Studies with a Reconstituted Muscle Glycolytic System

THE RATE AND EXTENT OF GLYCOLYSIS IN SIMULATED POST-MORTEM CONDITIONS

By ROBERT K. SCOPES*

A.R.C. Meat Research Institute, Langford, Bristol BS18 7DY, U.K.

(Received 4 February 1974)

The reconstituted glycolytic system described previously (Scopes, 1973) was used to simulate post-mortem glycolytic metabolism in muscle. The effects of the following factors have been investigated: ATPase (adenosine triphosphatase) amount, AMP deaminase amount, percentage of the phosphorylase in the *a* form and the effect of diluting the glycolytic enzyme complex as a whole. It was confirmed that the rate of metabolism was solely dependent on the amount of ATPase present and that various concentrations of the glycolytic enzymes had no effect over a wide range encompassing the variation found in anatomically different muscles. The extent of metabolism, represented by the value of the 'ultimate' pH, depended markedly on the amount of phosphorylase in the *a* form; as little as 1% of the *a* form resulted in a considerably lower pH than in its absence. To a lesser extent the amount of AMP deaminase also affected the ultimate pH, but this was probably only significant for comparisons of genetically distinct muscles with widely differing amounts of AMP deaminase. The reconstituted system behaved almost identically with regard to post-mortem glycolytic metabolism compared with intact muscle tissue. It is concluded that the controlling effectors found with the reconstituted system apply to intact muscle also.

The rate and extent of glycolytic metabolism and other changes in post-mortem muscle is of particular interest to the meat industry, since it has an important bearing on the quality of the product. Extensive studies of the post-mortem changes in muscle have been made to elucidate the important controlling factors. The results not only have practical significance but also demonstrate many fundamental features of muscle structure, function and glycolytic metabolism.

Analytical investigation of the post-mortem glycolytic metabolism in intact muscle (Bendall, 1960; Cassens & Newbold, 1966, 1967; Newbold & Scopes, 1967; Kastenschmidt *et al.*, 1968), frozen and thawed muscle (Scopes & Newbold, 1968) and muscle-mince preparations, to which could be added various compounds (Newbold & Scopes, 1971*a,b*; R. P. Newbold & R. K. Scopes, unpublished work), has led to an understanding of the controlling steps in the metabolic behaviour.

The principal features of post-mortem metabolism are as follows: there is a linear pH fall with time over most of the period, with a quite rapid levelling out at the end, at which point the 'ultimate', or lowest pH, is reached. Metabolites subsequent to hexose mono-

phosphates are normally present in only low quantities, but hexose monophosphate amounts usually increase somewhat, reaching a peak of as much as 10 $\mu\text{mol/g}$ when the pH is about 6.0, the magnitude of this peak being greatest at low temperatures (Newbold & Scopes, 1967). This indicates an imbalance between phosphorylase and phosphofructokinase activities. Since Ca^{2+} release into the sarcoplasm has been implicated at low temperatures (Newbold, 1966), conversion of phosphorylase *b* into the *a* form under the influence of Ca^{2+} (Brostrom *et al.*, 1971) most likely explains this occurrence. Very high hexose monophosphate amounts occur in fast-metabolizing pig muscles (Kastenschmidt *et al.*, 1968), no doubt also owing to substantial amounts of phosphorylase *a* being present. Deamination of adenine nucleotides occurs steadily, with a maximum rate around pH 6.2, but metabolism continues even after nearly all the adenine mononucleotides have been lost, until the pH reaches its ultimate value. This is usually close to 5.5, though it can vary in the presence of residual glycogen from about 5.8 down to values even below 5.0 (Lawrie *et al.*, 1958).

More detailed investigations of the post-mortem metabolism with whole muscle preparations are confounded by such things as compartmentalization, interconversion of phosphorylase forms and varia-

* Present address: Department of Biochemistry, La Trobe University, Bundoora, Vic. 3083, Australia.

tions between animals. The reconstituted glycolytic system described by Scopes (1973) can be used to simulate post-mortem conditions without any of these complicating side effects. Moreover, the amount of various controlling enzymes can be varied at will, even to the stage of omitting an enzyme if required.

Scopes (1974) described the metabolism occurring in simulated tetanic contractions, in which a very high rate of ATP splitting occurs. Post-mortem muscle differs from this situation only in degrees; the ATP-splitting rate is orders of magnitude smaller, but it also results in stimulation first of creatine kinase (EC 2.7.3.2), and then of glycolysis to regenerate the lost ATP. Whereas in tetanic contraction the events are too fast to allow the acidification to be entirely neutralized by the blood carrying away waste products, in post-mortem muscle acidification occurs because there is no blood flow. Thus in the simulated system the pH is allowed to fall. This pH fall is a direct measure of glycolysis since the buffering species present in muscle (carnosine and/or anserine, Pi, phosphate esters and protein) cause a linear fall in pH with acid and lactate production during glycolysis (Bendall, 1960; Newbold & Scopes, 1967).

The fall in pH *post mortem* has three important effects on the glycolytic and related enzymes. First, it alters the apparent equilibrium of acid-dependent reactions, such as that catalysed by creatine kinase. Secondly, it may cause considerable changes in activity of enzymes through altering V_{max} , K_m , or the effectiveness of various activators and inhibitors. Thirdly, it may cause denaturation, especially at elevated temperatures. The combination of these effects cause the observed post-mortem glycolytic changes in muscle. In particular the extent of glycolysis is partly determined by a feedback loop; a factor resulting in cessation of glycolysis is the low pH, which itself is the result of the preceding glycolysis.

The present paper reports results obtained with the reconstituted glycolytic system to simulate post-

mortem muscle. The amounts of the key enzymes were varied and the results indicate which factors are of major importance in determining both the rate and the extent of pH decrease in post-mortem muscle.

Materials and Methods

Procedures for preparing the reconstituted glycolytic system and analysing the metabolic process were as described previously (Scopes, 1972*a,b*; 1973, 1974). In addition to the compounds listed, in these experiments the buffers included histidine (30mM) and extra potassium acetate (40mM) to produce a pH fall that was approximately linear with lactate production. Also more glycogen was added initially (60–70mM-glucose equivalents) to ensure that exhaustion of glycogen was not a limiting factor. AMP deaminase was included in the experiments at the amount indicated, otherwise the activities of the enzymes were as listed previously (Scopes, 1973).

Results

One of the earlier results with the reconstituted glycolytic system is illustrated in Table 1. A myofibrillar preparation (Bendall, 1961) was used as ATPase (adenosine triphosphatase). Except for a small rise in fructose diphosphate towards the end and the rather slow terminal metabolism, the general features resemble metabolism in intact muscle. Numerous other experiments were carried out with myofibrils and sarcoplasmic reticulum preparations as the ATPases. The main feature of these was the unpredictability of the ultimate pH, which sometimes was as low as 5.4, and at other times not less than 6.3. But by the time the ultimate pH was reached whatever its value, all the adenine mononucleotide had been deaminated; thus variability in the amount of AMP and the activity of AMP deaminase could clearly be significant factors.

After these early experiments, the factors that had

Table 1. *Progress of simulated post-mortem metabolism*

A myofibril suspension was used as the ATPase in the presence of 0.1 mM-Ca²⁺. Concentrations are in mM. Temperature, 30°C.

Time (min)	pH	Phosphocreatine	ATP	IMP	Hexose monophosphate	Fructose diphosphate	Lactate
2	7.19	10.8	4.6	0.1	0.5	0.01	9
25	6.70	2.9	3.7	0.9	4.7	0.02	25
41	6.30	0.5	1.1	3.8	3.8	0.02	39
86	5.93	0	0.1	4.3	5.6	0.04	49
144	5.75	0	0.04	4.5	5.6	0.71	53
288	5.56	0	0	4.5	4.5	0.34	60
1000	5.51 (ultimate)	0	0	4.6	4.9	0.02	68

been causing variability were identified and individually investigated as described below. Because of its stability, kinetic characteristics and ease of handling, the potato ATPase (high-ratio ATPase/ADPase apyrase; EC 3.6.1.5) was used in all these experiments. Variability was virtually eliminated within a given batch of glycolysing samples, with the ultimate pH reproducible to ± 0.01 pH unit [for example a batch of nine identical samples, individually mixed, gave ultimate pH values of 5.38 ± 0.005 (S.E.M.)]. Reproducibility over the long term was ± 0.02 pH unit.

Effect of various amounts of AMP deaminase

It was anticipated that the higher the amount of AMP deaminase in the mixture, the faster would IMP be produced, causing cessation of metabolism sooner (i.e. a higher ultimate pH). Results of two sets of experiments in which the amount of AMP deaminase was varied over a wide range are shown in Fig. 1 and Table 2. These show that the anticipated effect did indeed occur, although it was only slight, with the highest AMP deaminase amount giving an ultimate pH about 0.1 unit higher than the lowest value. This difference was statistically highly significant ($P < 0.001$), however, in view of the reproducibility of the results.

Fig. 1. pH fall in simulated post-mortem conditions, with various amounts of AMP deaminase present

The ATPase amount was 1.5 units/ml, and the temperature was 37°C. Phosphorylase *a* content was about 0.1% of the total phosphorylase. AMP deaminase amounts were: curve A, 6; curve B, 12; curve C, 25; curve D, 50; curve E, 100; curve F, 200 units/ml.

Table 2. *Effect of variation of AMP deaminase concentration on the ultimate pH*

The ATPase used (5 units/ml) was the potato enzyme. Phosphorylase *a* content was 0.5% of the total phosphorylase, and the temperature was 30°C. The experiment was repeated twice with reproducibility of ± 0.02 pH unit.

AMP deaminase (units/ml) (mean of three experiments)	Ultimate pH (at 60 min)
4	5.41
10	5.42
25	5.44
50	5.47
100	5.50

In addition to the results in Fig. 1, another mixture was set up at the same time in which there was no AMP deaminase. The pH fell even further (ultimate value 5.38), but the metabolism was considerably altered. The AMP concentration, normally barely detectable at the sensitivity available (0.01 mM), increased steadily, reaching a maximum of over 4 mM; only towards the end was there a slight decrease owing to trace contamination with AMP deaminase. Analysis of the intermediates showed that this AMP was able to activate the phosphorylase *b*, as there was a substantial concentration of hexose monophosphate, but it was not able to activate phosphofructokinase, as the concentration of fructose diphosphate was virtually zero. It appears that under these conditions (pH 6.5 and lower; [AMP] > 0.2 mM), phosphofructokinase is actually inhibited by AMP, perhaps by competing with the substrate MgATP^{2-} . This effect has also been indicated in kinetic studies on purified phosphofructokinase (R. K. Scopes, unpublished work).

Effect of phosphorylase a

The considerable effect of only small amounts of phosphorylase *a* on the development of the 'resting metabolic state' has been demonstrated previously (Scopes 1973). For the same reasons the presence of phosphorylase *a* is also likely to affect the post-mortem metabolism substantially, as it enables glycolytic resynthesis of ATP to occur at lower amounts of AMP by removing the absolute requirement for AMP in glycogenolysis. Several of the experiments have pointed to the large effect that the amount of phosphorylase *a* can have on the ultimate pH [e.g. compare the ultimate values in Fig. 1 (0.1% phosphorylase in the *a* form) and Table 2 (0.5% *a* form); also the curves F and G in Fig. 2]. An experiment was set up in which the percentage of phosphorylase in the *a* form varied from zero (<0.1%) to 2%, other conditions being identical; the ATPase was present at 3 units/ml and AMP deaminase at 40 units/ml, both measured at 37°C.

Table 3. *Effect of the presence of phosphorylase a on simulated post-mortem metabolism*

The ATPase amount was 3 units/ml, AMP deaminase was 40 units/ml, the temperature was 37°C and the pH fell below 6.0 at between 40 and 45 min in each case.

Phosphorylase <i>a</i> (% of total phosphorylase)	Ultimate pH (at 100 min)	Hexose monophos- phate at pH 6.0 (mM)	% of adenine mononucleo- tide deaminated by pH 6.0
<0.1	5.74	0.25	90
0.25	5.57	1.4	79
0.5	5.47	2.2	74
1.0	5.34	5.7	65
2.0	5.32	10.5	62

The important features of this experiment are summarized in Table 3. In all cases the ultimate pH was reached in 70–80 min. Even 0.25% of the phosphorylase in the *a* form had a substantial effect in decreasing the ultimate pH; the differences in pH are highly significant in view of the reproducibility of ± 0.01 pH unit mentioned previously. Even with 0.25% phosphorylase *a* the hexose monophosphate concentration (at pH 6.0) was higher. The greatest effect was with the largest amount of phosphorylase *a* (2%), giving an ultimate pH of 5.32. Since experiments in which the phosphorylase *a* content was as high as 40% have not given ultimate pH values below 5.3, it seems likely that (at this ATPase concentration) the 2% phosphorylase *a* was producing a maximal effect. Also shown in Table 3 is the extent of deamination that had occurred by the time the pH had reached 6.0. Higher values of glycolytic potential (i.e. higher phosphorylase *a* content) resulted in less deamination by a given stage in the progress of metabolism.

Effect of the amount of ATPase on the rate and extent of pH fall

Many experiments have been carried out in this category and the results are typified by the one described in detail below. In view of the requirement for some phosphorylase *a* to achieve an ultimate pH in the range occurring in intact muscle, the enzyme mixture contained 1% of phosphorylase in the *a* form. AMP deaminase was present at 80 units/ml (measured at 37°C) and the only variable was the amount of ATPase (potato enzyme) added after the preincubation period of 20 min. During this period somewhat more than half of the creatine was phosphorylated, and the pH fell to about 7.0 in each case. Then the appropriate amount of a concentrated solution of ATPase was added, the pH measured by direct immersion of the electrode in the glycolysing solution, and samples were taken for analyses at

Fig. 2. *pH fall in simulated post-mortem conditions, with various amounts of ATPase present*

The AMP deaminase amount was 50 units/ml and the temperature was 37°C. The phosphorylase *a* content was 1% of the total phosphorylase in curves A–F and 10% in curve G. ATPase (potato enzyme) amounts were: curve A, zero; curve B, 0.5; curve C, 1.0; curve D, 2; curve E, 4; curve F, 8; curve G, 8 units/ml. Further details of this experiment are given in Table 4. Fig. 2(a) shows the pH changes up to 6 h and Fig. 2(b) illustrates the changes during the first 15 min for: Δ = curve A; $\blacktriangle$ = curve D; $\circ$ = curve F; $\bullet$ = curve G.

appropriate intervals. The pH changes after adding the ATPase are summarized in Fig. 2 and Table 4. With no ATPase the pH continued to fall for 30 min before reaching an almost steady state just below pH 6.8. However, the pH in such experiments always continued to fall at a very slow rate. With added ATPase the rate of pH fall was virtually linear after the initial slower rate, right down to close to the ultimate pH; in this way it mimicked the situation in intact muscle very closely. The slopes of the curves increased in proportion to the ATPase present, demonstrating that the rate was directly proportional to the amount of ATPase. The ultimate pH values of the two slowest metabolizing mixtures (which are in the range normally found in muscle *post mortem* at 37°C) were identical, but as the ATPase amount

Table 4. *Effect of ATPase amount on simulated post-mortem metabolism*

The AMP deaminase amount was 80 units/ml, the temperature 37°C and potato ATPase was used. Phosphorylase *a* was 1% of the total phosphorylase, except in G. Plots of pH against time are given in Fig. 2.

	ATPase activity at 37°C (units/ml)	Rate of pH fall (pH units/h)	Ultimate pH	Hexose monophosphate at pH 6.0 (mm)	% of adenine mononucleotide deaminated by pH 6.0
A	0	0.02	—	—	—
B	0.5	0.29	5.52	12.1	70
C	1.0	0.54	5.52	8.6	69
D	2	1.17	5.55	4.5	68
E	4	2.4	5.61	0.85	81
F	8	4.0	5.66	0.18	96
G	8*	4.8	5.40	7.6	68

* Plus additional phosphorylase *a* to make it 10% of total phosphorylase.

increased so the ultimate pH values rose. At the faster rates it was clear that the phosphorylase *a* alone was not sufficient to maintain the high hexose monophosphate concentrations that are necessary for rapid glycolytic resynthesis of ATP. Phosphorylase activity was increasingly required, and this could only be achieved by a rise in AMP concentration; this resulted in more rapid deamination of adenine nucleotides. The lack of hexose monophosphates at higher ATPase rates is demonstrated by their measurement at the time the pH passed 6.0, which is shown in Table 4. Expt. G was a repeat of Expt. F, but this time extra phosphorylase *a* (10% of the total, i.e. 8 units/ml at 37°C) was added simultaneously with the ATPase. The pH fell rapidly and steadily, reaching a lower pH than any other, and stopped very suddenly. Moreover, the hexose monophosphate concentration was much higher and much less of the adenine nucleotides had been deaminated by pH 6.0. Copious denaturation occurs below pH 5.5 at 37°C (Scopes, 1964), and this caused a slight rise in pH after the ultimate pH had been reached (Bendall & Wismer-Pedersen, 1962). An interesting feature is more clearly illustrated in Fig. 2(b), an enlargement of the first few minutes illustrated in Fig. 2(a). It demonstrates a rise in pH during the first few minutes in the case of the high ATPase/low phosphorylase *a* Expt. F. This is caused by the fact that before glycolysis is activated the only net reaction occurring is:

where *x* is the degree of ionization of the second hydroxyl of P_i in the particular conditions. For pH of about 7.05 and $I = 0.16 \text{ mol/l}$, $x = 0.7$. Thus for every phosphocreatine split, $0.3 H^+$ ions were absorbed and the pH increased. Analysis confirmed that in the first 2 min very little lactate had been produced and about 12 mm-phosphocreatine was lost; the latter

would be sufficient to cause a pH increase of nearly 0.1. The small amount of glycolysis that had by then occurred caused some acidification; the observed pH increase was 0.05. The phenomenon of increasing pH has been observed in intact living muscle during a series of twitches (Distèche, 1960, quoted in Bendall, 1969). In Expt. B, with a much lower rate of ATP turnover, glycolytic resynthesis of ATP had already commenced by the time any significant phosphocreatine loss had occurred and so the pH fell continuously. For Expts. C, D and E in Fig. 2, the steady fall in pH was preceded by a significant lag period. This lag is also observed in intact muscle *post mortem* (Cassens & Newbold, 1967). But in Expt. G, the extra phosphorylase 'pushed' glycolysis into action immediately and the pH fell with hardly any lag before the maximum rate was reached. Comparing Expts. F and G, not only were the hexose monophosphate amounts higher throughout in Expt. G, but the phosphocreatine amount and ATP also remained higher for longer.

Importance of glycolytic enzyme amount on rate of pH fall

All experiments described above have used the reconstituted glycolytic system as a protein concentration of 40 mg/ml, comparable with the concentrations of enzymes in intact muscle. Clearly the concentrations of most of the enzymes are in excess by several orders of magnitude of the amount required to achieve the throughput of glycolysis obtained with, say, 0.5–1 unit of ATPase present/ml, the amount normally observed in post-mortem muscle. An investigation was made of the effect of diluting the glycolytic complex as a whole, everything else remaining the same, including ATPase. The AMP deaminase used in this experiment was mixed with the ATPase, and so was not varied. As the glycolytic potential was decreased by dilution, higher concentrations of ADP and AMP were needed to activate the glyco-

Fig. 3. pH fall in simulated post-mortem conditions, with various amounts of the glycolytic enzyme complex present

The ATPase amount was 1 unit/ml, AMP deaminase 10 units/ml, the temperature 30°C and the phosphorylase a content 0.2% of the total phosphorylase. The different curves are offset on the time-scale for clarity. Concentrations of the glycolytic enzyme complex were: ○, 40 mg/ml; ●, 13 mg/ml; △, 4 mg/ml; ▲, 1.3 mg/ml; □, 0.4 mg/ml.

lytic enzymes, so deamination occurred sooner. This resulted in increasingly higher ultimate pH values as the glycolytic complex was diluted; however, this did not affect the initial rate of pH fall. The experiment was carried out at 30°C with 1 unit of ATPase/ml and 10 units of AMP deaminase/ml. Fig. 3 illustrates the results obtained; the curves have been offset on the time-scale for clarity. Even 10× dilution made no difference in rates, and only caused a higher ultimate pH for the reason outlined above. At 30× dilution the rate was still the same down to about pH 6.5, where excessive loss of adenine nucleotide began to slow metabolism down. Even at 100× dilution, the system did its best to keep up with ATP splitting despite the fact that there was only 0.5 unit each of phosphorylase and phosphofructokinase present/ml, barely enough to maintain the ATP amount even if both enzymes were fully activated. Thus within a wide range, the concentration of the glycolytic enzymes as a whole does not affect the overall rate of glycolysis; the throughput is maintained by alterations in the concentration of activating metabolites. The rate is determined solely by the ATPase present.

Discussion

The quality of the product in the meat industry is frequently adversely affected by changes occurring during the 24 h or so after slaughter of the animal,

changes that are largely brought about by the post-mortem glycolytic metabolism. This metabolism normally results in conversion of glycogen into lactate, and a pH fall from over 7.0 to around 5.5. Both the rate and extent of this metabolism are important and deviations from normal usually result in a product which for one reason or another is undesirable. Deviations that occur include abnormally high or low ultimate pH values and excessively fast pH fall.

It is clear that the rate of glycolysis must depend on the rate of ATP degradation, and whatever factors are controlling the activities of the key enzymes phosphorylase and phosphofructokinase must themselves be determined by the rates of the ATPase systems present. The experiments illustrated in Figs. 2 and 3 and Table 4 confirm this fact, the rates being directly proportional to the amounts of ATPase present and independent of the concentrations of the glycolytic enzymes. This is so over a wide range of conditions encompassing all the variations of rates found in muscles *post mortem*. The different amounts of glycolytic enzymes found in different muscles, especially between 'red' and 'white' muscles (Pette & Bücher, 1963) are also encompassed, indicating that the amount of glycolytic enzymes present does not affect the rate of post-mortem metabolism.

The experiment in which no ATPase was present illustrated an interesting point. Theoretically the pH should not have continued to fall after the maximum phosphocreatine amount had been reached, but in practice it always did, with a gradual increase in lactate concentration. There must have been some system present capable of slowly hydrolysing ATP; it is unlikely that the rate found, 0.02 μmol of ATP split/min per ml, was due to spontaneous hydrolysis, so other factors must be considered. There is the possibility of a contaminating soluble ATPase in the reconstituted glycolytic enzyme mixture, but the ATPase activity of the kinases present is more likely to be involved, i.e. 'nothing kinase' activity. Both phosphofructokinase (Uyeda, 1970) and creatine kinase (Sasa & Noda, 1964) have ATPase activities of the order of 10⁻⁵ times their normal kinase activities, at least in the absence of normal substrate and there is good reason to suppose that the other kinases present (myokinase, phosphoglycerate kinase and pyruvate kinase) would also have comparable ATPase activity. A considerable proportion of the soluble ATPase activity could thus be due to these 'nothing kinase' activities. A further possible source may be sulphenyl glyceraldehyde phosphate dehydrogenase, which undoubtedly was present, acting in the way described by Allison & Benitez (1972).

The factors determining the extent of pH fall have not been clearly defined previously. Experiments with minced muscle preparations have shown that

maintenance of NAD concentrations and high P_i amounts favour low ultimate pH values, whereas dilution with iso-osmotic KCl leads to rapid cessation of metabolism at a relatively high pH (Newbold & Lee, 1965; Newbold & Scopes, 1971*a,b*). The present results with the reconstituted system demonstrate that the amount of phosphorylase *a* present is the most important factor, although other things being constant, varying AMP deaminase or ATPase amounts also affect the ultimate pH slightly. One may conclude that dilution of a mince preparation with KCl alone leads to complete conversion of phosphorylase into the *b* form, compared with an undiluted mince which maintains a small amount of phosphorylase in the *a* form.

Extrapolating the present results to the situation in intact muscle, it would appear that a low ultimate pH is caused by the phosphorylase *a* content being greater than normal. The reason for this can only be guessed at; some differences in the ability of the sarcoplasmic reticulum to maintain Ca^{2+} concentrations below the amount for activation of phosphorylase *b* kinase may be involved. Fig. 2 indicates that the ATPase activity itself is not very important in determining the ultimate pH, unless a high ATPase activity is associated with a low phosphorylase *a* content. The latter is unlikely to occur in post-mortem muscle, since all evidence indicates that a high ATPase activity (i.e. fast pH fall), found particularly in pig muscles, is due to Ca^{2+} activation of myofibrillar ATPase (Kastenschmidt, 1970), and is accompanied by high phosphorylase *a* content owing to activation of phosphorylase *b* kinase. The latter can be deduced from the very high concentration of hexose monophosphate in such muscles. The effect of temperature on certain bovine and ovine muscles, in which exposure to low temperatures shortly after death causes contraction, is also ascribed to Ca^{2+} being released, activating the myofibrillar ATPases and phosphorylase *b* kinase. Thus hexose monophosphate amounts were highest at the lowest temperature during post-mortem glycolysis (Newbold & Scopes, 1967). But in these cases the presumed higher content of phosphorylase *a* was not sufficient to counteract other slowing effects of low temperature itself, and ultimate pH values were usually higher at the low temperatures.

The experiments described here, with the exception of the early results in Table 1, used an ATPase from potato for controlling the rate of pH fall. This enzyme is ideal in many ways; it has a flat pH-activity curve, a high affinity for $MgATP^{2-}$ (Valenzuela *et al.*, 1973) and is not inhibited in any way by other compounds present. The ATPases acting naturally *post mortem* are more complex and not clearly defined. Thus, although myofibrillar ATPase has much the greatest potential, it may be involved only slightly *post mortem* because of its requirement for Ca^{2+} ion activation.

The ATPases of the sarcoplasmic reticulum, both basal and Ca^{2+} -pump linked, are involved to some extent, but are probably not very active at the lower pH values.

Other possible contributors to the overall ATPase activity are the cyclic systems such as phosphofructokinase-fructose diphosphatase, phosphorylase-glycogen synthetase and related enzymes, and phosphorylase *b* kinase-phosphorylase *a* phosphatase. With all of these, conditions that lead to activation of one enzyme result in inactivation of the other. An experiment, in which there was no other ATPase present, but a purified preparation of muscle fructose diphosphatase (Fernando *et al.*, 1968) at a concentration five times that occurring normally was present, did not indicate any additional rate of pH fall over the residual rate that occurs in such conditions. This is in contrast with the results of Bloxham *et al.* (1973), who used liver fructose diphosphatase, which is less sensitive to AMP inhibition. Thus ATPase activity owing to the first of these cyclic systems does not occur under these conditions.

The residual 'nothing kinase' rate could also contribute to post-mortem muscle ATPase activity. The slowest rates occurring naturally must be due to a combination of sources, but abnormally high rates are probably mainly caused by Ca^{2+} release into the sarcoplasm, activating both the sarcoplasmic reticulum Ca^{2+} -pump and the myofibrillar ATPase.

In conclusion, in post-mortem muscle the rate of pH fall is entirely due to the ATPase activity in the muscle. The extent of pH fall (the ultimate pH) appears to be controlled mainly by the amount of phosphorylase *a* acting in the tissue, but some small effect of various amounts of AMP deaminase may also be involved, particularly when comparing anatomically different muscles. Denaturation or complete inactivation of any particular enzyme cannot be involved at pH values above which it has been found to be active. In the reconstituted glycolytic system the lowest pH at which the most susceptible enzyme, phosphofructokinase, is still acting at 37°C is 5.35, lower than the normal range of ultimate pH values found. Since the temperatures in post-mortem muscle in slaughterhouse conditions are usually considerably lower than 37°C, it is improbable that denaturation *in situ* normally plays a part in determining the ultimate pH of meat. But for exceptionally low pH values reported (Lawrie *et al.*, 1958), denaturation inactivation cannot be ruled out.

References

- Allison, W. S. & Benitez, L. V. (1972) *Proc. Nat. Acad. Sci. U.S.* **69**, 3004-3008
- Bendall, J. R. (1960) in *Structure and Function of Muscle* (Bourne, G. H., ed.), vol. 3, pp. 227-274, Academic Press, London and New York
- Bendall, J. R. (1961) *Biochem. J.* **81**, 520-535

- Bendall, J. R. (1969) *Muscles, Molecules and Movement*, p. 163, Heinemann, London
- Bendall, J. R. & Wismer-Pedersen, J. (1962) *J. Food Sci.* **27**, 144–159
- Brostrom, C. O., Hunkeler, F. L. & Krebs, E. G. (1971) *J. Biol. Chem.* **246**, 1961–1967
- Bloxham, D. P., Clark, M. G., Holland, P. C. & Lardy, H. A. (1973) *Biochem. J.* **134**, 581–587
- Cassens, R. G. & Newbold, R. P. (1966) *J. Sci. Food Agr.* **17**, 254–256
- Cassens, R. G. & Newbold, R. P. (1967) *J. Food Sci.* **32**, 13–14
- Distèche, A. (1960) *Mem. Acad. Roy. Med. Belg.* **32**, 1–33
- Fernando, J., Enser, M., Pontremoli, S. & Horecker, B. L. (1968) *Arch. Biochem. Biophys.* **126**, 599–606
- Kastenschmidt, L. L. (1970) in *Physiology and Biochemistry of Muscle as a Food* (Briskey, E. J., Cassens, R. G. & Marsh, B. V., eds.), vol. 2, pp. 735–753, University of Wisconsin Press, Madison
- Kastenschmidt, L. L., Hoekstra, W. G. & Briskey, E. J. (1968) *J. Food Sci.* **33**, 151–158
- Lawrie, R. A., Gatherum, R. P. & Hale, H. P. (1958) *Nature (London)* **182**, 807–808
- Lawrie, R. A., Manners, D. J. & Wright, A. (1959) *Biochem. J.* **73**, 485–490
- Newbold, R. P. (1966) in *Physiology and Biochemistry of Muscle as a Food* (Briskey, E. J., Cassens, R. G. & Trautman, J. C., eds.), vol. 1, pp. 213–224, University of Wisconsin Press, Madison
- Newbold, R. P. & Lee, C. A. (1965) *Biochem. J.* **97**, 1–6
- Newbold, R. P. & Scopes, R. K. (1967) *Biochem. J.* **105**, 127–136
- Newbold, R. P. & Scopes, R. K. (1971a) *J. Food Sci.* **36**, 209–214
- Newbold, R. P. & Scopes, R. K. (1971b) *J. Food Sci.* **36**, 215–218
- Pette, D. & Bücher, T. (1963) *Hoppe-Seyler's Z. Physiol. Chem.* **331**, 180–195
- Sasa, T. & Noda, L. (1964) *Biochim. Biophys. Acta* **81**, 270–279
- Scopes, R. K. (1964) *Biochem. J.* **91**, 201–207
- Scopes, R. K. (1972a) *Anal. Biochem.* **49**, 73–87
- Scopes, R. K. (1972b) *Anal. Biochem.* **49**, 88–94
- Scopes, R. K. (1973) *Biochem. J.* **134**, 197–208
- Scopes, R. K. (1974) *Biochem. J.* **138**, 119–123
- Scopes, R. K. & Newbold, R. P. (1968) *Biochem. J.* **109**, 197–202
- Uyeda, K. (1970) *J. Biol. Chem.* **245**, 2268–2275
- Valenzuela, M. A., del Campo, G., Marin, E. & Traverso-Cori, A. (1973) *Biochem. J.* **133**, 755–763