3:1

The influence of temperature on post mortem changes in porcine muscles

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Excessive chilling of beef shortly after slaughter results in muscle shortening (cold shortening) leading to tough meat with an increased drip loss. On small pieces cold shortening can be also induced in porcine muscles post mortem. In practice, however, i.e. in a carcass, cold shortening of pork muscles is minimal, as the post mortem changes are occuring much faster in porcine than in bovine muscles. Under the "slowest conditions" that is at 11°C in porcine M. cleidooccipitalis the rigor mortis starts about 2 - 3 hours post mortem being complete at 6 - 7 hours post mortem. In bovine sternomandibularis muscle the full rigor under the same temperature conditions has developed at about 18 hours.

Whereas all principal biochemical changes post mortem in normal porcine muscles are occuring much faster than in beef, they follow a similar pattern. Below 10°C cold shortening takes place, above 20°C rigor shortening occurs. Shortening increases the drip loss of pork but has no influence on cooking loss of meat or on the water binding capacity of salted products.

Very rapid post mortem changes in pork may result in PSE meat. PSE meat, showing no shortening of muscles is characterized by a pale colour and an early and intensive exudation of drip. Both detrimental characteristics can be minimized by rapid chilling of PSE muscles, even after they have reached their final pH ($pH < 5.8$) at high temperatures ($> 38°C$). The earlier the chilling starts, the faster the temperature drops, and the higher pH_1 is, the more effective is the treatment. Even chilling after 3 hours post mortem has a recognizable effect. The reason for this improvement of PSE meat by chilling is most probably due to a slow rate of denaturation of muscle proteins under the prevailing conditions of high temperature (38 - 41°C) and low pH (< 5.8) which can be prevented or minimized by rapid chilling preferably done by hot boning.

3:2

Ultrastructure in electrically stimulated dark cutting beef early post mortem

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Dark cutting beef having a high ultimate pH is known to be more tender than normal beef having an ultimate pH of about 5.5. Electrical stimulation has been shown to have a tenderizing effect on normal beef but not on dark cutting beef (DFD).

In the present study ultrastructural changes early post mortem were followed in one dark cutting carcass having an ultimate pH of 6.65. M. longissimus dorsi was removed from one side of the carcass immediately before and from the other side immediately after low voltage stimulation. Samples were taken at 2, 3, 4 and 6 hours post mortem and prepared for transmission electron microscopy. Changes in pH were followed during the first two days and shear force was evaluated 7 days post-slaughter. Results showed that electrical stimulation hastened the pH drop slightly, but the difference between the non-stimulated and electrically stimulated muscle never exceeded 0.2 pH units.

The electron micrographs showed that the non-stimulated dark cutting samples looked fairly normal up until 4 hours. At 6 hours some changes in Z-discs and I-bands could be seen, and part of the muscle seemed to be in rigor. In the electrically stimulated DFD muscle some changes of Z-discs could be seen at as early as 2 hours. At 6 hours pronounced changes were seen in the form of heavy contractions and complete disorganization of the tissue. The normal striated pattern of muscle was completely obliterated in large parts of the tissue. This picture has neither been reported in normal carcasses, even after ageing for 9 days, nor in electrically stimulated normal carcasses aged for 24 hours. The changes could have been caused by a combined effect of supercontractions and proteolytic activity.

The shear force values for the non-stimulated and electrically stimulated dark cutting meat were 2.45 (0.59) kg and 2.70 (0.77) kg respectively (standard deviation within brackets). There was no significant effect of electrical stimulation, but both values could be considered as being indicative of very tender meat.

It can be concluded that electrically stimulated dark cutting meat, early post mortem, developed an ultrastructure partly characterized by heavy contractions and complete disorganization, a picture not previously reported for normal carcasses. However, an increase in tenderness due to electrical stimulation was not recorded.

3:3

Meat quality in beef heifers slaughtered at oestrus

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The influence of oestrus and the associated heightened level of physical activity on the occurrence of carcass bruising and dark-cutting beef was examined. Resting muscle and blood samples were taken from twenty-eight Hereford cross heifers. Oestrus was synchronised and animal behaviour was monitored on a video recorder. The particular behaviours examined were number of mounts, number of times mounted on and the number of falls. From these behaviours the length of oestrus was calculated and the time elapsed (if any) between the end of oestrus and slaughter. The cattle were slaughtered at different stages of oestrus, i.e. after varying amounts of oestrus activity. Results are presented for ten inactive animals with less than 35 interactions per animal (slaughtered in early oestrus) and eighteen active animals with between 42 and 213 interactions per animal (slaughtered in late oestrus).

Muscle glycogen fell to forty-four per cent of the resting value in the active animals ($P < 0.001$) and plasma CPK increased three fold ($P < 0.001$). These constituents were unchanged in the inactive group. The mean ultimate pH (M. longissimus dorsi) was 5.92 in the active group and the range was 5.54 to 6.76, and 7 of the 18 active animals had pH_u values above 6.0. The mean pH_u in the inactive group was 5.48 (5.42 to 5.52). Oestrus had no significant effect on heart rate, rectal temperature or plasma glucose concentration.

Muscle glycogen concentration at 40 min post mortem was highly correlated with number of mounts ($r = -0.85$, $P < 0.001$) and the number of times an animal was mounted on ($r = -0.71$, $P < 0.001$). Oestrus behaviours were also significantly correlated with plasma CPK, muscle ultimate pH value and carcass bruising.

These results support our recent observation that dark-cutting in a group of commercial beef heifers was significantly correlated with the presence of oestrus at slaughter. The present results also indicate that dark-cutting was related to the physical activity that occurs during oestrus as distinct from physiological changes associated with ovulation.

3:4

Effect of electrostimulation on meat tenderness

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Date on the changes in the composition of myofibrillar proteins are presented for electrostimulated meat which had been stored subfrozen. Optimal electrostimulation parameters were found to be:
voltage - 150 V, frequency - 25 Hz, time cycle - 72 s. Electrophoresis on polyacrylamide gels containing sodium dodecyl sulphate (SDS) was used for identifying the protein composition of the meat. Storage of electrostimulated meat at subcryoscopic temperatures was found to result in qualitative changes in the myofibrillar proteins. The appearance of a protein fraction with a molecular mass of 30,000 shows improved meat tenderness.

3:5

Post mortem energy metabolism in muscles studied by non-invasive phosphorus-31 nuclear magnetic resonance

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Phosphorus-31 Nuclear Magnetic Resonance (^{31}P-NMR) was utilized to follow non-invasively the post mortem metabolism of the major phosphorylated metabolites in muscles from slaughter carcasses.

In a first series of experiments M. longissimus dorsi was hot boned from intact beef carcasses before and after low voltage electrical stimulation, within 10 minutes of stunning, and kept at room temperature. Spectra were obtained at 103.2 MHz on a home-made Fourier transform spectrometer equipped with a 6T magnet. Glycolytic pathway metabolites were also analyzed separately using enzymatic methods. In additon to adenosine-5'-triphosphate (ATP), creatine phosphate (CP) and inorganic phosphate (P_i) considerable amounts of glucose-6-phosphate and fructose-6-phosphate as well as glycerol-3-phosphate were detected. ATP was mainly present as a Mg^{2+} ATP complex. Adenosine-5'-diphosphate appeared to be mainly bound to muscle proteins. A good quantitative agreement was found for the levels of ATP, CP and sugar phosphates, when estimated by NMR or enzymatic assays. The response to electrical stimulation varied considerably between individual carcasses, from no immediate effect to an immediate pH drop of 0.8. We noted that electrical stimulation had the greatest effect on muscles having a high CP/ATP ratio. The effect on muscles having low CP levels was very small. Since the chemical shifts of the P_i and sugar phosphate resonances are a function of pH, the intracellular pH could be directly deduced from the NMR spectra. Values obtained in this manner were, within the errors of both methods, the same as those determined in iodoacetate/KCl homogenates. The pH gradients within the tissue never exceeded 0.3 pH units.

In a second set of experiments pre rigor muscle samples were frozen in liquid nitrogen, kept at -80°C, and thawed in the spectrometer. No ordinary spectra could be observed in the solid state but upon thawing (between -5° and 0°C) good spectra were obtained. Upon thawing we observed a very fast consumption of CP, followed by ATP depletion and a temporary reduction in the P_i signal.

In a third series of experiments pre rigor muscles from pig and lamb were studied in the spectrometer. Light muscle (M. adductor) showed a faster p.m. metabolism than dark muscle (M. semimembranosus) in both species. Mechanical damage, e.g. homogenization, caused an extremely fast depletion of high energy phosphates.

It was concluded that ^{31}P-NMR provides a useful complement to existing methods of studying of post mortem metabolism, due to its non-invasive nature plus the fact that some of the NMR parameters are sensitive to the intracellular environment.

3:6

Light scattering from muscle during the onset of rigor mortis

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The light scattering power of muscle depends strongly upon its physiological state. Thus relaxed muscle scatters less than muscle in rigor and extensive water loss from pig muscle in rigor is associated with meat of very high scattering power.

We aim to understand the extent to which various structures within the muscle fibre contribute to the overall scattering power of the muscle.

We have employed a combination of optical and mechanical measurements to observe the changes which take place as muscle enters the rigor state. Multiwavelength measurements enable scattering changes to be distinguished from changes in absorption and these permit a detailed comparison between the amplitude and kinetics of the scattering changes and the mechanical (stiffness) changes which attend the onset of rigor mortis. The kinetics of the scattering increase which attends rigor mortis closely parallel the mechanical changes which occur. In single fibres the maximum rigor-induced scattering increase is about two-fold measured at an angle of 90°. Moreover, in these single muscle fibre preparations, variations in sarcomere length cause similar variations in the amplitude of both the scattering and mechanical change; both diminish as the sarcomere length is increased towards a position of no-overlap between the thick and thin filaments.

An attempt has been made to quantify the contribution of the sarcoplasmic reticulum and other cell membranes to the scattering power of small bundles of fibres and of single muscle fibres in rigor. Disruption of the membranes with non-ionic detergents causes only a small (~10%) drop in the scatter from such preparations in physiological saline.

We conclude that the formation of "rigor bonds" between the thick and thin filaments of the contractile apparatus is a principal cause of the light scattering exhibited by meat and that membranes contribute rather little.

3:7

Endogenous calmodulin, Ca^{2+} and phospholipase A_2 activity and their relationships to halothane sensitivity in young and adult pigs

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Adult halothane-sensitive pigs have previously been shown to have an enhanced mitochondrial phospholipase A_2 activity and a significantly higher level of sarcoplasmic Ca^{2+} in M. longissimus dorsi post-mortem than adult halothane-insensitive pigs. This paper reports the endogenous calmodulin, Ca^{2+} and phospholipase A_2 activity in young (3-4 weeks) and adult (23 weeks) pigs and the factor(s) responsible for the enhanced phospholipase A_2 activity in M. longissimus dorsi mitochondria of halothane-sensitive pigs. Mitochondrial calmodulin was estimated on the basis of its ability to stimulate calmodulin-deficient phosphodiesterase activity, phospholipase A_2 activity by the formation of fatty acids, oxygen uptake with a Clark oxygen electrode and Ca^{2+} either by the formation of a Ca^{2+}-murexide complex or by atomic absorption.

The values of endogenous calmodulin, Ca^{2+} and phospholipase A_2 activity in young halothane-sensitive line and adult halothane-insensitive pigs were not significantly different, but these values were considerably lower than those of adult halothane-sensitive pigs. M. longissimus dorsi of young halothane-sensitive line pigs, in contrast to adults, showed normal rate of decline in muscle pH post-mortem.

Mitochondrial phospholipase A_2 activity is calmodulin-dependent since its activity was inhibited by low concentrations of trifluoperazine, an inhibitor of calmodulin-dependent enzymes. This inhibitor was also effective in preventing the Ca^{2+}-induced large amplitude swelling of mitochondria, in inhibiting the formation of fatty acids and in preventing the uncoupling of mitochondria by exogenous Ca^{2+} during succinate oxidation at high temperature (e.g. $40^{o}C$).

The present report shows that a higher than normal amount of calmodulin is responsible for the enhanced mitochondrial phospholipase A_2 activity in adult halothane-sensitive pigs and that the calmodulin, Ca^{2+}-dependent phospholipase A_2 activity is closely associated with the formation of PSE in M. longissimus dorsi, which is not observed in young pigs.

3:8

Histological traits of two muscles of lambs as affected by age in comparison with some muscles of Buffaloes

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The structural variations of the longissimus dorsi (LD) and biceps femoris (BF) muscles of 9 Ossimi lambs slaughtered at the ages of 8, 10 and 12 months (3 animals each), and 3 buffaloes at the age of 18 months were studied. The effect of cold-storage and frozen-storage which started immediately after slaughter, was investigated. The differences in sarcomere lengths (SL) between the two muscles were smaller in lambs than in buffaloes. The opposite was found for the muscle fibre diameter (FD). After rigor mortis the contraction of the fibres was more pronounced in the BF than in the LD and in the older animals compared with the younger ones. As a result of cold-storage the increase of FD and decrease of SL were more evident in the BF than in the LD muscle indicating a greater degree of contraction. After aging, the increase of SL and decrease of FD were greater in the LD than in the BF, showing a slower rate of relaxation and aging in the latter case. A period of 6 days storage at $4^{o}C$ was enough to cause the aging of the lamb as indicated by the appearance of the fibre breaks and the appearance of granular substances, but was not enough for buffaloes meat which showed only a small number of breaks with no granular substances. The changes in the muscle tissue due to frozen-storage were much greater for the older animals than the younger ones, for the BF muscle than the LD muscle, and for the buffalo meat than the lamb.

3:9

Sarcomere length measurement by laser diffraction and light microscopy.

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Treatments of carcasses that change muscle sarcomere length (SL), change meat tenderness. A convenient method to measure SL uses the diffraction pattern produced by muscle fibres placed in a beam of monochromatic laser light. This method however may give results differing from those obtained by light microscopy (Varcoe & Jones, 1983) especially after electrical stimulation (George et al., 1980). We have compared both methods in routine analysis.
One year old bulls were slaughtered and differences in SL induced by temperature conditioning electrical stimulation, rapid cooling or hot boning of carcass halves. Muscle samples (1 - 2 g, 3 x 2 x 2 cm^3) were taken at various times (up to one week at 2°C after slaughtering) from Longissimus dorsi 1st - 3d rib (14 samples LD1) and 6th - 7th rib (8 samples, LD2), Infraspinatus (8 samples, IF) Semitendinosus (12 samples, ST), Tensor fasciae latae (8 samples, TF), Gastrocnemius (9 samples, G) and occasional other muscles. Samples were fixed in gluteraldehyde and SL determined by light microscopy and laser diffraction. In microscopy 5 to 15 sarcomeres were observed in 20 different fibres for each sample. In laser diffraction 20 different diffraction line distances were measured.
SL from laser diffraction for LD1 (1.21-3.24 µ), LD2 (1.69 - 2.09 µ), TF (2.22 - 3.63 µ) and G (1.98 - 3.57 µ) were not significantly different from values derived from light microscopy. Laser diffraction gave significantly higher values however with IF. (Paired t-test $p < 0.05$). Regression of microscopic values (Y) on laser values (X) for all data (n = 73) gives:
$Y = 0.361 + 0.802\ X$ ($R = 0.91$, $RSD = 0.19$). The regression coefficient and constant differ significantly from 1 and 0 respectively. These results are similar to other work (Varcoe & Jones, 1983). Laser diffraction does not differ from light microscopy however in measuring changes in SL induced by various treatments. Such changes can be expressed as the ratio SL treated muscle/SL control muscle with: Y(laser ratio) = 0.99 X (microscopic ratio) $R = 0.85$ $RSD = 0.10$ (n = 72)
Also laser measurements show less variability than microscopic measurements.

VARCOE G. & S.D.M. JONES Can. Inst. Food Sci. Technol. J. 16, 82 - 83, 1983
GEORGE A.R., J.R. BENDALL & R.C.D. JONES Meat Sci. 4, 51 - 68, 1980

3:10

Swelling of whole meat and myofibrils - as measured by pulse-NMR

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In this report the applicability of pulse-NMR-measurements with regard to water holding in meat is shown with some representative investigations on the swelling of whole meat and myofibrils. By measuring the transverse relaxation time (T_2) of water protons in meat, with the help of pulse-NMR, the water distribution within the meat system can be mapped. Multiexponential decay of T_2 has been observed where each T_2 has been interpreted as representing a discernible domain of water. Relaxation data were analyzed in multiexponential decay by curve decomposition.

Muscle tissue from M. longissimus dorsi (young bull) was taken 2-5 days post mortem. For swelling studies the whole meat tissue was placed in buffer [100 mM KCl, 20 mM K-phosphate (pH 7.0), 1 mM EDTA and 1 mM NaN_3] or 0.8 M NaCl solution. Myofibrils from muscle tissue were prepared according to the procedure for the determination of myofibrillar fragmentation index. For swelling studies the myofibrils were resuspended in buffer, 0.4 M NaCl or 0.8 M NaCl solution.

The water proton relaxation of the whole meat sample could be fitted to a 3 process relaxation, where the relaxation was dominated (86%) by a relaxation with T_2 around 35 m.sec. Approximately 12% of the water protons had a decay of $T_2 \approx 120$ m.sec. and the last 1-2% was characterized by a T_2-value ranging from 600 to 800 m.sec. When the whole meat was equilibrated with buffer no significant changes in the water distribution could be observed. Swelling in 0.8 M NaCl of the whole meat caused a small upshift of the main relaxation to $T_2 \approx 40$ m.sec.

When the myofibrils were isolated from the whole meat tissue, the 35 m.sec. relaxation process vanished and was substituted for a much slower relaxation process varying from 111 to 118 m.sec. Almost all water protons relaxed according to this process. The observations made are interesting in two respects, firstly, they indicate that most of the water in meat is held within the myofibrils, secondly, when myofibrils are extracted from the tissue a substantial swelling of the myofibrils occurs. This points out the role the sarcolemma and the endomysium have in inhibiting swelling of the myofibrils.

Moreover, when the myofibrils were resuspended in salt solutions of different molarity (0.4 and 0.8 M) further noticable swelling occurred, much more pronounced than in the whole meat. Evidently, for substantial swelling of the myofibrils to occur through salt addition, the myofibrils should have been laid bare. The 0.8 M NaCl solution was most effective in causing swelling.

3:11

Histochemical characteristics of muscles of various pH_1 for pasteurized tinned ham

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Intensive selection of pigs aimed at promoting fast growth and good muscle development brought about a decrease in their adaptability to changes occurring in the environmental conditions for various reasons. An enhancement of metabolic processes occurring in the animals induces a decline in the technological quality of meat, e.g. PSE and DFD. Histochemical investigations carried out on mature muscles of meat pigs revealed, among other things, that intensive feeding and restricted amount of exercise favour the formation of muscles rich in cells in which anaerobic energetic processes prevail. Such muscles used for production of pasteurized ham give a product of unsatisfactory consistency and non-uniform colouring, varying from grey pink to dark red. In spite of careful selection of muscles of uniform colour before canning, after thermal processing 70% of the final product had colour characteristics which did not comply with the standard. Searching for reasons, the selection of muscles based on the pH_1 criterion has been tried in order to find out if it will act as a guide to obtaining products of standard colour.

The experiments were performed on 28 white pigs of meat type, weighing 110 kg before slaughter. At 45 min and 24 h after slaughter, the muscles were examined to determine pH_1, R = IMP/ATP, total pigment and myoglobin content, and after pickling, the nitrosopigment content in the final product. Histological evaluation was based on routine hematoxylin and eosin staining; glycogen was determined qualitatively, as well as LDH and SDH activities.

The results showed greater pH variation in normal muscles than in PSE muscles, and an inverse relationship between R and pH_1. The routine hematoxylin and eosin staining of the muscle tissue revealed the presence of hypertrophied fibres of a watery character in about 80% of preparations. Only in two cases were no PSE cells found. In hypertrophied fibres, a separation of sarcoplasm from sarcolemma, a lowered glycogen level, and a drop in LDH and SDH activities were observed.

These findings suggest that pH_1 measurement is not a satisfactory criterion by which to select normal and PSE muscles as the hypertrophied fibres characteristic of PSE muscles also occurred in a great number in normal muscles.

3:12

A study into combination ham products microstructure

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Results are given of the study of the structure of traditional (control) and combination (test) ham products using light microscopy and scanning electron microscopy.

Samples for light microscopy were fixed in 20% neutral formalin. Sections, cut on a freezing microtone, were stained with haematoxylin and eosin or with sudan III. Photomicrographs at a magnification of x 90 were prepared using an MBI-15 microscope.

For scanning electron microscopy samples fixed in formalin were dehydrated in alcohol or frozen, lyophilized (vacuum dried) and coated with a thin layer of gold. The samples were examined in a JEOL ISM-50A scanning electron microscope at magnifications of x 2500 to x 12500, and micrographs prepared.

During the filling of the casing with raw traditional ham product the meat pieces were bound together by protein released from the muscle fibres, the sarcolemma (cell membrane) of which had been broken down.

Combination ham products also revealed a binding substance between the pieces of raw meat. By light microscopy this was seen to be in the form of a compact fibrous mass.

Soy protein was not identified by light microscopy.

3:13

Variable response of beef myofibrils to salt solutions

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Meat takes up water when treated with saline solutions during processing. Polyphosphates act synergistically and aid in the production of the sticky exudate that causes meat pieces to bind together on cooking. Offer and Trinick (Meat Science, 8, 245-281 (1983)) have shown that myofibrils isolated from rabbit psoas muscle take up water by lateral expansion in the presence of salt, and that pyrophosphate promotes swelling at lower salt and extraction of myosin from the A-band. They also found that there was considerable variability in the amount of swelling and A-band extraction, particularly between preparations of myofibrils. This work suggests very strongly that the myofibril is the site of water uptake in meat, and suggests a cause for variability in water uptake during processing.

We have addressed the following questions: (1) do the results from rabbit apply to other species such as beef? (2) does pyrophosphate, believed to be the active component of polyphosphate, affect the maximum water uptake by myofibrils achieved at high salt concentrations? (3) what is the cause of the variability in swelling?

Myofibrils were isolated from vacuum-packed muscle that had been held at 10°C for 24 h post mortem at a sarcomere length of about 2.7 μm. For phase contrast light microscopy, myofibrils were suspended between the bars of a gold electron microscope grid attached to the underside of the coverslip. This ensured that the myofibrils were freely bathed in saline solutions on all sides with no impediment to swelling, and eliminates the possibility of the variability being due to this cause. Many individual myofibrils were photographed during irrigation with a series of increasing salt concentrations from 0.1M to 1.0M NaCl, with or without 10mM pyrophosphate, in 1mM $MgCl_2$, 10mM Na acetate pH 5.5 at about 20°C.

Beef sternomandibularis myofibrils behave qualitatively in the same way as those from rabbit psoas. In salt alone there is swelling mainly at salt concentrations above 0.5M and some removal of A-band material largely from its centre. In the presence of pyrophosphate, swelling commences below 0.5M NaCl and extraction of the A-band is usually from the edges inwards and is more complete than with salt alone. Quantitative measurements of myofibrillar swelling show that the majority of beef myofibrils increase in volume by between 0 and 100% in 1.0M NaCl, with a mean value of about 50%. Pyrophosphate has little effect on the maximum amount of swelling, at the high salt concentration. Thus the myofibrils swell sufficiently for them to be the main site of water uptake in salt-treated beef. Variability is found within a single myofibril preparation, and in this respect beef is different from rabbit, in which variation was observed by Offer and Trinick chiefly between preparations. Thus, while some myofibrils triple in volume, others do not swell at all; while the A-band is totally removed in some myofibrils, in others it resists extraction, Z-disc and A-band sometimes swell equally, but sometimes only the A-band swells. This variability occurs even in fresh material, so conditioning is unlikely to be its cause. We suspect that myofibrils from different muscle fibre types differ in their behaviour and that this may be the origin of the variability observed in myofibril behaviour.

3:14

The agreement of ATPase with immunology for typing myofibers of chicken skeletal muscle

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Antibodies to the myosin heavy chain (MHC) of fast-twitch and slow-tonic myofibers of chicken skeletal muscle were prepared and purified by affinity-column chromatography. Gel-electrophoresis-derived-enzyme-linked-immunosorbent-assay (GEDELISA) was used to show the presence of antigenically distinct MHC's in fast-twitch and slow-tonic myofibers and to prove the specificity of the anti-fast and anti-slow antibodies. Immunofluorescent staining revealed that anti-fast antibodies stained only histochemically fast myofibers while anti-slow antibodies stained only histochemically slow myofibers. Immunofluorescent typing of chicken skeletal muscle myofibers, based on a single specific characteristic (MHC) directly related to speed of contraction, provides evidence for the efficacy of the histochemical procedure for myofibrillar adenosine triphosphatase (ATPase) as an accurate method of myofiber typing in the chicken.

3:15

Nutritional digestibility of insoluble collagen - influence of hydrochloric acid

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Collagen in meat and meat products is generally considered nutritionally digestible only after prior denaturation to gelatin, e.g. by cooking. This is often explained by the fact that collagen is known to be very resistant to proteolytic enzymes when studied in vitro.

In the present investigation the digestion of insoluble collagen (extracted bovine Achilles tendon), gelatin (swine skin) and meat (0.4% collagen of total protein content) were studied in vitro and in vivo.

In the in vitro assays samples were incubated at 37°C in pepsin/HCl of different pH for 2 hours, in some instances followed by a treatment with pancreatin at pH 6.8 for 2 hours precipitated by trichloroacetic acid (25 w/v %). Crude porcine enzymes were used. The digestibility was evaluated as nitrogen solubility. The in vivo assay was performed as nitrogen balance studies on growing rats and true digestibility was evaluated by analyses of faeces. The rat assay was performed with and without oral administration of Omeprazol®, inhibiting the gastric acid secretion.

The in vitro study showed that the solubility of collagen was constant at a level of about 90% in pepsin/HCl when the pH was between 1.3 and 2.0. At a pH of around 2.5 a prominent decrease in solubility was obtained resulting in a value of 37% at pH 3.2. Further treatment with pancreatin increased the solubility to only a small extent. The in vivo results showed a decrease in the digestibility from 95% without, to 70% with the inhibition of gastric acid secretion. Both gelatin and meat samples showed complete solubilities after treatment with pepsin at a low pH, when followed by pancreatin. Only a slight decrease of the solubilities was obtained when the pH was increased. In vivo digestibilities of gelatin and meat were both more than 97%, in spite of inhibition of the gastric acid secretion.

The study showed that production of hydrochloric acid was important for the digestion of insoluble collagen, but not for gelatin and meat. The role of hydrochloric acid (1) in swelling of the collagen fibre, (2) in giving an optimal pH for the action of pepsin and (3) in the lowering of the denaturation temperature of collagen is discussed.

It is concluded that insoluble collagen is gelatinized and digested to a high extent without prior denaturation during the conditions occurring in the gastrointestinal tract. Hydrochloric acid has an important function in the digestion of collagen, but not for gelatin and meat. This is true for studies in vitro as well as in vivo.

3:16

Proteolytic action of lysosomal proteinases on the myofibrillar structure. Comparaison with the CaANP effects and the post mortem changes

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Myofibrillar proteins from rabbit and bovine muscles were treated with the main lysosomal proteinases (cathepsins D, B, L and H) and the consequent changes were investigated by SDS - polyacrylamide gel electrophoresis.
On rabbit muscle myofibrils, cathepsin D degraded myosin heavy chain (MHC), troponine I (TN-I) and to a lesser extent troponin T (TN-T), ß-tropomyosin (ß-TM) and myosin light chain 2 (LC2). Concomitantly to the decrease in the MHC band, we noted the appearance of two main proteolytic fragments with molecular weight (Mw) of 130 Kd and 95 Kd. In addition, a 27 Kd component was generated. With myofibrillar proteins from either bovine Longissimus dorsi or Rectus abdominis muscles cathepsin D hydrolyzed MHC, TN-I and LC2. Degradation of MHC was concomitant with an increase in the intensity of five bands with a Mw in the range of 120 to 130 Kd for the heaviest two ones and of 90 to 100 Kd for the others. As for rabbit muscle myofibrils a 27 Kd breakdown component was produced. Cathepsin B seemed to be less efficient than cathepsin D. Nevertheless, this proteinase hydrolyzed rabbit muscle MHC (with a concomitant appearance of proteolytic fragments with Mw of about 80 Kd, 90 Kd, 95 Kd and 130 Kd), TN-T, TN-I and ß-TM. With bovine muscle myofibrils this enzyme degraded MHC leading to the appearance of two bands with Mw of 120 and 130 Kd respectively. TN-T, TN-I, ß-TM and LC1 were digested. Whereas two components (Mw 26 Kd and 27 Kd) were released by cathepsin B from rabbit muscle myofibrils, only the 27 Kd was observed in bovine muscle treated myofibrils.
Cathepsin L led to drastic changes in the pattern myofibrillar proteins from rabbit muscle. MHC, TN-T, TN-I, TN-C, ß-TM, LC1 and LC2 were almost completely digested. As MHC disappeared, an intensification of three bands running as components with Mw of about 80 Kd, 95 Kd and 130 Kd respectively was observed. Between LC1 and α-TM, two hydrolytic products (Mw 26 Kd and 27 Kd) appeared, the last being the major one. Similarly, the myofibrillar proteins of bovine muscles were extensively degraded, most proteins being affected (MHC, TN-T, TN-I, TN-C, LC1, LC2, ß-TM, α-actinin). MHC hydrolysis was concomitant with the appearance of several subfragments. Two of them run just below C-protein and the others run below α-actinin. Either in rabbit or in bovine muscle myofibrils, one (27 Kd) or two (26 and 27 Kd) proteolytic fragments were released. These fragments disappeared further if the treatment with cathepsin L was prolonged.
Cathepsin H hardly hydrolyzed myofibrillar proteins of neither rabbit nor bovine muscles. The main change observed with the three substrates was the release of a 27 Kd component.

These results are discussed with regards to the Calcium-Activated-Neutral Proteinase (CaANP) effects and to the post mortem changes known to occur at the level of the myofibrillar proteins.

Bovine muscular collagen breakdown by a collagenase from Achromobacter iophagus

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The ability of a collagenase produced by a non pathogenic bacterium, Achromobacter iophagus, to degrade muscle collagen was studied. The effects of incubation conditions (pH, temperature) and substrate were investigated in relationship to the possible use of this enzyme in the specific tenderization of collagen rich muscles.

The efficiency of the enzymatic breakdown has been studied on isolated intramuscular collagen from Pectoralis profundus and Sternomandibularis muscles of animals aged between 16 months and 10 years. Collagen degradation was followed by its solubilization and the change in thermal isometric contraction properties of the fibres. The enzyme exhibits a strong pH dependence for all substrates tested with a rapid decrease in activity when the pH drops from 7.4 to 5.5. A mean 70% reduction in solubilization ability was observed with optimum incubation temperature and time. As regards incubation temperature, the maximum activity is obtained between 37 and 45°C for a 16 hour assay. A rapid thermal inactivation occurs above 45°C and no activity can be detected after a 15 min treatment at 55°C. The collagen solubilization decreases rapidly when the temperature is lowered to 30 and 20°C. Among the other incubation factors, we observe a very strong time effect: a five hour incubation is needed to reach 80% of the maximum solubilization with an intermediate crosslinked substrate. An optimum salt concentration is also required.

The properties of the collagen in turn affect its solubilization by the enzyme. An increase in substrate thermal stability (crosslinking) increases the time required to reach a constant solubilisation level with intramuscular collagen. In contrast, the epimysial collagen is much more resistant to proteolysis than intramuscular tissue from muscles of the same animal and surprisingly no effect of substrate thermal stability on its degradation is observed.

The activity of the Achromobacter iophagus collagenase is at least equivalent to that of Clostridium histolyticum on muscle collagen. The incubation conditions for maximum activity can be summarized as follows: pH $\geq$ 6, temperature 37-42°C, time > 5 hours, [NaCl] 0.2 M. Concerning the substrate, an increase in crosslinking reduces the speed of intramuscular collagen breakdown.

3:18

Proteolysis of sarcoplasmic proteins during in vitro incubation of bovine muscle extracts

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Proteolytic activity ascribed to a wide variety of proteinases has been demonstrated in extracts of muscle tissue. This activity may be important in protein turnover in vivo, in conditioning of muscle as meat and/or in flavour development during cooking of meat. The prevailing conditions in each situation are widely different yet involving the same range of proteins and of proteinases. In the work to be presented here the proteolytic activity of sarcoplasmic proteinases on sarcoplasmic proteins has been examined.

Extraction of muscle in 0.04 M buffer provided the preparation of sarcoplasmic proteins. Using synthetic substrates the extract was shown to exhibit a variety of activities attributed to known soluble and lysosomal proteinases. The proteins contained in the extract were characterised using SDS-PAG-Electrophoresis. Muscle extracts were incubated at 0° for periods up to 72 hr. The nature and extent of proteolysis was assessed from alterations in the SDS-PAGE profiles.

Muscle extracts were prepared by two procedures. A single muscle sample was extracted in Universal buffer pH 6.5, the extract divided into four aliquots, which were then adjusted to pH 5.0, 6.0, 7.0, 8.0 respectively. Alternatively individual muscle samples were extracted in buffers of pH 5.0, 6.0, 7.0 and 8.0.

With the exception of the extract made in buffer pH 5.0 all extracts showed similar protein profiles. After 24 hr incubation at 37° alterations in the protein profiles were indicative of proteolytic activity at pH 5.0, 7.0 and 8.0. Longer incubation at pH 6.0 was required to show detectable proteolysis. At all pH values longer incubation resulted progressively in more proteolysis. The nature of the proteins undergoing degradation and of the products thereof and the effects of proteinase inhibitors on the reactions involved are under investigation.

3:19

The action of Cathepsin L and Ca-activated neutral proteases on myofibrillar proteins

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We have examined the ability of proteolytic enzymes, Cathepsin L and Ca-activated neutral proteases to degrade myofibrils in order to assess what contributions these enzymes make to the structural degradation occurring in the conditioning of meat.

Cathepsin L is a cysteine lysozomal proteinase that requires thiol compounds for activation. It is optimally active against proteins within the range pH 5.5 to pH 6.0. The enzyme was purified to a high specific activity from extracts of rabbit liver by the method of Mason et al. (1984).

Two Ca-activated neutral proteases are present in the cytosol. One enzyme requires 100 μM Ca^{2+} while the other requires 1.5 mM Ca^{2+} for optimum activity. The optimum pH for both enzymes is pH 7.0. The two enzymes were purified from rabbit muscle in four stages: separation on DEAE Sephacel, hydrophobic chromatography on Phenyl-Sepharose, gel filtration on Sephacryl S-200, and finally on phenyl Sepharose again.

Rabbit longissimus dorsi myofibrils were treated with Cathepsin L, SDS gel electrophoresis showed that troponin T and troponin I were degraded and a polypeptide of MW 30,000 produced. Proteins with a molecular weight greater than myosin heavy chain (MW 200,000) were also degraded. In these respects Cathepsin L gave similar results to that obtained by the action of the Ca-activated neutral proteases.

However, there was also a loss of myosin heavy chain in myofibrils treated with Cathepsin L, which was also observed in myofibrils prepared from meat which had been conditioned at temperatures greater than 30°C. Ca-activated neutral proteases do not attack myosin.

Cathepsin L and Ca-activated neutral proteases removed Z-lines from myofibrils but unlike the action of Ca-activated neutral proteases, Cathepsin L did not release α-actinin into the supernatant. The loss of Z-lines by Cathepsin L, therefore, may be the result of the degradation of α-actinin. Desmin was degraded only by Ca-activated neutral proteases.

The evidence suggests that in meat conditioning, both Cathepsin L and Ca-activated neutral proteases could contribute to the proteolytic modification of the myofibrillar structure. Since the ultimate pH of meat is about pH 5.5 the conditions are more ideally suited to Cathepsin L. On the other hand, the absence of Ca is known to retard the degradation of myofibrils during conditioning. It is possible that Cathepsin L may succeed the Ca-activated neutral proteases in post-mortem proteolysis as the pH falls during the development of rigor especially following electrical stimulation which promotes the early rupture of lysosomes.

3:20

Ageing of Bovine Muscle: Desmin Degradation Observed via Enzyme Linked Immuno Sorbent Assay (ELISA)

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The 55,000 dalton protein desmin from bovine skeletal muscle is known to be degraded by proteolytic action during post mortem storage of beef concomitant to the increase in meat tenderness. Desmin is located in the Z-lines of the striated muscle myofibril and it probably acts as a structural framework component throughout the fiber.

In this study a 55,000 dalton protein was extracted from ox sternomandibularis muscle and purified using preparative Sodium Dodecyl Sulphate Polyacrylamide Gel Electrophoresis (SDS-PAGE). Antibodies from rabbits immunized with this protein stained the Z-lines in indirect immunofluorescence microscopy of myofibrils. When immunizing with a fraction of muscle proteins soluble in 1 M KI, the resulting antisera was shown to contain antibodies against the purified 55,000 dalton protein using gel precipitation techniques and Enzyme Linked Immuno Sorbent Assay (ELISA). The KI extract was analyzed using SDS-PAGE and desmin was found totally absent from the gel. This observation suggests that desmin is degraded during ageing and the resulting proteolytic fragments are soluble in 1 M KI in contrast to the intact protein. In an attempt to employ this result as an assay for postmortem cytoskeletal breakdown the concentration of proteolytic fragments was measured using competitive ELISA.

Ox sternomandibularis muscle was sampled immediatly post mortem and up to 6 days later. The samples were homogenized and separated in two salt soluble fractions: concentrated KI soluble and guanidine-HCl soluble. The guanidine fractions were analyzed using SDS-PAGE and the KI fraction were assayed for their ability to inhibit the desmin anti-desmin immunochemical reaction.

During 6 days at 15°C the desmin content of the guanidine fraction decreased 70% and the content of inhibitor in the KI fraction increased 3.6 times.

As the degradation of the cytoskeleton is related to increase of tenderness during ageing of meat, the observations described are believed to be promising for use as an index of meat ageing.

3:21

Isolation and characterisation of native titin

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Titin is a new and unusual protein that is present in muscle in large quantities (≃ 10% of the myofibrillar mass) and is likely to form a system of longitudinal elastic filaments within the sarcomere (Wang, 1982). Locker and Wild (1982) have proposed that such filaments are a key factor affecting the tenderness of cooked meat. Based on a high ionic strength extraction and a purification procedure involving precipitation of myosin in low salt followed by column chromatography, we have recently devised a method which for the first time allows isolation of titin without exposure to denaturing solvents.

Sedimentation velocity experiments on such purified native preparations indicate that the titin molecule is highly assymetric with a sedimentation coefficient of 13.4S. Electron microscopy of rotary-shadowed titin molecules reveals string-like structures with a diameter of about 40Å and lengths up to 8000Å. Differences were observed depending upon whether such specimens were layered or sprayed onto the mica substrate; we tentatively ascribe these to elasticity in titin revealed by the shearing forces that accompany spraying. In accord with this, the circular dichroism spectrum of titin suggests that its secondary structure is largely random coil, a conformation characteristic of elastic proteins such as elastin.

Negatively stained titin also shows string-like structures, but these can now be seen to have an appearance similar to a string of beads, the distance between successive beads being about 40Å. Structures similar to these have also been observed associated with negatively stained separated native thick filaments, both alongside the cross-bridge regions and in coils near the filament ends. The 40Å spacing of these strings is also very similar to the striation periodicity of end-filaments, (Trinick, 1981), recently identified structures at the tips of thick filaments, suggesting that end-filaments are composed of titin. The relationship between titin and the various models of elastic connecting filaments will be discussed.

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3:22

The influence of the biochemical condition of the raw material on meat processing

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Due to the modern trends in animal breeding, an improvement of growing rate and muscle mass has been achieved. On the other hand, the high stress susceptibility of pigs and the associated poor meat quality causes difficulties in meat processing. For the detection and identification of stress susceptible pigs, the halothane test, blood enzyme tests and genetic control are the methods commonly used. However the practical application of these methods is difficult due to the high costs and complexity.

In the framework of our research work, the frequency of occurrence of poor quality meat was investigated in the four most common Hungarian breeds:- KAHYB, HUNGAHYB, TETRA-S, and HUNGARIAN BIG WHITE. 6-month old pigs weighing 100-105 kg designated for canned ham production were investigated. At the same time, some biochemical characteristics of the blood were also measured.

Blood samples were collected at slaughter, pH_1 and pH_2 values measured, and some factors connected with changes of red blood cells and hemoglobin determined; also the colour of the semimembranosus muscle was measured. A rapid biochemical test for blood based on these measurements was elaborated and its correlation with meat characteristics and CK-activity studied by multidimensional statistical analysis.

It was concluded that the new biochemical test is very suitable for the prediction of poor meat quality, and may be used in the selection of animals on the big animal farms.

3:23

Membrane Integrity and Meat Quality

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Membrane defects have been suggested as the primary cause of porcine stress susceptibility, halothane sensitivity and malignant hyperthermia. A generalised membrane defect may explain elevated serum enzyme levels eg CPK, LDH, greater erythrocyte fragility and excessive mitochondrial swelling in stress susceptible pigs. The permeability of membranes, the activity of membrane bound enzymes (eg Ca^{2+} - activated ATPase), hormone specificity and osmotic fragility may be related to membrane fluidity. Studies on liposomes have established that fluidity is affected by 1) mole ratio of cholesterol to phospholipid 2) chain length and degree of unsaturation of the phospholipid acyl chain and 3) mole ratio of phosphatidyl choline to sphingomyelin.

The objective of this study was to investigate how the composition of synthetic model membranes (liposomes) and mitochondrial membranes affected their fluidity. Microviscosity which is inversely related to fluidity was measured by a fluorescence polarising technique using 1,6-Diphenyl-1,3,5-hexatriene (DPH) as a fluorescence probe. In the first series of experiments the effects of altering the cholesterol : phospholipid ratio on the fluidity of liposomes was investigated. It was found that as the cholesterol content was increased the microviscosity increased.

Mitochondrial membranes were prepared from both stress sensitive pigs (pH_1 = 5.75) and stress resistant pigs (pH_1 = 6.45) and the cholesterol : phospholipid ratio and microviscosity measured. The cholesterol : phospholipid ratio in stress resistant pigs (0.087) was higher than in stress sensitive pigs (0.064). There were also differences in the fatty acid composition of the mitochondrial preparations. The microviscosity measured at temperatures over the range 5 to 50°C was higher in mitochondrial preparations from stress resistant pigs than stress sensitive pigs. The lipid components of the mitochondrial preparations were extracted and incorporated into liposomes by sonication. The microviscosity of liposomes prepared from stress resistant pigs was higher than for liposomes prepared from stress sensitive pigs.

These results indicate how changes in the lipid components of mitochondrial membranes can affect their fluidity. Changes in the lipid components of membranes may be responsible for a generalised membrane defect which could play a major role in cellular control mechanisms and thus meat quality.

3:24

Relationship of collagen content, type and cross-linking with texture of different muscles

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In the past it has proved difficult to assess the contribution of collagen to meat texture using direct biochemical methods due to the difficulty of separating the different hierarchical connective tissue structures in muscle. We have developed a method which allows the purification of the epimysium (EP), perimysium (P) and endomysium (EN) of muscle in quantitative amounts so that further analysis can be carried out.

EP is dissected from each muscle before treatment and then P and EN are prepared after a brief homogenisation in a low ionic strength calcium buffer by sieving through a 1 mm mesh copper sieve. P is retained as gross connective tissue on the grid whilst the endomysil fraction passes through. Detailed biochemical analysis showed that these three connective tissue fractions were quite heavily contaminated with myosin and actin even after exhaustive washing with salt solutions.

We therefore designed a simple washing system using the detergent sodium dodecyl sulphate (SDS) which allowed us to obtain very clean samples of EP, P and EN. These were then used for estimation of collagen fibre size, collagen content, the relative proportions of the major collagen types I and III and collagen cross-link content. We chose six bovine muscles varying in toughness and texture (psoas major [PM], longissimus dorsi [LD], semitendinosus [ST], pectoralis profundis [PP], gastrocnemius [G] and sternomandibularis [SMD]) for our initial studies.

We were able to corroborate previous work (2) which had shown a correlation between gross collagen content and toughness and we demonstrated a relationship between perimysial collagen fibre size and toughness. The four better quality muscles (PM, LD, ST and PP) had similar fibre diameters (approx. 50 nm) whereas the tougher muscles (G and SMD) had much larger fibres (75 - 95 nm). We found similar ratios of type I to type III collagen in the perimysia and endomysia of all the muscles studied showing that this parameter has little influence on toughness. Collagen cross-links were shown to be of considerable importance in determining toughness, as the content of heat-stable cross-links was always significantly higher in tougher muscles. For example, the P of sternomandibularis contained as much as twice the content of heat-stable cross-link (relative to the labile cross-links) when compared to psoas major whilst the EN of this muscle contained a similarly elevated proportion of this cross-link compared to the quality muscles.

A complex mechanism for the role of collagen in determining meat texture can be proposed from our results involving shrinkage of major tracts of connective tissue in the EP and P during cooking, shrinkage of basement membrane collagen down onto the contents of the endomysial sheath (both effects leading to water loss), and shearing of perimysial collagen sheets and P/EN junctions on mechanical challenge. Our hypothesis will be presented at the meeting.

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3:25

The nitrogen content of beef muscles

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The nitrogen content of beef was determined by applying the macro Kjeldahl method to samples of muscles completely trimmed of external fat and aponeurosis. At first, (group A), 411 samples were considered (10 animals and 58 different muscles); from the first group was studied a second group (group B) of 270 samples (10 animals x 27 muscles). The animals varied in carcass weight, age and conformation (carcass weight = 316.2 $\pm$ 66.9 kg, age 51.2 $\pm$ 23.7 months, conformation score (EAAP method) = 9.1 $\pm$ 3.3).

The samples were analysed for nitrogen and dry matter and the fat was estimated from the dry matter content. In each group the results of nitrogen determination were expressed as a percentage of fresh weight (N), or on an estimated fat free basis (N_{FE}) or as a percentage of dry matter (N_{DM}).

The average of (N) was, in group A, 3.41 + 0.17 with a range of 2.85 to 3.93 and, in group B, 3.41 + 0.17 with a range of 2.85 to 3.78. Analysis of variance indicated a significant effect ($P < 0.001$) of both animals and muscles. The highest contents of (N) were found in Adductor, Semimembranosus and Longissimus dorsi muscles and the lowest in Diaphragma medialis, Transversus abdominis, Serratus ventralis pars cervicis.

The average value of N_{FE} was 3.46 $\pm$ 0.18 in group A (range 2.90 - 3.93) $\pm$ and 3.47 $\pm$ 0.19 in group B (range 2.90 - 3.91). The effects of animals and muscles on N_{FE} were both highly significant. The correlation between (N) and (N_{FE}) was 0.956.

The average value of N_{DM} was 13.97 $\pm$ 0.97 (range 9.83 - 16.87) in group A and 13.88 $\pm$ 0.96 (range 10.03 - 16.15) in group B.

The consequences of the variation of N_{FE} on the value of the nitrogen factor normally used by analysts to calculate the raw meat content of products made from pure beef are discussed as well as the importance of the variation of N_{DM} on the cost of the nitrogen nutrient intake from different beef cuts.

:26

Extractability of native nitroso heme pigments from cured meat

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Nitrosomyoglobin (NOMb), metmyoglobin (MetMb) and their heat-denatured pigments (DNOMb and DMetMb) were prepared from a reaction medium containing myoglobin (Mb) and sodium nitrite with and without sodium ascorbate, and their extractability with 75% acetone from processed meat products was investigated. No significant difference in absorption spectrum of the 75% acetone extract could be observed between NOMb and DNOMb, and they had the same optical density at 395 nm, one of the absorption maxima. MetMb and DMetMb could not be extracted with 75% acetone, and it was confirmed that native nitroso heme pigments (NOHP) could be quantitatively extracted with 75% acetone from processed meat products in the same manner as denatured NOHP.

Consequently, an examination was made of the extractability of native NOHP with water from commercial raw ham and cured meat, following the 75% acetone procedure, and the effect of an endogenous factor on the extractability was noted. Cured meat was prepared by adding nitrite, ascorbate and sodium chloride to porcine skeletal muscle at pH 5.0~6.5. The percentage of water extracted NOHP to the total NOHP was expressed as the extractability of native NOHP. NOHP content was estimated from the absorbance at 395 nm of the 75% acetone extract. Most of the heme pigments were extracted with water from the uncured raw meat. In the commercial raw hams tested, the extractability of native NOHP was generally low, ranging from 8 to 75%. The extractability of native NOHP increased in proportion to pH at the time of curing. Even when the pH of the meat cured at 5.5 rose to 6.5 at the time of water extraction, there was only a slight increase in extractability. The extractability of NOMb added to the raw meat swiftly decreased at pH 5.5, but at pH 6.5, the NOMb could all be extracted without any adsorption on to the meat.

Myofibrils from porcine skeletal muscle were cured with nitrite, ascorbate and sodium chloride in the presence of Mb. The extractability of NOMb at pH 5.0~6.5 changed in a manner similar to that for cured meat. Variation in the extractability of NOMb added to the myofibrils with pH was also similar to that observed for the raw meat.

On the basis of the above data, the decline in extractability of native NOHP from cured meat with a decrease in pH was concluded to be due to an interaction between native NOHP and the myofibrils in the cured meat.

3:27

Relative effects of sex and anabolic agents on veal muscle characteristics

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The relative effects of sex and anabolic agents (trenbolone acetate-oestradiol) on veal muscle characteristics were studied in animals exhibiting similar growth rates. The Longissimus dorsi muscles of 6 Norman-breed veal carcasses (average carcass weight : 120 kg, age at slaughter : 5 months) were analyzed in each of the 4 sub-groups (male control, male treated, female control, female treated).

Eight muscle characteristics were analyzed at 24 h post mortem namely:

- basic parameters such as dry matter (DM), total enthalpy change during a standard heating cycle using differential scanning calorimetry (DSC) and NMR measurements: a single T_1 relaxation time and two T_2 values: T_2a (short time) and T_2b (long time).
- collagen was analyzed for total content on a dry weight basis (CC) and for its thermal stability through the maximum isometric tension (MIT) generated during a standard heating cycle.
- the myofibrillar structure was characterized by its enzymatic equipment (EE) based on the percentage of the different myosin isoforms present in the Longissimus dorsi muscles.

The sex influences specifically DM and CC, the dry matter being higher (+ 0.9% abs. value) and collagen content lower (-14.6%) in female than in male muscles. A significant difference in the percentage of the myosin isoform I between male and female hormone treated group was found but no overall significant sex effect was revealed by the other parameters.

The hormone effect was most pronounced on DM, DSC, MIT, EE and T_2b measurements. The anabolic agents decreased significantly the dry matter (-0.4% abs. value) and the thermal stability of collagen (-14.5%) in both sex groups; for collagen the females gave a more significant response. The myosin isoforms pattern is also affected by the hormone treatment: the fast isozyme II is decreased whereas the fast form V is slightly increased. The longest T_2 relaxation time (T_2b) is increased by the treatment in both sex groups. No significant hormone effect was found on the other muscle characteristics CC, T_1 and T_2a.

Among the parameters studied, the most discriminating for sex effect are the two quantitative characteristics: dry matter and collagen content, and for anabolic treatment effect two qualitative properties of the structural protein fraction: the pattern of myosin isoforms and the collagen stability.

3:28

Characterisation of the pattern of fatty acids from different animals

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In our institute the quality of meat and fat from several species has been investigated. These investigations included the determination of fatty acids in different kinds of fat. The data presented here summarize the results regarding the most important and characteristic fatty acids.

We analysed fats from beef, pork, sheep, rabbit, chicken and kid and evaluated statistically the differences in the content of several fatty acids between various kinds of tissue (subcutaneous, inter- and intramuscular) and between their position in the carcass. Other sources of variance were breed, feeding and category. Patterns of fatty acids were evaluated after esterification of fatty acids by the boron-trifluoride method and gas-chromatographic determination.

After statistical interpretation by multiple analysis of variance (SPSS, Anova) the per cent portions of different influences were evaluated. For palmitic, stearic, oleic and linoleic acid there were significant differences caused by tissue and localisation of fat for all species. The outer layers of depot fat had the highest content of unsaturated fatty acids, the inner depot fat the smallest. Pattern of intramuscular fat was not as highly influenced by feeding as the subcutaneous and intermuscular fat. Besides this result the per cent portions of sources of variance for beef showed a significant influence by category (young bulls, heifers, very young cows). Unexplained influences were from about 30% (stearic acid) to 80% (palmitic and linoleic acid) for beef.

For pigs the greatest influences came from feeding. There was not only the kind of fat effective but to a great extent also the total amount of feeding. Unexplained influences varied between 13% (linoleic acid) to 39% (stearic acid).

Inner fat from chicken is influenced mainly from derivation (44% for linoleic acid), unexplained influences varied from 49% (linoleic acid) to 99% (stearic acid). We want to emphasize that the standard deviation altogether was very low. The influences of grading classes and sex were negligible (pork, sheep). Fat and its technological properties can be characterized by two values: the mean value of carbon numbers and double bonds. There is a direct connection to the saponification and iodine value.

Fatty acids with branched chains and/or uneven carbon numbers are of special interest for biochemists. Between different species great differences in the content of margaric acid (C-17) occur. Highest contents of this fatty acid were found in fat from sheep, lowest in those from chicken.

3:29

Chemical composition of pork and mutton in Egypt

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The chemical composition of meat obtained from the hind quarter i.e., the hind legs, of male animals of sheep and pigs, about 8 months age (after 2.5 hours of slaughtering) was investigated. Mutton showed higher moisture and protein contents compared to pork, which contained higher content of fat. The specific gravity was slightly higher for lard than sheep fat, which may indicate higher fatty acids content in lard. The same trend was noticed for refractive index, which may refer to higher unsaturated fatty acids content in lard as mentioned by Sokolov, (1965). The melting point of sheep fat was higher than that of lard, while peroxide, T.B.A., acid, iodine and saponification values were higher in lard than sheep fat which may assure higher content of unsaturated fatty acids in lard.

The fatty acids composition of 2-monoglycerides and triglycerides was determined by gas liquid chromatography. Lard was characterised by high percentage of saturated fatty acids specially palmitic acid at the 2-monoglycerides. The palmitic acid enrichment factor of lard was higher than sheep fat, while the unsaturation ratio was low for lard. Other ratios based on the fatty acids composition of 2-monoglycerides were determined. The ratio of total C_{16}/total C_{18} was considerably high in case of lard than sheep fat.

The glyceride pattern of lard and sheep fat, calculated by Vander Wal,(1960) was determined, where the USU/SUS ratio was higher in lard than sheep fat.

Fractionation of triglycerides, by thin layer chromatography was carried out, where lard gave 10 separable bands, while sheep fat showed 4 highly saturated ones.

The photo-micrographs of lard and sheep fat crystals were also investigated. Sheep fat crystallized in characteristic fan-like tufts, with more or less pointed ends, needle-like and crystals of lard were chisel-like shaped.

3:30

Incidence and quality characteristics of dark cutting in Belgian beef.

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In two slaughterhouses a total number of 2296 animals including 807 bulls, 853 steers and 636 cows were systematically examined for DFD incidence.
Information about age, breed, sex, distance of transport, lairage time before slaughter, carcass weight and electrical stimulation was obtained for each animal.
PH_{24} measurements were made in the LD muscle.
Muscles with a $pH_{24} \geq 6.20$ were classified as being DFD. Muscles with $5.80 \leq pH_{24} < 6.20$ were classified as being intermediate. All other carcasses were considered to be of normal meat quality ($pH_{24} < 5.80$).
DFD occurred in 3.6% of the bulls, in 2.4% of the steers and in 7.2% of the cows.
The mean pH_{24} values in the LD from these dark cutting bulls, steers and cows were respectively 6.53, 6.42 and 6.49 while for normal animals these values were respectively 5.51, 5.50 and 5.52.
A high incidence of DFD was observed particularly in young bulls and old lean cows (lowest weight classes).
PH_{24} measurements in 15 important muscles of the hind- and forequarter of a number of dark cutters showed that the LD muscle in particular but also the biceps femoris, the semitendinosus and the gracilis were DFD susceptible.
From 30 DFD carcasses measurements of water-holding capacity, colour, myoglobin oxidation and tenderness of the LD muscle were compared with those of normal LD muscles.
The cooking loss of DFD meat was approximately 15% lower than that of normal meat. Dark cutting beef had lower lightness values. The lower redness and yellowness values resulted in a more purple hue and in a considerable decrease of saturation.
In low-pH meat, 4.5% of the total myoglobin content was oxidized to metMb 48h post mortem. Following a refrigerated storage period of 8 days metMb amounted up to 14.2%.
In the DFD meat samples, however, metMb represented already 28.0% of the total myoglobin content within 48h post mortem. Such an amount of metMb renders the fresh meat unacceptable to the consumer.
DFD meat was markedly more tender than beef of normal pH.

3:31

Some physical and chemical studies on drip originating from frozen buffalo and camel meat

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This investigation was carried out to gain information on the drip obtained from frozen buffalo and camel meat. The results showed that the rate of drip and colour increase were usually greater for the camel meat than the buffalo meat. Higher quantities of solids were found in the drip from the buffalo meat than the camel meat. The nitrogen content of the drip increased progressively with frozen storage time; the nitrogen loss from the meat in the drip was related closely to the loss of solids. It was noted also that the ether extractable matter and the pH value increased slightly, especially at the end of the storage period. The drip contained appreciable amounts of certain minerals which indicated the loss of nutritional value upon thawing. The quantity of minerals in the drip was affected by the type of meat, time of storage and particular mineral. Each drip had more total saturated fatty acids and lower total unsaturated fatty acids than the respective meats.

3:32

The relationship between metabolic and contractile types of pig muscles and the water holding capacity of meat

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We have studied how the water holding capacitv of pork meat is related to the metabolic and contractile types of the muscles, without the effect of the 24 hours post mortem pH factor. We have characterized the metabolic and contractile types of 30 pig muscles and made the following distinctions:

- glycolytic fast twitch white muscles, such as the longissimus dorsi,
- oxidative slow twitch red muscles, such as the infraspinatus, supraspinatus, and
- intermediate muscles such as the rectus femoris.

We measured the water holding capacity of the above muscles by centrifugation at 200 *g* of a mixture of minced muscle and a solution of 0.15 ionic strength, the pH of which varied from approximately 4 to 8.

When the muscles are raw, with an identical pH, there is little difference between muscles of the different metabolic and contractile types. The supraspinatus, infraspinatus muscles show a slightly higher water holding capacity than the longissimus dorsi group. However, the results were different with cooked meat: cooking leads to a lower water holding capacity for slow red muscles (infraspinatus, supraspinatus) than it does for fast twitch muscles (longissimus dorsi).

In addition, we studied the contribution of the different muscular components to the water holding capacity, as well as the change in water holding capacity over a period of one to twenty four hours post mortem. Thus we could show the prevailing role of the sarcoplasmic proteins. Their addition to the myofibrillar proteins increases significantly the water holding capacity of these proteins. This effect diminishes if the sarcoplasmic proteins are denatured by the action of the pH and/or the temperature.

3:33

The relationship between water holding capacity and pH in the swine muscle post mortem

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In previous research we demonstrated a relationship between low water holding capacity of the swine muscle 24 hours after slaughter (WHC-24) and dry-cured ham defects. We subsequently found that the WHC-24 is related to the pH determined 1 hour (pH-1) and 24 hours (pH-24) after slaughter, and that the WHC after 1 hour (WHC-1) is related to the pH-1; we also noted muscle with a low or very low pH-1 without PSE condition.

The aim of the present work is to evaluate the prevalence of pig carcasses with PSE or abnormal muscle by measuring the pH-1 and WHC-1, and to investigate the glycolytic metabolism in these muscles by histochemical (glycogen amount and phosphorylase activity) and biochemical (lactic acid amount) tests.

We found a high percentage of pig carcasses with longissimus dorsi muscle which had a very low pH-1 and WHC-1, PSE condition and also a very fast glycolytic metabolism i.e. a very low glycogen content, very low phosphorylase activity and high level of lactic acid 1 hour after slaughter. We also found a marked prevalence of carcasses with longissimus dorsi muscle which had a low or very low pH-1, a good WHC-1, and quite a variation in the rate of the glycolytic metabolism, with or without PSE condition.

3:34

Some physical and chemical studies on buffalo and camel meat during cold storage

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This work was carried out to study the effect of cold storage at 4°C for 7 days on some physical and chemical composition of buffalo and camel meat. The results showed that after cold storage the tenderness was increased, while water holding capacity decreased slightly. The tenderness and water holding capacity of buffalo meat were better than for camel meat during cold storage. Buffalo meat was darker than that of camel meat.

It was noticed that during the aging process at 4°C for 7 days the moisture content, crude protein, pH value, collagen and elastin decreased. The retention % of collagen and elastin in buffalo meat were 83.20 and 77.66; and in camel meat were 85.78 and 88.89, respectively. After aging of the meat, the total soluble nitrogen as well as the soluble protein nitrogen increased.

The results showed that, in the presence of free amino acids in the fresh samples did not vary greatly and depended on the meat source. Cold storage of both types of meat caused the detection of some free amino acids such as methionine, tryptophane, glutamic acid and serine.