Die Bedeutung von Ca^{2+}-Ionen für den Einfluß der Temperatur auf den ATP-und Glykogen-Abbau im zerkleinerten Rindermuskel post mortem

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Das hohe Wasserbindungsvermögen von schlachtfrischem Rindfleisch kann über Monate durch rasches Einfrieren von gesalzenem oder ungesalzenem zerkleinertem Muskelgewebe vor Eintritt des ATP-Abbaues aufrecht erhalten werden. Bei der Verarbeitung des in dieser Weise eingefrorenen Rindfleisches ohne vorheriges Auftauen erhält man Würste von ausgezeichneter Qualität. Hierbei kommt es darauf an, den ATP-Abbau während des Kühlens und Gefrierens so klein wie möglich zu halten. Daher untersuchten wir den Einfluß der Temperatur zwischen +24°C und -6°C auf den ATP-Umsatz im zerkleinerten Sternomandibularis-Muskel von Bullen.
Ist NaCl nicht zugegen, so nehmen die Geschwindigkeiten von ATP-Abbau und Glykolyse mit fallender Temperatur bis etwa +6°C ab, um dann bei weiterer Kühlung wieder anzusteigen und bei etwa -1°C ein Maximum zu erreichen. Die Zunahme des ATP-Turnover bei Temperaturen unter +6°C beruht auf einer Freisetzung von Ca^{2+}-Ionen aus Zellorganellen, da Zusatz von EGTA die Geschwindigkeitsmaxima bei -1°C eliminiert und zu einer kontinuierlichen Abnahme des ATP-Turnover mit sinkender Temperatur führt.

Wird zerkleinerter schlachtfrischer Rindermuskel gesalzen (2 % NaCl), so nehmen die Geschwindigkeiten der ATP-Hydrolyse und der Glykolyse mit sinkender Temperatur kontinuierlich ab, bis das Fleisch gefroren ist. Oberhalb von etwa +3°C verläuft der ATP-Abbau innerhalb der ersten Stunden post mortem im gesalzenen Gewebe rascher als im ungesalzenen. Es wird angenommen, daß dieser Effekt auf einer Freisetzung von Ca^{2+}-Ionen aus Zellorganellen durch Austausch gegen Na^{+}-Ionen beruht. In der Tat senkt ein Zusatz von EGTA zum gesalzenen schlachtfrischen Muskel die Geschwindigkeit des ATP-Abbaues auf ein Niveau, wie es im ungesalzenen zerkleinerten Gewebe festzustellen ist. Die Geschwindigkeit des ATP-Abbaues kann beim Abkühlen des gesalzenen schlachtfrischen Fleisches natürlich nicht zunehmen, wenn bereits der größte Teil der Ca^{2+}-Ionen aus den Organellen durch Austausch gegen Na^{+}-Ionen freigesetzt ist.

The Role of Ca^{2+} in the Temperature Effect on the Postmortem Breakdown of ATP and Glycogen in Minced Bovine Muscle

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The high water-holding capacity of prerigor beef can be preserved for months by rapid freezing of minced salted or unsalted muscle before onset of the breakdown of ATP. If beef frozen in this way is processed without preceeding thawing, sausages of excellent quality are obtained. It is important to ensure that the depletion of ATP during cooling and freezing is kept to a minimum. Therefore, the influence of temperature between +24° and -6°C on the ATP turnover in minced sternomandibularis muscles of bulls was studied.

If NaCl is not present, the rates of ATP breakdown and glycolysis decrease with falling temperature to about +6°C but then increase with further cooling, reaching a maximum rate at about -1°C. The rise in the ATP turnover at temperatures below +6°C is due to a release of Ca^{2+} ions from cell organelles because addition of EGTA eliminates the rate maxima at -1°C and results in a contineous decline of ATP turnover with falling temperature.

If minced bovine muscle is salted (2% NaCl) in the prerigor state, the rates of ATP hydrolysis and glycolysis decrease with falling temperature until the meat is frozen. Above about +3°C the ATP depletion is faster in salted tissue than in unsalted one in the first hours postmortem. It is suggested that this effect is due to a release of Ca^{2+} ions from cell organelles by exchange against Na^{+} ions. Addition of EGTA to salted prerigor muscle indeed lowers the rate of ATP breakdown to the level observed in unsalted minced muscle. It is obvious that during cooling of salted beef the rate of ATP depletion cannot increase because the Ca^{2+} ions are already released from the organelles by exchange against Na^{+} ions

L'importance des ions Ca^{2+} pour l'influence de la temperature sur la dégradation ATP et glycogène dans le muscle bovin concassé post mortem

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La haute capacité du boeuf qui est fraichement abattu à fixer de l'eau peut etre maintenir pour des mois en congelant rapidement le tissu musculaire concassé, salé ou non salé avant le commencement de la dégradation ATP. En utilisant boeuf congelé de cette manière sans l'avez décongelé avant vous receverez de la charcuterie d'une qualité excellente. Il est important à tenir la dégradation ATP aussi mince que possible pendant la réfrigération et la congélation. C'est pourquoi nous avons examiné l'influence de la temperature entre -24°C et -6°C sur le turnover ATP dans le muscle sternomandibularis concassé des taureaux.

Si NaCl n'est pas présent, les vitesses de la dégradation ATP et de la glycolyse diminuent à temperature tombante à environ +6°C, montant encore en continuant la réfrigération et pour attirer un maximum à environ -1°C. L'augmentation du turnover ATP aux temperatures sous +6°C est basée sur un dégagement des ions Ca^{2+} des substructures cellulaires, car une adjonction de EGTA élimine les maxima de vitesse à -1°C et mène à une diminution continuante du turnover ATP avec une temperature tombante.

En salant le muscle bovin concassé qui est fraîchement abattu (2% NaCl) les vitesses de la hydrolyse ATP et de la glycolyse diminuent avec une temperature tombante d'une facon continuante jusqu'à ce que la viande soit congeler. Si la temperature est plus d'environ +3°C pendant les premières heures post mortem la dégradation ATP se passe plus vite dans un tissu salé que dans un tissu non salé. Il est supposé que cet effet est basé sur un dégagement des ions Ca^{2+} des substructures cellulaires par échange contre ions Na^{+}. En effet l'adjonction de EGTA au muscle salé et fraîchement abattu la vitesse de la dégradation ATP diminue à un niveau comme il peut être constaté dans un tissu concassé non salé. Naturellement en réfrigerant la viande fraîchement abattue et salée la vitesse de la dégradation ATP ne peut pas augmenter si la plupart des ions Ca^{2+} des substructures cellulaires est déjà dégagée par échange contre ions Na^{+}.

Важность Ca^{++}ионов для влияния температуры на разложение АТФ и гликогена в измельченной говяжей мышце после смерти.

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Высокая способность связывания воды парной говядины, может быть сохранена месяцами, при быстром замораживании соленных или несоленных измельченных мышечных тканей до наступления разложения АТФ. При переработке таким способом замороженной говядины, без предварительного оттаивания, получаются колбасы отличного качества. Слишком важно напомнить, что разложение АТФ,во время охлаждения и замораживания, было как можно менее. Вследствие того и исследовались влияния температуры в границах +24°C и -6°C на разложение АТФ в измельченней парной мышце Стерномандибуларис быков.

Если не имеется поваренной соли уменьшается быстрота разложения АТФ и гликолиза при понижении температуры до около +6°C, чтобы тогда при продолжении охлаждения вновь увеличилась и при около -1°C достигла максимум.

Повышение "turn-over " АТФ при температуре ниже +6°C основывается на освобождении Ca^{++} ионов из клеточных органел, так как прибавка ЭГТА исключает максимум скорости и при -1°C приводит до постоянного уменьшения "turn-over " АТФ при понижении температуры.

Если измельченную парную говяжую мышцу засолим (2% поваренной соли), постоянно уменьшается быстрота гидролиза АТФ и гликолиза с уменьшением температуры, пока мясо не заморазится. Свыше около +3°C разложение АТФ совершается в границах первых часов после смерти в засоленной ткани быстрее, чем в незасоленной . Предполагается, что этот эффект лежит в освобождении ионов калция из клеточных органел через размену с ионами натрия. То есть, добавление ЭГТА засоленной парной мышце уменьшает быстроту разложения АТФ до нивоа, который утверден в незасоленной измельченной ткани. Быстроту разложения АТФ,при охлаждении засоленного мяса, сразу после убоя, не можно естественно повышать, если уже самая большая часть ионов калция из органел освобождена путем размены с ионами натрия.

The Role of Ca^{2+} in the Temperature Effect on the Postmortem Breakdown of ATP and Glycogen in Ground Bovine Muscle

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Introduction

For certain types of processing bovine muscle is ground, salted, and frozen or freeze-dried in the prerigor state in order to preserve the high water-holding capacity of the prerigor beef for a longer period (HAMM, 1972, 1973, 1977a; HAMM and POTTHAST, 1975). In these types of processing it is important to ensure that the breakdown of adenosine triphosphate (ATP) during cooling and freezing is kept to a minimum. For this reason, the influence of cooling and freezing of ground prerigor beef, either salted or unsalted, on the breakdown of ATP and glycogen was studied. For the purpose of comparison freezing of non-ground muscle was also included in these studies.

Material and Methods

Samples from the sternomandibularis muscle of bull carcasses were taken immediately after slaughter. For each temperature studied a muscle slice (150 g), a 50 g sample of ground muscle (4,5 mm plate) and a 50 g sample of ground and salted (2 % NaCl) muscle were sealed in polypropylene pouches. The pouches were immersed in the methanol bath of a cryostate which was adjusted to the particular temperature (20-30 min p.m.). After 10-20 min the center of the sample had reached the bath temperature (zero time). After an immersion period of 3 or 3.5 hrs the samples were analyzed.

Lactate, which was also determined in the fresh sample before immersion in the cryostat, was enzymatically determined after HOHORST (1970). The ATP breakdown was followed by means of the "R value" after the method of HONIKEL and FISCHER (1977). With this photometric procedure the extent of the enzymatic transformation of ATP into inosine derivatives is measured. A low "R value" indicates a high ATP concentration and vice versa.

Results and Discussion

1. Influence of temperature on the postmortem changes in intact and ground tissue

In absence of added NaCl, the effect of temperature on the postmortem changes in the ground muscle samples follows a pattern similar to that of the non-minced tissue (Fig.1). In all samples, between -1° and -6° C, i.e. at freezing temperatures, the rates of ATP breakdown and glycolysis decrease strongly with falling temperature. Maximum rates in ATP depletion and lactate accumulation at -3°C as they were observed by BEHNKE et al. (1973) in the same type of bovine muscle were not found in our experiments.

The decrease in the rate of ATP and glycogen breakdown, i.e. in the ATP turnover (Fig.1 and 2) and in the rate of pH decline (Fig.2), by lowering the temperature from +24°C to about +6°C is certainly due to the normal influence of temperature on enzymatic reactions.

Increase in the rate of ATP turnover between +6° and -1°C in the intact muscle (Fig.1) is wellknown to be associated with the "cold shortening" phenomenon, i.e. the toughening of meat by rapid cooling of prerigor meat (for references see BENDALL, 1973; HONIKEL, 1975; HAMM, 1976). The finding of BENDALL (1973) that during "cold shortening" the ATP level remains rather constant because of a fast resynthesis of ATP from ADP by glycolysis is confirmed by our results with non-ground tissue.

The "cold shortening" phenomenon is explained by the influence of temperature on the membrane system of the sarcoplasmic reticulum (SR). Below about +10°C decreasing temperature is supposed to cause an increasing inactivation of the ATP driven calcium pump which transports Ca^{2+} ions from the sarcoplasma across the membrane into the SR. Therefore, calcium ions are released from the sarcotubular system into the sarcoplasma following the fall in ion concentration; they activate the myosin ATPase and, consequently, initiate the onset of rigor mortis (BENDALL, 1973). It could be also possible that calcium ions released postmortem from mitochondria by anoxia cannot be accumulate in the SR at lower temperatures (LEHNINGER et al., 1967)

It is interesting that also with ground muscle a remarkable increase in the ATP turnover between +6°C and -1°C was obtained (Fig. 1 and 2). Grinding causes an acceleration of the breakdown of ATP and glycogen at temperatures above about +3°C and -1°C respectively. This effect was already observed in our earlier work (VAN HOOF and HAMM, 1971; HAMM, 1977b).

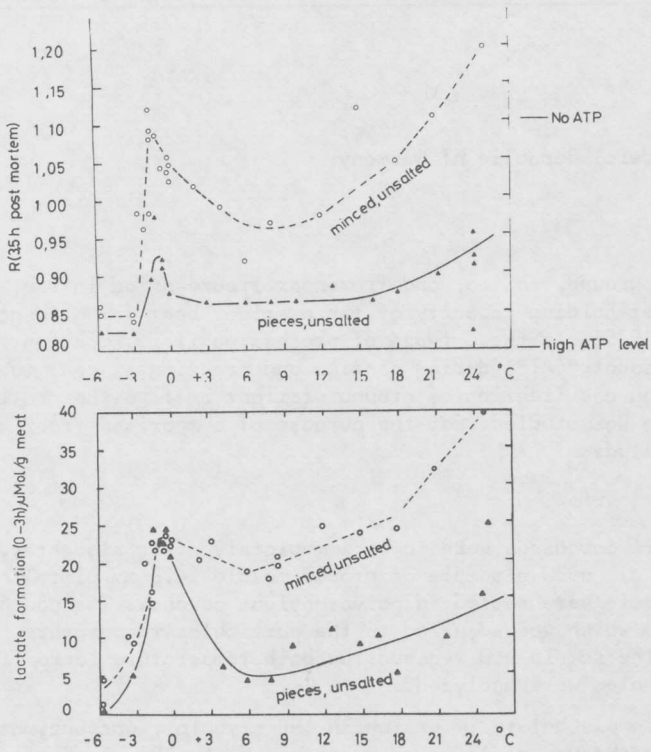

Fig.1: Influence of temperature on the postmortem breakdown of ATP ("R value") (above) and lactate formation (below) in nonground ("pieces") and ground ("minced") bovine sternomandibularis muscle.

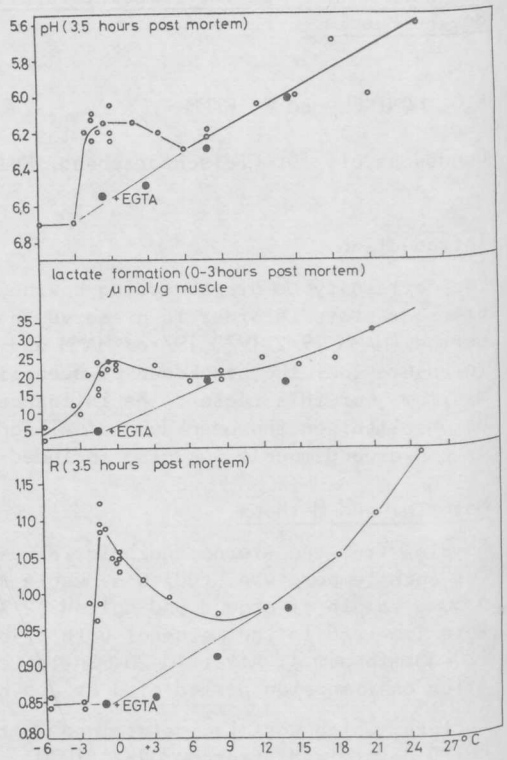

Fig.2: Effect of temperature on biochemical postmortem changes in homogenates of bovine sternomandibularis muscle as influenced by EGTA (15 µmoles/g homogenate)

The marked maximum of the rate of ATP breakdown at -1°C suggests that at higher temperatures the calcium pump of the SR is effective also in the ground muscle. In contrast to the intact muscle, the ATP disappears very rapidly at -1°C because 3 hours p.m. most of the glycogen is metabolized in the ground muscle (HAMM and VAN HOOF, 1971) and, therefore, further resynthesis of ATP cannot occur.

In order to eludicate the role of calcium ions released from subcellular membrane systems in the effect of temperature on the biochemical postmortem changes, 15 µmoles of the calcium sequestering agent EGTA were added to 1 g homogenate at different temperatures between -1° and +14°C. In the presence of EGTA the rates of lactate formation of decline in pH and of ATP depletion (R value) decrease contineously with falling temperature without the rate maxima at -1°C observed in the control samples (Fig. 2).

The result can be regarded as an evidence for the postulated role of calcium ions released from subcellular structures in the temperature effect on ATP turnover and pH changes.

2. Influence of temperature on the postmortem changes in ground tissue in presence of NaCl

The pattern of the temperature effect on the postmortem changes in salted, ground muscle is completely different from that found with unsalted ground tissue (Fig. 3). The rates of ATP breakdown and lactate formation decrease contineously with falling temperature until the tissue is frozen. No maxima at -1°C were observed.

At tempratures = +3°C salting of the ground muscle causes an increase in the rate of ATP depletion. This was already observed in our earlier work (VAN HOOF and HAMM, 1973; HAMM, 1977b) and explained by an exchange of Ca^{2+}ions from the SR against Na^{+}ions of the NaCl. The calcium ions so released accelerate the ATP breakdown by additional activation of myosin ATPase. If most of the Ca^{2+}ions present in the SR or other subcellular structures are released by the effect of added NaCl, lowering the temperature should not be able to cause an additional release of calcium and, consequently, should not result in an increase of the rate of ATP depletion. This corresponds exactly to our observation that with salted ground muscle there is no maximum of ATP breakdown at -1°C (Fig.3). Lowering the temperature from +24°C down to the freezing point of tissue results in the normal contineous reduction of the rate of enzymatic reactions.

The observation that the addition of salt lowers the rate of glycolysis post mortem even at temperatures above +6°C confirms earlier results according to which the glycolysis in minced muscle is

inhibited by NaCl (VAN HOOF and HAMM, 1973; DALRYMPLE and HAMM, 1974). Those results were obtained with bovine longissimus muscle and with rabbit muscle.

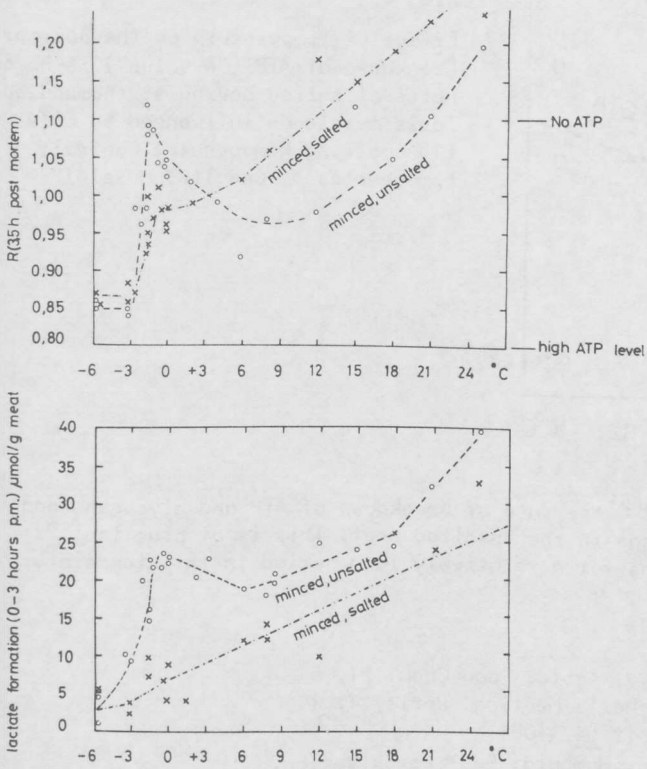

Fig. 3:

Influence of temperature on the postmortem breakdown of ATP ("R value") (above) and lactate formation (below) in unsalted and salted ground (minced) bovine sternomandibularis muscle

NEWBOLD and SCOPES (1971) made a similar observation when they studied the influence of dilution of muscle homogenates by 0.16 M KCl. They suggested that KCl might inhibit one of the first steps of glycogenolysis. We postulated that addition of alkaline chloride causes some inhibition of most of the glycolytic enzymes (DALRYMPLE and HAMM, 1974).

With regard to the inhibition of enzymes by elevated ionic strength the question is of interest why between +4°C and the freezing of tissue (below -1°C) the rate of ATP breakdown in the ground muscle is lower in the presence than in the absence of NaCl. This phenomenon can be explained by the fact that increase in the ionic strength by addition of monovalent ions normally alters the physical and enzymatic properties of proteins. Salting of muscle tissue in the rigor or postrigor state - when all calcium releasable after death is released - inhibits the ATPase as we have shown in earlier experiments (HAMM and VAN HOOF, 1974). NaCl seems to exert two effects on prerigor muscle: (a) activation of ATPase by an increased release of calcium ions from subcellular structures and (b) lowering the ATPase activity by the elevated ionic strength. The latter (b) effect will diminuish the former one (a) and will become predominant as soon as most of the releasable calcium ions are released by lowering temperature.

As we have suggested above, the increase in the rate of ATP-breakdown during the first 3 hours p.m. in the ground muscle by addition of NaCl at temperatures above +3°C (see Fig. 3) is due to an increased release of calcium ions from subcellular membrane systems. In order to proove this theory, EGTA (15 µmoles per 1 g homogenate) was added to salted and unsalted ground prerigor sternomandibularis muscle of bulls. Again, for each temperature studied the samples were obtained from another animal. Addition of EGTA to the salted tissue caused a considerable decrease in the rate of ATP breakdown to inosine compounds (R value) in the temperature range measured (Fig. 4). At temperatures above +4°C the rate of ATP breakdown in the salted samples with added EGTA was about the same as in the control samples without NaCl. No significant effect of EGTA on the rate of lactate formation in the salted ground muscle tissue could be observed in this temperature range.

These results can be regarded as an convincing evidence for the release of calcium ions by addition of NaCl to the prerigor muscle and for the effect of the released Ca^{2+} on the breakdown of ATP.

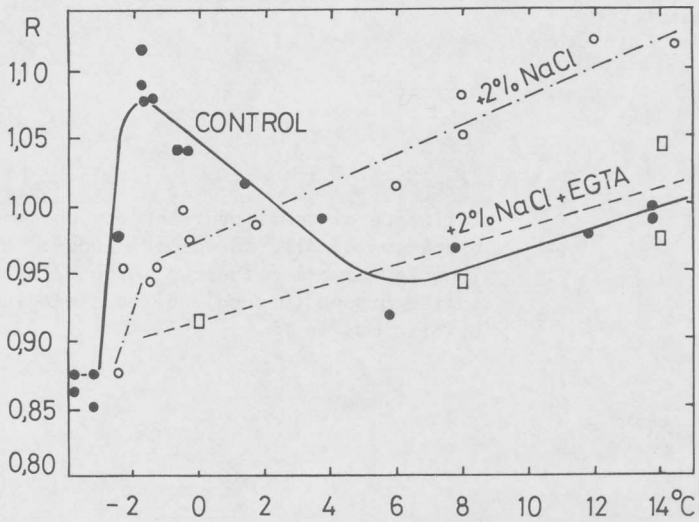

Fig. 4:

Effect of temperature on the postmortem breakdown of ATP ("R value") in homogenates of salted bovine sternomandibularis muscle as influenced by EGTA (15 μmoles/g homogenate. Control: homogenates of unsalted muscle).

As it was shown in this paper, between 0° and -3°C the rate of breakdown of ATP and glycogen (and of the decline in pH) is slower in the salted than in the unsalted beef. This is of practical interest because during freezing the meat remains for a relatively long period in this temperature range.

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