Post-Mortem Glycolysis in Skeletal Muscle

THE EXTENT OF GLYCOLYSIS IN DILUTED PREPARATION OF MAMMALIAN MUSCLE

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1. Sheep-, rabbit- and ox-muscle minces prepared soon after slaughter were diluted with 1 vol. of 0.16 M-potassium chloride in the absence (potassium chloride mince) and presence of added cofactor or glycolysable substrate, and the effects on the ultimate pH were examined. 2. Changes in the concentrations of glycogen and lactate and the concentrations of some phosphorus-containing fractions were determined in ox-muscle preparations. 3. Glycolysis ceased at appreciably higher pH in the potassium chloride mince than in undiluted mince. The inclusion of glycogen, ATP, ADP, NAD or magnesium chloride in the diluent had little effect on the ultimate pH of the diluted mince. 4. Lactic acid production continued at lower pH values in diluted mince containing added glucose 1-phosphate, fructose 1,6-diphosphate or glucose plus hexokinase than in potassium chloride mince. 5. The evidence points to failure of the phosphorylase step being responsible for the dilution effect.

When adequate glycogen is present, post-mortem glycolysis in mammalian muscle frequently results in the pH falling to an ultimate value of 5.4–5.5. However, in some muscles the ultimate pH is appreciably higher than this (Lawrie, 1955; Howard & Lawrie, 1957; Lawrie, Manners & Wright, 1959). Excessively low values (below 5.1) have also been noted by Lawrie, Gatherum & Hale (1958) in pig muscle.

Little is known of the factors, other than inadequate glycogen, that determine the extent of glycolysis and hence the ultimate pH in post-mortem muscle. Bate-Smith & Bendall (1949) noted that an ultimate pH value of 5.3 appeared to be a limiting value, below which glycolysis was completely inhibited. They considered that the most probable explanation for this was that one or other of the enzymes involved in glycolysis (possibly phosphofructokinase) was increasingly inhibited as the pH fell. However, unless the pH-dependence of such inhibition varies considerably among muscles, it is not apparent why glycolysis should cease in some muscles at appreciably higher pH values. Briskey & Lawrie (1961) have suggested that in these muscles either the phosphorylase is inactivated more readily or the glycogen is less accessible to attack.

In the present study, glycolysing muscle minces were diluted in the presence or absence of added

cofactor or glycolysable substrate. The effects of such treatments on the ultimate pH were established, and in some instances glycogen utilization, lactic acid production and the concentrations of some phosphorus-containing fractions were measured. It was found that glycolysis in minced muscle diluted with an equal volume of 0.16 M-potassium chloride ceases at a pH appreciably higher than the ultimate pH normally reached by undiluted mince. Evidence is provided that this is due to failure of the phosphorylase step in the diluted mince.

MATERIALS AND METHODS

Chemicals. ADP (sodium salt), ATP (disodium salt), fructose 1,6-diphosphate (sodium salt), glucose 1-phosphate (dipotassium salt), NAD (β -DPN) and yeast hexokinase (type III) were obtained from the Sigma Chemical Co. (St Louis, Mo., U.S.A.), and glycogen (shellfish) was obtained from Mann Research Laboratories (New York, N.Y., U.S.A.). Other chemicals used were A.R. grade, and distilled water that had been passed through a mixed-bed deionizer was used throughout.

Muscle samples. Bovine neck muscles (sternomandibularis) were collected from the local abattoir about 30 min. after slaughter. Longissimus dorsi muscles from rams that had been anaesthetized by intravenous injection of thiopentone sodium before slaughter were obtained from the C.S.I.R.O. Division of Animal Physiology about 10 min. after death. In both instances the muscles were wrapped in

plastic bags and transported without refrigeration to the laboratory, a journey occupying about 30 min.

Rabbit muscles (*longissimus dorsi*) were obtained from New Zealand White rabbits killed by decapitation after intraperitoneal injection of thiopentone sodium.

Muscle preparations. Muscle samples were freed from gross fat and connective tissue and passed twice through a mincer fitted with a plate having $\frac{3}{16}$ in. holes. Samples of mince (2.5 g. when pH changes only were studied, and 20–40 g. when other changes were also examined) were quickly weighed, solutions of the required composition added (1 ml. of diluent/g. of mince used) and the mixtures then rapidly dispersed by stirring. Ox-muscle minces were diluted about 90 min. *post mortem*, sheep-muscle minces about 70 min. *post mortem* and rabbit-muscle minces 20–25 min. *post mortem*.

Mince diluted with 0.16 M-KCl containing no other additive is referred to as 'potassium chloride mince'. Solutions containing other additives (in 0.16 M-KCl) were prepared within 1 hr. before the start of an experiment and, when substances having some buffering capacity were included, the pH was adjusted to 6.6–6.7 with 0.5 N-HCl or 0.5 N-KOH.

All experiments were carried out in air at room temperature (20–25°). After the initial dispersion of the mince in the diluent, the samples were left undisturbed except for a brief mixing immediately before sampling or reading the pH.

Undiluted minces were kept in N₂ at room temperature throughout the day. Usually the ultimate pH was reached on the same day and the residual glycogen content (i.e. the glycogen remaining when the ultimate pH was reached) was then determined. When this was not possible the samples were stored at 5° overnight and examined next morning.

Intact muscle samples were kept in N₂ at room temperature throughout the day and stored at 5° overnight. Ultimate pH values were measured 24 hr. *post mortem*.

In two of the experiments estimates were made of the numbers of viable bacteria present in the potassium chloride minces 6 hr. after preparation, and of those in the corresponding undiluted ox-muscle minces. Colony counts after incubation in nutrient agar for 5 days at 25° indicated that the muscle preparations in one of the experiments contained less than 3×10^5 and in the other less than 2×10^5 viable bacteria/g.

Measurement of pH. Measurements of pH were made with a Radiometer (Copenhagen, Denmark) model 22 pH-meter, scale expander and combined electrode (GK 2021C). The pH of diluted minces was measured at about 30 min. intervals, starting soon after preparation. With intact muscle or undiluted mince, about 1 g. of tissue was homogenized with about 10 ml. of 5 mM-sodium iodoacetate in a Marsh-Snow homogenizer (Marsh & Snow, 1950) for the determination of pH. This was done at intervals of 45–60 min.

Sampling for chemical estimations. Glycogen utilization, lactic acid production and the concentrations of phosphorus-containing fractions were measured in ox-muscle preparations only. Samples were examined at three different times. In each experiment, initial and final samples were taken, the former a few minutes after the preparation of the diluted minces and the latter when the ultimate pH was reached or closely approached in the preparation requiring the longest time for this to occur. Intermediate samples were taken from potassium chloride minces within about 30 min. after they had reached their ultimate pH. From each of the other

preparations an intermediate sample was taken when the preparation had attained a pH close to the ultimate pH of the corresponding potassium chloride mince.

Chemical estimations. Glycogen was estimated with anthrone reagent by the method of Carroll, Longley & Roe (1956) after precipitation with 95% (v/v) ethanol from hot 30% (w/v) KOH digests and one reprecipitation (Good, Kramer & Somogyi, 1933). After the addition of the ethanol and heating to boiling, the precipitates were allowed to settle at 1° overnight.

Trichloroacetic acid extracts (for lactic acid and phosphorus analyses) were prepared by homogenizing samples (about 1 g. of mince, or 2 g. of diluted mince) with 15–20 ml. of ice-cold 10% (w/v) trichloroacetic acid for 30 sec. in a Bühler homogenizer. After filtration the extracts were immediately neutralized to phenolphthalein with 2 N-NaOH and stored at 1°.

Lactic acid was determined in triplicate by the method of Barker & Summerson (1941).

Total acid-soluble P and orthophosphate P were determined by the method of Allen (1940). Acid-labile P, alkali-labile P and triose phosphate P were estimated as described by Bailey & Marsh (1952), acid-labile P being the inorganic phosphorus released from sources other than creatine phosphate during hydrolysis in N-HCl at 100° for 10 min., and alkali-labile P being the increase in inorganic phosphorus after hydrolysis in 0.2 N-NaOH at 100° for 3 min. The increase in inorganic phosphorus after hydrolysis in N-NaOH at room temperature for 20 min. represents triose phosphate P.

The procedures described permitted the expression of lactic acid and P values in terms of total soluble P content of the extract. To relate these values to weight of muscle preparation, independent analyses of total acid-soluble P were carried out on weighed samples of each of the muscle preparations under examination.

RESULTS

Changes occurring in undiluted minces

With the exceptions noted below, the pH values of 20 ox-, nine sheep- and three rabbit-muscle minces at the time of dilution were between 6.5 and 6.7. The pH of four of the ox-muscle minces at this time was about 6.4 and that of four of the ox-muscle minces and one of the sheep-muscle minces was about 6.8. The mean pH values were: ox-muscle mince, 6.60; sheep-muscle mince, 6.58; rabbit-muscle mince, 6.62.

Comparison of intact and minced portions of eight ox-muscle and two sheep-muscle samples showed that mincing did not affect the ultimate pH.

The ultimate pH values of the minces varied considerably (Table 1), and were reached in 3½ hr. or longer with ox and sheep muscle, and in less than 3 hr. with rabbit muscle.

The rabbit muscles contained little or no residual glycogen. On the other hand, the ten sheep-muscle preparations contained 0.9–10.0 mg./g., the one muscle that did not reach an ultimate pH below 5.60 containing 4.2 mg./g. The 11 ox-muscle

preparations examined for residual glycogen contained 0.6–6.4 mg./g. and had ultimate pH values ranging from 5.51 to 5.78. Hence cessation of glycolysis in any of these ox-muscle or sheep-muscle preparations was not due to exhaustion of the glycogen supply.

There was no correlation between ultimate pH and residual glycogen. The ultimate pH of the ox-muscle mince containing the smallest amount of residual glycogen was 5.78 and of that containing the largest amount 5.72.

Table 1. *Distribution of ultimate pH values of muscle minces*

Sheep and rabbit longissimus dorsi muscles and ox sternomandibularis muscles were minced soon after slaughter and the ultimate pH of each mince was determined as described in the Materials and Methods section.

Ultimate pH	No. of samples		
	Sheep muscle	Ox muscle	Rabbit muscle
5.31–5.40	3	2	0
5.41–5.50	4	1	0
5.51–5.60	2	5	0
5.61–5.70	0	5	2
5.71–5.80	1	6	2
5.81–5.90	0	1	0
5.91–6.00	0	2	0
Total	10	22	4
Mean ultimate pH ...	5.46	5.68	5.71

The mean ultimate pH (5.68) of minced ox sternomandibularis (22 muscles) was appreciably lower than that reported by Howard & Lawrie (1957) for ox sternocephalicus stored for 12–14 weeks at -10° (5.93). The mean ultimate pH (5.46) of sheep longissimus dorsi (ten preparations) was almost identical with that reported by Howard & Lawrie (1957) for ox longissimus dorsi (5.48), and was lower than that of ox sternomandibularis muscles ($P < 0.001$).

In minced ox muscle (ten experiments) the acid-labile P at the ultimate pH (5.4–5.8) amounted to $1.5 \mu\text{g. atoms/g.}$ or less, and the alkali-labile P was in the range $0.5\text{--}3.0 \mu\text{g. atoms/g.}$ In four of these minces the ultimate pH was about 5.7–5.8 and the residual alkali-labile P was $0.5\text{--}1.5 \mu\text{g. atoms/g.}$ The remaining minces reached ultimate pH values of 5.60 or lower and, in five of them, the residual alkali-labile P was $1.5\text{--}3.0 \mu\text{g. atoms/g.}$ Triose phosphate was not detected in ox-muscle minces at the ultimate pH (four experiments).

Changes occurring in diluted minces

Information on ultimate pH was obtained on comparable preparations of ox and sheep muscles, and on a few occasions on analogous rabbit-muscle preparations. When complete results were available, the effects of various treatments on ultimate pH were found to be similar for all species.

Effect of diluting with potassium chloride. Regardless of the ultimate pH of the undiluted mince, that of the corresponding potassium chloride mince was

Table 2. *Effects of dilution, with and without the addition of glycogen, on ultimate pH, glycogen utilization, lactic acid production and concentrations of some phosphorus-containing fractions in minced muscle preparations*

Samples (1 part by wt.) of freshly prepared ox-muscle mince were diluted with 1 vol. of diluent. Samples of each preparation were taken for glycogen estimation and trichloroacetic acid extraction 6 min. after dilution, at an intermediate time (determined as described in the Materials and Methods section) and 386 min. after dilution. When the final samples were taken the pH had not changed in the diluted minces for about 230 min. and in the undiluted mince for about 30 min.

Preparation	Diluent	Total acid-soluble P ($\mu\text{g. atoms/g.}$)	Sample	pH	Amount (in 1 g. of preparation)				
					Glycogen (as μmoles of glucose)	Lactic acid (μmoles)	Inorganic P ($\mu\text{g. atoms}$)	Alkali- labile P ($\mu\text{g. atoms}$)	Acid- labile P ($\mu\text{g. atoms}$)
Undiluted mince	None	53.0	Initial	6.69	34.5	35.0	23.0	2.0	10.5
			Intermediate	5.88	12.5	81.0	38.5	1.5	3.0
			Final	5.68	5.0	92.0	42.5	0.5	0.5
Potassium chloride mince	0.16 M-KCl	27.0	Initial	6.54	17.5	18.0	11.5	1.0	5.0
			Intermediate	5.95	10.0	34.5	20.5	0.5	0.5
			Final	5.95	9.5	35.0	21.5	0.5	0
Potassium chloride mince with added glycogen	0.16 M-KCl containing 0.9% (w/v) of glycogen	27.5	Initial	6.54	44.5	18.0	12.5	1.0	5.0
			Intermediate	5.91	36.5	35.5	20.5	0.5	0.5
			Final	5.91	36.0	35.5	22.0	0.5	0.5

Table 3. *Effects of adding glycolysable substrates other than glycogen to glycolysing muscle preparations*

Diluted ox-muscle minces were prepared as described in Table 1 with diluents previously adjusted to pH 6.60–6.65. Initial trichloroacetic acid extracts were made 4 min. after dilution and initial glycogen determinations 12 min. after dilution. Final samples were taken 333 min. after dilution, by which time the pH had remained unchanged in the undiluted mince for about 60 min., in the potassium chloride mince with added glycogen for about 180 min. and in the glucose–hexokinase mince for about 120 min. In the hexose monophosphate mince and hexose diphosphate mince the pH had fallen by about 0.06 unit in the last 60 min. before the final sample was taken and continued to fall slowly after the final sample was taken to ultimate pH values of 5.57 and 5.56 respectively.

Preparation	Diluent	Total acid-soluble P (μ g.atoms/ g.)	Sample	pH	Amount (in 1 g. of preparation)					
					Glycogen (as μ moles of glucose)	Lactic acid (μ moles)	In- organic P (μ g.- atoms)	Triose phos- phate P (μ g.atoms)	Alkali- labile P (μ g.- atoms)	Acid- labile P (μ g.- atoms)
Undiluted mince	None	55.5	Initial	6.73*		34.5	17.0	0.5	5.5	14.0
				6.68†	47.5					
			Intermediate	5.90	26.0	84.5	33.5	0	3.0	6.0
			Final	5.38	10.0	112.0	41.0	0	2.5	1.5
Potassium chloride mince with added glycogen	0.16M-KCl containing 1.5% (w/v) of glycogen	28.5	Initial	6.64*		18.0	9.5	0.5	2.5	7.0
				6.57†	70.0					
			Intermediate	5.85	58.5	44.5	22.5	0	0.5	0.5
			Final	5.85	59.0	43.5	22.5	0	0.5	0.5
Hexose mono- phosphate mince	52 mM-Glucose 1-phosphate in 0.16M- KCl	55.0	Initial	6.50*		19.0	7.0	1.0	18.5	11.5
				6.38†	26.0					
			Intermediate	5.82	23.5	53.5	19.5	1.5	20.5	10.5
			Final	5.60	22.0	63.5	32.5	1.0	13.0	6.5
Hexose diphos- phate mince	30 mM-Fruc- tose 1,6-di- phosphate in 0.16M- KCl	58.5	Initial	6.56*		19.0	9.5	2.0	25.5	17.4
				6.48†	25.5					
			Intermediate	5.80	10.0	57.0	30.5	1.0	16.0	7.5
			Final	5.61	10.0	65.5	39.5	1.0	9.5	5.0
Glucose–hexo- kinase mince	56 mM-Glucose and 0.02% (w/v) hexo- kinase in 0.16M-KCl	29.0	Initial	6.55*		19.0	9.0	0.5	4.0	7.0
				6.28†	25.5					
			Intermediate	5.73	26.0	42.0	5.0	1.0	12.5	6.0
			Final	5.45	25.0	54.5	17.5	0.5	4.5	2.5

* 4 min. after dilution.

† 12 min. after dilution.

within the range 5.82–6.05. In two instances, where the ultimate pH of the undiluted mince was about 6.0, that of the potassium chloride mince was essentially the same, but in all other instances the ultimate pH of the potassium chloride mince was higher than that of the undiluted mince and was reached sooner. The effect is referred to below as the 'dilution effect'.

In potassium chloride minces both glycogen utilization and lactic acid production ceased at a higher pH than in undiluted minces, and residual acid-labile P and alkali-labile P concentrations were very low (Table 2).

Effect of adding glycogen. The ultimate pH values reached in the undiluted minces indicated that, with few exceptions, the corresponding potassium chloride minces contained sufficient glycogen to permit the pH to fall below 5.8. Appreciable amounts of

glycogen were also found in potassium chloride minces that had reached their ultimate pH.

The ultimate pH attained when glycogen (up to 30 mg./ml.) was included in the diluent was only slightly lower than that of the corresponding potassium chloride mince. In addition, the amounts of glycogen used and of lactic acid produced were similar in the presence and absence of added glycogen, as also were the acid-labile P and alkali-labile P at the ultimate pH (see Table 2).

It follows that, provided that there is enough glycogen to permit the pH to fall to about 5.8, glycogen concentration is relatively unimportant in the dilution effect. Consequently it is not considered necessary in the sections below to distinguish between potassium chloride minces and similar preparations containing added glycogen.

Effect of adding cofactors of glycolysis. Effects on

the ultimate pH due to inclusion in the diluent of ATP (3 or 6mm), ADP (4 or 8mm) or NAD (2.5 or 5mm), with or without glycogen (10mg./ml.), were small. The effect of adding a magnesium salt was examined in ox- and sheep-muscle preparations only. Inclusion of magnesium chloride in the diluent resulted in some lowering of the ultimate pH, an effect that increased with increasing concentration of added magnesium salt; but even when the diluent was 20mm in magnesium chloride (a concentration of Mg^{2+} higher than that in muscle) the ultimate pH was lowered by only about 0.1pH unit. These results indicate that the dilution effect is not due simply to dilution of one or other of these cofactors.

Effects of adding glycolysable substrates other than glycogen. Inclusion in the diluent of glucose 1-phosphate (26 or 52mm), fructose 1,6-diphosphate (30 or 37.5mm) or glucose (56mm) plus hexokinase (0.2–0.4mg./ml.), to give 'hexose monophosphate mince', hexose diphosphate mince' and 'glucose-hexokinase mince' respectively, resulted in a marked lowering of the ultimate pH (see Table 3).

The ultimate pH of a hexose monophosphate mince or a hexose diphosphate mince was always lower than that of the corresponding potassium chloride mince but was usually somewhat higher than that of the corresponding glucose-hexokinase mince (see Table 3).

Analyses of hexose monophosphate minces, hexose diphosphate minces and glucose-hexokinase minces and of the corresponding potassium chloride minces and undiluted minces were made on four occasions, only two of which included the determination of triose phosphate P. Similar results were obtained on each occasion: one set is given in Table 3. In one experiment acid-labile P and alkali-labile P in all preparations showed no further change, or had decreased only slightly, 12hr. (at 5°) after the final samples (see the Materials and Methods section) had been taken.

The diluted preparations containing added glycolysable substrates other than glycogen appeared to differ in their ability to utilize glycogen. In all of them, however, the glycogen content remained virtually unchanged after the pH had fallen below about 5.8, though lactic acid was still produced. At the same time the concentration of alkali-labile P fell, but appreciable amounts still remained when the ultimate pH was reached.

DISCUSSION

Acid-labile and alkali-labile phosphates. Under the conditions of hydrolysis used, 60–100% of the phosphorus of each of the glycolytic intermediates from glucose 6-phosphate to triose phosphate inclusive is known to be alkali-labile. This is taken into account in the discussion that follows, as also is

the fact that, although it is not the only possible source of acid-labile P, glucose 1-phosphate contributes essentially 100% of its phosphorus to this fraction (Cori, Colowick & Cori, 1937).

Utilization of glucose 1-phosphate in diluted minces. In those preparations (i.e. the hexose monophosphate minces) to which glucose 1-phosphate was added, its concentration fell rapidly. For instance, Table 3 shows that, 4min. after dilution, the amount of acid-labile P present in the hexose monophosphate mince (11.5 $\mu g.$ atoms/g.) was considerably lower than the total P added as glucose 1-phosphate (26.5 $\mu g.$ atoms/g.). Also, the concentration of alkali-labile P in this initial sample was considerably higher than in the corresponding potassium chloride mince. These findings demonstrate that the phosphoglucomutase step was not the rate-limiting one for glycolysis in diluted minces at pH 6.5–6.6.

Interruption of the glycolytic sequence in potassium chloride minces. The fact that in hexose monophosphate minces, hexose diphosphate minces and glucose-hexokinase minces lactic acid could still be produced and that the alkali-labile P concentration continued to decrease, even though glycogen was no longer being utilized (i.e. after the pH fell to about 5.8), is of particular significance. The lactic acid formed below pH 5.8 was presumably derived from one of the hexose 6-phosphates or from hexose diphosphate. Triose phosphate represented only a small part of the alkali-labile P at any time (see Table 3) and changes in its concentration could not account for the considerably larger decrease in alkali-labile P concentration observed. It is clear therefore that in potassium chloride minces a block had developed at one of the early steps in the glycolytic sequence by the time the pH had fallen to about 5.8. The very low concentration of alkali-labile P in these preparations at the ultimate pH implies that the production of glucose 6-phosphate had ceased, and hence that the block had occurred at either the phosphoglucomutase or the phosphorylase step.

Failure of the phosphorylase step. The equilibrium state in phosphorylase systems is defined by the ratio of inorganic P to glucose 1-phosphate P. The results obtained in the present work do not permit calculation of this ratio. However, some evidence that the block in the glycolytic sequence in potassium chloride minces occurred at the phosphorylase step can be adduced by considering the ratio of inorganic P to acid-labile P at the ultimate pH. This ratio is, in all probability, lower than the ratio of inorganic P to glucose 1-phosphate P, since it is likely that phosphates other than glucose 1-phosphate also made contributions to the residual acid-labile P.

The relation between equilibrium ratio and pH

has been studied for animal (muscle, liver, brain) phosphorylase in the pH range 7.6–6.0 (Cori & Cori, 1940; Cori, Cori & Green, 1943) and for plant (potato) phosphorylase in the pH range 7.0–5.0 (Hanes, 1940). Where these ranges overlap the two systems are closely similar in their pH relationships. Hanes & Maskell (1942) deduced, however, that the two systems were probably not identical in all respects.

In the present work several of the potassium chloride minces reached ultimate pH values close to 6, at which pH the equilibrium ratio for the phosphorylase system is about 6:1. However, the ratio of inorganic P to acid-labile P in potassium chloride minces at the ultimate pH was always greater than 30:1 (ten experiments). If in these preparations the block in the glycolytic sequence had been at the phosphoglucomutase step, equilibrium would have been established at the phosphorylase step. It may be concluded therefore that the block was at the phosphorylase step.

Reasons for the failure of the phosphorylase step in potassium chloride minces at a pH of about 5.8 or higher are not apparent. Glycogen and inorganic phosphate were present, and the pH conditions were not such as to preclude phosphorylase activity, since in the undiluted minces glycogen utilization continued at pH values below 5.8.

In the ten undiluted minces examined the ratio of inorganic P to acid-labile P was 25:1 or higher (see Tables 2 and 3 for examples). These ratios were considerably higher than the equilibrium ratios that could be expected for the phosphorylase system at pH values between 5.4 and 5.8. It may be argued therefore that in undiluted minces too the phosphorylase system was not active at the ultimate pH.

Other factors determining the ultimate pH of muscle preparations. The ability of diluted minces to convert some glycolytic intermediates into lactic acid after the pH had fallen below 5.8 suggests that the ultimate pH of potassium chloride minces depended not only on the pH at which glycogen utilization ceased but also on the concentrations, at this point, of these intermediates.

The presence of appreciable amounts of residual alkali-labile P in hexose monophosphate minces, hexose diphosphate minces and glucose–hexokinase minces, and in many of the undiluted minces, suggests that the ultimate pH of these was determined by the failure of one of the enzyme systems responsible for acid production either from one of the hexose 6-phosphates or from hexose diphosphate. The results give no clear indication of which step was involved, nor do they provide an explanation for the appreciable variability noted among these preparations in the pH at which utilization of alkali-labile phosphates ceased.

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