The aerobic/anaerobic transition of glucose metabolism in *Trypanosoma brucei*

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The ratio of glycerol to pyruvate produced by *T. brucei* incubated with glucose at various oxygen tensions has been used as an index of the aerobic and anaerobic pathways of glucose metabolism. A minimal model is presented which fits the observed data. The value of the notional K of the aerobic/anaerobic transition from the model is close to that of the K_m of trypanosomal glycerophosphate oxidase. The anaerobic pathway appears to be almost completely inoperative at oxygen tensions in the range of those found in venous and arterial blood.

Trypanosoma brucei *Glucose* *Glycolysis* *Oxygen* *Glycerol* *Pyruvate*

1. INTRODUCTION

African trypanosomes of the *brucei* group undergo a complex life cycle involving the salivary glands and midgut of the insect vector and the bloodstream of the mammalian host. The bloodstream form of these trypanosomes has neither lipid nor carbohydrate reserves, nor does the mitochondrion possess a competent citric acid cycle. Glucose is the most important nutrient for these cells and the major and perhaps sole energy-yielding metabolic pathway is glycolysis (review [1]). Bloodstream trypanosomes contain no lactate dehydrogenase [2] and the NADH formed in the glyceraldehyde phosphate dehydrogenase step is reoxidized to NAD via a 3GP shunt [3] involving NAD-linked 3GP dehydrogenase which is localized in the glycosome [4] and mitochondrial 3GP oxidase [5].

Thus under aerobic conditions glucose is metabolized almost completely to pyruvate, approx. 2 mol pyruvate being produced per mol glucose [6–9]. Under anaerobic conditions or in the presence of an inhibitor of 3GP oxidase, such as salicylhydroxamic acid [8], glucose is metabolized to equimolar quantities of glycerol and pyruvate [6–9].

We wished to assess the relative importance of the aerobic and anaerobic pathways of glucose metabolism in trypanosomes in vivo; i.e., in the bloodstream of the mammalian host. However, the free oxygen concentrations of venous and arterial blood do not correspond to those of total anaerobiosis and aerobiosis. To learn more about the nature of the aerobic/anaerobic transition we investigated the dependence on O_2 concentration of the ratio of glycerol to pyruvate produced by these cells at oxygen tensions in the physiologically significant range which is intermediate to the extremes of full aerobiosis and anaerobiosis.

2. EXPERIMENTAL

Enzymes and cofactors were obtained from Sigma, London. All other chemicals were of Analar grade.

Cells of the long slender form of *T. brucei* were isolated from the blood of 250 g Wistar rats 71 h

Abbreviations: 3GP, L-glycerol-3-phosphate; DHAP, dihydroxyactone phosphate; Po_2, partial pressure of oxygen

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after infection with 10^7 cells of strain MITat 1.1 (obtained from Dr H.P. Voorheis of Trinity College Dublin) by intraperitoneal injection. The cells were separated from blood components by centrifugation ($600 \times g$) for 10 min at 4°C and further purified on a short column of DEAE cellulose in isotonic phosphate-buffered saline (pH 8.0) containing 10 mM glucose (Buffer A), as described by Lanham and Godfrey [10]. Cell counts were performed on a Neubauer haemocytometer. Glycerol and pyruvate were estimated in the same assay, from extent of NADH oxidation in the presence of lactate dehydrogenase, pyruvate kinase, phospho-*enol*pyruvate and ATP. Glycerol kinase was added last for the determination of glycerol [11]. The assays were performed in triplicate and were standardized by using glycerol/pyruvate solutions of known concentration. Glucose was determined by using hexokinase and glucose-6-phosphate dehydrogenase as in [11].

Incubations at various oxygen tensions were carried out at 37°C in RPMI medium 1640 or in RPMI salts medium, initial pH 8.1, incorporating 150 mg% bovine serum albumin. These media have been shown to be capable of maintaining glycolysis and cell motility of *T. brucei* over the time periods used [9]. Seventy-five ml of the medium was equilibrated with the appropriate N_2/O_2 mixture in a 3-necked flask fitted with a gas inlet/outlet tube, probe type oxygen electrode (L.H. Fermentation, Model 507) and a rubber septum through which a large-bore syringe needle was inserted below the surface of the medium. The gas mixture, prehumidified by prior passage through water, was bubbled through the medium via a sintered tube at 200 ml/min. Oxygen concentration was monitored continuously and did not vary by more than ± 2 mmHg Po_2 during the incubations. Cells were introduced through the needle to give a final concentration of 2×10^7 cells/ml. At predetermined times 1-ml samples were withdrawn via the needle. A small amount was retained for cell count and the remainder quickly spun in a microfuge; this operation took less than 90 s. The cell-free supernatant was assayed for glycerol and pyruvate. In some experiments glucose was also assayed to confirm that the sum of glycerol and pyruvate could account for the glucose consumed. Metabolic competence of the cells in the withdrawn samples was confirmed by measuring the respiration rates in Buffer A using a Clark oxygen electrode.

3. RESULTS AND DISCUSSION

3.1. *Glycerol and pyruvate production*

Sample results for three oxygen tensions are shown in fig.1. It can be seen that the rate of production of glycerol plus pyruvate is about the same at all Po_2 values and that glycerol is produced at all oxygen tensions. Under anaerobic conditions glycerol and pyruvate are produced at roughly equal rates, glycerol production slightly exceeding that of pyruvate. At higher Po_2 values pyruvate is the major product but there is also a small but significant rate of glycerol production.

The ratio glycerol:pyruvate in the medium after 60 min incubation is plotted against Po_2 in fig.2, this ratio being a convenient way of expressing the relative involvement of the aerobic and anaerobic pathways of glucose metabolism. The ratio is independent of cell count and also of sampling time between 30 and 75 min. As seen in fig.2 the glycerol:pyruvate ratio remains constant at approx. 0.1 from a Po_2 of 160 mmHg down to below 40 mmHg from which it rises to a limiting value of approx. 1 under anaerobic conditions.

3.2. *Analysis of the aerobic/anaerobic transition*

At high oxygen tensions the 3GP shunt operates between the glycosome and the mitochondrion and provides a means for regenerating the DHAP reduced by NADH. Thus the equivalent of both triose phosphates can be converted to pyruvate resulting in 2 mol pyruvate per mol glucose. Under anaerobic conditions the 3GP shunt is inoperative. The DHAP reduced to 3GP cannot be regenerated and the 3GP is dephosphorylated and excreted from the cell as glycerol in a 1:1 ratio with pyruvate.

This scheme can be described by the following minimal model:

$$\text{glucose} + O_2 \longrightarrow 2\ \text{pyruvate} + H_2O \quad (1)$$

$$\text{glucose} \longrightarrow \text{pyruvate} + \text{glycerol} \quad (2)$$

where (1) and (2) represent the aerobic and anaerobic pathways, respectively.

Let V = rate of pyruvate production via pathway (1) under total aerobiosis, and v = rate of pyruvate

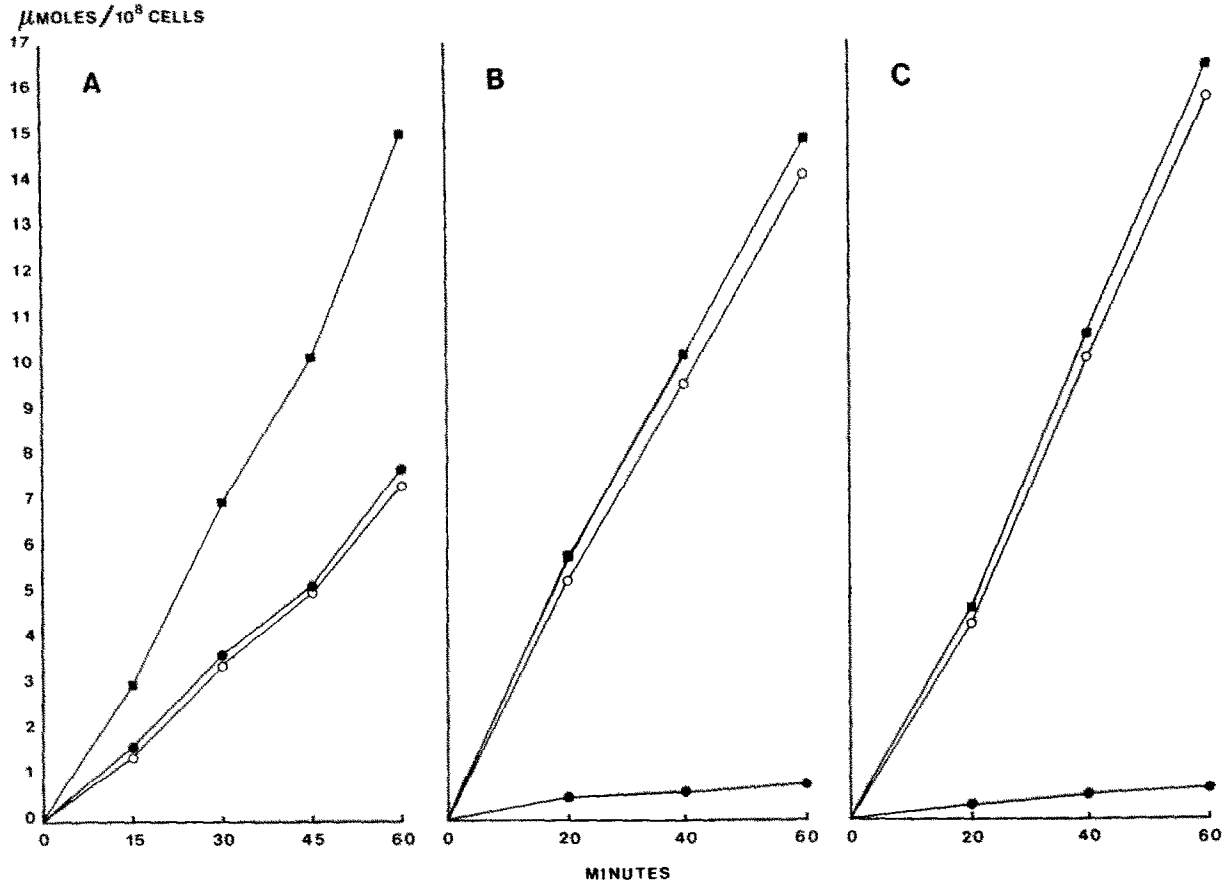

Fig.1. Glycerol and pyruvate production from *T. brucei*. For details see text. Po_2: 0 mmHg (A); 16 mmHg (B); 160 mmHg (C). (Glycerol) + (pyruvate) (■); (glycerol) (●); pyruvate (○).

production via pathway (1) under partial aerobiosis.

It is reported [8] and we have confirmed that bloodstream trypanosomes utilize glucose at about the same rate under aerobic or anaerobic conditions. This holds also at intermediate oxygen tensions. Thus $(V - v)/2$ = rate of glycerol (or pyruvate) production via pathway (2) at any level of aerobiosis.

As the total rate of production of glycerol plus pyruvate is constant it therefore follows that

$$\frac{\text{(glycerol)}}{\text{(glycerol)} + \text{(pyruvate)}} = \frac{V - v}{2V}$$

and

$$\frac{\text{(glycerol)}}{\text{(pyruvate)}} = \frac{V - v}{V + v} \qquad (3)$$

If we assume that the rate of pyruvate production via pathway (1) follows a Michaelian dependence on O_2 concentration:

$$v = \frac{V(O_2)}{K + (O_2)}$$

where K is a notional K_m of the aerobic pathway for oxygen, then by substitution into (3) we obtain

$$v = \frac{K}{K + 2(O_2)} \qquad (4)$$

Eqn 4 tends to zero at high O_2 concentrations and becomes unity when $(O_2) = 0$.

In fact we find that small but significant amounts of glycerol in excess of that predicted by theory are produced at all levels of aerobiosis, even when gassing with 95% oxygen. Glycerol : pyruvate ratios greater than one under anaerobic conditions

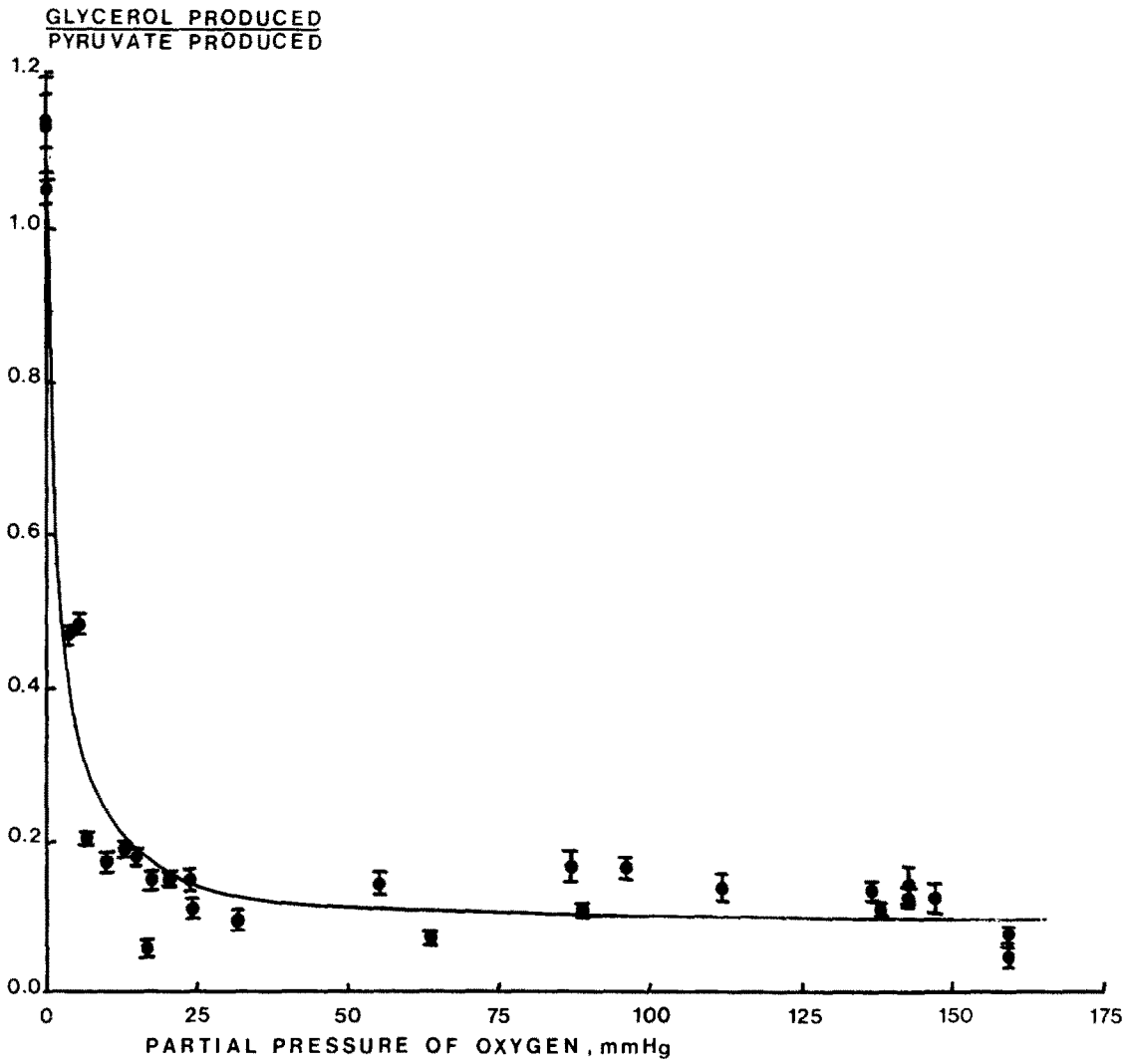

Fig.2. Ratio of glycerol:pyruvate concentrations in the medium of *T. brucei* incubated with glucose at various Po_2. The procedure is described in the text. The points are experimental, the bars representing standard errors of estimation. The curve is the best fit by least squares to the data of glycerol/pyruvate = $K/(K + 2(O_2)) + C$; $C = 0.09$, $K = 3.6 \pm 0.8$ mmHg.

reported by other workers [7,13,14] were consistent with our results. Under aerobic conditions glycerol:pyruvate ratios as low as 0.02 have been reported [6]; however, ratios as high as 0.24 and 0.4 were obtained by Ryley [7,13] for *T. rhodesiense* and *T. brucei*. Similarly, values of 0.11 and 0.1 were found by Grant and Fulton [6] and by Fairlamb and Bowman [15]. These latter workers ascribed the presence of glycerol in aerobic incubations to transient anaerobiosis experienced by the cells during workup but this is unlikely to be the case in the present study. Insufficient information is available to speculate profitably on the failure of the accepted pathways of glucose catabolism to account for all the glycerol found. Whatever the cause, we have corrected for this constant amount of glycerol by modifying eqn 4 to:

$$v = \frac{K}{K + 2(O_2)} + C$$

where C represents the effect of the 'aerobic glycerol' on the glycerol:pyruvate ratio; the equation was fitted to the data in fig.2.

4. CONCLUSION

Two conclusions can be drawn from these results. First, the value of K obtained from the model is 3.6 ± 0.8 mmHg which corresponds to an oxygen concentration of $5.0\,\mu$M. This may be compared to values of K_m in the range $2–8\,\mu$M reported for 3GP oxidase in *T. brucei* and *T. evansi* [16]. We are aware of the difficulties pointed out by Fisher [17] involved in interpretation of K_m values of intact cells. Nevertheless the close agreement between the notional K of the aerobic pathway reported here and the K_m of trypanosomal 3GP oxidase provides evidence for this enzyme as the control point in the aerobic/anaerobic transition and implies rate limitation at this point for the aerobic pathway at low oxygen tensions. This is consistent with the large increase in intracellular 3GP levels observed in *T. brucei* incubated with glucose under anaerobic conditions [18].

Secondly, the Po_2 of arterial blood is 100 mmHg and that of venous blood is 40 mmHg [19] and our results show that the glycerol:pyruvate ratio remains virtually constant in this range at a value of approx. 0.1. It thus appears that the anaerobic pathway of glucose metabolism of *T. brucei* has little if any physiological significance in the bloodstream of the mammalian host.

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