CHANGES IN THE STRUCTURAL PROTEINS AND THE SHEAR VALUES OF MUSCLE AS A RESULT OF PROLONGED HIGH PRESSURE TREATMENT

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SUMMARY

Beef semimembranosus muscle samples, in rigor either stretched or contracted, were heat treated at 30°C or 60°C for up to 24 h with and without the application of pressure (150 MPa). Treatment effects on Warner-Bratzler shear force values of samples, their SDS gel electrophoresis patterns, and their appearances under the scanning (SEM) and transmission (TEM) electron microscopes, were examined.

Compared to heat treatment alone, 30°C heat plus pressure treatment of contracted samples resulted in a steady moderate decrease in shear values as treatment time increased. At 60°C with pressure for 4 or 24 h a large decrease to small shear values occurred. A presumed pressure-accelerated enzymatic breakdown of myosin was inferred from an examination of SDS gel patterns. Myosin breakdown also appeared likely from the disruption of thick filaments seen under the TEM. However, from a consideration of the available evidence, it is thought that the presumed enzymatic mechanism may not be the sole mechanism involved in pressure-heat tenderisation.

Evidence of connective tissue disruption seen in the scanning electron micrographs of stretched muscle samples treated at 60°C for 24 h is consistent with a decrease in the shear values of these samples.

Because pressure treatment at 60°C rapidly destroys the influence of the myofibrillar component on toughness, but reduces that of the connective tissues relatively very slowly, if at all, the treatment can be used to estimate the influence of each of these components on the toughness of meat.

INTRODUCTION

At this Laboratory the effects of high pressure treatment on muscle have been studied to explore the possible technological usefulness of such treatments, as well as to increase the understanding of muscle when used as a food (Bouton et al. 1977; Macfarlane 1973; 1974; 1985; Macfarlane and McKenzie 1976). In connection with the latter application, a pressure-heat treatment has been developed which has been interpreted as destroying the influence of the myofibrillar component on toughness but having little or no effect on that of the connective tissues (Bouton et al. 1977). Such a treatment can be used to estimate the contribution of the myofibrillar proteins and the connective tissues to cooked meat toughness.

To further explore the mechanisms involved in pressure-heat treatment, meat has been pressure treated

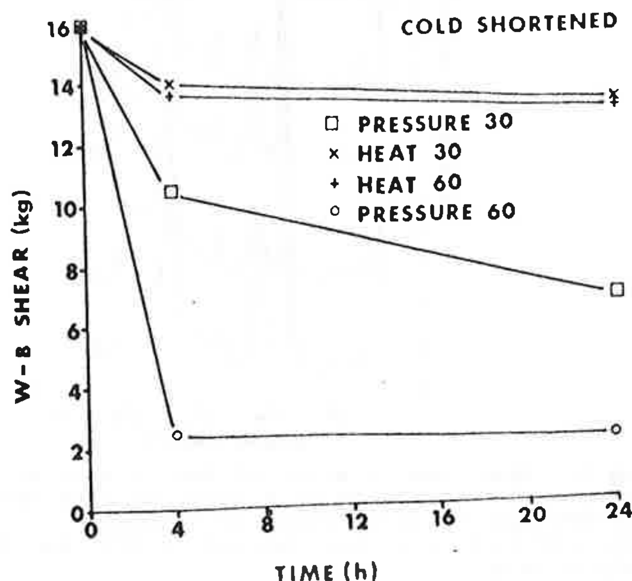

Fig 1: Warner-Bratzler shear force values for samples of contracted (cold shortened) muscle that were cooked at 80°C for 1 h after application of the treatment as indicated for the times shown.
Least significant difference (P = 0.05): 4.0 kg.

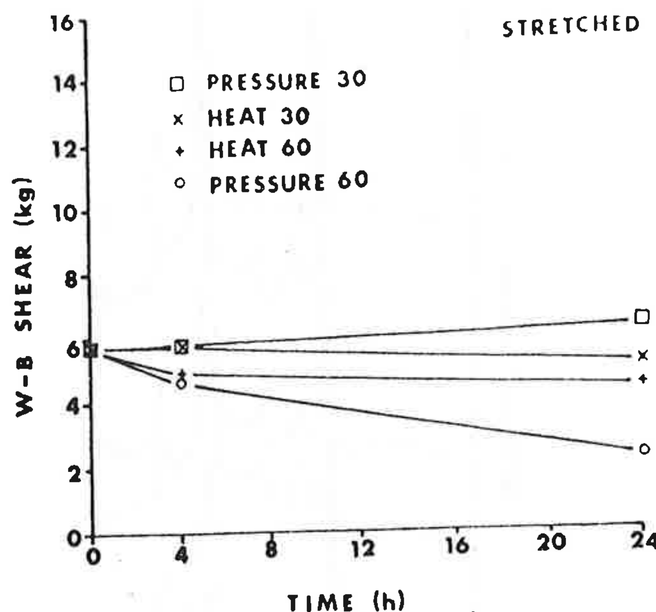

Fig 2: Warner-Bratzler shear force values for samples of stretched muscle that were cooked at 80°C for 1 h after application of the treatments as indicated for the times shown.
Least significant difference (P = 0.05): 2.4 kg.

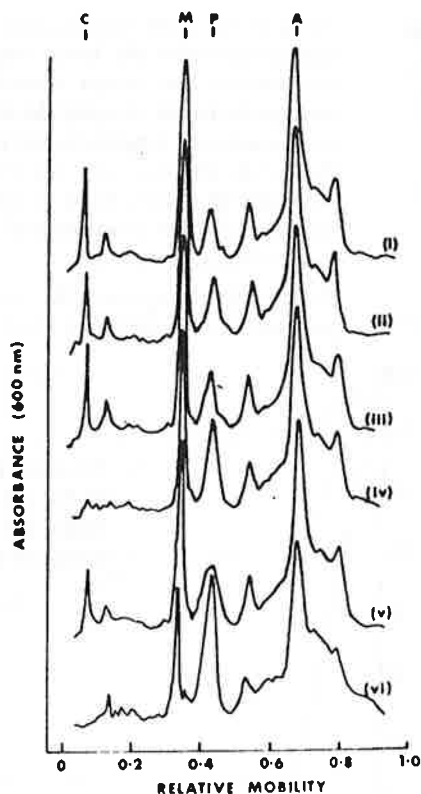

Fig 3: Densitometer scans of SDS gels (2.6% acrylamide) obtained from extracts of samples of beef semitendinosus muscles that had been treated as follows:
(i), (iii) and (v): heat treated at 30°C for 1, 4 or 24 h respectively.
(ii), (iv), (vi): pressure treated at 30°C for 1, 4 or 24 h respectively.
Peak identification: A-actin; C-connectin; M-myosin; P-145000 MW component(s).

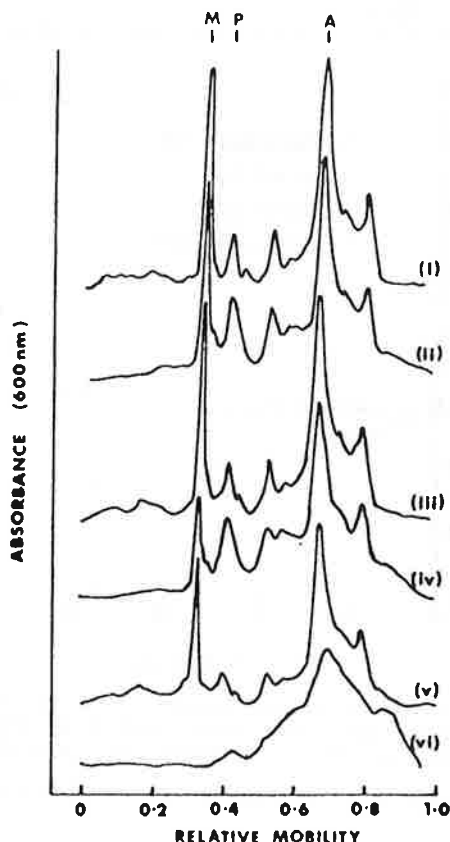

Fig 4: As for Fig 3, except treatment temperature was 60°C.

at 30° and 60°C and the effects on Warner-Bratzler peak shear values, SDS gel electrophoresis patterns, and ultrastructure investigated.

EXPERIMENTAL METHODS

From one side of each of three beef carcasses the semimembranosus muscle was excised within one hour of slaughter, placed in a polyethylene bag and immersed in iced water for two days to allow it to cold shorten and enter rigor. The other side of each of the carcasses was aitchbone hung ('tenderstretched' - to maintain the semimembranosus muscle in a stretched condition during onset of rigor), and placed in a chiller. The semimembranosus muscle of each of these sides was boned out approximately three days post-slaughter. The shortened and the stretched muscles were then cut into samples approximately 7 cm (in the direction of the fibres) x 7 x 4 cm which were individually sealed in polyethylene bags. The samples were stored frozen at -20°C until required for treatment, when they were placed in a chiller at +2°C for 24 h to thaw.

Pressure treatment was at 150 MPa and was carried out as described by Macfarlane and McKenzie (1986) except the pressure vessel had a chamber 11.4 cm in diameter and 35.6 cm in length.

SDS gel electrophoresis and Warner-Bratzler shear measurements were carried out as described by King et al. (1981) and Bouton et al. (1975) respectively.

Analysis of variance was used to test for significance between treatment effects.

Transmission electron microscopy and scanning electron microscopy of samples taken directly after heat or heat-under-pressure treatments, i.e. without subsequent cooking, followed the procedures described by Rowe (1978, 1984).

RESULTS AND DISCUSSION

Warner-Bratzler shear values

W.B. peak shear values for contracted muscle samples are shown in Fig 1, and for stretched muscle in Fig 2. Contracted muscle treated at 30°C produced results consistent with those reported by Macfarlane and McKenzie (1986). Thus shear values for the heat only treated samples changed little even after treatment for 24 h, but those for pressure-treated samples showed a significant decrease. However, this decrease was not as great as that achieved

by pressure treatment at 60°C, when it can be seen that both 4 and 24 h treatments produced low shear values. Shear values for the non-pressure-treated 60°C samples were similar to those for the 30°C samples.

Untreated samples of stretched muscle (Fig 2) gave much lower shear values than did the corresponding contracted samples, reflecting the low influence of the myofibrillar proteins on the toughness of the stretched samples. Compared to the shear values for the untreated samples, only those for the 60°C, 24 h pressure treatments were significantly decreased. It appeared likely that this decrease was in the connective tissue component of toughness as the myofibrillar influence in stretched muscle is already low.

SDS gel electrophoresis

No pronounced differences were apparent between the SDS gel electrophoresis patterns from the contracted and the stretched muscle samples, and only the results obtained from stretched muscle are illustrated here.

For samples treated at 30°C, it can be seen (Fig 3) that with increase in duration of treatment, in the pressurised samples the peak for myosin decreased while that at approximately M.W. 145000 (peak P) increased, so that by 24 h, the latter peak was the most pronounced. Therefore it appears that pressure treatment results in myosin breakdown giving a fragment(s) of approximately 145000 M.W. In the 24 h pressure-treated samples, compared to the non-pressure-treated samples there appeared to be an increase in the components giving rise to the hump on the low molecular weight side of the actin peak. This hump possibly was due to other fragments of myosin associated with the presumed cleavage that gives rise to the fragment(s) of peak P. It can also be seen that pressure treatment accelerated the breakdown of connectin (peak C).

Pressure treatment at 60°C for 1 or 4 h appeared to result in an increase in the size of peak P. However at 4 h there was a large decrease in the size of the peak for myosin, and in addition to the breakdown of myosin, this reduction might be due to heat treatment reducing its dye-binding ability. That the dye-binding ability of myosin is reduced by heat treatment at 60°C appears likely from consideration of the results for heat treatment at 24 h. Thus from Fig 4 it is evident that the size of the peak for myosin was greatly reduced but from Fig 1 it can be seen that there was little reduction in the high shear values for the samples of contracted muscle. From the latter observation it is inferred that the myosin molecules of thick filaments were largely intact, a view substantiated by the TEM appearance of myosin. However staining of myosin appears to be little affected by heat and pressure-heat treatments for 1 h (Fig 4). The latter pressure-heat treatment virtually eliminates the myofibrillar influence on toughness, but does not result in as great an apparent yield of peak P as is achieved in 30°C pressure treatments that have much smaller effects on toughness. Therefore it appears likely that another mechanism is involved in pressure-tenderisation.

Ultrastructure

Structural effects brought about by heat/pressure treatments can be categorised either as connective tissue or myofibrillar effects. There is a somewhat surprising difference in the effect of pressure treatment at 60°C for 24 h between the stretched and the contracted samples. The perimysial collagen component is almost totally removed (Fig 5A). This is consistent with the finding reported above that 60°C 24 h pressure treatment reduced the shear values of stretched samples. All the other treatments examined in this experiment produced no obvious dramatic degradation of the perimysial connective tissue, see, eg. Figs 5 B, C and D.

In stretched samples pressure treatment at 30°C even for 1 h produced changes in the A band of the sarcomeres such that they are apparently more fragile. This fragility apparently results from a weakening in the region of the pseudo-H zone. Fig 6A shows the A bands of a 30°C 1 h pressure treated sample with breaks at the pseudo-H zone (double arrows). These fractured A bands are not seen in the heat treated only samples. This weakening of the A band in the pseudo-H zone appears to be linked with a loss of M-line material from this region, eg. Fig 6B shows a 30°C, 24 h pressure treated sample. However the myosin rod portions of the thick filaments are still *in situ* and can be seen spanning the pseudo-H zone.

At 60°C pressure treatment appears to be structurally more destructive, even after treatment for only 1 h. Figs 6 C and D show the 1 h and 4 h 60°C pressure treatment samples. There is evidence of extensive A-band fragility, apparently to a greater extent than in the samples pressure treated at 30°C. Double arrows, Figs 6 C and D, indicate breaks at the pseudo-H zone. Just about every A band is involved. In addition the actin filaments of the I band appear less substantial. TEM confirms this greater, pseudo-H zone fragility (Fig 6E). There appears to be no myosin filaments crossing the normal region for the pseudo-H zone, leaving this region apparently cleared. The remainder of the myosin filaments appear fragmented.

In contracted samples the pressure-heat treatments have less obvious structural effects. The extensive overlap of actin and myosin filaments in shortened sarcomeres mask some of the features which would otherwise be visible, particularly in SEM micrographs. Heat treatment without pressure at either 60°C or 30°C has very little visible effect on the myofibrils (eg. Fig 7A). With pressure, however, as in the stretched samples the thick filaments of the A bands are progressively weakened. The fragility, as interpreted by an increased tendency to break at the pseudo-H zone, becomes progressively more common with longer time and higher temperature, eg. Figs 7 B, C and D.

In samples subjected to the 30°C 24 h pressure treatment the rod portions of myosin molecules remain in the pseudo H zone (Fig 6B). However pressure treatment at 60°C for 1 h clears this region (Fig 6C). If this clearance indicated advanced enzymatic action that resulted in the pressure-tenderising effect and that also produced peak P, then a larger peak would be expected from the 60° than the 30°C treatment. That this expectation is not the case is a further indication that the enzymic action responsible for the production of peak P is not primarily responsible for pressure tenderisation at 60°C.

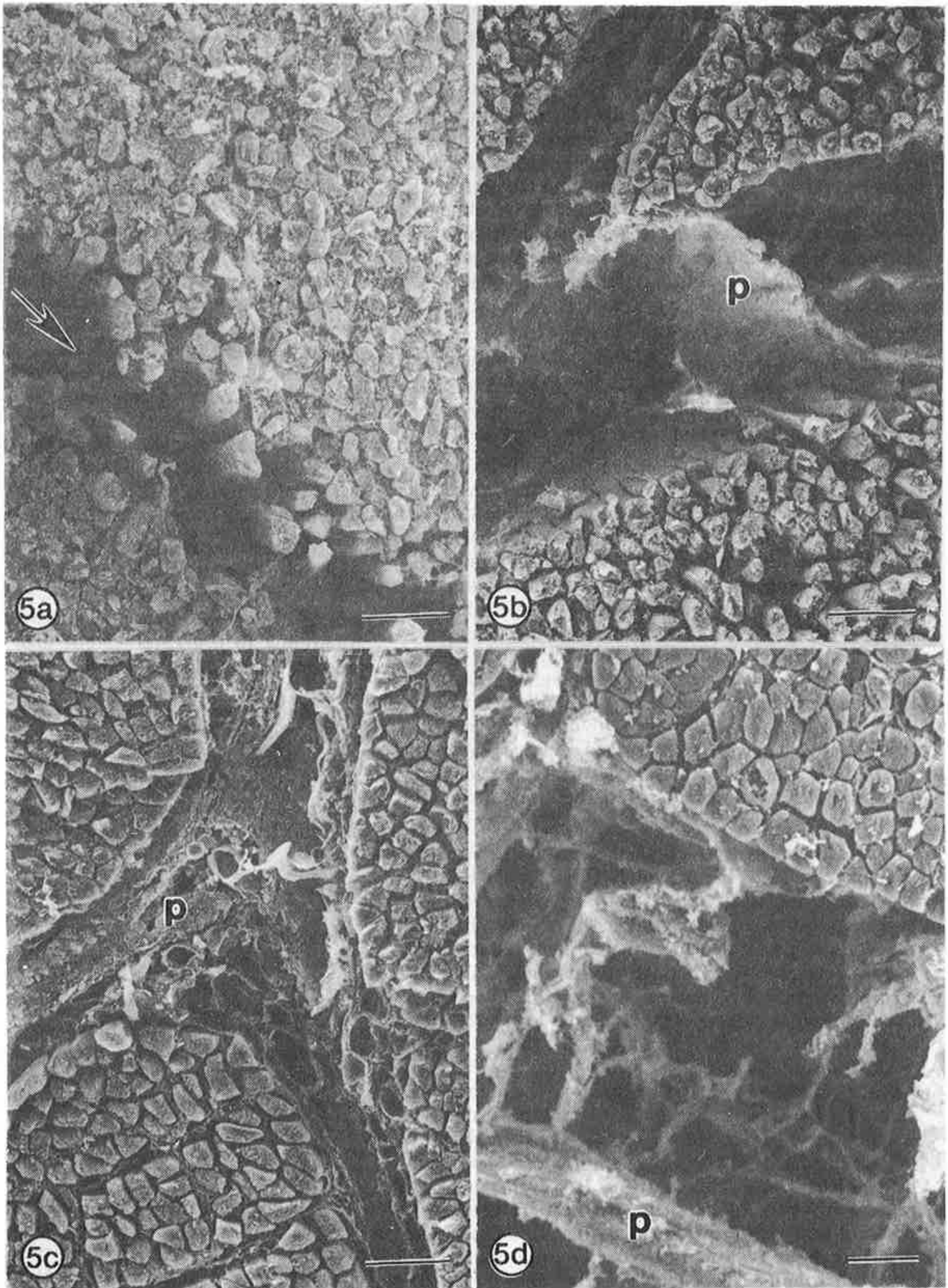

Fig 5: SEM micrographs of transversely cut bovine semimembranosus muscle. A, stretched sample, pressure, 60°C for 24 h. Arrow indicates region between two muscle fibre bundles where perimysial tissue would be expected. B, stretched sample, 60°C for 24 h. C, stretched sample, pressure, 30°C for 24 h. D, cold shortened sample, pressure, 60°C for 24 h. P = perimysium, bars = 100 μm.

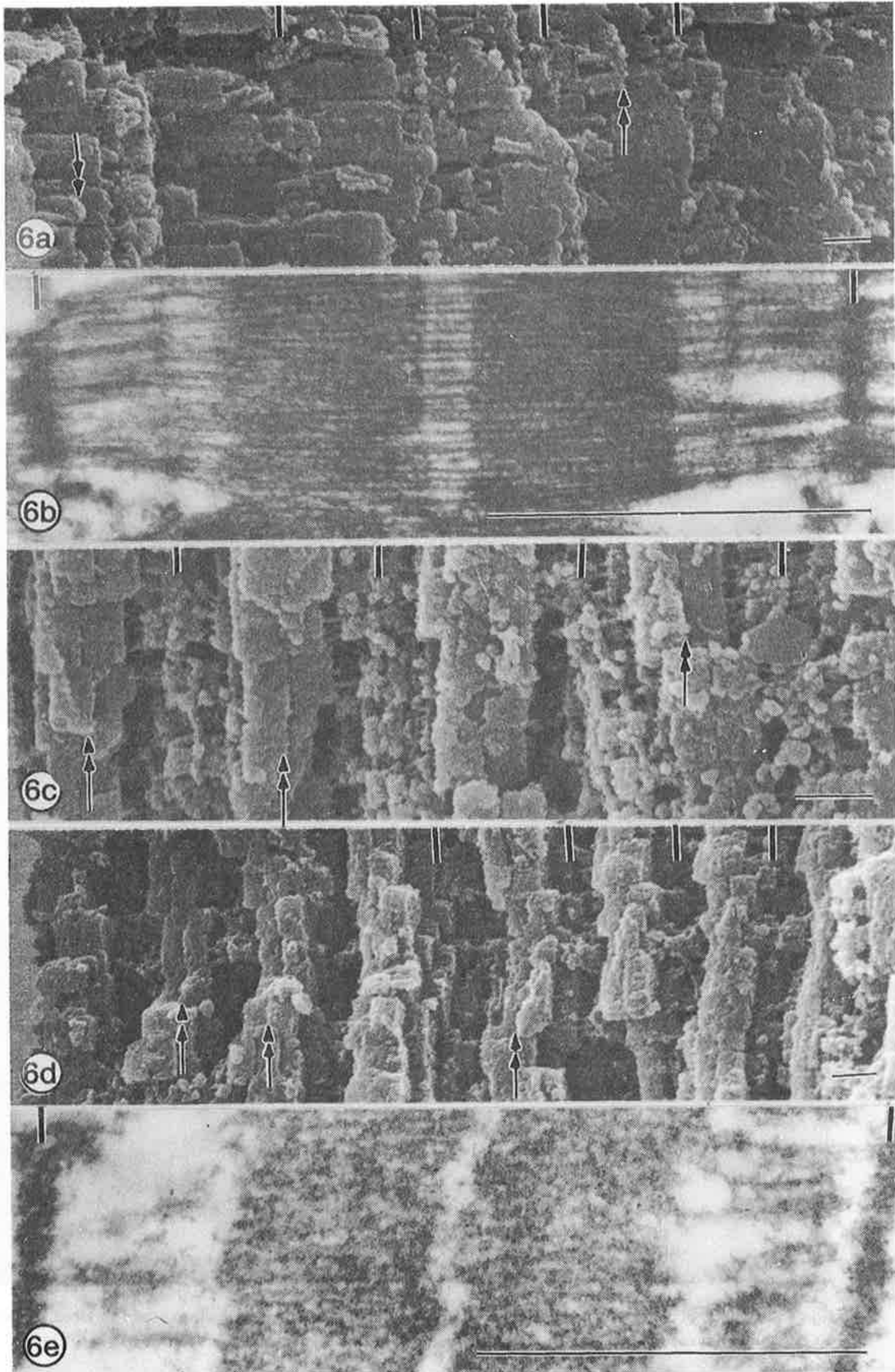

Fig 6: SEM and TEM views of longitudinally exposed myofibrils of stretched bovine semimembranosus muscle. A, SEM sample, pressure, 30°C for 1 h. B, TEM sample, pressure, 30°C for 24 h. C, SEM, pressure, 60°C for 1 h. D, SEM pressure, 60°C for 4 h. E, TEM, pressure, 60°C for 24 h. Vertical bars at tops of micrographs indicate the sarcomere repeat patterns. Double arrows indicate regions of breaks in pseudo - H zone of A bands. Bars = 1 μm.

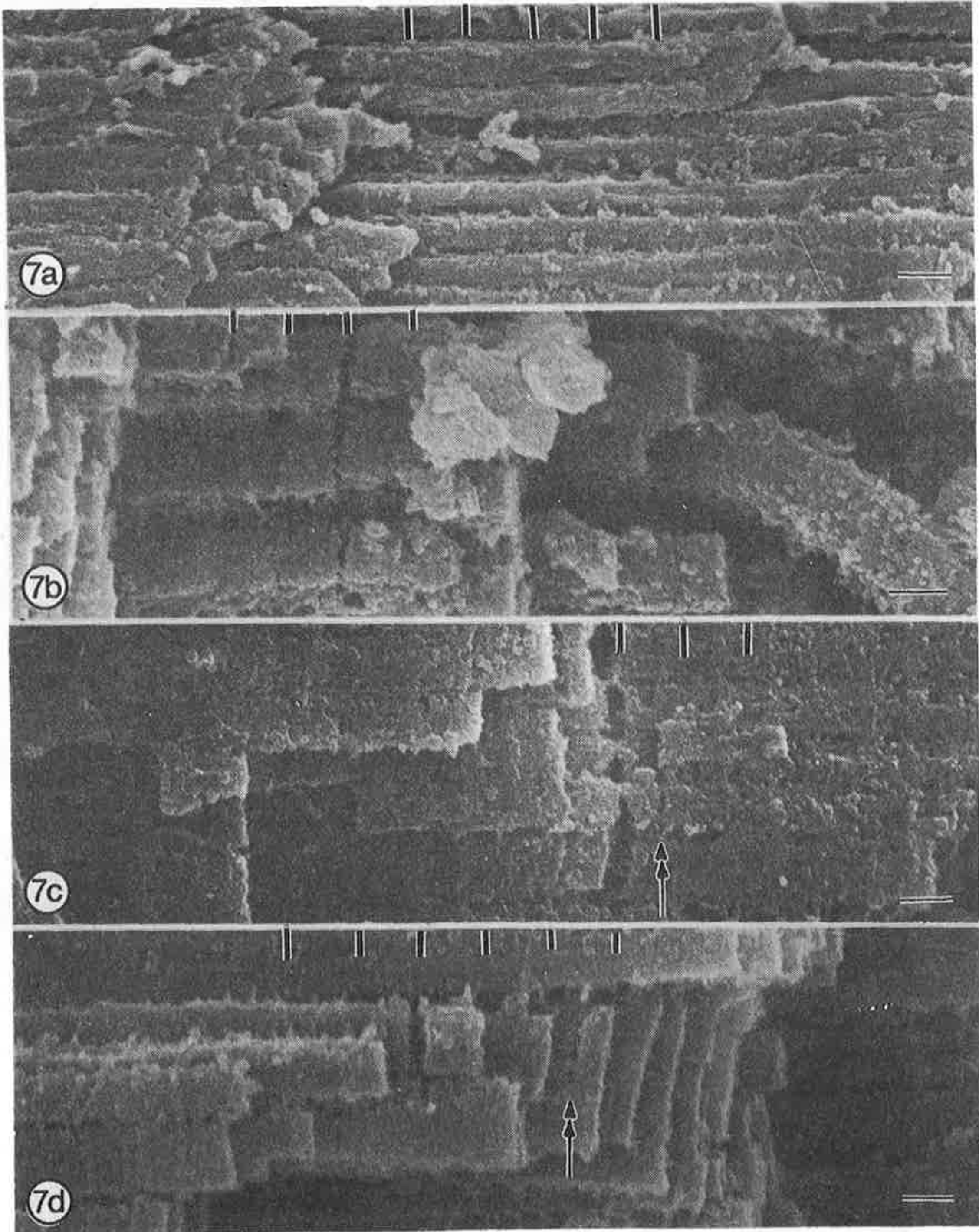

Fig 7: SEM views of cold shortened samples showing the myofibrils in longitudinally exposed surfaces. A, pressure, 60°C for 1 h. B, pressure, 60°C for 4 h. C, pressure, 60°C for 24 h. Vertical bars and arrows as Fig 6. Bar = 1 μm.

ACKNOWLEDGEMENT

This work was supported in part by funds provided from meat industry research levies administered by the Australian Meat and Livestock Research and Development Committee.

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