POSTMORTEM BREAKDOWN OF ATP AND GLYCOGEN IN GROUND MUSCLE: A REVIEW

R. HAMM

Department of Chemistry and Physics, Federal Institute of Meat Research, Kulmbach, Federal Republic of Germany

(Received: 15 February, 1976)

SUMMARY

The postmortem metabolism of ATP and glycogen in ground bovine muscle (in some cases also in rabbit muscle) was studied.

At pH 7 protons released during postmortem glycolysis are bound by the phosphorylation of ADP to ATP and are liberated during enzymic hydrolysis of ATP. At lower pH values the protons are immediately released during glycolysis. Not more than 10% of the total drop in pH postmortem is due to protons liberated by the hydrolysis of ATP present in the tissue at death. Half of the buffering capacity of bovine muscle is caused by the myofibrillar proteins.

The myofibrillar ATPases, rather than the membrane ATPases, seem to predominate in hydrolysing ATP postmortem in the intact as well as in the ground muscle. The rate of the breakdown of ATP determines the rate of postmortem glycolysis. Phosphofructokinase and, to a lesser extent, phosphorylase play the major role in the control of glycolytic metabolite levels in ground muscle. The mean values of glycogen recovery, obtained by measuring all glycolytic metabolites and lactate, indicate a general stoichiometric relationship in ground tissue, although several muscles did not fit in the scheme.

Grinding of the prerigor muscle causes an accelerated hydrolysis of ATP and ADP, resulting in a faster increase in the IMP concentration and in an accelerated glycolysis. The increase in the turnover of ATP by grinding might be due to a faster release of Ca^{++} ions from the damaged sarcoplasmic reticulum.

Addition of sodium chloride (2–4%) to the ground prerigor muscle causes an increase in the rate of the breakdown of ATP to IMP. NaCl changes the steady-state of glycolysis without a major change in the rate of glycogen breakdown. The stimulation of phosphofructokinase observed is probably due to the faster disappearance of ATP in the presence of NaCl. The faster turnover of ATP could be due to an enhanced release of Ca^{++} ions from the sarcoplasmic reticulum by exchange against Na^{+}.

Meat Science (1) (1977)—
Printed in Great Britain

After several hours postmortem an inhibition of glycolysis occurs in the salted tissue which is probably due to a denaturation of glycolytic enzymes by the combined effect of low pH (<6) and high ionic strength. The high water-holding capacity of prerigor salted ground beef does not decrease postmortem in spite of the high rate of ATP breakdown. This effect can be explained by an inhibition of rigor mortis in the fibre fragments caused by the combined effect of ATP, high pH and salt ions.

Addition of diphosphate to the prerigor ground tissue in the absence or presence of added NaCl results in an acceleration of ATP and glycogen breakdown. A hypothesis for the high ATP turnover is discussed. The higher rate of glycogen breakdown in the presence of diphosphate is caused mainly by an acceleration of the phosphofructokinase step. A rate limitation, by diphosphate, in the glyceraldehyde-3-phosphate dehydrogenase step and/or the following glycolytic steps was also observed.

1. INTRODUCTION

The biochemical reactions which occur in intact muscles of meat animals after death have been studied in many investigations, but few authors have looked at the postmortem changes in ground muscle. Postmortem changes in muscle, minced in the prerigor state, are of increasing practical interest since processing of meat immediately after slaughter, *i.e.* without intermediate refrigeration, is of economical advantage. Processing of beef before the onset of rigor mortis provides an excellent quality in sausages made from such meat (Hamm, 1972, 1973). Muscle in the prerigor state has a high WHC,* and better fat emulsification properties than muscle in the rigor or postrigor states, thus producing a sausage product with reduced moisture loss and less rendering out of the fat when cooked. These superior processing properties of prerigor muscle are directly related to its high level of ATP which results in a more relaxed state and greater myofibrillar hydration and solubility (Hamm, 1972). These properties, favourable for meat processing, are lost during the development of rigor mortis but can be retained for several days by salting the ground prerigor muscle, or for several months by freezing the prerigor ground muscle in the absence or presence of salt (Hamm, 1972, 1973, 1975*b*), or even for years by lyophilisation of the prerigor salted ground meat (Hamm & Potthast, 1975).

Much of the research work on postmortem changes in prerigor ground meat has been done during recent years in our laboratory, and published in German. This

* *Abbreviations.* ATP: adenosine triphosphate; ADP: adenosine diphosphate; AMP: adenosine monophosphate; IMP: inosine monophosphate; G-1-P: glucose-1-phosphate; G-6-P: glucose-6-phosphate; F-6-P: fructose-6-phosphate; FDP: fructose-1,6-diphosphate; DAP: dihydroxyacetone phosphate; GPA: glyceraldehyde-3-phosphate; 3-PGA: 3-phosphoglycerate; 2-PGA: 2-phosphoglycerate; PEP: phosphoenolpyruvate; P_i: inorganic phosphate; PFK: phosphofructokinase; GAPDH: glyceraldehyde-3-phosphate dehydrogenase; PK: pyruvate kinase; LDH: lactate dehydrogenase; p.m.: postmortem; SR: sarcoplasmic reticulum; WHC: water-holding capacity.

paper presents a review of these investigations. It does not include our work on postmortem changes during freezing and thawing of ground prerigor muscle (Van Hoof & Hamm, 1971; Dalrymple & Hamm, 1975*b*), and our studies on the influence of water activity on postmortem changes in ground muscle, freeze-dried in the prerigor state (Hamm *et al.*, 1974; Potthast, 1972; Potthast *et al.*, 1975, 1976). This research will be discussed later in a review of postmortem changes in meat at low temperatures.

The regulatory enzymes which control ATP metabolism and glycolysis in the living tissue are still active in the muscle postmortem, but these enzymic mechanisms are not able to maintain the ante-mortem levels of ATP and glycogen because the oxygen supply of the cell is stopped as soon as the blood circulation is interrupted by death of the animal. The lack of the aerobic ATP synthesis from ADP in the muscle mitochondria results in an anaerobic depletion of glycogen and consequently in a disappearance of ATP within a few hours p.m. In this respect, there is no major difference between intact and ground tissue but only a difference in the rate of these postmortem changes, as demonstrated by the results discussed in this review. Apparently soon after death the permeability of the cell membranes is changed and the membrane potentials are abolished. So, destruction of the membranes by mincing cannot influence the pattern but only the rate of postmortem metabolism. We also studied the influence of sodium chloride and diphosphate (pyrophosphate) on postmortem changes in ground muscle with regard to the prerigor processing procedures mentioned above. For a better understanding of postmortem ATP and glycogen metabolism, as well as of changes in pH and buffer capacity p.m., these topics of general interest are discussed on the basis of our results, in the first sections of this review.

2. MATERIALS AND METHODS

2.1. *Material*

Most of our experiments were carried out with bovine *longissimus dorsi* muscle, in some cases also with bovine *sternomandibularis* muscle; and with red and white muscles of rabbits. Muscles were obtained about an hour after death, trimmed of external fat and connective tissue, ground through an electric meat grinder (once through a plate with 4·5-mm or 6-mm holes) and stored at 2–4°C.

2.2. *Determination of ATP and its metabolites*

In the perchloric acid extract of the tissue, ATP, ADP, AMP, IMP, inosine and hypoxanthine were determined by the TLC procedure of Potthast & Hamm (1969) (Potthast, 1974). In this method, ATP and its metabolites are well separated on silica gel HF_{254} using a solvent mixture of ethylene glycol monoethyl ether–isoamyl alcohol–octyl alcohol–NH_3–water (70:30:10:45:30). The quantitative evaluation

of the chromatogram is carried out directly from the TLC plate using spectrofluorimetry or UV spectrophotometry. The ATP values obtained by this method were occasionally controlled by the enzymic assay of ATP (*cf.* Bergmeyer, 1972). The agreement between both methods was always excellent (Potthast & Hamm, 1969; Hamm & Van Hoof, 1971); and also with salted tissue (Van Hoof & Hamm, 1973*a*). The accuracy of the results obtained by the TLC procedure can be readily checked in each analysis because the metabolites mentioned above are formed in the postmortem muscle almost completely by the breakdown of ATP. Thus, the sum of the molar concentrations of all ATP metabolites, including ATP, must be the same at all times p.m.; if this is not the case, an experimental error exists. During storage of intact muscle tissue for 72 hours p.m., in samples taken at nine different times p.m., an average of ATP plus metabolites of 100·0 ± 2·4% of the value immediately after death was found. In a corresponding storage experiment with ground muscle tissue, an average of 99·9 ± 4·4% was found (Hamm & Van Hoof, 1971). These results clearly show that the TLC procedure of Potthast and Hamm is a reliable method for studying the complete postmortem breakdown of ATP to hypoxanthine.

2.3. *Determination of glycogen and its metabolites*

In some of our work (papers with Van Hoof as co-author), glycogen was determined by means of the method of Potthast (Potthast, 1972; Potthast *et al.*, 1976). In this procedure the tissue is digested by 30% KOH at about 100°C. The glycogen is precipitated by ethanol and hydrolysed to glucose by 2N H_2SO_4. In this hydrolysate the glucose is determined enzymically.

In the work with Dalrymple a method for the extraction of glycogen and its metabolites from a single meat sample was used (Dalrymple & Hamm, 1973). Perchloric acid homogenates of muscle are mixed with amyloglucosidase which extracts the glycogen and reduces it to glucose. The glucose is then analysed with specific enzymes. Compared with the KOH-ethanol glycogen extraction method the amyloglucosidase method indicates higher levels of glycogen which are attributed to protein-bound glycogen and ethanol soluble carbohydrate not determined by the KOH-ethanol method (Dalrymple & Hamm, 1973). The use of perchloric acid homogenates allows the determination of metabolites in the protein-free extract of the same homogenate. The metabolites glucose, G-1-P, G-6-P, F-6-P, FDP, GAP, DAP, 3-PGA, PEP, pyruvate and lactate were analysed according to procedures in Bergmeyer (1972).

3. THE REASON FOR THE DROP IN pH OF MUSCLE POSTMORTEM

The drop of pH in muscle postmortem from ≥7 to about 5·5 is usually explained by the glycolytic breakdown of one glucose moiety of glycogen to two molecules

of lactic acid according to reaction (1):

$$C_6H_{10}O_5 + H_2O \rightarrow 2\text{lactate}^- + 2H^+ \quad (1)$$

Of course, this net formula does not explain in which of the numerous steps of glycolysis the protons are released. Furthermore, the hydrolysis of the ATP present in the tissue at the time of death also could contribute to the increase in hydrogen ions in meat p.m. Therefore, the question arises which postmortem reactions are responsible for the decrease of pH.

In order to answer this question, the complete reaction chains of glycolysis and ATP metabolism p.m., shown in Fig. 1 and Table 1, have to be considered.

As Fig. 1 shows, protons are released within the glycolytic chain before the formation of lactate from pyruvate. However, at pH values around 7, these protons are bound during the phosphorylation of ADP to ATP (reaction (4), Table 1).

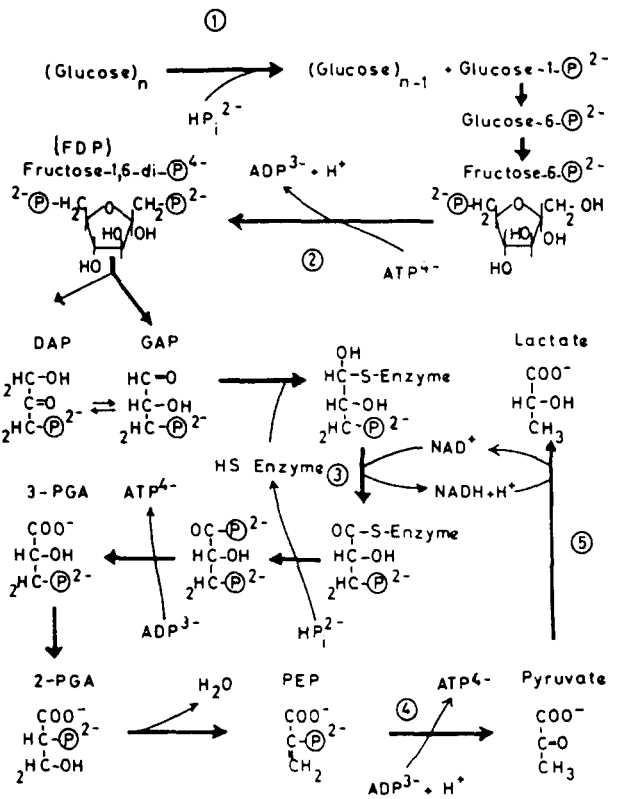


Fig. 1. Scheme of glycolysis in the pH range 7·0–7·5 with reference to the release and uptake of protons (Honikel & Hamm, 1974*a*). Ⓟ $^{2-}$ = —O—PO_3^{2-}.

Therefore, in net effect, no protons are released during the glycolysis itself (reaction (2)):

$$C_6H_{10}O_5 + 3HPO_4^{2-} + 3MgADP^- + H^+ \rightarrow 2\,\text{lactate}^- + 3MgATP^{2-} + 2H_2O \quad \text{(see footnote to Table 1).} \qquad (2)$$

The protons released during glycolysis in muscle p.m. at pH 7 do not originate from the glycolytic chain itself; they are formed by the enzymic hydrolysis of ATP (reaction (4)). By summing reactions (2) and (4) the net reaction (1) is obtained.

TABLE 1

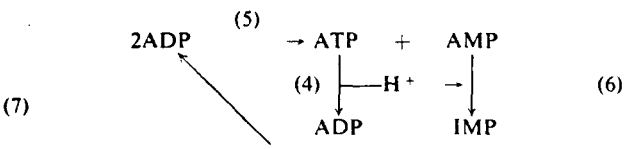
POSTMORTEM METABOLISM OF CP AND ATP IN MUSCLE AT pH 7*

$$H^+ + CP^{2-} + MgADP^- \leftrightharpoons \text{creatine} + MgATP^{2-} \qquad (3)$$

$$MgATP^{2-} + H_2O \leftrightharpoons MgADP^- + P_i^{2-} + H^+ \qquad (4)$$

$$2MgADP^- \rightarrow MgATP^{2-} + AMP^{2-} + Mg^{2+} \qquad (5)$$

$$AMP^{2-} + H^+ + H_2O \rightarrow IMP^{2-} + NH_4^+ \qquad (6)$$



* At pH 7, the exact negative charges of AMP, IMP and P_i are a little lower than two (Scopes, 1971). But whole numbers have been used rather than fractions for indicating the charges. The small error involved does not influence the correctness of the conclusions drawn in the text.

The further metabolism of ADP to IMP p.m., including the action of the myokinase reaction (5) and deamination (6), does not result in a net change of proton concentration at pH 7 ((7), Table 1).

Apart from glycolysis, the enzymic hydrolysis of the ATP present in muscle at the time of death also contributes to the release of protons p.m. at pH 7 (reaction (4)). The formation of ATP from CP and ADP present at the time of death (reaction (3)), and the hydrolysis of this ATP (reaction (4)), result in a net change of protons of zero.

On the basis of these facts and of the normal concentrations of CP, ATP and glycogen in the living beef *longissimus dorsi* muscle, Honikel & Hamm (1974*a*) calculated the amount of protons released by glycolysis and ATP metabolism p.m. at pH 7 (Table 2). More than 90% of the release of protons at this pH is due to glycolysis. The remaining protons originate from the hydrolysis of ATP present at the time of death.

During the breakdown of glycogen and ATP p.m. the pH drops from 7 to about 5.5. Between pH 5 and 6 ADP does not bind protons during phosphorylation and, on the other hand, protons are not released during ATP hydrolysis:

$$MgADP^- + H_2PO_4^- \rightleftharpoons MgATP^{2-} + H_2O \qquad (8)$$

TABLE 2

RELEASE AND UPTAKE OF PROTONS IN MUSCLE TISSUE POSTMORTEM AT pH 7 (Honikel & Hamm, 1974*a*)

*μmoles/g tissue at the time of death**	*Reaction*	*Release (+) or uptake (−) of protons μmoles H^+/g*
20 CP	Formation of ATP from CP and ADP [3]	−20
	Hydrolysis of ATP formed by [3] ([4])	+20
6 ATP	Hydrolysis of ATP present in the tissue at death [4]	− 6
40 glucose units	Glycolysis (breakdown of glycogen to lactate) [1]	−80
—	Breakdown of ADP to IMP [7]	± 0
	Sum	+86†

* Normal concentration in bovine *longissimus* muscle.
† At pH 7, the actual value might be somewhat lower because of the small error caused by the use of whole figures for expressing the charges (*cf.* footnote to Table 1).

In consideration of this fact and of some changes in the release and binding of protons in the glycolytic chain at pH ≤ 6 (Honikel & Hamm, 1974*a*), the net reaction of glycolysis is changed from (2) to (9):

$$C_6H_{10}O_5 + 3MgADP^- + 3H_2PO_4^- \rightarrow 2\,lactate^- + 3MgATP^{2-} + 2H^+ + 2H_2O \quad (9)$$

Thus the protons are released during glycolysis itself and not by ATP hydrolysis. Between pH 5·5 and 6 the reactions (7) result in the binding of protons because protons are not released by ATP hydrolysis ((4) becomes (8)). Consequently, at pH values ≤ 6 glycolysis by itself contributes to the drop of pH p.m. even more than at pH 7 (Honikel & Hamm, 1974*a*).

In agreement with these considerations it was found that under certain conditions in the presence of high concentrations of Ca^{++}, which inhibit postmortem glycolysis in rabbit muscle but accelerate ATP breakdown strongly, a marked drop of muscle pH does not occur (Honikel & Hamm, 1974*a*).

4. ON THE BUFFERING CAPACITY OF MEAT AND ITS CHANGES POSTMORTEM

If the pH value of muscle tissue at different times p.m. is plotted against the corresponding lactate value, usually a linear curve is obtained. Mincing of the tissue does not significantly change this curve (Fig. 2). It is by no means self-evident that the relationship between the linear magnitude of lactate concentration and the logarithmic magnitude of pH is linear. Actually, the pH–lactate curve between pH 7 and 5·5 represents the almost linear medium part of the S-shaped titration curve of muscle tissue (Honikel & Hamm, 1974*b*). Shape and position of this curve are determined by the buffering capacity of the tissue.

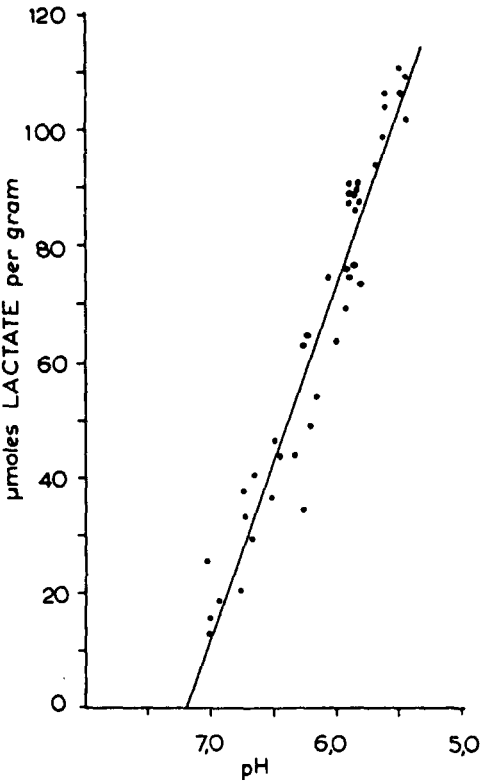


Fig. 2. Relationship between the formation of lactate and the drop of pH in minced bovine muscle postmortem (Hamm & Van Hoof, 1971).

Many years ago Bate-Smith (1938) tried to determine the contribution of several tissue components to the buffering capacity (B.C.) of meat postrigor. He found that, in addition to certain phosphate compounds, the dipeptides carnosine and anserine, as well as proteins, contribute essentially to the B.C. of meat. Hamm (1958) also pointed out the importance of the muscle proteins for the B.C. of meat; he found differences in B.C. between prerigor and postrigor tissue. In spite of these findings, the importance of the muscle proteins for the total B.C. of meat was still underestimated (*e.g.* Bendall, 1973*a*).

More recently, Honikel & Hamm (1974*b*) investigated the influence of different buffering substances present in muscle on the B.C. of meat immediately after slaughter and 4 days p.m. Some of the results are listed in Table 3. Soluble non-protein compounds such as nucleotides, glucose-6-phosphate, l-glycero-phosphate, inorganic phosphate, carnosine, anserine, lactate and hydrogen carbonate account for half of the B.C. of the fresh muscle (pH 7). The other half of the B.C. is due to

the myofibrillar proteins. Four days p.m. (pH 5·6) the contribution of the myofibrillar proteins to the total B.C. is even higher; at this pH the non-protein compounds have a lower, the myofibrillar a slightly higher, B.C. than at pH 7.

Honikel & Hamm (1974*b*) found a very good agreement between the B.C. data calculated from the concentration and pK values of muscle constituents, and the data calculated from the titration of tissue homogenates and extracts. These results demonstrate the great importance of myofibrillar proteins for the buffering capacity of meat.

TABLE 3

CONTRIBUTION OF THE DIFFERENT FRACTIONS OF MUSCLE TISSUE (BOVINE *L. dorsi*) TO THE TOTAL BUFFERING CAPACITY OF TISSUE (Honikel & Hamm, 1974*b*)

Fraction	*pH*	*Days p.m.*	*Buffering capacity* μ*moles* H^+/*g tissue*/*pH*	*Percent of the total buffering capacity*
Total tissue	7	0	48	100
	6·0	0	47	100
	5.5	4	42	100
Myofibrils	7	0	21·5	45
	6·0	0	18·5	39
	5·5	4	24	57
Soluble proteins	7	0	3·5	7
	6·0	0	5·5	12
	5·5	4	5	12
Protein-free extract	7	0	23	48
	6·0	0	23	49
	5·5	4	13	31

5. BREAKDOWN OF ATP AND GLYCOGEN IN GROUND MUSCLE POSTMORTEM: GENERAL ASPECTS

5.1. *Loss of ATP*

The rate and the extent of the breakdown of ATP in muscle p.m., which is of importance for the quality of meat, is determined by the activity of the tissue ATPases and by the formation of ATP from ADP during glycolysis (Fig. 1). Scopes (1971) concluded from experiments with model systems that the rate of the ATP loss in bovine muscle p.m. is primarily due to the activity of the AMP deaminase (5'-adenylate aminohydrolase) (reaction (6)). In all of our experiments, changes in the rate of ATP loss were accompanied by a corresponding change in the rate of formation of IMP, the AMP concentration being relatively constant (Hamm & Van Hoof, 1971, 1974; Van Hoof & Hamm, 1973*a*, *b*). Changes in ADP concentration p.m. can be caused by the hydrolysis of ATP to ADP (reaction (4)) and/or by the use of ADP for ATP synthesis either by glycolysis (Fig. 1), by the myokinase reaction (5) or by the creatine phosphokinase reaction (3) (*see* Section 5.3). In our experiments, the disappearance of ADP during the first 12 hours p.m. occurred faster the higher the rate of the ATP breakdown. In all cases, however, ADP was

not completely metabolised. After 24 hours p.m. the level of ADP remained constant at about 0·3 to 0·8 μmoles/g wet tissue (Hamm & Van Hoof, 1971, 1974; Van Hoof & Hamm, 1973*a*, *b*). This was also observed by other authors (Newbold & Scopes, 1967; Valin & Charpentier, 1969). Valin & Charpentier (1969) showed that this remaining ADP is completely bound to myofibrillar proteins. The question as to whether this fraction of ADP participates in the binding between actin and myosin at the rigor state of myofibrils is not yet answered.

The enzymic dephosphorylation of IMP p.m. to inosine and then to hypoxanthine occurs at a very low rate. In bovine *longissimus dorsi* muscle, changes in the rate of ATP breakdown caused by different influences had only a slight effect on the rate of hypoxanthine formation within 72 hours p.m. (Hamm & Van Hoof, 1971; Van Hoof & Hamm, 1973*a*, *b*).

5.2. *Type of ATPase effective postmortem*

Several ATPases, hydrolysing ATP according to reaction (4), exist in the muscle cell, *e.g.* in the membranes of sarcoplasmic reticulum, mitochondria, sarcolemma, etc., and in the myofibrils (*cf.* Greaser *et al.*, 1969; Van Hoof & Hamm, 1973*a*). The question arises which of these ATPases are responsible for the ATP breakdown p.m. Hamm *et al.* (1973) had tried a new approach to this problem. Thin slices of rabbit skeletal muscle were incubated in different media immediately after death. K^+- and Na^+-ions in concentrations of 0·1 and 0·15M did not increase the breakdown of ATP. Ouabain, an inhibitor for the Na^+, K^+-stimulated membrane ATPase, did not influence the rate of ATP breakdown. Oligomycin, which influences the mitochondrial ATP metabolism, had no significant effect, either. The incubation in 3×10^{-3}M quinidine sulphate had also no effect. This substance inhibits the sarcoplasmic reticulum ATPase, as the authors demonstrated with isolated sarcoplasmic reticulum preparations. 0·03–0·05M $CaCl_2$ showed a strong stimulating effect on the ATP breakdown at the beginning of incubation (*see also* Table 4, Nos 7 and 8). The stimulating effect of 0·05 $MgCl_2$, although not occurring immediately after death, increased strongly within a few hours p.m. (Dalrymple & Hamm, 1974*b*; Hamm *et al.*, 1973). These effects of Ca^{++} and Mg^{++} can be explained by the transition from the Ca^{++}-activated myosin ATPase to the Mg^{++}-activated actomyosin ATPase during the development of rigor mortis.

Hamm *et al.* (1973) concluded from these results that the myofibrillar ATPase rather than membrane ATPase or sarcoplasmic reticulum ATPase predominates in hydrolysing ATP in skeletal muscle p.m. In these experiments, however, the real turnover of ATP (*see* Section 5.3) was not known because the degree of glycolysis occurring at the same time was not determined.

These experiments were thus repeated in our laboratory.

5.3. *Turnover of ATP; change postmortem*

The rate of the breakdown of ATP p.m., measured by the decrease in ATP

concentration in the tissue, does not necessarily reflect the degree of the ATP turnover in muscle and is, therefore, no measure of the ATPase activity of the tissue. A constant level of ATP, for instance, can be due either to a complete inhibition of ATPase or to a fast ATP turnover which restores the hydrolysed ATP via glycolysis by phosphorylation of ADP to ATP (Fig. 1). The latter case is given, for example, in the process of 'cold shortening' (Bendall, 1973*b*). The actual ATPase activity p.m. can be approximately evaluated by the calculation of the ATP turnover from the decrease in ATP concentration in the tissue (Δ[ATP]) and the increase in lactate whereby the appearance of one mole lactate corresponds to the formation of 1·5-mole ATP (reaction (2)):

$$\text{ATP turnover} = [\text{ATP hydrolysed}] = \Delta[\text{ATP}] + 1{\cdot}5\Delta\,[\text{lactate}] \qquad (10)$$

In our experiments, discussed in this review, the formation of ATP from CP and ADP (creatine phosphokinase reaction (3)) did not require to be taken into consideration because at the beginning of the postmortem experiments—mostly 1 to 2 hours p.m.—CP was no longer present in the tissue.

Within the first three hours, we observed an ATP turnover between 0·15 (intact tissue) and 0·48 μmoles ATP $\cdot$ min^{-1} $\cdot$ g^{-1} (ground tissue) (Table 4, Nos 1, 2, 3, 5, 6, 7). These values are similar to the rate with which the 'non-contractile' myosin ATPase splits ATP in muscle at 25°C ($\sim$0·37 μmoles $\cdot$ min^{-1} $\cdot$ g^{-1}: Newbold & Scopes, 1971*a*; Bendall, 1973*a*).

Between 3 and 5 hours p.m. the turnover of ATP was much slower than between 0 and 3 hours, namely as low as 0·11–0·13 μmoles ATP $\cdot$ min^{-1} $\cdot$ g^{-1} (Table 4). This is not due to an inactivation of ATPases because ATP added to the minced postrigor muscle is split at a relatively high rate even at pH 5·5 (*see* below). The low rate of ATP hydrolysis after 3 hours p.m. is certainly due to the decrease in the concentration of substrates (ATP and glycogen). With decreasing ATP concentration, the turnover of ATP in minced muscle decreases (postrigor) (Hamm & Van Hoof, 1974).

It should be mentioned that sometimes we found bovine *longissimus dorsi* muscles with an unusually fast postmortem breakdown of ATP and glycogen, similar to that in PSE porcine muscle (Hamm & Van Hoof, 1970). The estimated postmortem turnover of ATP in intact muscles of this type is about 0·6 μmoles ATP $\cdot$ min^{-1} $\cdot$ g^{-1}, *i.e.* four times faster than in normal muscle.

After development of rigor mortis, the breakdown of ATP added to the ground bovine muscle in physiological concentration was about one hundred times faster than the postmortem breakdown of ATP present in the tissue at the time of death (Hamm & Van Hoof, 1974). The turnover of ATP at comparable ATP concentration and pH was ten times faster in the ground muscle postrigor than prerigor (Table 4, No. 9). According to Bendall (1973*a*), the 'contractile' ATPase (actomyosin ATPase) of bovine muscle splits ATP at 1°C with a rate of 1·3 μmol ATP $\cdot$ min^{-1} $\cdot$ g^{-1} (at 37°C: 180 μmoles $\cdot$ min^{-1} $\cdot$ g^{-1}). The value of about

TABLE 4

POSTMORTEM TURNOVER OF ATP IN BOVINE *longissimus* MUSCLE (EXCEPT SAMPLE NOS. 7 AND 8: RABBIT MUSCLE). STORAGE p.m. AT 4°C

No.	*State of tissue*	*Addition of*	*pH of tissue after hours p.m.*		*Turnover of ATP μmoles min⁻¹ g⁻¹ (according to eqn [10]) in the range of*		*Reference*
			3	6	0–3 *h p.m.*	3–6 *h p.m.*	
1	intact	—	6·7	6·5	0·15	0·13	Hamm & Van Hoof (1971)
2	ground	—	6·1	5·8	0·48	0·13	
3	ground	—	6·0	5·8	0·46	0·13	Van Hoof & Hamm (1973*a*)
4	ground	2% NaCl	6·1	5·8	0·52	0·11	
5	ground	—	6·6	5·8	0·46	0·13	Van Hoof & Hamm (1973*b*)
6	ground	—	—	—	0·43	0·11	Hamm & Van Hoof (1970)
7	slices	0·15M Tris	6·5	6·3	0·15	0·29	Honikel & Hamm (1974*a*)
8	slices	0·05M $CaCl_2$	5·9	5·7	0·27	0·12	

Addition of $5 \cdot 10^{-3}$M ATP to postrigor muscle

No.	*State of tissue*	*Addition of*	*pH before addition of ATP*	*ATP turnover within 5 min after addition of ATP*	*Reference*
9	ground	—	5·5	1·8	Hamm & Van Hoof (1974)
10	ground	—	6·9*	2·0	
11	ground	2% NaCl	5·5	0·9	
12	ground	2% NaCl	6·9*	1·5	

* pH adjusted by addition of NaOH.

2 μmoles ATP · min^{-1} · g^{-1} found by us in the postrigor muscle with $5 \cdot 10^{-3}$M added ATP at pH 6·9 and 10–15°C (Hamm & Van Hoof, 1974) seems to be very close to the activity of the 'contractile' ATPase. Thus, from these results also, it can be concluded that, during rigor mortis, the myosin ATPase is transformed into actomyosin ATPase and that this transformation governs the ATPase activity of the muscle tissue p.m.

5.4. *Glycolysis*

The enzymic hydrolysis of ATP initiates and controls the breakdown of glycogen in muscle tissue not only *in vivo* but also postmortem. Using model mixtures of substrates and enzymes, Scopes (1971) could demonstrate that glycolysis occurs only if ATP is dephosphorylysed. The physiological purpose of glycolysis is to maintain the ATP concentration in the muscle cell at a constant level. The rate of glycolysis p.m. is primarily determined by the rate of the hydrolysis of ATP (Bendall, 1973*a*) which influences mainly the phosphorylase (E.C. 2.7.1.1) reaction (Fig. 1, step ①) and the PFK (E.C. 2.7.1.11) reaction

(Fig. 1, step ②): decrease of the ATP level stimulates these reactions, and vice versa. Furthermore, high concentrations of ATP inhibit the PK (E.C. 2.7.1.40) step (Fig. 1, step ④).

This feedback interaction between ATP metabolism and glycolysis does not mean that the rate of glycolysis in muscle p.m. corresponds necessarily to the rate of ATP loss. The example of 'cold shortening' has already been mentioned. Another example is the influence of Ca^{++} ions on the postmortem changes in rabbit muscle slices observed by Honikel & Hamm (1974*a*). High Ca^{++} concentration (0·15M $CaCl_2$) caused an increase in the rate of ATP breakdown p.m. but a considerable inhibition of glycolysis. On the other hand, at an added concentration of 0·05M $CaCl_2$, the rate not only of the ATP loss increased but also of glycolysis (Table 5). It seems to be somewhat surprising that, at an ATP concentration of '0',

TABLE 5

INFLUENCE OF 0·05M $CaCl_2$ ON THE BREAKDOWN OF ATP AND GLYCOGEN IN RABBIT MUSCLE POSTMORTEM. SLICES OF THE *longissimus* MUSCLE WERE INCUBATED WITH (a) 0·17M NaCl + 0·15M TRIS BUFFER (pH 7·2); (b) 0·05M $CaCl_2$ + 0·03M TRIS BUFFER (pH 7·2) (Honikel & Hamm, 1974*a*)

Incubation medium	*Time p.m. h*	*ATP µmoles/g*	*Glycogen µmoles/g**
Not incubated†	0	4·2	78
0·17M NaCl	1	4·2	78
	3	2·8	67
	6	0·7	50
	24	0	34
0·05M $CaCl_2$	1	0·15	61
	3	0	52
	6	0	34
	24	0	25

* Expressed in glucose units.
† 25 min p.m.

glycogen is still broken down and lactate is formed (Table 5). Apparently, in this case the ATP synthesised from ADP by glycolysis is immediately hydrolysed at such a rate that the ATP level in the muscle cell remains at a very low, not detectable level. The turnover of ATP calculated by eqn (10) was greatly increased by the addition of 0·05M $CaCl_2$ (Table 4, Nos 7 and 8).

During freezing of ground muscle tissue prerigor, the ATP level remained almost constant but glycolysis occurred to a considerable extent (Dalrymple & Hamm, 1975*b*; Van Hoof & Hamm, 1971).

Dalrymple & Hamm (1975*a*) studied the postmortem glycolysis of prerigor ground bovine and rabbit muscle. In that investigation, the postmortem levels of all glycolytic metabolites, including glycogen and free glucose, were presented—probably for the first time. Glycogen, glucose, G-6-P, F-6-P and lactate make up more than 95% of the total glycogen metabolites in muscle at all times p.m.

Different muscles and species can have large varieties in glycolytic rate and also in metabolite levels. The cessation of normal glycolysis p.m. results from the inactivation of phosphorylase and PFK, as evidenced by the remaining glycogen and normal hexose phosphate levels. This inactivation results from limiting levels of adenosine nucleotides and/or low pH (Bendall, 1973*a*).

High and constant levels of G-6-P and F-6-P in comparison to the concentration of metabolites (except lactate), after the PFK step, demonstrate that PFK and, to a lesser extent, phosphorylase play the dominant role in the control of metabolite levels in ground muscle (Dalrymple & Hamm, 1975*a*). The maximum activities of each of the control enzymes and LDH in ground muscle are presented in Table 7. The steady state is readily seen.

The mean value of glycogen recovery as lactate, and as intermediates and lactate, indicated a general stoichiometric relationship in ground tissue, although in several muscles this was not the case, as indicated by the large standard deviation (Table 6).

TABLE 6

RECOVERY OF TOTAL METABOLITES, GLYCOGEN AS LACTATE, AND GLYCOGEN AS INTERMEDIATES PLUS LACTATE IN 22 GROUND SAMPLES OF BOVINE *longissimus dorsi* (8) AND *sternomandibularis* (10) AND RABBIT WHITE MUSCLE (4)
(Dalrymple & Hamm, 1975*a*)

Metabolites	*Concentration**			*Recovery* %
	Initial	*End*	*Change*	
Glycogen	39·7 ± 13·0	7·3 ± 6·6	−32·4 ± 9·4	—
Lactate	15·0 ± 7·7	44·4 ± 5·8	+29·6 ± 5·3	94·1 ± 24·6
Intermediates and lactate	27·0 ± 8·9	54·8 ± 6·4	+27·8 ± 5·8	89·1 ± 15·9
Total metabolites	66·7 ± 9·2	62·1 ± 8·1	− 4·6 ± 5·3	93·4 ± 7·5

* All concentrations are expressed in μmoles of glucose equivalents per gramme wet muscle.

Bendall (1973*b*) reported, for intact bovine muscle, a conversion of glycogen to lactate (after allowing for glucose production) of 95·8% ± 30%, a value which is very much in line with our results presented in Table 6. Although there was some variation, the large majority of samples demonstrated a stoichiometric sum of metabolites (Table 6), which is in agreement with the results by Bodwell *et al.* (1965), for the sum of glycogen, reducing sugars and lactate in the intact tissue of bovine *longissimus*.

6. INFLUENCE OF GRINDING OF BOVINE MUSCLE ON THE BREAKDOWN OF ATP AND GLYCOGEN POSTMORTEM

Grinding of the *longissimus dorsi* muscle within 1 hour p.m. caused an accelerated breakdown of ATP (and ADP), resulting in a faster increase in the IMP concentration (Hamm & Van Hoof, 1971) (Fig. 3). However, after 72 hours the same level

of IMP was found in the ground and in the intact muscle; in both cases ATP was completely hydrolysed. Soon after the disappearance of ATP the concentration of IMP reached a maximum, an effect which was observed also in other experiments (Hamm & Van Hoof, 1970; Van Hoof & Hamm, 1973*a*, *b*).

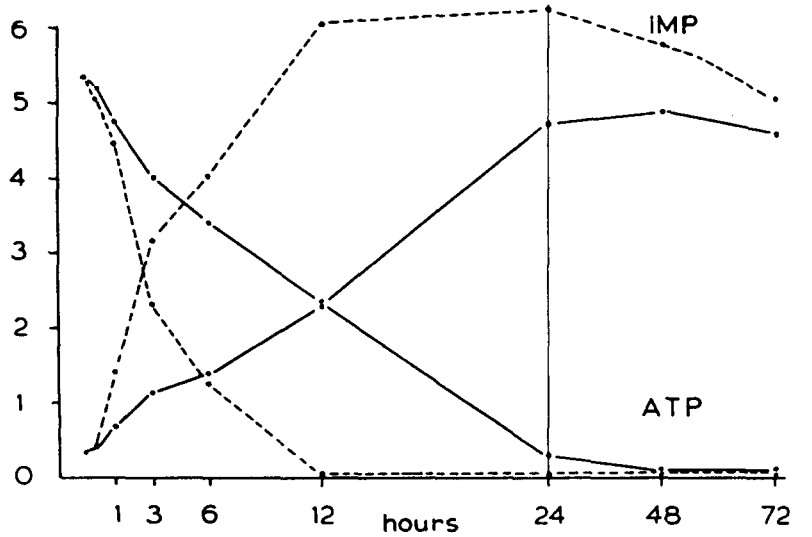


Fig. 3. Influence of grinding of bovine muscle on the breakdown of ATP to IMP postmortem (storage at 4°C). ●——●: intact muscle; ●---●: ground muscle. Ordinate: μmoles/g (Hamm & Van Hoof, 1971).

As was expected, the acceleration of the breakdown of ATP by grinding caused a faster glycolysis (Fig. 4) and a higher rate of the decline of pH. However, after 72 hours p.m. in the intact muscle, as well as in the ground tissue, the same ultimate values of glycogen, lactate and pH were found.

An acceleration of the decrease in ATP and pH (besides other metabolites) in bovine muscle by grinding was also observed by Newbold & Scopes (1971*a*).

The formation of 6·0 μmoles lactate/g wet tissue lowered the pH by 0·1 unit, a value which was not influenced by mincing and which is in agreement with the result of Newbold & Scopes (1967) obtained with intact bovine *sternomandibularis* muscle.

Under the conditions used (grinding once in a meat grinder, storage at 4°C), the grinding of bovine muscle caused a remarkable increase in the turnover of ATP within the first 3 hours p.m. (Table 4, Nos 1 and 2); this indicates an increase in ATPase activity on grinding. After 3 hours p.m., the velocity of the ATP turnover was about the same, in intact and minced tissue (Table 4, Nos 1 and 2). According to Hamm & Van Hoof (1971), the effect of grinding might be due to damage of the SR of the muscle cell, resulting in a release of Ca^{++} ions from the SR membrane

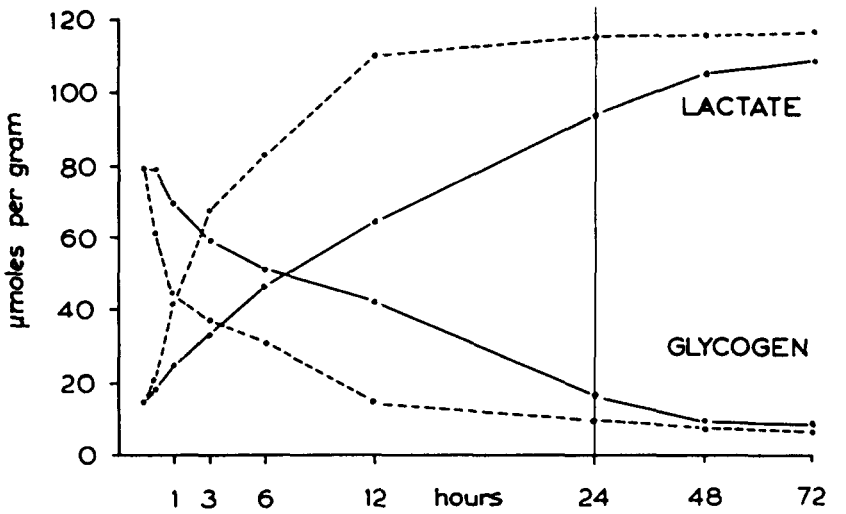


Fig. 4. Influence of grinding of bovine muscle on the breakdown of glycogen (expressed in glucose units) to lactate postmortem (storage at 4°C). ●——●: intact tissue; ●---●: ground tissue (Hamm & Van Hoof, 1971).

and causing an activation of the myosin ATPase. In the intact muscle, the Ca^{++} ions are also released from the SR, but at a lower rate; therefore after some hours p.m. the ATP turnover in intact and ground tissue is about the same.

A threefold increase in the postmortem rates of both glycogen breakdown and ATP loss by mincing was also observed with prerigor Atlantic cod (*Gadus morhua*) (Nowlan & Dyer, 1974). It should be mentioned that in fillets of carp (*Cyprinus carpio*) ATP is metabolised to hypoxanthine much faster than in the whole fish (Hamm & Masic, 1971).

7. THE INFLUENCE OF SODIUM CHLORIDE ON POSTMORTEM CHANGES IN GROUND BOVINE MUSCLE

It is of advantage to grind and salt beef for sausage production immediately after slaughter in order to use the high WHC of muscle tissue before the onset of rigor mortis. Beef has to be salted before much ATP and glycogen are broken down (Hamm, 1972). With regard to such processing procedures, the influence of NaCl on the breakdown of ATP and glycogen p.m. is of interest.

Addition of 2% NaCl to ground prerigor beef muscle caused an increase in the rate of the breakdown of ATP (and ADP) to IMP (Fig. 5) and a faster increase in the level of P_i. Increasing the NaCl concentration from 2 to 4% enhanced this effect (Van Hoof & Hamm, 1973*a*).

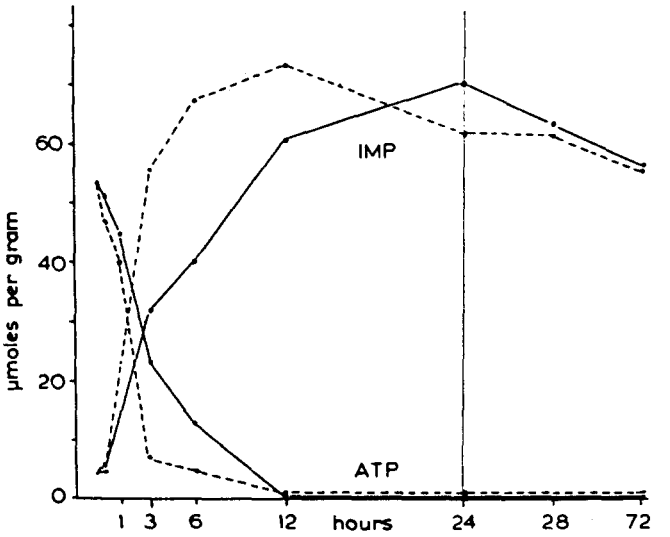


Fig. 5. Influence of added NaCl (2%) on the breakdown of ATP to IMP in ground bovine muscle postmortem (~15°C). ●——●: without NaCl; ●---●: with NaCl (Van Hoof & Hamm, 1973*a*).

Addition of 2% NaCl to ground prerigor beef muscle did not influence the rate of glycogen breakdown during the first hours p.m. but increased somewhat the rate of lactate build-up during this period (Fig. 6) (Van Hoof & Hamm, 1973*a*; Dalrymple & Hamm, 1974*a*).

Contrary to the non-salted tissue, the levels of hexose monophosphates (G-1-P, G-6-P and F-6-P) in the salted ground muscle decreased quickly immediately after addition of NaCl and remained at a low level until the end of glycolysis. In the non-salted ground beef the levels of the metabolites FDP, DAP and GAP continued to be very low during storage p.m.; addition of NaCl to the prerigor tissue resulted in an increase of these metabolites within the first hours p.m. The concentration of the metabolites 3-PG, 2-PG and PEP was not effected by NaCl. Dalrymple & Hamm (1974*a*) concluded from these results that the PFK activity during the first hours p.m. is higher in the presence of NaCl than in the absence of the salt. Thus, NaCl changes the steady state without a major change in the rate of glycogen breakdown. The stimulation of PFK activity is probably due to the faster disappearance of ATP in the presence of NaCl (Fig. 5).

The turnover of ATP in ground muscle during the first 3 hours p.m. is increased by the addition of NaCl (Table 4, Nos 3 and 4). The following influences could be responsible for the effect of NaCl on turnover and loss of ATP: (1) activation of membrane ATPases by Na^+ ions; (2) activation of 5'-adenylic acid aminohydrolase by NaCl; (3) enhanced release of Ca^{++} ions from the SR by exchange against Na^+.

After discussion of the literature, Van Hoof & Hamm (1973*a*) came to the conclusion that the last effect seems to be the most probable. This theory was supported by a later paper of Hamm & Van Hoof (1974) in which they showed that NaCl does not stimulate but inhibits the turnover of ATP added to ground postrigor muscle (Table 4, Nos 9–12). In this case, exchange of Na^+ against Ca^{++} cannot be of influence because the postmortem release of Ca^{++} ions from membrane systems will be completed; addition of NaCl also exerted the inhibiting effect observed with isolated myofibrillar ATPases in this range of ionic strength by many authors (literature, *cf.* Van Hoof & Hamm, 1973*a*).

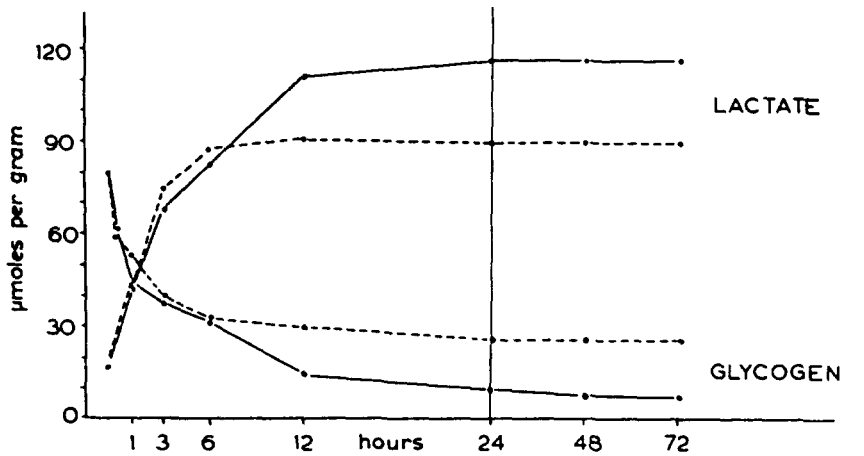


Fig. 6. Influence of added NaCl (2%) on the breakdown of glycogen (expressed in glucose units) to lactate in ground bovine muscle postmortem (~15°C). ●——●: without NaCl; ●---●: with NaCl (Dalrymple & Hamm, 1974*a*).

As mentioned above, addition of 2% NaCl had some stimulating effect on the build-up of lactate within the first hours p.m. After this period, however, NaCl inhibits the breakdown of glycogen and the formation of lactate (Van Hoof & Hamm, 1973*a*; Dalrymple & Hamm, 1974*a*) (Fig. 6). After 24 hours, an almost complete cessation of glycolysis occurred, the residual glycogen being as high as 30 μmoles glucose units/g tissue (Van Hoof & Hamm, 1973*a*). This effect was enhanced by increasing the NaCl concentration up to 4%.

The findings of Newbold & Lee (1965) and Newbold & Scopes (1971*a*) are comparable with these results. From their pH and ATP data it can be seen that by dilution of muscle homogenates with 0·15M KCl (1:1) lactate formation p.m. at first was not influenced: later it was inhibited; whereas KCl stimulated the breakdown of ATP. The authors concluded from their experiments, with and without added co-factors (G-1-P, FDP, glucose and hexokinase), that one of the first steps of glycolysis is blocked in the presence of KCl as soon as the pH falls to about 5·8.

Dalrymple & Hamm (1974*a*) suggested that the observed inhibition of glycloysis by NaCl, occurring after 6–9 hours p.m. (in ground beef), is due to the simultaneous effects of low pH and high ionic strength which cause a denaturation of enzyme proteins. The inhibition of glycolytic enzymes occurs in the presence of NaCl at a higher pH (about 5·8) than in the absence of NaCl (about 5·5).

In general, breakdown of ATP p.m. results in the development of rigor mortis which causes a marked decrease in WHC of meat (Hamm, 1972). Therefore, it is surprising that salting of ground meat prerigor prevents the drop of WHC, although ATP is split even at a higher rate (Hamm, 1972). It could be demonstrated by rheological measurements that the WHC in prerigor salted meat does not decrease because the onset of rigor mortis in the fibre fragments is prevented (Hamm & Rede, 1972; Hamm, 1975). This is probably due to a strong electrostatic repulsion between adjacent protein molecules caused by the initial combined effect of ATP, high pH and high ionic strength. Hamm & Van Hoof (1974) simulated exactly the same effect by the addition of ATP to postrigor ground bovine muscle in the absence and presence of 2% NaCl. At pH 6·9 breakdown of ATP resulted in a contraction of fibre fragments and in a decrease in WHC, provided that no NaCl was added. NaCl prevented the contraction of fibre fragments as well as the decrease in WHC. In the presence of NaCl, addition of ATP showed only 'softening' and strong hydrating effects on the myofibrillar systems, in spite of a complete enzymatic breakdown. These results support the concept that the combined effect of ATP, high pH and salt ions keeps the actin and myosin filaments so far apart that the breakdown of ATP cannot cause an association of filaments (Hamm, 1972).

It should be mentioned that the enzymic hydrolysis of ATP p.m. causes a decrease in the solubility of myofibrillar proteins not only in the absence but also in the presence of NaCl (Hamm, 1958). This observation was confirmed by Hamm & Grabowska (1976) by means of sephadex gel chromatography and SDS polyacrylamide gel electrophoresis. The fact that the protein solubility decreases p.m. in the presence of NaCl, although the high WHC and softness of myofibrillar fragments is not changed, shows that dissolution experiments do not reflect the real state of myofibrillar proteins *in situ*. Apparently, under certain conditions, actin and myosin cannot interact *in situ* but they may react with each other during the extraction process.

8. INFLUENCE OF DIPHOSPHATE (PYROPHOSPHATE) ON THE BREAKDOWN OF ATP AND GLYCOGEN IN GROUND BOVINE MUSCLE POSTMORTEM

The myofibrillar ATPases are activated by Ca^{++} and/or Mg^{++} ions. Van Hoof & Hamm (1973*b*) expected that addition of inorganic diphosphate (DP) to the ground prerigor muscle would inhibit ATP hydrolysis to a certain extent by sequestering the bivalent cations and by a competitive inhibition of the ATPases. Such an effect

should prevent rigor mortis in the fibre fragments and, therefore, the postmortem loss of WHC. But the results did not follow these expectations.

Beef was ground within 1 hour p.m. and salted with 2% NaCl with and without addition of 0·3, 0·5 and 1·0% sodium diphosphate. In other experiments, 0·5% DP were added without addition of NaCl. An addition of 0·5 and 1·0% DP resulted in an acceleration of the breakdown of ATP to IMP and inosine (Fig. 7). This effect

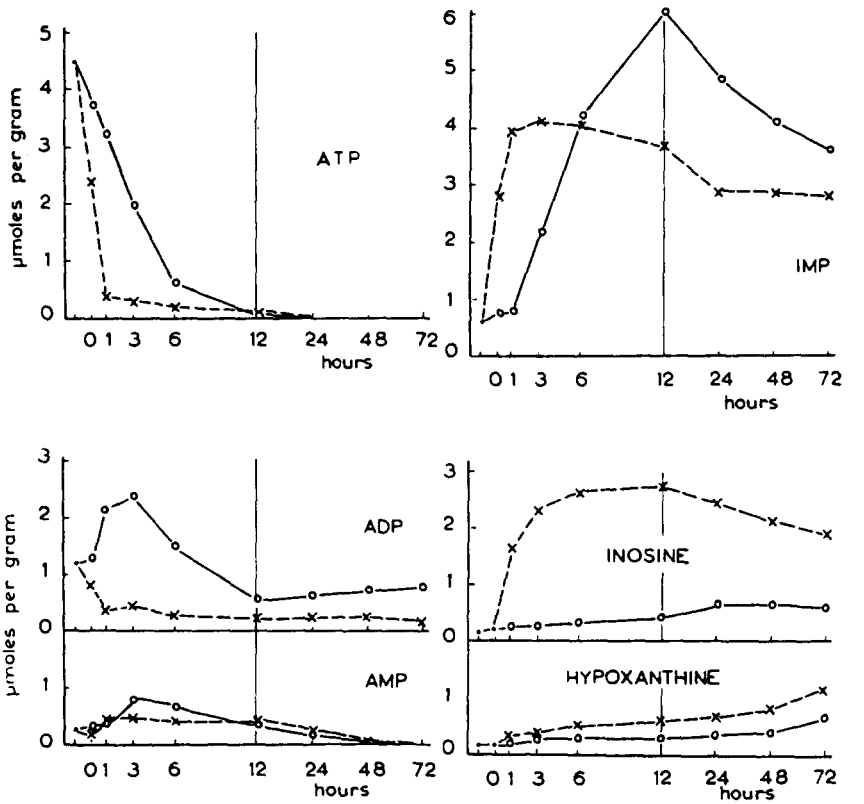


Fig. 7. Influence of sodium diphosphate added to ground bovine muscle in absence of NaCl on the levels of ATP, ADP, IMP, AMP, inosine and hypoxanthine in tissue at different times post-mortem. ○——○: no added DP; ×---×: 1·0% DP (Van Hoof & Hamm, 1973*b*).

of DP was virtually the same without and with added NaCl. 0·3% DP did not exert a marked effect (Van Hoof & Hamm, 1973*b*).

In the samples with added DP the rate of glycogen breakdown was higher than in the control sample (Fig. 8). In the DP samples the ultimate concentration of glycogen was lower (Van Hoof & Hamm, 1973*b*; Dalrymple & Hamm, 1974*b*). From the effect that DP increases not only the rate of the disappearance of ATP

but also the rate of breakdown of glycogen it can be concluded that DP enhances the turnover of ATP in ground bovine muscle.

Why does DP cause a stimulation but no inhibition of ATP hydrolysis p.m.? The molar concentrations of added DP exceeded by far the molar levels of free $[Ca^{++} + Mg^{++}]$ in bovine muscle. These are about 5·7mM at the time of death and about 7·5mM 48 hours p.m. (Hamm, 1962). The dissociation constant of CaDP is apparently not low enough to bring the $[Ca^{++}]$ down to a level below

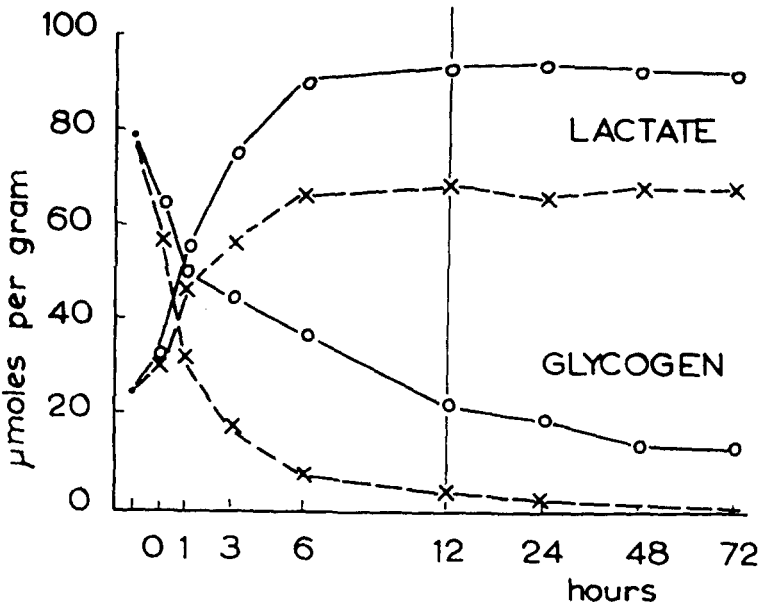


Fig. 8. Influence of sodium diphosphate added to ground bovine muscle in the absence of NaCl on the levels of glycogen (expressed in glucose units) and lactate in tissue at different times postmortem. ○——○: no added DP; ×---×: 1·0% DP (Van Hoof & Hamm, 1973*b*).

10^{-5}M which would be necessary for suppressing the activation of myosin ATPase by the Ca^{++} released postmortem from the SR. DP concentrations of 0·5 or 1·0% should be sufficient to cause a partial inhibition of the actomyosin ATPase but such an influence cannot become effective before actomyosin formation p.m. (*see* Section 5.2), and would not explain an acceleration of ATP hydrolysis during the first hour p.m. As mentioned above (*see* Section 7), NaCl increases the ATP breakdown in muscle p.m.; but the increase in the Na^+ concentration by the addition of 0·5–1·0% sodium diphosphate is too small to cause such an effect (Van Hoof & Hamm, 1973*b*).

Van Hoof & Hamm (1973*b*) suggested an explanation of the effect of DP on ATP hydrolysis which is based on the results of E. W. Taylor's group (Finlaysen &

Taylor, 1969; Taylor *et al.*, 1970). Mg^{++} ions inhibit myosin ATPase in the absence of actin by stabilising the enzyme-product (=ADP) complex (EP). The activating effect of DP could be due to binding of Mg^{++} to DP, causing a loss of stability of EP. Therefore, the rate limiting step III in the following reaction would be accelerated:

$$E + S \underset{}{\overset{I}{\rightleftharpoons}} ES \xrightarrow{II} EP \xrightarrow{III} E + P \quad (E = \text{enzyme}; S = \text{substrate}; P = \text{product})$$

A number of facts which support this theory were discussed by Van Hoof & Hamm (1973*b*). But it is still uncertain why DP does not inhibit reaction (1).

It is not likely that the stimulation of ATP hydrolysis by DP is primarily due to an activation of 5'-adenylate aminohydrolase (*see* Section 5.1) because DP tends to inhibit this enzyme. Their results led Van Hoof & Hamm (1973*b*) to the opinion that the rate of the breakdown of ADP to inosine is determined by the rate of ATP breakdown.

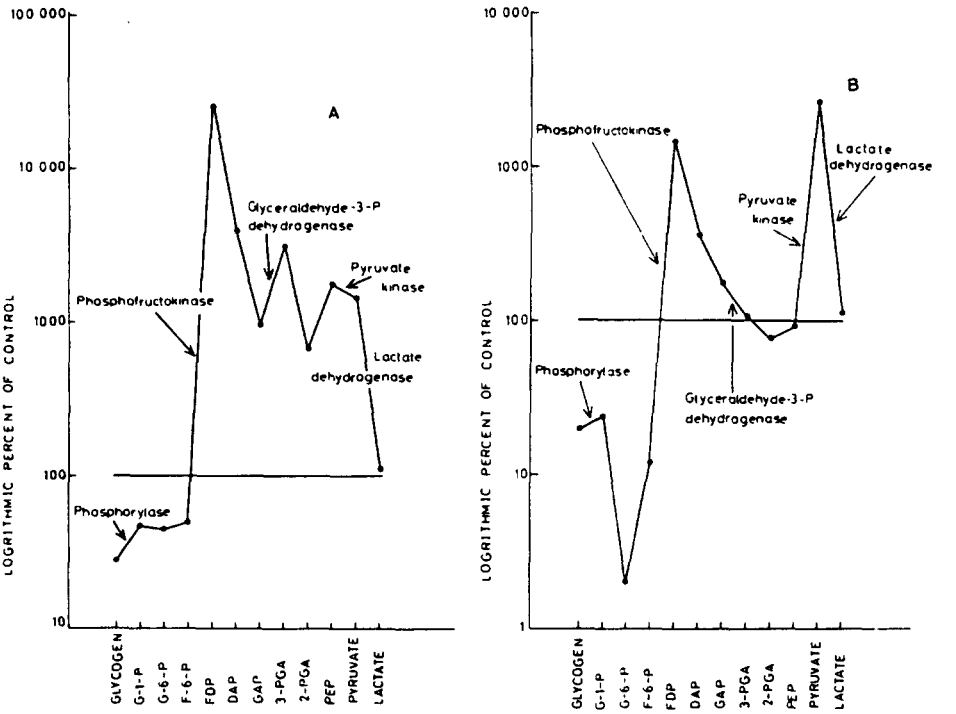


Fig. 9. 'Crossover plot' comparing the concentration of glycolytic metabolites in bovine *sternomandibularis* muscle samples 1 h (A) and 24 h (B) after mixing with either distilled water or DP solution (1·0% DP in the mixture). Control values (distilled water) are given as 100%. The values for the DP samples are shown as their percent of the control value. Percent of control scale is logarithmic. Each point represents the mean of three samples (Dalrymple & Hamm, 1974*b*).

The addition of DP to ground prerigor muscle has a very pronounced effect on glycolytic metabolites. Figure 9 shows the 'cross over plot' for the samples 1 and 48 hours p.m., which gives the percent changes in the metabolite concentrations of the DP samples as compared to the control samples whose metabolite concentrations are designated as 100%. A control site can be identified by a crossover in the metabolic pattern between a relative depletion and a relative accumulation of intermediates, or vice versa.

Dalrymple & Hamm (1974*b*) interpreted their results as follows. The lowering of the ATP level, which takes place very rapidly with added DP (Fig. 7), causes a release of the ATP inhibition of PFK (*cf.* Fig. 1, step ②), thus resulting in greatly increased PFK activity in these samples (Fig. 9). Since P_i is also a known activator of PFK, the activation of PFK could be partially due to P_i because the activity of the tissue DPase (E.C. 3.6.1) is high enough to form sufficient P_i from the added DP (Neraal & Hamm, 1973) to cause this effect within the first hour after DP addition. The build-up of FDP, due to P_i and ATP activation of PFK and rate limitation of GAPDH (Fig. 9) (*cf.* Fig. 1, step ③), is further enhanced because FDP is a powerful activator of PFK. Thus the increase in PFK activity becomes autocatalytic in nature. Addition of DP causes some blockage of flux at the LDH step (Fig. 9) (*cf.* Fig. 1, step ⑤). Thus, in some samples, there was a much lower amount of lactate build-up in spite of a greater glycogen disappearance (Van Hoof & Hamm, 1973*b*). The necessary NAD^+ required to reduce the blockage at GAPDH is not produced fast enough at the LDH step, due to some rate limitation also at that step. This blockage of LDH (Fig. 9) could be due to a lack of NADH. A lack of both NAD^+ and NADH would indicate that a possible DP or P_i activation of NAD deamination takes place. NAD^+ and NADH have been shown to rapidly disappear from minced postmortem muscle due to the action of NADase (E.C. 3.2.2.5) (Newbold & Scopes, 1971*b*) but the NAD^+/NADH ratio remained constant during the disappearance (Bernofsky & Pankow, 1973).

TABLE 7

THE MAXIMUM ACTIVITIES OF THE GLYCOLYTIC CONTROL ENZYMES AND LDH, MEASURED AS THE FORMATION OF PRODUCT DURING THE FIRST HOUR OF THE EXPERIMENTAL PERIOD. ACTIVITY IS EXPRESSED IN μmoles OF PRODUCT FORMED IN GLUCOSE EQUIVALENTS/h/g OF UNDILUTED MUSCLE. THE PERCENT INCREASE IN ACTIVITY DUE TO THE ADDITION OF 1% SODIUM DIPHOSPHATE (DP) IS ALSO GIVEN

(Dalrymple & Hamm, 1974*b*)

Enzyme	*Activity*		*Percent change in activity*
	Control	*DP*	
Phosphorylase	6·62	15·68	+237
PFK	9·95	21·68	+218
GAPDH	9·95	14·64	+147
PK	9·94	13·76	+138
LDH	9·94	13·19	+133

Using the activities in Table 7, Dalrymple & Hamm (1974*b*) calculated ATP production during the same time period. From this calculation it becomes evident that, in spite of an increase to over twice the rate of glycogen breakdown in the DP samples, the production of ATP is only a little higher than in the control. This effect, in conjunction with a higher ATPase activity, causes an almost total depletion of ATP within an hour after addition of DP, as shown in Fig. 7.

REFERENCES

BATE-SMITH, E. C. (1938). *J. Physiol.*, **92**, 336.
BENDALL, J. R. (1973*a*). In *The structure and function of muscle*, 2nd Ed., G. H. Bourne, II, 243. New York: Academic Press.
BENDALL, J. R. (1973*b*). Proc. 19th Meet. European Meat Res. Workers, Paris, **1**, 1.
BERGMEYER, H. O. (1972). *Methoden der enzymatischen Analyse*. 2nd Ed. Verlag Chemie. Weinheim, Bergstr, Germany.
BERNOFSKY, C. & PANKOW, M. (1973). *Arch. Biochem. Biophys.*, **156**, 143.
BODWELL, C. E., PEARSON, A. M. & SPOONER, M. E. (1965). *J. Fd Sci.*, **30**, 760.
DALRYMPLE, R. H. & HAMM, R. (1973). *J. Fd Technol.*, **8**, 439.
DALRYMPLE, R. H. & HAMM, R. (1974*a*). *Fleischwirtschaft*, **54**, 1084.
DALRYMPLE, R. H. & HAMM, R. (1974*b*). *J. Fd Sci.*, **39**, 1218.
DALRYMPLE, R. H. & HAMM, R. (1975*a*). *J. Fd Sci.*, **40**, 850.
DALRYMPLE, R. H. & HAMM, R. (1975*b*). *Zeitschr. Lebensmitt. Untersuch. Forsch.*, **158**, 333.
FINLAYSEN, B. & TAYLOR, E. W. (1969). *Biochem.*, **8**, 802.
GREASER, C., CASSENS, R. G., BRISKEY, E. J. & HOEKSTRA, W. G. (1969). *J. Fd Sci.*, **34**, 120.
HAMM, R. (1958). *Zeitschr. Lebensmitt. Untersuch. Forsch.*, **107**, 1.
HAMM, R. (1962). *Zeitschr. Lebensmitt. Untersuch. Forsch.*, **117**, 132.
HAMM, R. (1972). *Kolloidchemie des Fleisches*. Berlin, Hamburg: Paul Parey Verlag.
HAMM, R. (1973). *Fleischwirtschaft*, **53**, 93.
HAMM, R. (1975*a*). *J. Texture Sci.*, **3**, 182.
HAMM, R. (1975*b*). In *Meat*, eds. D. J. A. Cole & R. A. Lawrie, 231. London: Butterworths.
HAMM, R., DALRYMPLE, R. H. & HONIKEL, K. O. (1973). Proc. 19th Meet. European Meat Res. Workers, Paris, **1**, 73.
HAMM, R. & GRABOWSKA, J. (1976). *Fleischwirtschaft* (in preparation).
HAMM, R. & MASIC, D. (1971). *Arch. Fischereiwiss.*, **22**, 121.
HAMM, R. & POTTHAST, K. (1975). *Fleischwirtschaft*, **55**, 87.
HAMM, R., POTTHAST, K. & ACKER, L. (1974). *Zeitschr. Lebensmitt. Untersuch. Forsch.*, **154**, 73.
HAMM, R. & REDE, R. (1972). *Fleischwirtschaft*, **52**, 331.
HAMM, R. & VAN HOOF, J. (1970). *Fleischwirtschaft*, **50**, 218.
HAMM, R. & VAN HOOF, J. (1971). *Zeitschr. Lebensmitt. Untersuch. Forsch.*, **147**, 193.
HAMM, R. & VAN HOOF, J. (1974). *Zeitschr. Lebensmitt. Untersuch. Forsch.*, **156**, 87.
HONIKEL, K. O. & HAMM, R. (1974*a*). *Fleischwirtschaft*, **54**, 557.
HONIKEL, K. O. & HAMM, R. (1974*b*). *Zeitschr. Lebensmitt. Untersuch. Forsch.*, **156**, 145.
NERAAL, R. & HAMM, R. (1973). Proc. 19th Meet. European Meat Res. Workers, Paris, **4**, 1419.
NEWBOLD, R. P. & LEE, C. A. (1965). *Biochem. J.*, **97**, 1.
NEWBOLD, R. P. & SCOPES, R. K. (1967). *Biochem. J.*, **105**, 127.
NEWBOLD, R. P. & SCOPES, R. K. (1971*a*). *J. Fd Sci.*, **36**, 209.
NEWBOLD, R. P. & SCOPES, R. K. (1971*b*). *J. Fd Sci.*, **36**, 215.
NOWLAN, S. S. & DYER, W. J. (1974). *J. Fish. Res. Board Can.*, **31**, 473.
POTTHAST, K. (1972). Thesis. Universitat Münster.
POTTHAST, K. (1974). *J. Chromatog.*, **88**, 168.
POTTHAST, K. & HAMM, R. (1969). *J. Chromatog.*, **42**, 558.
POTTHAST, K., HAMM, R. & ACKER, L. (1975). In *Water relations in food*, ed. R. B. Duckworth, 365. London: Academic Press.
POTTHAST, K., HAMM, R. & ACKER, L. (1976). *Zeitschr. Lebensmitt. Untersuch. Forsch.* (in press).
SCOPES, R. K. (1971). Proc. 17th Meet. European Meat Res. Workers, Bristol, p. 14.

TAYLOR, E. W., LYMN, R. W. & MOLL, O. (1970). *Biochem.*, **9**, 2989.
VALIN, C. & CHARPENTIER, J. (1969). *Ann. Biol. Anim. Biochim. Biophys.*, **9**, 555.
VAN HOOF, J. & HAMM, R. (1971). *Fleischwirtschaft*, **51**, 964.
VAN HOOF, J. & HAMM, R. (1973*a*). *Zeitschr. Lebensmitt. Untersuch. Forsch.*, **150**, 282.
VAN HOOF, J. & HAMM, R. (1973*b*). *Zeitschr. Lebensmitt. Untersuch. Forsch.*, **153**, 271.