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EFFECT OF HIGH HYDROSTATIC PRESSURE ON MEAT MICROSTRUCTURE

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Abstract

Bovine longissimus muscle was prerigor pressure treated at 103.5 MNm^{-2} at 37°C for 2 min and immediately sampled, fixed and examined by light microscopy, scanning and transmission electron microscopy. Parameters like pH, Warner-Bratzler shear force values and sarcomere length were measured and related to the microscopic observations. Pressure treated samples have shorter sarcomere length, lower pH and W-B values. Physical changes include separation of sarcolemmal and endomysial sheath, contraction bands, disruption of myofibrillar structure and increased interfibrillar and intermyofibrillar spaces. At the subcellular levels, disappearance of glycogen granules, appearance of swollen mitochondria, sarcoplasmic reticulum and in some cases ruptured mitochondria were observed. These morphological changes in mitochondria and sarcoplasmic reticulum should furnish the additional Ca^{2+} to account for the pressure induced contraction. The interactions between the chemical and physical effects should account for the tenderizing effect of pressure treatment.

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Introduction

Cold shortening in prerigor chilled muscle with its associated negative effect on meat tenderness (Locker and Hagyard, 1963) is a serious problem in the meat industry. Meat scientists have responded to this problem with different degrees of success. Techniques such as mechanical tenderization, delayed chilling, electrical stimulation and hydrostatic pressurization (Macfarlane, 1973; Busch et al., 1967; Smith et al., 1971; Carse, 1973) were suggested to eliminate or minimize this problem and hopefully render meat acceptable to consumers.

Prerigor pressurization (PRP) of ovine or bovine muscles has profound effects on the physico-chemical properties of meat (Macfarlane, 1973; Kennick et al., 1980). Rapid drop in pH, accelerated postmortem glycolysis, reduced W-B shear force values and higher tenderness ratings were all reported for the pressure treated sample over the corresponding control.

Studies involving pressurization of isolated protein components of myofilaments have demonstrated an F-G transformation of actin (Ikkai and Ooi, 1966) and depolymerization of myosin (Joseph and Harrington, 1968). Pressure in the order of 150 MNm^{-2} caused sarcoplasmic reticulum (SR) to lose its extra adenosine triphosphatase (ATPase) with no effect on the basal ATPase (Horgan, 1981).

Electron microscopy techniques were successfully used to study ultrastructural changes of aged meat (Schaller and Powrie, 1971; Eino and Stanley, 1973), heat induced changes (Leander et al., 1980; Parrish et al., 1973) and changes induced by electrical stimulation of carcasses (Will et al., 1980; Savell et al., 1978).

Although all pressure treatment studies demonstrated its tenderizing effect (Macfarlane, 1973; Kennick et al., 1980; Bouton et al., 1977) yet the mechanism by which it does so has not been conclusively established. Macfarlane and Morton (1978), using the electron microscope, observed absence of M-line, loss of integrity and aggregation of I-band filaments as a result of postrigor pressure treatment. Consequently the purpose of the current study was to a) investigate the PRP induced changes in meat microstructure; b) relate these changes to our earlier observations on the physico-chemical and organoleptic properties; and c) over all to shed light on

the mechanism by which PRP treatment tenderizes meat.

Material and Methods

Sample preparation and treatment

Samples were obtained from 5 steers of Good and Choice grade (approx wt 470 kg) slaughtered at our laboratory. Immediately after slaughter, skinning, evisceration and washing of the carcass (approx 25-35 min), the longissimus muscle (12th thoracic to the 2nd lumbar vertebrae) from one side of each carcass was excised, vacuum packed in Cry-O-Vac bags (Cry-O-Vac with R5 Series single stage rotary vacuum pump, Busch Inc.) and placed in a water bath at 37 ± 1°C for 10-15 min to equilibrate to the treatment temperature. Thereafter, the samples were transferred to a pre-heated pressure chamber (10.2 cm in diameter and 30.5 cm long) filled with water at 37 ± 1°C, as a working fluid. The chamber was then tightly closed and a pressure of 103.5 MNm^{-2} (1030 kg/cm^2) was applied and maintained for 2 min. After release of the pressure, the samples were removed and stored in a cooler at 1 ± 1°C. Matching muscles on the opposite side of each carcass were left on the carcass and stored along with the treated samples until they were sampled at appropriate times postmortem.

The pH of the samples was measured with a Corning pH Meter Model 125 fitted with an Orion combined glass electrode (Orion Res. Inc.). The glass electrode was inserted in a freshly made incision (approx 3-5 cm deep) each time pH was taken.

Sarcomere length of the samples was measured 24 hr postmortem. About 5 g of finely chopped muscle tissue were blended with 50 ml of 0.25 M sucrose solution for 30 sec at low speed in a Waring blender. Immediately after blending, 1-2 drops of this suspension were dispersed on a glass slide, covered with a cover slip and examined with a phase contrast microscope equipped with a filar micrometer. The length of 10 sarcomeres in each of 25 randomly selected myofibrils was measured and their mean was taken to be the sarcomere length of the sample and expressed in micrometer (μm).

Shear force measurements were made 7 days postmortem. The samples were placed in polyethylene bags and cooked in a water bath at 80 ± 1°C for 40 min to an internal temperature of 70 ± 1°C. After the samples were cooled to 45°C, rectangular pieces 1.25 x 0.8 cm with their axis parallel to the fibers were removed and sheared in a Warner-Bratzler shear device. For taste panel assessment (panel consisted of 10 trained judges), each sample was cut into 10 serving portions with a given judge always receiving the same section from each sample. The same 10 judges served on all panels.

Electron microscopy

Within 1½ hr postmortem samples (approx 2x3x1 mm) were removed, with a razor blade, from the control and PRP treated muscles and fixed immediately in a 3% glutaraldehyde in 0.1 M phosphate buffer (pH 7.2). After 4 hr the samples were then transferred to a fresh phosphate buffer for a further 12 hr.

Samples for transmisson electron microscope (TEM) were placed for 1 hr in 1% osmium tetroxide (OsO_4) in 0.1 M phosphate buffer (pH 7.2), followed by successive dehydration in 50, 70, 90 and 100% acetone solutions and stained with a saturated solution of uranyl acetate in 70% acetone. Specimens were infiltrated, embedded in Spurr's epoxy formulation and cured for 12 hr at 70°C. Silver-grey sections were cut, using a diamond knife, on a Porter-Blum MT-2 Ultramicrotome, stained with Reynold's lead citrate and examined with a Philips EM-300 transmission electron microscope.

Fixed samples of tissue recovered after the selection of TEM samples were placed successively for 30 min in 50, 70, 90 and 100% acetone then followed by a similar treatment in 50, 70, 90 and 100% trichlorotrifluoroethane in acetone. From absolute trichlorotrifluoroethane, samples were critical point dried in absolute monochlorotrifluoromethane. Samples were then mounted on aluminum planchets and rotary coated with approximately 100 A° of 60:40 gold-palladium in a Varian VE-10 vacuum evaporator. The coated samples were viewed and photographed in an AMRAY 1000 A scanning electron microscopy (SEM) at an accelerating voltage of 20 kV. Images were recorded on Polaroid type 55 film.

Statistical Analysis

Since the design of the experiment utilizes a paired comparison, the data were analyzed by the paired t-test (Steel and Torrie, 1960).

Results and Discussion

As shown in Table 1, the physico-chemical and organoleptic properties were affected by the pressure treatment. The pH value and W-B shear force values of the PRP treated samples were significantly lower than the corresponding control.

Table 1 - Effect of hydrostatic pressure on the physico-chemical and organoleptic properties of bovine longissimus muscle.

Item	Treatment	
	Control	PRP[a]
pH[b]	6.78 ± 0.36[d]	5.73 ± 0.32[e]
W-B (kg/cm^2)	8.2 ± 0.40[d]	2.9 ± 0.24[e]
Tenderness rating[c]	5.35 ± 0.30[d]	6.7 ± 0.45[e]
Sarcomere length (μm)	1.95 ± 0.15	1.78 ± 0.20

[a] PRP = prerigor pressurization (103.5 MNm^{-2}, 37°C, 2 min).

[b] pH of the control and pressure treated samples measured immediately after the treatment.

[c] n/mean = 10j x 5 rep. = 50j/mean, tenderness rating was based on an eight-point scale, desirability increasing with score.

[d,e] values in the same row bearing different superscript are significantly different ($P<.05$).

Also the taste panel assessment was in the favor of the pressure treated samples. Sarcomeres from the pressure treated samples were 8.2% shorter than those from the control samples.

All the electron micrographs (Figs 1-6) show detectable differences between the control and PRP treated samples. It appears that the PRP treatment has its effects both at the cellular and subcellular levels.

SEM micrographs of the control samples show the muscle fibers to be intact without any damage on their surfaces (Fig 1a). On the other hand the SEM micrograph of PRP treated samples reveals distorted endomysial and sarcolemmal sheath (Fig 1b), interfibrillar spaces (Fig 1b), intermyofibrillar spaces (Fig 1c) and globular material on the surface of the fibers (Fig 2). The nature of the globular material is not known but thought to be disintegrated collagen fibers and/or coagulated sarcoplasmic protein (Cheng and Parrish, 1976).

The banding pattern, the different components of the sarcomere and the subcellular fractions are intact and distinct in the micrograph (Fig 3a) of the control sample. Most of the ultrastructural changes induced by PRP treatment can be pointed out from Fig 3b. The mitochondria and sarcoplasmic reticulum are shown to be swollen. The triads and T-systems can be easily identified in the control sample whereas in the treated samples only remnants of triads can be seen. Effect of pressure on the Z-line is very prominent where some breaks along its entire length can be seen and in some instances the material of the Z-line is almost removed. The degradation of the Z-line is thought to be caused by a protease endogenous to the muscle fiber and that its activity is Ca^{2+} dependent (Busch et al., 1972; Olson et al., 1977). Myofibrils isolated from at-death bovine muscles and incubated with a calcium activated factor (CAF) lost their Z-line in 3 min (Olson et al., 1977). Under our experimental conditions, it is likely that the Ca^{2+} concentration in the sarcoplasmic fluid increases as a result of the observed effect of pressure on the Ca^{2+} pumping systems (mitochondria and sarcoplasmic reticulum). Degradation of Z-lines has been implicated in postmortem meat tenderness (Olson et al., 1977, 1976; Parrish et al., 1973; Moller et al., 1973).

Another feature of the PRP treated sample was the disappearance of the H-zone and M-line characteristics of myofibrils. Macfarlane and Morton (1978) reported a similar finding in post-rigor pressure treated (100 MNm^{-2} for 60 min at 25°C) ovine semimembranosus muscle. Since the disappearance of the H-zone was observed in shortened sarcomeres as well as in stretched ones, it is not likely that the material of the H-zone was removed.* Rather, it suggested that in the case of stretched sarcomeres the H-zone was extended to the entire length of the A-band as a result of the thin filaments pulling away . In the shortened sarcomere the thin filament pull in towards the center of the A-band to the extent it shaded all the H-band area thus making it indistinguishable from the rest of the A-band.

The clearly dense areas of glycogen-like granules apparent in the control samples (Fig 4a) cannot be seen in the PRP treated samples (Fig 4b). This observation agrees well with the pH results presented in Table 1. The drop of pH in the PRP treated muscle could be explained by the accelerated breakdown of glycogen to lactic acid according to the following reaction:

$$C_6H_{10}O_5 + H_2O \longrightarrow 2\ \text{lactate}^- + 2\ H^+ \quad (1).$$

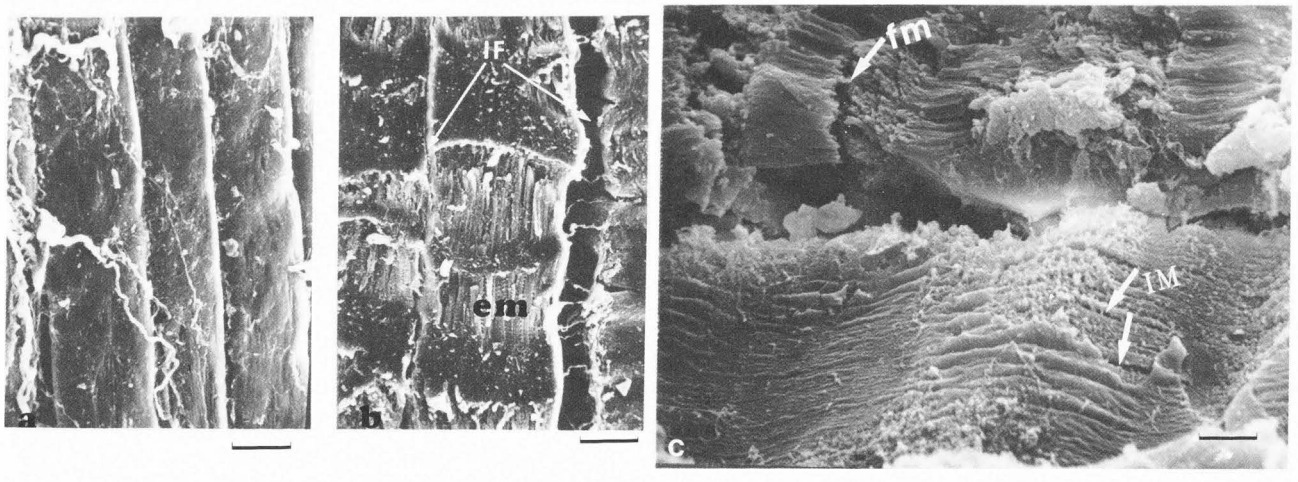


Fig 1 - Scanning electron micrographs of bovine longissimus muscle sampled and fixed 1½ hr postmortem: a) Longitudinal section of muscle fibers from the control sample. b) Same as (a) except the samples are PRP treated (103.5 MNm^{-2}, 37°C for 2 min). Note the damaged surface of the muscle fibers, exposed myofibrils (em), and interfibrillar spaces (IF). c) Same as in (b) except at high magnification, note intermyofibrillar spaces (IM) and fragmented myofibrils (fm). Bar in Fig 1a and b is 50.0 μm. Bar in Fig 1c is 2.0 μm.

*See Discussion with Reviewers.

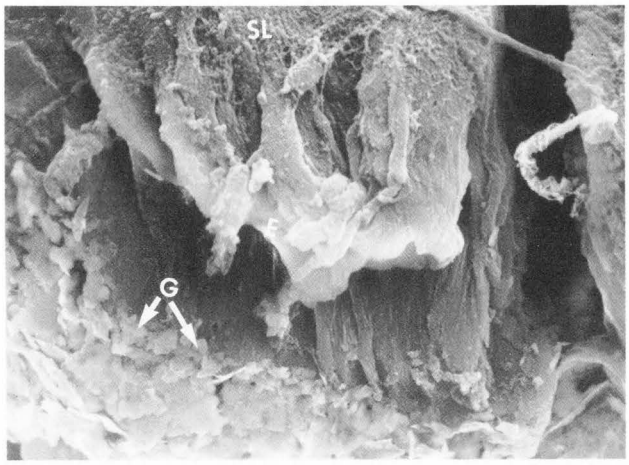


Fig 2 - Scanning electron microscopy micrograph of PRP treated (103.5 MNm^{-2}, 37°C for 2 min) longissimus muscle sampled and fixed 1½ hr postmortem. Note the globular material (G), endomysium (E) and the granular appearance of sarcolemma (SL). Bar is 2.0 μm.

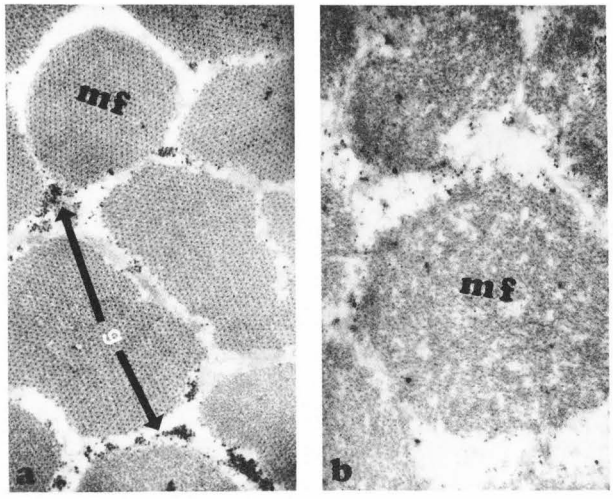
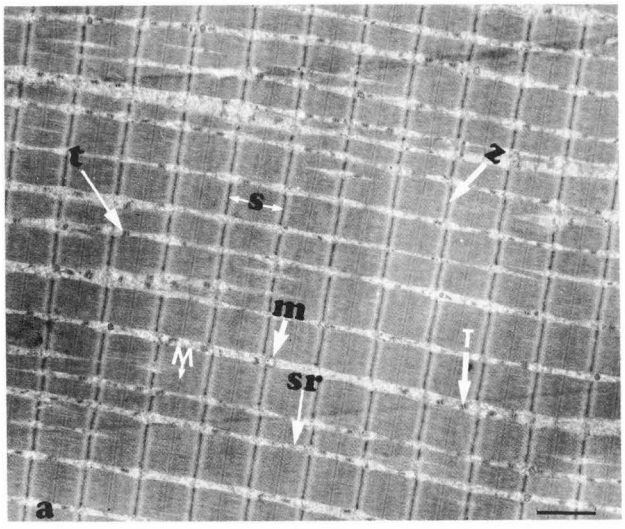


Fig 4 - Transmission electron micrograph of bovine longissimus muscle sampled and fixed 1½ hr postmortem. a) Cross section from the control sample, note the dense areas of glycogen-like granules (g) and the clear arrangement of thin filament around thick filament. b) Same as (a) except the samples are PRP treated (103.5 MNm^{-2}, at 37°C for 2 min). Note: only dispersed remnants of the dense area noted in (a) can be seen and the blurred appearance of filaments (mf). Bar is 0.5 μm.

The hexagonal arrangement of actin filament around the myosin filaments in the control sample (Figs 4a and 5a) can be seen clearly whereas in the treated sample such a feature is indistinct (Figs 4b and 5b). Several investigators have reported the sensitivity of actin to pressure treatments (Ikkai and Ooi, 1966; Ivanov et al., 1960; Macfarlane and Morton, 1978).

At high magnification (68,970X), TEM



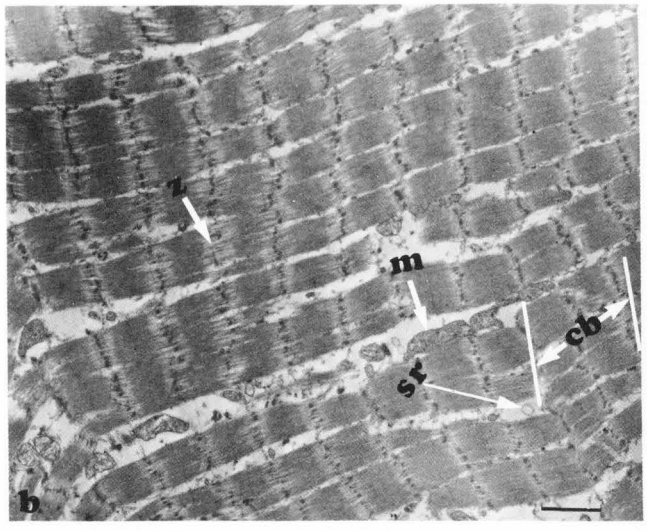


Fig 3 - Transmission electron micrographs of longissimus muscle sampled and fixed 1½ hr postmortem. a) Longitudinal section from the control sample, note the intact sarcomere components and the subcellular fractions. b) Same as (a) except the sample is PRP treated (103.5 MNm^{-2}, at 37°C for 2 min); note: swollen mitochondria (m), swollen sarcoplasmic reticulum (sr), disintegrated Z-lines, disappearance of H-zone, M-line, triads (t) and T-systems (T). cb = contraction band, S = sarcomere, bar is 2.0 μm.

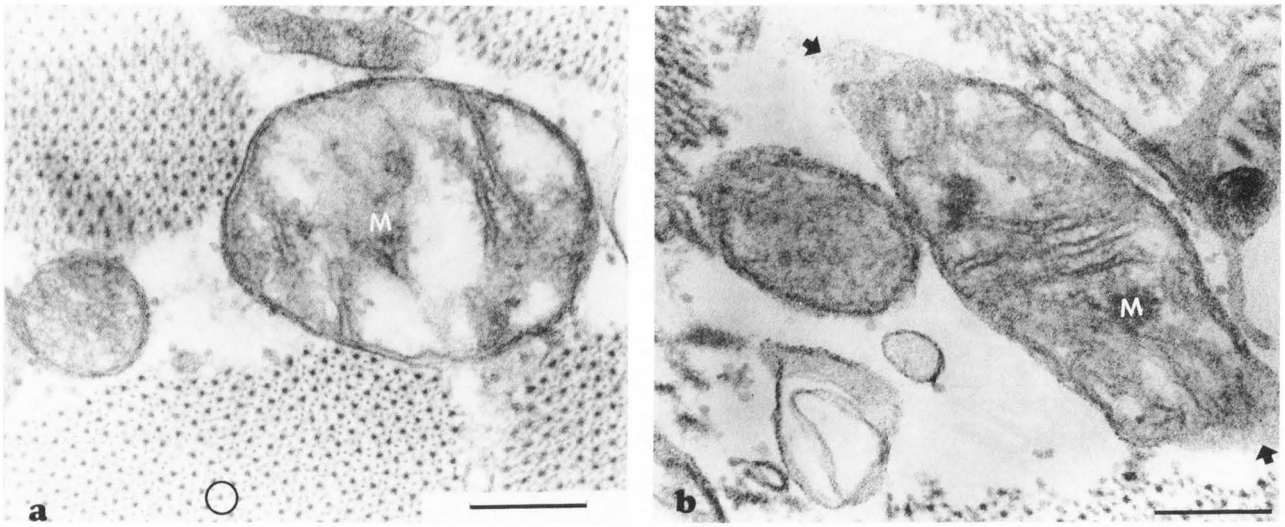


Fig 5 - Transmission electron micrographs of bovine longissimus muscle sampled and fixed 1½ hr post-mortem. a) Cross section from the control sample, note the clear hexagonal arrangement of thin filament around the thick filament (as in circle), mitochondria are intact (M). b) Same as (a) except the samples are PRP treated (103.5 MNm^{-2}, 37°C for 2 min). Note the mitochondrial membrane is almost burst at both ends of the mitochondria (arrows), also note the blurred appearance of the filaments. Bar is 0.3 μm.

micrographs (Fig 5b and 6b) show the mitochondrial membrane of the PRP sample to be ruptured whereas that of the control is intact (Fig 5a and 6a). Electrical stimulation had a similar effect on mitochondria (Will et al., 1980). In the presence of mitochondrial uncoupling agents or under anerobic conditions, chilled muscle strips shortened extensively (Buege and Marsh, 1975). Shortly after slaughter and exsanguination of the animal, muscle tissue becomes anoxic and this was even aggravated under our experimental conditions where the samples have to be vacuum packed prior to the treatment. This coupled with our observation above raises the possibility that the additional sarcoplasmic Ca^{2+} required for the pressure induced contraction came from mitochondria.

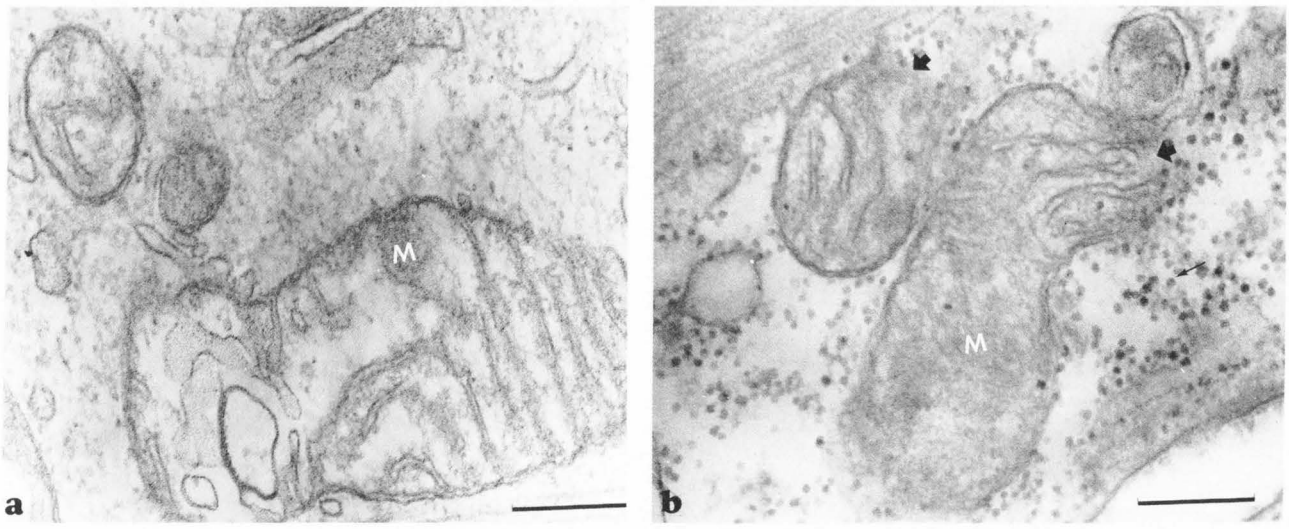


Fig 6 - Transmission electron micrograph of bovine longissimus muscle sampled and fixed 1½ hr post-mortem. a) Longitudinal section from control sample showing mitochondria (M) intact and normal. b) Same as (a) except the sample was PRP treated (103.5 MNm^{-2}, at 37°C for 2 min), note ruptured mitochondrial membrane (thick arrows) and the dense granules (thin arrows). The nature of these granules is unknown. Bar is 0.3 μm.

However, if the sarcoplasmic reticulum is not affected by PRP treatment it could have accumulated the Ca^{2+} released from mitochondria. Therefore, a combined effect of pressure on mitochondria and sarcoplasmic reticulum is more logical to explain the origin of the additional Ca^{2+} required for the pressure induced shortening.

The PRP treatment brought a substantial decrease in W-B shear force values and high tenderness ratings by the panelists. This could be attributed to the effect of pressure on the physical strength (endomysial and sarcolemmal sheath) of the muscle fibers and the disruption of the myofilament structure. Also, preliminary results at our laboratory indicated an increase in the free activity of lysosomal enzymes as the result of the pressure treatment. This could contribute to the observed improvement in tenderness.

Some of the ultrastructural changes observed in this study are likely to be aggravated upon cooking (heating) of the meat sample. Therefore, interaction between pressure treatment effects and cooking effects is another possible mechanism to account for the observed improvement in tenderness as a result of pressure treatment.

In conclusion, it is likely that more than one mechanism is responsible for the tenderizing effect of PRP treatment.

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Discussion with Reviewers

C.A. Voyle: In the experiments described in this paper pressure was applied for two minutes. Macfarlane and Morton (1978) applied pressure for sixty minutes and found that pressure applied for only a few minutes does not affect the ultrastructure significantly. How do the authors explain this discrepancy?
Authors: There is no discrepancy here, because the comparison is not valid. The Macfarlane and Morton (1978) experiment was on postrigor muscle whereas our experiment was on prerigor muscle. These are two different systems which are likely to differ in their degree and rate of response to the pressure treatment.

H.D. Geissinger: Is the appearance of exposed myofibrils in Fig 1b due to the PRP treatment, or due to the specimen preparation method?
Authors: No doubt, it is due to the PRP treatment for some good reasons: 1) the two samples (control and treated) were prepared similarly by a highly qualified experienced electron microscopy personnel; 2) the observation was made on more than one sample and on different muscles as well.

J.W. Savell: The authors discuss the apparent disappearance of glycogen-like granules in the PRP treatment and based on pH data, postmortem glycolysis has been accelerated. By what mechanism do the authors feel that postmortem glycolysis would be accelerated by PRP?
Authors: This is a very interesting question but difficult to answer at this stage of our research. We have speculations rather than a definite answer to share with you. It is likely that the treatment stimulates the activity of some of the glycolytic enzymes. For example the activity of phosphofructokinase increases at low ATP concentration. The conditions of the muscle postmortem and PRP treatment favor low ATP concentration. During pressurization there is an increased demand for energy resulting from extreme contraction (observed thereafter) to cope with this situation, it is likely that glycolysis was enhanced to compensate for energy used in the contraction process.

C.A. Voyle: The 37° equilibration treatment of prerigor muscle for 10-15 minutes prior to pressurizing the sample is likely to induce a contractile reaction in the muscle fibers with consequent structural disruption. Were any samples examined which had been heated but not pressurized in order to differentiate between the effects of these components of the treatment?
Authors: Actually we are not heating the samples, rather we are trying to maintain them at 37°C which is considered to be the physiological temperature of the carcass.

J.J. Macfarlane: Because at 1½ hour PM the control muscle would still be in the prerigor condition, was the sample restrained from contracting on removal from the muscle and before it was fixed?
R.J. Carroll: Were the muscles restrained during fixation?
C.A. Voyle: Were any precautions taken to avoid shortening of the muscle fibers during the removal of samples for EM at 1½ hr postmortem?
H.D. Geissinger: Why did you not pin out your samples before fixation?
Authors: The samples were not restrained. We do not expect the pressure treated sample to shorten during removal, however the control samples are likely to do so during removal but commercially hot boned meat is not restricted and we prefer to make our comparisons under commercial condition, in other words under practical conditions.

H.D. Geissinger: Were fractured myofibrils (Fig 1c) and interfibrillar spaces (Fig 1b) seen in the control samples?
Authors: The muscle fibers in Fig 1a lie side by side in a compacted manner so it is difficult to see any interfibrillar spaces. Fractured myofibril is a feature of the pressure treated sample (Fig 1c) and we did not observe it in the control sample (Fig 1a).

R.J. Carroll: How do the sarcomere lengths obtained by light microscopy compare with sarcomere lengths obtained from the transmission electron micrograph?
Authors: They compare favorably.

J.W. Savell: According to the Material and Methods section, there appears to be about 30 minutes between the end of the PRP treatment and the time at which the samples for electron microscopy were removed. Do the authors feel that the vast majority of the results observed between control and PRP treatments occurs during the 2 minute treatment or does the PRP process merely accelerate many of the physical and chemical events which may occur in the 30-plus minute period before the samples are fixed?
Authors: We strongly feel that most of the observed differences between control and pressure treated samples had occurred during the pressurization period. As an example we measured the pH of the treated sample immediately upon removal from the pressure chamber, in other words within 1-2 minutes post-pressurization.

R.J. Carroll: Why were the tissue samples fixed at pH 7.2 when the pH's of the tissue after treatment were 6.78 and 5.73?
Authors: It would have been a good idea to do so. But we preferred to use the standard procedure available in the literature for the preparation of muscle sample for the electron microscopy observation. Also, in doing wo we will be able to compare our findings with that in the literature.

H.D. Geissinger: You show some electron dense granules in Fig 6a, although these are not as electron dense as those in Fig 6b. What is the nature of the granules in Fig 6a?
Authors: We do not know the nature of these granules.

J.J. Macfarlane: I question whether much, if any, reliance can be placed on a mean value of sarcomere length in PRP treated samples. In my experience, in meat pressure treated so as to give the large decrease in shear values reported here, a large range of sarcomere lengths are present, from those contracted to form clots to those that are highly stretched. I presume a large variation in sarcomere lengths was also observed here - you referred to shortened and stretched sarcomeres in PRP treated samples. It is possible that the technique used for sarcomere length measurement was selective, perhaps because highly contracted and highly stretched sarcomere were destroyed or lost in the course of sample preparation.
Authors: Throughout the course of our investigation we had some difficulties in measuring the sarcomere length of the pressure treated samples. However it is beyond the scope of this study to enumerate these difficulties. Generally we do agree with your comment.

J.W. Savell: Based on the substantial and significant differences found in both Warner-Bratzler shear and sensory panel tenderness between the control and PRP treatments and the fact that the samples were evaluated at seven days postmortem, I wonder if there is a point in time where the tenderness of both treatments would be somewhat similar? There always appears to be a treatment X postmortem aging interaction in most studies, where postmortem treatments have been applied where the response to postmortem aging by the treated samples will be less than the response to postmortem aging by the control samples. Has postmortem aging of PRP meat been studied to learn the impact of treatments such as 3, 7, or 21 days would have on tenderness or the extent to which the magnitude of the difference between control and treated becomes less with increasing time?
Authors: We wondered the same thing but have not yet studied this aspect thoroughly. The preliminary results which we obtained on two samples indicated that up to 15 days of aging there was an obvious difference in tenderness in the favor of the pressure treated sample. Our plan is to extend the aging period to the point where the difference between control and PRP samples disappear, if ever this is going to happen.

J.J. Macfarlane: (P.77:...not likely that the material in the H-zone was removed). As the H-zone is thought to arise because of the absence of thin filaments, it seems odd to associate the absence of an H-zone with removal of material from the H-zone. You pointed out that in transverse sections of pressure treated muscle the thin actin filaments are indistinct. Therefore pressure presumably had a pronounced effect on the structure of the thin filaments and this effect may also account for the absence of an H-zone.
Authors: Thank you for your comment.