may be classed into extended (psoas), intermediate (biceps femoris, semitendinosus) and contracted groups (long. dorsi, semimembranosus, glutaeus medius) on the basis of mean post-rigor sarcomere lengths at 30 hrs. (Table 1). Measurements were also taken at 4 days and 10 days post-mortem but no changes in sarcomere length were observed.

Discussion: It is widely known that all meat muscles become more tender with ageing post-mortem, although some muscles do not become tender enough in 10 days to allow their use for grilling.

Tenderising during aging is clearly partially caused by the dissolution of ultrastructure reported here and elsewhere (Stromer et al. 1967, Davey et al. 1969). The degree of dissolution seems to be similar in the 6 muscles we have studied, and the residual differences in tenderness must be due to differences in other properties and components of the muscle. The small overlap between I and A filaments in the psoas is reasonably ascribed as the cause of consistent tenderness in this muscle. (Locker, 1960b). The stretching of the psoas and compression of the long. dorsi, semimembranosus and glutaeus may be ascribed to the position of the leg in the hung carcass. However the long. dorsi and glutaeus medius with well-contracted sarcomeres are preferred as tender grilling steaks, especially after ageing, while biceps femoris and semitendinosus have intermediate length sarcomeres and are generally tough. Clearly, sarcomere length is not simply related to toughness when comparing one muscle with another although within one muscle the relationship is straightforward and simply explained (Marsh & Leet 1966).

Voyle (1969) recorded shorter sarcomere lengths in straight "actively" contracted fibres compared with adjacent crimped "passively" contracted fibres in excised cold-shortened sternomandibularis muscle. We have been unable to demonstrate a simple relationship between fibre conformation and sarcomere length within any one muscle.

Contraction nodes in inidvidual fibres were described by Paul et al. (1944) and they appear to be widely distributed in our samples. They may be produced by local 'cold-shortening' or metabolic variations between different parts of one fibre. The adjacent extended regions may contribute to tenderness by weakening the fibres.

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Table 1: Sarcomere Lengths (μM) 30 hr. post-mortem

Muscle		Animal 1	Animal 2	Animal 3	Overall range and mean
Long. Dorsi	r.	1.860 - 1.450	1.800 - 1.500	2.200 - 1.450	2.200 - 1.450
	m.	1.703	1.672	1.768	1.714
Psoas	r.	3.270 - 2.660	3.350 - 2.810	3.540 - 3.060	3.540 - 2.660
	m.	3.036	3.123	2.998	3.050
Biceps Femoris	r.	2.210 - 1.705	2.165 - 1.714	1.991 - 1.726	2.210 - 1.705
	m.	1.935	1.890	1.884	1.903
Semitendinosus	r.	2.191 - 1.764	2.350 - 1.686	2.350 - 1.730	2.350 - 1.686
	m.	1.982	1.867	2.095	1.981
Semimembranosus	r.	1.850 - 1.510	1.840 - 1.450		1.850 - 1.450
	m.	1.723	1.736		1.730
Glutaeus Medius	r.	1.812 - 1.480	2.010 - 1.500		2.010 - 1.480
	m.	1.639	1.755		1.697

For each animal the mean calculated over 30 - 40 measurements. Sarc. length Semitendinosus and Glutaeus medius pre-rigor both 2.350 μ.

REFERENCES

Bendall, J.R. (1960) "Structure and Function of Muscle". Vol. 3. Ed. Bourne, G.H. Acad. Press Inc. New York.

Davey, C.L., Dickson, M.R. (1970) Studies in meat tenderness. 8. Ultrastructural changes in meat during ageing. J. Fd. Sci. 35, 56.

Davey, C.L., Gilbert, K.V. Gilbert (1966) Studies in meat tenderness. 2. Proteolysis and the ageing of beef. J. Fd. Sci. 31, 135.

Davey, C.L., Kuttel, H., Gilbert, K.V. (1967) Shortening as a factor in meat ageing. J. Fd. Technol. 2, 53.

Davey, C.L., Gilbert, K.V. (1969) Studies in meat tenderness. 7. Changes in the fine structure of meat during ageing. J. Fd. Sci. 34, 69.

Deatherage, F.E. (1963) The effect of water and inorganic salts on tenderness. Proc. Meat Tenderness Symposium 1963. Campbell Soup Company.

Drake, M.P., Giffee, J.W. (Jr.)., Ryer III, R., Harriman, H. (1957) Proteolytic enzyme activity in irradiation-sterilized meat. Science 125: 23.

Fukazawa, T., Briskey, E.J., Takahoski, F., Gasui, T. (1969) Treatment and post-mortem ageing effects on the Z-line of myofibrils from chicken pectoral muscle. J. Fd. Sci. 34, 606.

Franzini-Armstrong, C., Porter, K.R. (1964) The Z-disc of skeletal muscle fibres. Zeist für Zellforsch, 61, 661.

Glauert, A.M. (1965) Techniques for Electron Microscopy. (Ed. Kay, D.H.), 2nd edition. p 191. Blackwell Scientific Publications, Oxford and Edinburgh.

Goll, D.E., Henderson, D.W., Kline, E.A. (1964) Post-mortem changes in Physical and Chemical Properties of Bovine Muscle. J. Fd. Sci. 29, 590.

Goll, D.E. (1968) The resolution of rigor mortis. Proc. Twenty-first Annual Reciprocal Meats Conf., National Livestock and Meat Board, Chicago, 21, 16.

Henderson, D.W., Goll, D.E., Stromer, M.H. (1970) A comparison of shortening and Z-line degradation in post-mortem Bovine, Porcine and Rabbit Muscle. Am. J. Anat. 128, 117.

Herring, H.K., Cassens, R.G., Briskey, E.J. (1965a) Sarcomere length of free and restrained bovine muscles at low temp. as related to tenderness. J. Sci. Fd. Agric. 16, 379.

Herring, H.K., Cassens, R.G., Briskey, E.J. (1965b) Further studies on bovine muscle tenderness as influenced by carcass position, sarcomere length and fibre diameter. J. Fd. Sci. 30, 1049.

Herring, H.K., Cassens, R.G., Suess, G.C., Brungarat, V.H., Briskey, E.J. (1967) Tenderness and associated characteristics of stretched and contracted Bovine muscles. J. Fd. Sci. 32, 317.

Huxley, H.E. (1960) The Cell. Ed. Brachet, J., Mirsky, A.F. Acad. Press Inc. New York, 4, 366.

Jungk, R.A., Snyder, H.E., Goll, E.E., McConnell, K.G. (1967) Isometric tension changes and shortening in muscle strips during post-mortem ageing. J. Fd. Sci. 32, 158.

Locker, R.H. (1959) Striation patterns of ox muscle in rigor mortis. J. Biophysic. Biochem. Cytoc. 6, 419.

Locker, R.H. (1960a) Proteolysis in the Storage of Beef. J. Sci. Fd. Agric. 11, 520.

Locker, R.H. (1960b) Degree of muscular contraction as a factor in tenderness of beef. Food Research, 25, 304.

Locker, R.H., Hagyard, C.J. (1963) A cold shortening effect in beef muscles. J. Sci. Fd. Agric. 14, 787.

Lutalo-Bosa, A.J., Macrae, A.F. (1969) Hydrolytic enzymes in Bovine Skeletal muscle. 3. Activity of Some Catheptic enzymes. J. Fd. Sci. 34, 401.

Marsh, B.B., Lect, N.G. (1966) Studies in Meat Tenderness. III. The effects of cold shortening on tenderness. J. Fd. Sci. 31, 450.

McClain, P.E., Creed, G.J., Wiley, E.R., Hornstein, I. (1970) Effect of post-mortem ageing on isolation of intramuscular connective tissue. J. Fd. Sci. 35, 258.

Partmann, W. (1963) Post-mortem changes in chilled and frozen muscle. J. Fd. Sci.
Price, H.M., Howes, E.L., Sheldon, D.M., Hutson, O.D., Fitzgerald, R.T., Blumberg, J.M., Pearson, C.M. (1965) An improved biopsy technique for light and electron microscopic studies of human skeletal muscle. Lab. Invest. 14, 194.
Palade, G.E. (1952) J. Exp. Medicine, 95, 285.
Paul, P., Lowe, B., McGlurg, B.R. (1944) "Changes in Histol. Structure and palatability of beef during storage. Food Research, 9, 221.
Sabatini, D.D., Bensch, K., Barrnett, R.J. (1963) J. Cell Biol. 17, 19.
Sharp, J.G. (1963) Aseptic autolysis in rabbit and bovine muscle during storage at 37°C.
Steiner, G. (1939) Mechanical measurements of toughness in meat. 2. Fleisch -u-Milchhyg. 50, 61 and 74. Quoted in Bate-Smith, E.C. 1948. "Advances in Food Research" pp 1-38. Acad. Press, New York.
Stromer, M.H., Goll, D.E., Roth, L.E. Morphology of rigor-shortened Bovine muscle and the effect of Trypsin on pre and post-rigor myofibrils. J. Cell Biol. 34, 431.
Valin, C. (1970) Activation des enzymes lysosomales du muscle au cours de la maturation de la viande. Ann. Biol. anim. Bioch. Biophys., 10 (2) 313.
Voyle, C.A. (1969) Some observations on the histology of cold shortened muscle. J. Fd. Technol. 4, 275.
Wierbicki, E., Cahill, V.R., Kunkle, L.E., Closterman, E.W., Deatherage, F.E. Effect of castration on biochemistry and quality of beef. J. Agr. Fd. chem. 3, 244.