BBA 45546

THEORETICAL PHOSPHORYLATION RATES AFTER ADDITION OF A SMALL AMOUNT OF GLUCOSE TO INTACT ASCITES TUMOR CELLS

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(Received September 16th, 1966)

SUMMARY

Changes were measured in glycolytic and respiratory rates during the entire period of glycolysis and respiratory inhibition after addition of 0.08 or 0.15 mM glucose to Ehrlich ascites carcinoma cells in 54 mM phosphate buffer (pH 7.3) at 37°. Glycolytic products fully accounted for the glucose utilized.

Theoretical rates of glycolytic ATP synthesis were calculated from the rates of accumulation of glycolytic products, and rates of oxidative phosphorylation were calculated from respiratory rates, assuming a P:O ratio of 3.0. The maximum in the glycolytic phosphorylation rate curve preceded the minimum in the respiratory phosphorylation rate curve. As a consequence, the total phosphorylation rate curve was biphasic, first rising above, then falling below, and finally returning to the initial, pre-glucose rate. The area under the early rise approximately equalled the area above the later dip and corresponded to between 1 and 2 μmoles of ATP/ml cells. The low rate of change in the ATP content of the cells indicated that most of the change in phosphorylation rate represented changes in both ATP synthesis and ATP utilization.

It is hypothesized that ATP synthesized by glycolysis is more readily available to the ATP-utilizing systems. On addition of glucose, ATP is shifted from a respiratory to a glycolytic reservoir and a period of more rapid ATP utilization associated with a decrease in the level of endogenous substrates involved in the ATP-utilizing reactions ensues; after cessation of glycolysis, the process is reversed, and ATP utilization is slowed for a period while the endogenous substrates increase again.

INTRODUCTION

It has been shown previously that the theoretical rate of ATP synthesis, calculated from the rate of respiration and the rates of accumulation of glycolytic intermediates, increases above the initial, pre-glucose rate after addition of 0.77 mM glucose to cells incubated at 30° (ref. 1). At much lower levels of glucose (0.08 mM), there are distinct indications that the rate of ATP synthesis follows a biphasic curve after glucose addition, rising above the initial rate as glycolysis accelerates and then falling below this rate as glucose nears exhaustion and respiration begins to recover from inhibition[2]. The lag in the recovery of respiratory phosphorylation as glycolytic

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phosphorylation declines seemed well established, but the early rise in total phosphorylation rate was less certain because of errors in the respiration curve[2]. The present paper extends these studies to cells incubated at low glucose levels but a higher temperature (37°) and provides further evidence for the biphasic nature of the phosphorylation curve.

The experimental approach used is basically similar to the approaches used by LONBERG-HOLM, WU AND RACKER, CHANCE AND HESS to investigate the mechanisms controlling glycolysis and respiration in ascites tumor cells, since it depends on measurement of the kinetics of respiration and glycolytic intermediate accumulation after sudden introduction of glucose to cells aerobically metabolizing endogenous non-glycolytic substrates (see refs. 11, 15, 16, and 20, for example). The accumulation patterns and steady-state levels of intermediates observed in different laboratories have been compared previously[1]. The distinguishing features of the present work include the low level of glucose used (0.154 mM, or less), the balance between glucose utilized and products measured, and the simultaneous determinations of glycolytic and respiratory rates.

METHODS

Preparation of tumor cells

A hypotetraploid strain of Ehrlich ascites carcinoma cells was grown for 7–11 days in Swiss white mice and was prepared and incubated in a 54 mM phosphate buffer solution (phosphate-Locke, pH 7.3–7.4) as described previously[3,4].

Experimental procedures

1 ml of aerated tumor suspension (15–25 vol. % cells) was added to 5 ml of buffer, and after 60 sec, 0.5 ml of glucose in buffer (1 or 2 mM) was also added. At accurately timed intervals after glucose addition, 3 ml of ice-cold 14 % $HClO_4$ were rapidly blown into the suspensions. The $HClO_4$ extract was neutralized with 20 % KOH and left at 5° overnight; after removal of the $KClO_4$, these extracts were used directly for analyses involving enzymes. Additional filtrates were prepared from these with $Ba(OH)_2$ and $ZnSO_4$ for use in the lactate determination[1].

The Gilson Medical Electronics Oxygraph, Model K, was used with a bare, vibrating-reed platinum cathode for respiration studies, as described previously[1,5,6]. 1 ml of buffer was added to the chamber followed by 0.20 ml of the same tumor suspension used in the glycolysis experiment; after 60 sec, 0.10 ml of glucose solution was added and the recording was continued several minutes.

In experiments where glycolytic patterns in the multiple flasks were compared with those in the Oxygraph chamber, a 12-ml conical centrifuge tube containing 0.6 ml 14 % $HClO_4$ and fitted with a stopper holding 4-mm inlet and outlet tubing was used to empty the Oxygraph chamber. The outlet tubing was attached to a vacuum line which was then opened until the stream of air entering the inlet tubing displaced the surface of the $HClO_4$ without agitating it; the inlet tubing was inserted into the Oxygraph chamber at a predetermined time and the contents of the chamber were sucked into and mixed with the $HClO_4$ in less than 1 sec. The exact time of emptying could be determined from the Oxygraph tracing. The volume of suspension left behind in the chamber was negligible.

Estimation of metabolites

Glucose was estimated by means of a commercially prepared glucose oxidase–peroxidase–chromogen combination ('Glucostat')[1]. The various phosphorylated glycolytic intermediates as well as pyruvate, ADP, and ATP were determined by coupling specific enzymatic reactions with dehydrogenase reactions to reduce or oxidize pyridine nucleotides[1]. Lactate was estimated by the method of BARKER AND SUMMERSON[7]. An enzymatic method[8], used in a few experiments, gave results close to those obtained with the colorimetric method.

Materials

Most biochemicals were obtained from the Sigma Chemical Co. in the purest available form. All glycolytic enzymes used in the analytical procedures except hexokinase were secured in crystalline form from either Sigma Chemical Co. or from Boehringer und Soehne. Hexokinase (EC 2.7.1.1) was Sigma Type III or IV enzyme.

RESULTS

Typical metabolic patterns

Results obtained from 5 separate experiments are used in the calculations described below; the conditions used in these experiments are summarized in Table I.

TABLE I

EXPERIMENTAL CONDITIONS

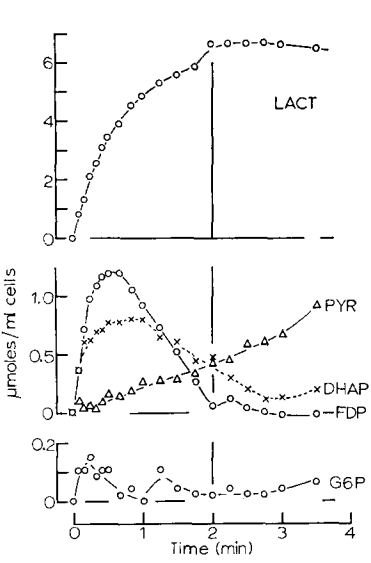
All experiments were carried out at 37° using a 54 mM isotonic phosphate buffer (pH 7.35) (phosphate-Locke solution). Respiratory rate given in μmoles O_2/ml cells per min.

Expt.	*Vol. % cells*	*Glucose*		*Endogenous respiratory rate*
		mM	*μmoles/ml cells*	
A	3.3	0.077	2.3	2.0
B	3.9	0.077	2.0	2.1
C	3.4	0.077	2.3	1.5
D	3.2	0.154	4.8	1.4
E	3.7	0.154	4.2	1.6

To illustrate the type of data used, the detailed results from one of these experiments (E) are presented in Figs. 1–3. The changes in the major accumulation products—lactate, fructose 1,6-diphosphate, and dihydroxyacetone phosphate—indicate that glycolysis is essentially over by 2 min, although minor changes continue after this time (Fig. 1). Glucose is exhausted by about 1 min (*cf.* Fig. 5, Expt. E); hence, lactate is accumulated between 1 and 2 min mainly at the expense of fructose 1,6-diphosphate and dihydroxyacetone phosphate. The continued increase in pyruvate after lactate has become constant implies an increase in the (NAD^+/NADH) ratio in the pyridine nucleotide pool associated with lactate dehydrogenase (EC 1.1.1.27) after the cessation of glycolysis[1].

Although the minor accumulation products contribute relatively little to the theoretical phosphorylation rates (see below), they provide sensitive indicators of

changes in glycolytic flux rates. Glucose 6-phosphate (Fig. 1), glyceraldehyde 3-phosphate, and 3-phosphoglyceric acid (Fig. 2) reach maxima within 20 sec, well before the maxima in fructose 1,6-diphosphate or dihydroxyacetone phosphate. A curious feature of this experiment is the secondary rise in 3-phosphoglyceric acid concomitant with a decline in 2-phosphoglyceric acid between 1 and 2 min. Possible interpretations of these observations will be considered in the DISCUSSION.



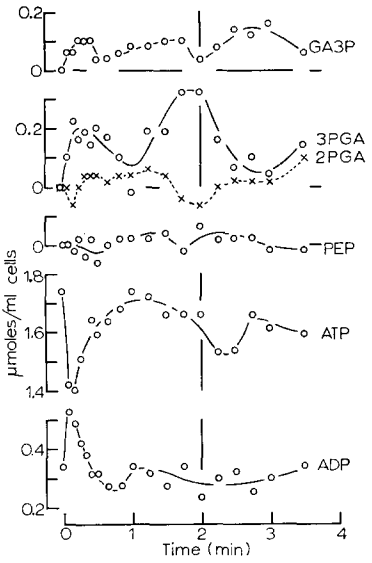


Fig. 1. Lactate (LACT), pyruvate (PYR), dihydroxyacetone phosphate (DHAP), fructose diphosphate (FDP), and glucose 6-phosphate (G6P) accumulation after glucose addition. Data from Expt. E (see Table I).

Fig. 2. Changes in glyceraldehyde 3-phosphate (GA3P), monophosphoglycerate (3PGA and 2PGA), phosphoenolpyruvate (PEP), ATP, and ADP after glucose addition. Data from Expt. E (see Table I).

The initial decline and recovery in ATP along with the rise and fall in ADP (Fig. 2) is a consistent finding in these experiments[1,2,9]. The changes in ATP are not exactly compensated by those in ADP, a consequence both of a change in AMP and an initial loss in total adenine mononucleotides[10,*].

The respiratory aspect of Expt. E is presented in Fig. 3. Curve B (upper frame) represents the rate after addition of buffer and Curve G, the rate after addition of glucose in the same volume of buffer; three values were averaged for each point. The difference curve, which should represent the effect of glucose alone, is given in the lower frame. Vertical bars give the mean deviation. A stimulation of respiration at 10 sec followed by a depression reaching maximum depth between 60 and 90 sec and then a recovery complete by 150 sec are apparent. Two differences between the curve in Fig. 3 and those described previously for lower temperatures[2] may be noted: the glucose-inhibited respiration returns completely to the rate observed in buffer; and respiration recovers more rapidly at 37°. In the detailed experiment at

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30° described earlier, respiration never regained its initial, pre-glucose rate; this was attributed to an acceleration of the initial rate because of a low initial ATP level[2]. In the 37° experiments, the cells were preaerated by shaking so that the initial ATP level would be high (Fig. 2). At both 30° and 23° the end of glycolysis often occurred earlier on the ascending limb of the respiratory rate curve than at 37°.

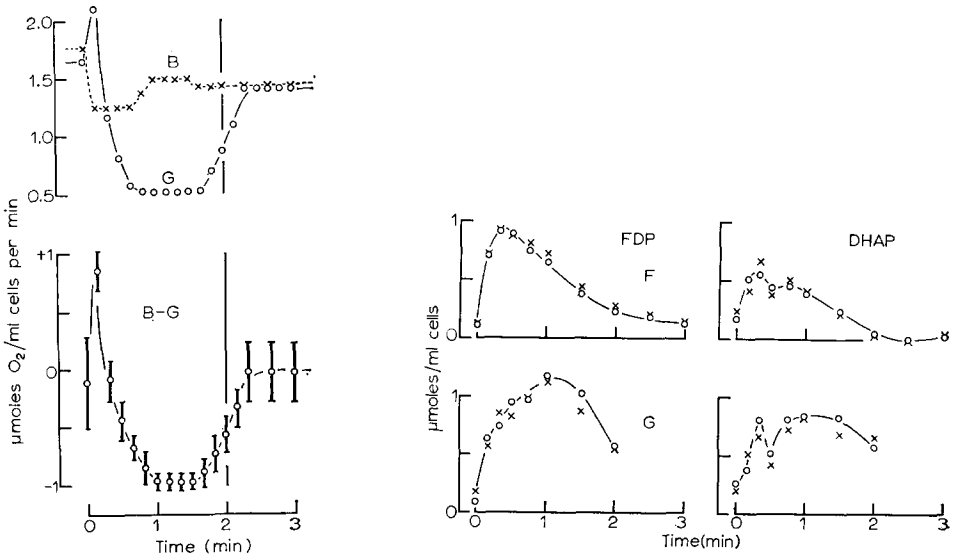

Fig. 3. Changes in respiratory rate after glucose addition. Data from Expt. E (see Table I). Upper frame: Curve B, respiration after buffer addition (average of 3 curves); Curve G, respiration after addition of glucose in buffer (average of 3 curves). Lower frame: difference between Curve B and Curve G in upper frame; vertical bars represent mean deviation of difference.

Fig. 4. Comparison of fructose diphosphate (FDP) and dihydroxyacetone phosphate (DHAP) accumulation in incubation flasks and Oxygraph chamber. Symbols: ○, values from multiple-flask incubation; ×, values from samples removed from Oxygraph chamber. Conditions: Expt. F, 3.4 vol. % cells, 0.154 mM glucose, 4.6 μmoles glucose/ml cells; Expt. G, 2.3 vol. % cells, 0.154 mM glucose, 6.7 μmoles glucose/ml cells. Other conditions as in Table I.

Glycolysis in the flasks and in the oxygraph chamber

An obvious problem in quantitative comparison of respiratory rate curves, obtained with the Oxygraph, and glycolytic rates, obtained by analyses of cells incubated in flasks, concerns the possible differences in the environments of the cells. The cells in the Oxygraph chamber were stirred by bubbling rather than shaking, exposed to a higher potassium concentration because of diffusion from a KCl bridge, and subjected to an electric current. Hence, control experiments in which the contents of the Oxygraph chamber were precipitated with $HClO_4$ and analyzed along with samples from a parallel flask incubation were carried out (see METHODS). Since the volume obtained from the chamber was small, only one or two analyses could be made from each extract. Hence, comparisons were based mostly on the fructose 1,6-diphosphate accumulation curves, which exhibited large, reproducible variations. The results of two such experiments (F and G) are summarized in Fig. 4. Values for fructose 1,6-diphosphate and dihydroxyacetone phosphate obtained by the usual procedure from the flask incubations are represented by circles and follow the expected types

of curves (see Fig. 1). Values obtained from the Oxygraph chamber contents, represented by crosses, are somewhat more scattered but fall close to the flask values. If a difference in accumulation patterns exists, it is too small to have an appreciable effect on the rate calculations.

Glucose balance curves

Calculation of glycolytic rates of phosphorylation depends on an assumption that all glucose passes simply and directly through glycolysis without contributions by or drains to non-glycolytic components. This assumption is generally supported by the data in Fig. 5. The lines represent glucose consumed (individual glucose points deviate by less than 0.1 μmole/ml cells) and the crosses represent the sum of all the measured intermediates. Except for Expt. B, where the product points are scattered and somewhat high, the summed products come to within 10% of the glucose consumed. The agreement is particularly good for Expt. E, which makes it a suitable experiment for the detailed comparisons considered below.

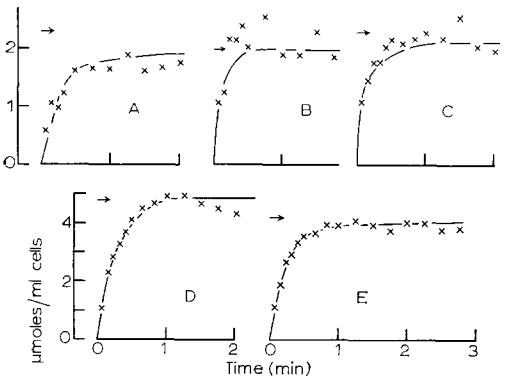

Fig. 5. Glucose balance curves. Expts. A–E (see Table I). Lines represent glucose utilized, determined from direct estimates; individual points deviated by less than 0.1 μmole from the line. Crosses represent the sum of the increases in measured glycolytic products.

Calculation of phosphorylation rates

Both glycolytic and respiratory phosphorylation rates are calculated as changes from the initial, endogenous rate. In the case of respiration, the mean rate of O_2 consumption after glucose addition, calculated from 3 to 5 Oxygraph tracings, is subtracted from the mean rate of consumption after addition of an equivalent volume of buffer (2 or 3 tracings). Use of this difference compensates for variations consequent from dilution and mixing effects and the resultant curve should represent effect attributable to the glucose alone[2]. The change in respiratory phosphorylation, assuming complete coupling, is then 6 times this difference, or $6\Delta d[O_2]/dt$.

Glycolytic phosphorylation is estimated from the accumulation rates of glycolytic products:

$$d/dt\,[\text{lactate} + \text{pyruvate}] - d/dt\,[\text{glucose 6-phosphate} + 2\ \text{fructose 1,6-diphosphate} + \text{dihydroxyacetone phosphate} + \text{glyceraldehyde 3-phosphate}]$$

As discussed previously[1,2,9], intermediates at the monophosphoglycerate stage are not included, since they do not represent a net change in ATP synthesis. Rates of accumulation are calculated from the point slopes of the smoothed curves such as

those illustrated in Figs. 1 and 2. This approach is valid when the glucose utilized is nearly accounted for by the measured products, as it is in these experiments (Fig. 5).

The change in the theoretical rate of phosphorylation by respiration is shown in Fig. 6 and may be compared with the corresponding rate of glycolytic phosphorylation in Fig. 7. Generally, as glycolytic phosphorylation increases, respiratory phosphorylation declines, and *vice versa*, but the maximum in the glycolytic

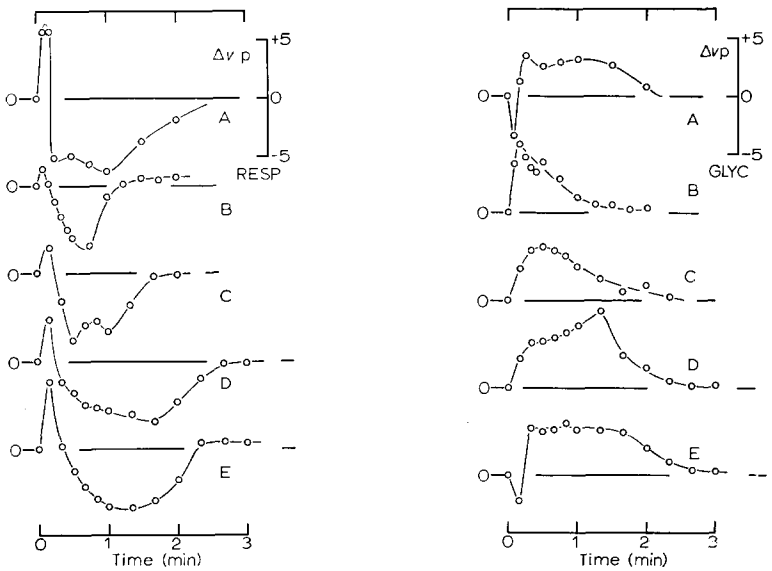

Fig. 6. Changes in the theoretical respiratory ATP synthesis rate after glucose addition. Expts. A–E. Curves represent change in rate of ATP synthesis from initial endogenous rate and were calculated by multiplying difference curves such as the (B—G) curve in the lower frame of Fig. 3 by 6. Total theoretical respiratory phosphorylation may be estimated by adding 6 times the endogenous respiratory rate given in Table I. Δv_P equals μmoles ATP/ml cells per min.

Fig. 7. Changes in the theoretical glycolytic ATP synthesis rate. Expts. A–E. Curves calculated from rates of accumulation of glycolytic intermediates, assuming complete coupling as described in text. Δv_P given in μmoles ATP/ml cells per min.

curves precedes the minimum in the respiratory curves. As a consequence, the sum of the two curves yield a biphasic total curve which rises above and then falls below the initial rate (Figs. 8 and 9). The area under the initial peak corresponds to between 1 and 2 μmoles of ATP synthesis and is approximately equaled by the area above the later dip (Table II). These areas also approach the levels of ATP in the cells. The total phosphorylation rate curve for Expt. E is presented separately in Fig. 9 so that the rate of ATP accumulation and the change in ATP content in the whole cell may be compared; similar results were obtained with the other experiments. After the initial sharp decline, the ATP accumulation rate, estimated from the slope of the ATP curve in Fig. 2, tends to follow the synthetic rate, although the variations are much smaller in magnitude (note difference in scale). The decline in ATP level, obtained by subtracting the individual values in Fig. 2 from the initial value, also follow the rate of ATP synthesis, indicating that ATP synthesis is highest when the ATP level

is lowest. This is the reverse of the relationship observed previously at higher glucose levels and lower temperatures[1] (see DISCUSSION).

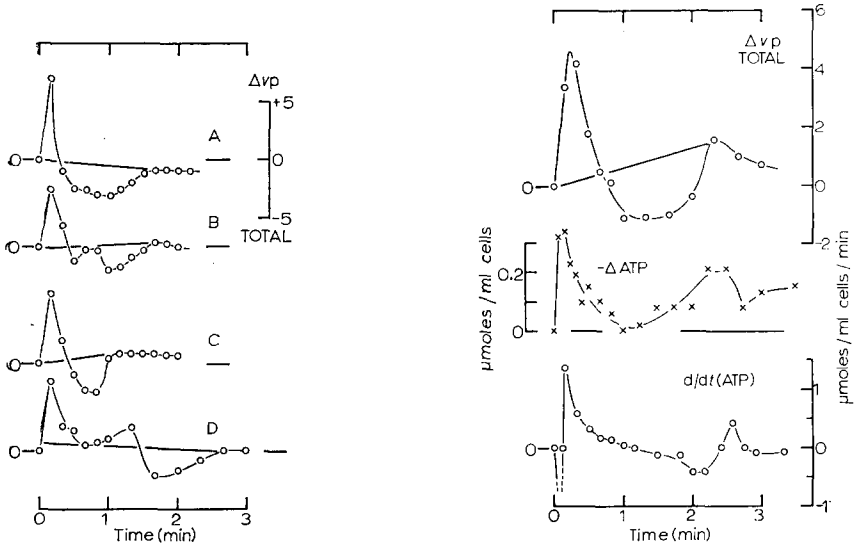

Fig. 8. Changes in the total theoretical ATP synthesis rate. Expts. A–D. Curves obtained by adding respiratory rate change curves (Fig. 6) to glycolytic rate curves (Fig. 7).

Fig. 9. Total theoretical ATP synthesis rate, decline in ATP level, and rate of change in ATP level in Expt. E. Top frame: change in total ATP synthesis rate, as described in Fig. 8. Middle frame: initial (pre-glucose) ATP level *minus* ATP level at time t; a positive value represents a decrease in ATP. Bottom frame: rate of change in ATP level.

TABLE II

PHOSPHORYLATION SHIFTS AFTER GLUCOSE ADDITION

Areas were calculated by integration between the portion of the Δv_P curve above the line and the line (early peak) and between the portion of the Δv_P curve below the line and the line (late dip) in the total phosphorylation rate curves shown in Figs. 8 and 9. The μmoles of ATP represents the amount present before glucose addition.

Expt.	*Areas (μmoles/ml cells)*		*μmoles of ATP/ml cells*
	Early peak	*Late dip*	
A	0.9	2.2	2.05
B	1.1	1.2	1.83
C	1.1	1.3	2.16
D	1.5	1.5	2.77
E	1.7	1.9	1.73

DISCUSSION

Correlations among glycolytic events in Expt. E

The early maximum in glucose 6-phosphate, first observed by LONBERG-HOLM[11], implies a decrease in hexokinase (EC 2.7.1.1) activity relative to phosphofructo-

kinase (EC 2.7.1.11), possibly because of a combined product inhibition of hexokinase[11] and ADP activation of phosphofructokinase[1]. Data obtained with varying glucose concentrations indicate that the initial rates of fructose 1,6-diphosphate and lactate accumulation increase as glucose is increased from 0.08 to 0.77 mM, whereas the rate of glucose 6-phosphate accumulation remains constant*; this suggests that hexokinase activity is sensitive to substrate concentration in this range, but that phosphofructokinase is somehow activated by the hexokinase reaction.

The early maximum in glyceraldehyde 3-phosphate despite the continued rise in dihydroxyacetone phosphate and declining rate of lactate accumulation suggests either a slowing of the triose phoshate isomerase (EC 5.3.1.1) or a partial separation of the glyceraldehyde 3-phosphate and dihydroxyacetone phosphate pools. The rate of lactate accumulation exceeds the rate of dihydroxyacetone phosphate accumulation at all times, indicating that a net conversion of dihydroxyacetone phosphate to glyceraldehyde 3-phosphate is taking place: If triose phosphate isomerase decreased in activity somewhat after 20 sec, a decline in glyceraldehyde 3-phosphate along with a continued rise in dihydroxyacetone phosphate might be expected. On the other hand, if a fraction of the dihydroxyacetone phosphate were being reduced to glycerol phosphate in the cytoplasm, shifted to the mitochondria, and reoxidized to dihydroxyacetone phosphate, then two separate pools of dihydroxyacetone phosphate might exist and its continued rise might reflect an increase in a mitochondrial pool unavailable to the isomerase. Indirect evidence for the existence of such a system in these cells has been reported previously[1,12].

The early maximum in 3-phosphoglyceric acid, also reported by LONBERG-HOLM[11], and confirmed in our laboratory for higher glucose concentrations[1], may reflect a brief period of non-glycolytic oxidation of glycolytically generated NADP (*cf.* ref. 1). The failure of 2-phosphoglyceric acid to follow the 3-phosphoglyceric acid suggests a sluggish monophosphoglycerate mutase (EC 2.7.3.5) and a straightforward application of the crossover theorem[13] to the period between 1 and 2 min implies that the mutase is the controlling step during this time. Whether the crossover theorem can be applied safely to a system replete with feedback loops and allosteric activations and inhibitions remains debatable, however. It should be noted that the secondary rise in 3-phosphoglyceric acid along with the fall in 2-phosphoglyceric acid does not occur in all experiments.

Interpretation of the total phosphorylation rate curves

The initial rise in the total theoretical rate of ATP synthesis, during the most active period of glycolysis, and the later depression, when glycolysis has almost ceased and respiration is beginning to recover, suggests that glycolytically generated ATP is more immediately available for the various ATP-utilizing reactions than oxidatively generated ATP[2]. The actual ATP level varies, but the rate of change in ATP content is usually much less than the change in the total rate of synthesis (Fig. 9) indicating that variations in the rate of synthesis are largely compensated by comparable variations in the rate of utilization. A tendency of the rate of ATP accumulation to follow the rate of synthesis, after the initial sharp decline, suggests that ATP accumulation reflects the rate of synthesis to some extent. However, the

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total rate of synthesis is inversely correlated with the ATP level itself, being highest when the ATP content of the cells is lowest (Fig. 9). This inverse correlation of synthetic rate and ATP level is the opposite of the relationship observed at higher glucose levels and lower temperatures, where higher rates of synthesis are associated with higher ATP levels[1]. The reversal of the correlation appears to be a function of the changing relationships among the velocities of phosphofructokinase (EC 2.7.1.11), lactate dehydrogenase (EC 1.1.1.27) and respiration with temperature, rather than a function of glucose concentration*.

A diagram of a hypothesis proposed to explain the biphasic phosphorylation rate curve is presented in Fig. 10. The hypothesis assumes that respiration (R) and glycolysis (G) supply different pools of ATP, that the rate of ATP utilization by conversion of endogenous X components to Y components controls the overall rate of ATP synthesis by respiration and glycolysis together, and that the enzymes catalyzing the reactions of the X components with ATP are more readily accessible to or have a higher affinity for the glycolytic ATP pool. The first two assumptions are by no means new. The separation of adenine mononucleotide pools into respiratory (presumedly mitochondrial) and glycolytic compartments has been suggested by several groups of investigators, including those associated with LYNEN[14], CHANCE[15], and RACKER[16]. The control of the ATP-synthesizing systems by ATP-utilizing systems is implied by JOHNSON's suggestion of control by ATP hydrolysis products[17] and is inherent in LYNEN's phosphate cycle[18]; it also seems well established experimentally in these cells by the work of QUASTEL AND BICKIS[19], at least for steady-state periods. The third assumption is introduced here to explain the bi phasic phosphorylation rate curve.

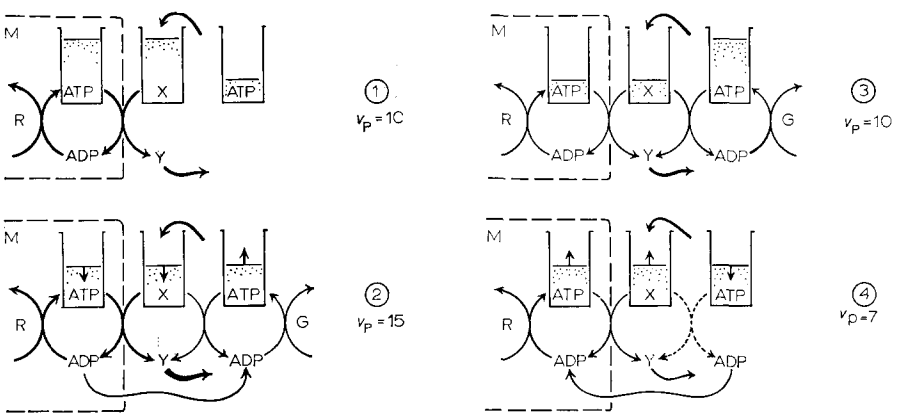

Fig. 10. Hypothetical explanation for biphasic ATP synthesis rate curve after addition of a small amount of glucose. Symbols: M, mitochondria; R, respiration supplying a respiratory ATP reservoir; X, endogenous components, supplied at a constant rate and reacting with ATP from either the respiratory or glycolytic reservoir to form ADP and Y components; G, glycolysis supplying a glycolytic ATP reservoir. *Stage 1*, steady state before addition of glucose; *Stage 2*, immediately after glucose addition when X-component reservoir is depleting because of a more rapid reaction with glycolytic ATP; *Stage 3*, new steady state where glycolytic and respiratory ATP are utilized at a balanced rate; *Stage 4*, after exhaustion of glycolytic intermediates but before respiration has completely recovered, when X-component reservoir is being replenished. Detailed description given in text.

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The suggested mechanism (Fig. 10) can be divided into four stages:

Stage 1. This represents the situation in cells before glucose addition. The X components are being supplied at a constant rate from endogenous sources and are being converted to Y components by phosphorylation with the reservoir of ATP maintained by respiration (R). The rates of ATP utilization and synthesis (v_P) are equal to 10 μmoles/ml cells per min, on the average. Since glycolysis is absent, the reservoir of ATP dependent on glycolysis is small.

Stage 2. Within 10 sec after addition of a low concentration of glucose, glycolysis is synthesizing ATP at a rate of about 5 μmoles/min and the glycolytic ATP reservoir is being expanded at the expense of the respiratory ATP reservoir. Since the X components can react more rapidly with the glycolytic ATP, at a given concentration, the reservoir of X components is being decreased and the total ATP synthesis and utilization rates are increased to near 15 μmoles/min. This stage corresponds to the upward spike in the phosphorylation rate curve; the area under this spike would be equivalent to the decrease in the X-component reservoir. It is assumed that the rate of formation of X components remains relatively constant and that the ADP can be rapidly equilibrated between the two compartments.

Stage 3. 20–40 sec after glucose addition the X components have decreased to the point where utilization of ATP in the respiratory reservoir is substantially slowed and the total rates of ATP synthesis and utilization have returned to near the original value. In certain experiments, such as B and D (Fig. 8), a transient steady state may be established at this stage. Most of the ATP has been transferred to the glycolytic reservoir.

Stage 4. After glucose nears exhaustion and glycolytic phosphorylation begins to slow because of the decline in glycolytic intermediates, the process in *Stage 2* begins to reverse. The ATP in the glycolytic reservoir is transferred back to the respiratory reservoir, *via* ADP, leading to some increase in the respiratory rate, but this increase is more than balanced by the slower phosphorylation of X components by the respiratory ATP reservoir. The net result is a shallow depression of the phosphorylation rate curve while the reservoir of X components expands to a volume which will support a more rapid phosphorylation by respiratory ATP. Eventually the whole system returns to *Stage 1.*

Although somewhat speculative, the suggested mechanism is consistent with the observations described above. It explains, for example, why the area under the initial spike is approximately equalled by the area above the later shallow depression (Table II). The postulated shift of ATP from a respiratory reservoir to a glycolytic reservoir after glucose addition is supported by observations with cells treated with iodoacetate, oxamate or 2,4-dinitrophenol. In iodoacetate or oxamate-poisoned cells, the total rate of ATP synthesis can be maintained for a period after addition of glucose by a compensatory increase in respiration, but the ATP level in the cells decreases sharply[5,*]. In contrast, when oxidative phosphorylation is uncoupled by dinitrophenol in glycolyzing cells, ATP synthesis is again maintained by a compensatory increase in glycolytic rate, but the ATP level remains high[3,16]. These observations suggest that either respiration or glycolysis can support ATP synthesis but that glycolysis is responsible for maintaining the ATP level after glucose addition; without

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glucose, it is apparent that respiration is maintaining the ATP level, since the level declines rapidly in non-glycolyzing cells exposed to dinitrophenol[3].

The proposal in Fig. 10 should be distinguished from those suggested to explain some of the shifts occurring in glycolytic intermediates in the Pasteur effect. The suggestion by LYNEN *et al.*[14] that hexokinase (EC 2.7.1.1) is more dependent on respiratory ATP while phosphofructokinase (EC 2.7.1.11) is more dependent on glycolytic ATP would not contradict our proposal, but neither is it implied by or required for our proposal. The machinery for regulating the rates of different steps within glycolysis is not considered here; the conversion of X to Y is meant to represent the variety of phosphorylative reactions involved in cell maintenance (protein synthesis, active transport, cell membrane construction, *etc.*). The proposal does run counter to the mitochondrial trapping of ATP during the initial stages of glycolysis suggested by CHANCE AND HESS[15] and elaborated by HESS[20], since it requires exactly the opposite, *i.e.* a movement of ATP out of the mitochondria. An argument against a mitochondrial trapping of ATP in our system has been presented previously[1]. It should be pointed out, however, that the glucose concentrations used in the experiments reported here were so low that the initial marked stimulation of respiration required by the mitochondrial trapping hypothesis was absent.

ACKNOWLEDGEMENTS

The authors gratefully acknowledge the skilled technical assistance of HELEN M. BURSON.

This work was supported by a grant from the National Institutes of Health, U.S. Department of Health, Education and Welfare (GM-11580).

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