Recent advances in Muscular Post-Mortem Biochemistry.

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Muscular post-mortem biochemistry is one of the fundamental blocks of meat science. It describes the transformation of muscle to meat. Most striking advances in our knowledge within this field have been made during the years after World War II. These have contributed generously to our understanding of meat as food. Many exellent reviews of the advances in the post-mortem biochemistry have been presented at these meat research conferences during the years as well as at other meetings, conferences and congresses devoted to food science, animal science or refrigeration. I shall today confine myself to a rather personal view of the latest advances within this field as a key note to further deliberations about the topic.

Relations between muscular post-mortem biochemistry and meat quality.

Thequality related everts in the immediate post mortem period is, 1) the development of tension in the muscle fibers as a result of the myofibrillar ATPase activity, and 2) the anaerobic glycolysis. If the tension in the fibers is not efficiently counter balanced by some kind of stretch on the muscle, the muscle fibers will contract. Toughness and decreased water holding capacity of the meat will be consequence.
The anaerobic glycolysis brings about a decrease in pH, dependent upon the amount of residual glycogen in the muscles, and occurs at various rates. The rate of glycolysis depends on the nervous and hormonal stimulation of the muscle fibers. In general will pH decrease result in a decrease in water binding capacity of the meat and, at least for certain muscles, a tendency towards increasing toughness. With increasing rate of post-mortem pH fall the effects on water holding capacity and toughness will greatly accelerate.
In the next phase of the post-mortem period we will see the aging process, which encompass a degradation of the myofibrillar structure. Within reasonable limits the aging process tend 1) to improve texture towards increasing tenderness and 2) increase water holding capacity.

Control of tension in muscle fibers.

An abundance of papers have been published about "cold shortening" and "thaw rigor" and discussed in excellent reviews (ex. Bendall 1973). In short, the results have shown that the rates of ATP turn over, which determines the delay phase of rigor mortis, is highly temperature dependent. High rates give short delay phase. When the delay phase in beef muscle is short, tension in the fibers at the onset of rigor is quite severe (Sink et al. 1965).
Cassens and Newbold (1967) showed that the delay phase of rigor increased as the temperature was lowered from 37°C to 15°C, but the phase decreased from 15°C to 1°C. In poultry is fiber shortening minimal at 12 - 18°C (Smith et al. 1969). When the muscles are kept at about 15°C until rigor has precipitated tension development is at a minimum and only minimum shortening will result even when the fibers are free to contract. This principle is used in the "hot boning" procedure. The development of tension, and length of the delay phase of rigor, are also influenced by ante-mortem factors as stress or death struggle, as well as through post-mortem administration of calcium chloride, dinitrofluorobenzene or iodoacetate. This applies to poultry breast meat (Khan 1975, Kahn and Nakamura 1970), to beef (Kahn and Lentz 1973) and to pork (Lister 1969). These factors or treatments usually accelerate glycolysis and ATP turn over .
The key factor in the control of delay phase is the release of calcium ions from the sarcoplasmic reticulum in the immediate ante- and post-mortem period. Treatments of the animals with tranquilizers or curare, which prevent the release of Ca by subduing nervous stimuli (Bendall 1966, Khan 1974, Sair et al. 1970), or by treatment of intact muscle with $MgCl_2$ immediately after death, minimize ATP turn over The result is delayed rigor and reduced fiber tension. Magnesium is known to prevent release of Ca from the sarcoplasmic reticulum by blocking the neuromuscular junctions. Ante-mortem injection of EDTA or EGTA, which sequester Ca, also tends to reduce rigor contraction (Weiner and Pearson 1966)
Electrical stimulation of muscle immediately post-mortem has been shown by a number of workers to accelerate the post-mortem processes (Harsham & Deatherage 1951, Hallund & Bendall 1965, Forrest & Briskey 1967, Carse 1973). Although this stimulation might give rise to increased muscle tension at rigor this appears not to be the case when the muscle is kept at 15°C.
The effect of fiber contraction on the meat quality is under practical conditions intimately linked to the effect of pH fall resulting from the post-mortem glycolysis. The rate and extend of the glycolysis is closely connected to the factors of importance for onset of rigor. We do, however, have specie differences in the net result of the post-mortem events on meat quality. In pork we observe variation in color and water holding capacity of the meat with PSE (pale-soft-exudative meat) as the consequence of accelerated glycolysis and fast onset of rigor. Variation in toughness of the cooked or roasted meat is not of major importance (Flynn and Bramblett 1975). In poultry and beef we see the combined effects of the rigor events as variations in toughness with relative minor impacts on color and water holding capacity although they can sometimes be easily observed (Troja & Niewiarowicz 1973)

Anaerobic glycolysis in pork muscle.

Certain pork muscles are remarkable by an extreemly rapid rate of post mortem glycolysis (Ludvigsen 1954, Wismer-Pedersen 1959). The phenomenon takes place in muscles with fairly high concentrations of white muscle fibers. It occurs when hereditary susceptible pigs are exposed to stress situations immediatly post-mortem and slaughtered with fairly high glycogen depots in the muscles (Briskey 1964, Wismer-Pedersen 1969, Sybesma 1972).

It is not clear, however, which mechanism in the muscles trigger off the rapid glycolysis. It might be an excessive stimulation of perfectly normal muscle fibers through the nervous system or through abnormal hormone levels affecting the blood supply to the fibers or directly stimulating the fibers (Lister et al. 1967, Topel 1969) In addition to the effect of these extracellular factors there is also the possibility that the stress susceptible pigs contain abnormal muscle fibers reacting excessively on normal stress stimulation.
Cooper et al (1969) thus reported that white and red fibers of stress-susceptible pigs had more intense ATPase and phosphorylase activity than of normal pigs. As prerequisite for glycolysis is a supply of ADP created through ATPase activity and a supply of glucose released from glycogen. Active phosphorylase is required for release of glucose from glycogen. The effect of epinephrine, released from the adrenal medulla into the blood when pigs are subjected to stress, interfere on muscle fiber metabolism at this point. Epinephrine stimulates adenyl cyclase which stimulates the formation of cyclic AMP. Cyclic AMP stimulates a protein kinase that activates phosphorylase kinase. The activated phosphorylase kinase converts inactive phosphorylase to active phosphorylase which starts the process of glycogen breakdown (Sutherland and Robison 1966, Topel 1975)
A positive correlation between rate of glycolysis and level of cyclic AMP would suggest that the rate-determining step was an epinephrine effect or immediately intracellular at the control point of c-AMP synthesis. If not, the rate- determining step could be due to an abnormality in the intrinsic enzyme system of the fibers. Ono, Topel and Althen (1976) found, that c-AMP levels were significantly higher in M. long. dorsi 3 minutes post-mortem for stress susceptible pigs than for controls, thereafter differences were not significant. In a paper to this session the authors have reported further observations to this point. Epinephrine appears thus to be a hormone of direct effect on the glycolytic metabolism of the pork muscle fibers.

Research is still going on with regard to abnormalities of muscle fibers in stress-susceptible pigs. Bembers & Satterlee (1975) have recently studied the properties of myoglobin from normal and PSE muscles. This is approbreate, as the anoxic effects of exsanguination may be considered an important factors in the onset of the rapid post-mortem glycolysis (Lister 1968). It was observed that myoglobin from PSE muscle exhibited a number of abnormalies, such as varying isoelectric points, rapid rate of autoxidation, and acid instability, but the question remained whether the abnormal myoglobin is a result of genetic change or a result of the rapid post-mortem pH fall.

What does the rapid glycolysis to the meat structure?

It is well-known that the PSE pork is characteristic by a reduced water holding capacity. This is a result of the rapid post-mortem glycolysis, but it is still not precisely known what changes the resulting rapid pH fall has inflicted on the meat proteins. Bendall and Wismer-Pedersen (1962) concluded from their experiments that the fibrillar proteins from PSE fibrils are not denatured or aggregated in the usual sense, but are probaly covered by a lager of denatured sarcoplasmic protein that is firmly bound to the surface of the myofilaments. Later authors, Penny (1967) and Yasui et al (1973) (1975) were able to show that natural actomyosin, myosin and fiber bundles prepared from rabbit muscle were considerably denatured under the pH-temperature conditions created by the rapid post-mortem pH fall. Loss of extractability and ATPase activity were used as criteria. Working with myofibrils from normal and PSE porcine muscle. Sung, Ito and Fukazawa (1976) found that normal myofibrils contracted instantaneously after the addition of Mg^{++} - ATP, while PSE fibrils under the same conditions contracted in part or did not contract. The normal myofibrils were from meat with pH higher than 5, 45, While those without ability to contract were from PSE muscle with pH lower than 5, 30. The authors suggest that the loss on contractility only partly can be explained by the pH related denaturation of the actin-myosin system. When the denaturation is measured by loss of extractability or ATPase activity straightline relationsships with pH from 5, 2 to 5, 7 were found. Loss of contractability occured however dramatically in the pH range 5, 3 - 5, 5. It is thus speculated that some changes might occur within myofibrils in PSE muscle so that contraction might be inhibited by an unknown factor other than the inactivation of ATPase activity.
These changes in the properties of the PSE muscle fibers must be considered as pH induced post-mortem phenomeria. Champion, Eikelenboon and Cassens (1974) studied thus the isometric tension of thibialis-anterior muscles from normal and stress susceptible pigs. They observed that on electrical stimulation developped the muscle from the stress-susceptible pigs considerable tension but showed abnormalities suggesting that the regulation of the Ca concentration by the sarcoplasmic reticulum was faulty.

Structural changes in myofibrils during aging.

Post mortem tenderization, or resolution of rigor, has been related to structural changes in the myofibrils, and to changes in myofibrillar proteins, during aging (Goll et al. 1970, 1974, Davey and Dickson 1970, Hay et al. 1973).
By examination with electron microscopy one observe ultrastructural changes in myofibrils including degradation of the Z-line. For example did Hay et al. 1973 examine chicken breast muscle up to 168 hours post-mortem. At 3 hours had the H-zones disappeared and the Z-lines and A-I junctions were more diffuse than in "at death" samples. After 48 hours had the Z-lines lost their pre-rigor appearance becoming diffused and ruptured. Deterioration continued through 168 hours giving myofilaments an appearance of granularity. These changes are similar to those reported for bovine and porcine muscle, however the changes occur more rapid in poultry muscle. The tendency for myofibrils to fragment when mechanical stress is applied (Juel Møller et al 1973) has been used to evaluate structural deterioration during aging. Fragmentation may namely result from weakening of bonds between the actin-containing filaments and the Z-line material. In general it might be concluded for chicken as well as bovine muscle on basis of this technique that the major structural deterioration of the myofibrils occur at the level of the Z-line.
Scanning electron micrographs of poultry myofibrils during aging have besides deterioration of the Z-line also revealed development of space between fibrils indicating loss of lateral attachments (Johnson and Bowers 1976). A similar observation has earlier been made by Davey and Dirkson (1970), who related the tenderiza-

tion of the meat to weakening of lateral attachments. These observations suggest that the M-line material is affected by the aging proces.

Aging effect on myofibrillar proteins.

The changes in myofibrillar proteins during aging has frequently been studied by use of sodium dodecyl sulfate in conjunction with acrylamide gel electrophoresis. The most spectacular result is the appearance of a 30.000 dalton protein band (Hay et al 1973, Penny 1974, Samejima and Wolfe 1976). It is probably a result of troponin degradation (Dabrowska et al. 1973).
An often debated question is whether or not the linkages between the myosin-and actin containing filaments are weakened or ruptured during aging. Fujimaki et al. (1965) found that actomyosin prepared from aged rabbit muscle was more easily dissociated by ATP than the actomyosin prepared from fresh muscle Dumont and Valin (1973) concluded from solubilization studies a loosening of the actin-myosin bonds during aging of beef. Their results have been used, in part, as a basis for the hypothesis that the filament interaction undergoes a weakening during aging. Later studies on superprecipitation behavoir and ATPase activity of chicken actomyosin at 0 and 168 hours post-mortem (Wolfe and Samejima (1976) have shown that aging has no effect on the dissociation of actin and myosin. These results seem incompatible with the hypothesis that the actin-myosin interaction undergoes a "weakening" during post-mortem aging. The often reported slippage or stretching of sarcomeres during aging of meat is thus more likely due to breakage in the Z-line area.

Active enzymes in the aging proces.

The mechanism by which the changes in the aging myofibrils occur is at present eagerly investigated. Many observations have shown that the presence of Ca greatly accelerate the degradation in Z-line area most likely because of its activation of a proteolytic enzyme system (Busch et al. 1972, Penny et al. 1974). Parrish et al. 1975, reported at this meeting last year that the degradation is due to a Ca activated proteolytic enzyme endogenous to muscle. Lysozomal cathepsins have been suggested active in the degradations of the troponins resulting in the 30.000 dalton protein (Drabrowska et al. 1973). In general may the lysosomal enzymes aid the increase in tenderness during aging. Dutson and Lawrie (1974) have thus found a correlation between soluble β-glucuronidase levels and tenderness.
Calcium is thus found to activate the enzyme system, which increase the rate of the rigor process as well as that which act on the myofibrils in the aging process. However the release of Ca from the sarcoplasmic reticulum due to nervous stimuli immediately post-mortem does not appear to activate the enzyme system which cause degradation of Z-lines. In chicken muscle, at least, only post-rigor increase in Ca content appears to produce tenderness. Under normal conditions, the Ca accumulating ability of the sarcoplasmic reticulum membranes decreases as the pH drops during rigor and thus Ca is released. If this release of Ca is sufficient to activate the enzyme system causing dissosiation of Z-lines further addition of extrinsic Ca would appear unnecessary to aid tenderization (Khan & Kim 1975).

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