Ultrastructural *post mortem* changes in myofibrillar structure of normal and halothane sensitive pigs

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SUMMARY: Ultrastructural modifications were studied in relation with the analysis of calcium localization at different *post mortem* times in order to determine the myofibrillar lysis that occurs in ageing meat from normal and halothane sensitive pigs. *Longissimus dorsi* muscle from pigs tested for halothane susceptibility was used. Samples were taken periodically for pH measurements, conventional electron microscopic examination and intracellular calcium precipitation. As expected, the rate of *post mortem* pH fall was higher in muscle from halothane sensitive pigs. The striation pattern of normal muscle exhibited numerous transversal fragmentations of myofibrils as soon as 24 h *post mortem*. This phenomenon is much more discrete in muscle from halothane susceptible pigs even after 8 days *post mortem*. This difference can be related to the lack of tenderization during the maturation processes and to the low organoleptic properties described by other authors as characteristics of PSE meat.

INTRODUCTION: Physical, chemical and structural *post mortem* changes have been well characterized in muscle (Bendall, 1973 Penny, 1980), but the tenderization process of meat remains a yet unresolved mechanism. Storage of meat is known to improve meat tenderness and is associated with ultrastructural changes in the myofibrillar structure. Proteolysis of muscle proteins is thought to be responsible for these changes, mainly via calcium dependent enzymes (Ouali, 1990). Halothane susceptible pigs often show a fast rate of *post mortem* pH fall and a rapid *rigor mortis* onset. This subsequently causes pale, soft and exudative (PSE) meat. The organoleptic properties of this meat are altered for a yet unexplained reason (Buchter and Zeuthen, 1971 ; Touraille and Monin, 1982)

In this study, we analysed the ultrastructural degradation of the myofibrillar structures in ageing muscles from normal and from halothane sensitive pigs. Intracellular calcium localization has also been analysed in order to determine its possible involvement in the proteolysis that occurs *post mortem* in muscles from these animals.

MATERIALS AND METHODS *Animals:* Three halothane susceptible pigs from Piétrain x Large White breed and two pure Large White pigs as control were used in this study. 200 g of muscle were removed from the *Longissimus dorsi* of each animal within 5 min after exsanguination. Samples were kept during 4 h at room temperature and then stored at 4 °C up to 8 days *post mortem*.

pH measurement : The pH of muscle tissue was determined by direct measurement with a pH electrode in the excised muscle. The pH was measured every 15 minutes during the first five hours after slaughter and at 24 hours *post mortem*.

Sample preparation for ultrastructural studies: Muscle strips were removed from excised muscle tissue within 10 min after slaughter, then at 4 h, 24 h and 8 days post mortem. Small blocks (1 - 2 mm3) were cut in the muscle strips and immersed in the fixative.

Two fixatives were used, depending on the planned observation : 1) for ultrastructural calcium localization : the calcium precipitation was performed with potassium pyroantimonate - osmium tetroxide solution. Samples were fixed during 4 h at room temperature in a freshly prepared 2.5% potassium pyroantimonate solution (Merck, Darmstadt) in 1% OsO4 solution brought to pH 7.6 with 0.01 N acetic acid, 2) for routine microscopy : the samples were fixed in 2% glutaraldehyde in cacodylate buffer 0.1 M, pH 7.2 at room temperature for 30 min and then at 4 °C for 4 h. A post-fixation was performed in 1% osmium tetroxide in 0.1 M cacodylate buffer for 1 h.

The fixed samples were then rapidly rinsed in distilled water and dehydrated in ethanol before embedding in epoxy resin.

Ultrathin sections (70-80 nm) were stained with uranyl acetate-lead citrate for routine microscopic studies or kept unstained for calcium observations. The observations were made with a Philips EM 400 electron microscope at an accelerating voltage of 80 KV.

RESULTS AND DISCUSSION *Post mortem pH fall :* The rate of pH fall in *Longissimus dorsi* from the five animals is illustrated in figure 1. For halothane susceptible pigs, the ultimate value of pH 5.5 is reached after 30 to 75 min *post mortem*. This rapid drop in pH is known to be a property of muscle that lead to PSE meat as expected in halothane sensitive pigs. In muscles from Large white pigs, the ultimate pH value is obtained at 4 to 5 h post mortem.

Ultrastructural localization of intracellular calcium: With potassium pyroantimonate precipitation procedure, electron opaque pyroantimonate salts are formed in the tissues and are observed as fine precipitates in thin sections. These precipitates are localized in myofibrils and in terminal

sternae of sarcoplasmic reticulum in samples fixed just after slaughter (Fig. 2-3). Then, as pH decreases, the precipitates increase in myoplasmic spaces seen between the myofibrils. After a long storage period, 8 days at 4°C, broken myofibrils are observed in samples from normal pigs (Fig. 4). The disruptions are located at the N-line level. The tissue is disorganized and large clear spaces are seen between myofibrils. In this tissue, calcium precipitates are very discrete in myofibrils and the sarcoplasmic compartment is almost precipitate free. At the opposite, muscle from halothane susceptible pigs exhibits well preserved myofibrillar structures. There is no broken myofibrils in these tissues, even after 8 days of storage. Calcium precipitates are still observed on N-lines of myofibrils and heavy precipitates are maintained in between myofibrils (Fig. 5). Increased calcium precipitates have already been described in bovine muscle during rigor onset (Vignon et al, 1989). In pigs, our results show that this calcium delocalization also occurs, but much faster than in bovine muscle, usually within the first 4 h post mortem. For halothane susceptible pigs, this phenomenon can occur at the first sampling time, about 10 min after slaughter. This increased level of intracellular calcium could be responsible for an accelerated ATP turnover in PSE muscle. Increase in free calcium could also be thought to activate cytosolic calcium dependent proteinases known to be involved in the tenderizing process of meat (Ouali et al, 1983). However, our results suggest that the enzymatic systems responsible for this process are partly inhibited in muscles from halothane sensitive pigs since no disruption in myofibrils are noticed, although these muscles exhibited high cytosolic level of calcium early *post mortem.*

Ultrastructural changes in stored muscles : In normal muscle stored during 8 days at 4 °C, myofibrils undergo strong changes. I-bands and Z-lines are the most susceptible structures to degradation. As already described, the ultrastructure of the myofibrils in these muscles presents the following characteristics:

- weak Z-lines and the loss of their transversal alignment (Davey et al, 1969 ; Dutson et al, 1974 ; Abott et al, 1971)
- disruptions in myofibrils at the junction of I filaments and Z-lines (Gann and Merkel, 1978 ; Penny , 1980)

These degradations are already observed at 24 h *post mortem* in muscle from normal pigs (Fig. 6). At the opposite, the degradation is more discrete in muscles from halothane sensitive pigs, even after a prolonged storage period of 8 days. No disruption in myofibrils was observed although the weakening of Z-line still occured in these tissues (Fig.7). It therefore seems that proteolysis of the structure located at the level of N-lines in myofibrils is inhibited in PSE meat.

CONCLUSIONS: Muscles from halothane susceptible pigs which exhibit an early high intracellular calcium level *post mortem* maintain a well preserved myofibrillar structure after a prolonged storage period. Assuming that myofibrillar disruptions are related to an increased tenderness of meat, we propose that this lack of degradation could be responsible for the toughness of meat from halothane sensitive pigs already mentioned by Touraille and Monin (1982). Since halothane susceptible pigs frequently produce meat with PSE properties, our results might explain the lack of tenderization in ageing PSE meat as described by Buchter and Zeuthen (1971).

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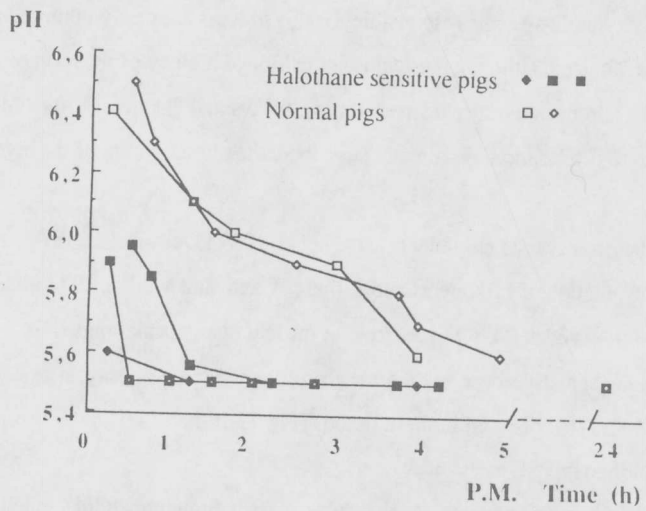
Fig.1: Post mortem pH fall in Longissimus muscles from halothane sensitive and normal pigs.



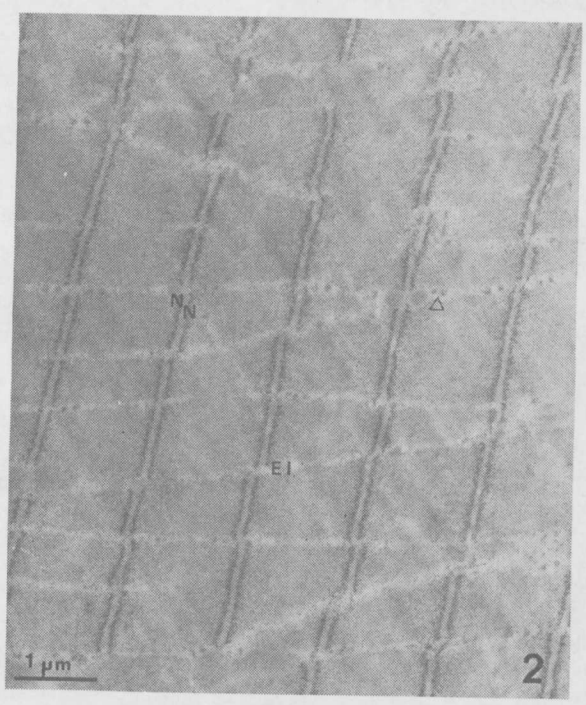


Fig. 2 and 3 : Electron micrographs of unstained ultrathin sections from longissimus dorsi fixed with potassium pyro-antimonate 1h post mortem. Calcium localization is seen as electron-dense precipitates. Fig. 2 : in muscle from a normal pig, calcium precipitates are in myofibrils, mainly on N-lines (N). There is no precipitate in intermyofibrilar spaces (EI), except at the location of terminal cisternae from sarcoplasmic reticulum (arrows).Fig. 3 : in muscle from halothane susceptible pig, calcium precipitates are seen on N-lines (N) and in intermyofibrilar spaces (EI).

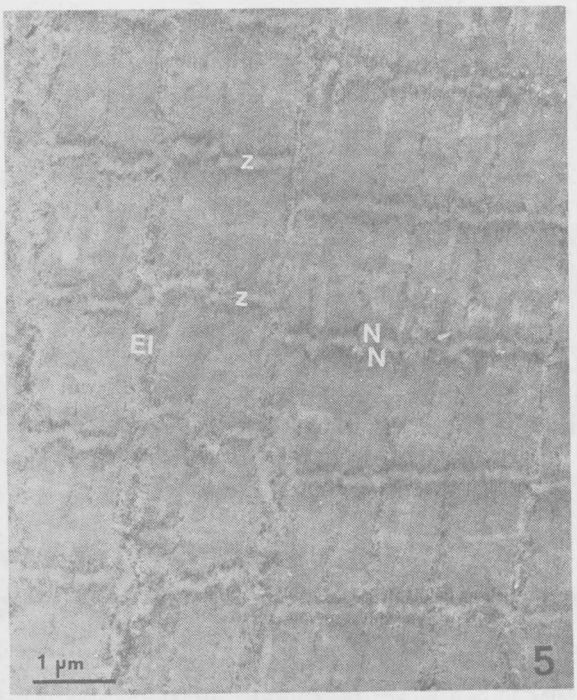


Fig. 4 and 5 : Electron micrographs of sections from longissimus dorsi fixed with potassium pyro-antimonate 8 days post mortem. Fig. 4 : in muscle from normal pig, calcium precipitates are lighter than in earlier sampling time and disruptions in myofibrils are observed in a region close to the Z-lines (Z). Fig. 5 : in muscle from halothane susceptible pig, calcium precipitates on N-lines (N) and in intermyofibrilar space (EI) are maintained as compared with earlier sampling time. No broken myofibrils can be seen.

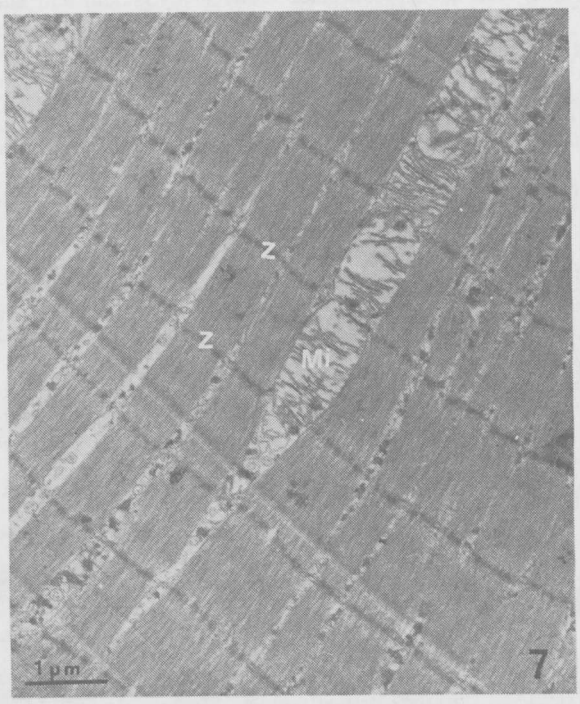


Fig. 6 and 7 : Electron micrographs of sections stained with uranyl acetate-lead citrate. Fig. 6 : section from a normal pig muscle fixed at 24h postmortem. Note the Z-lines disruptions (arrow-head) and the myofibrils degradations. Fig. 7 : section from a halothane susceptible pig muscle fixed at 8 days. The striation pattern is well preserved. Mi: mitochondria.