cytes is 250 times faster than its utilization. Reference [3] does indeed state that glucose transport in erythrocytes exceeds glucose utilization, and they cite [8] as a source of this information. However, the studies in [8] only measured glucose penetration in fresh blood while another paper was cited regarding the rate of glucose utilization. The authors in [8] state that glucose permeation depended on such variables as pH and temperature, and they cite another study which reported that the glucose permeation rate dropped in cell suspensions kept at 20 °C. How all these previous studies relate to blood stored at 4 °C for several days to a few weeks and assayed under completely different conditions is totally unclear. Our data would suggest that in stored blood assayed under our conditions [2], glucose transport cannot compensate for the rapid depletion of glucose caused by incubating red cells with ferricyanide over a 10 min period. In addition, findings similar to those we reported (Fig. 4 of [2]) regarding the effect of ferricyanide on the concentration of the various intermediates in glycolysis have been previously reported [9]. Furthermore, one of us (P. Arese, unpublished work) has also observed that diamide causes a similar change in the concentrations of the various glycolytic intermediates, except that in fresh blood no net lactate production was observed.

Finally, the apparent discrepancy between Miwa *et al.* and ourselves concerning the amount of lactate production observed in control cells may be reconciled by two observations. First, as mentioned above, our studies were conducted on stored blood. Depending on the length of storage in high-phosphate, glucose- and inosine-containing medium, erythrocytes will conduct glycolysis at vastly different rates. This observation is mentioned as a footnote in our paper [2]. Second, the data presented in Tables II–IV of [2] report the total concentration of lactate at the end of the incubation period, and not the rate of lactate production during the incubation. Because prior to oxidant stimulation all cells within an experiment contained the same lactate concentration, the increases in lactate concentration at the end of the 10 min stimulation period were interpreted to reflect the stimulation of lactate production by the oxidants.

In conclusion, we would like to state that the data we reported [2] are correct. As discussed above, related observations have been reported by others [4–6]. We believe that the failure of Miwa *et al.* [1] to repeat our observations may reflect differences in the age or storage conditions of the red cells.

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1. Miwa, I., Fukatsu, H. & Okuda, J. (1992) *Biochem. J.* **283**, 621–622
2. Harrison, M. L., Rathinavelu, P., Arese, P., Geahlen, R. L. & Low, P. S. (1991) *J. Biol. Chem.* **266**, 4106–4111
3. Elbrink, J. & Bihler, I. (1975) *Science* **188**, 1177–1184
4. Mishra, R. K. & Passow, H. (1969) *J. Membr. Biol.* **1**, 214–224
5. Arese, P., Bosia, G. P., Pescarmona, G. P. & Till, U. (1977) *Acta Biol. Med. Germ.* **36**, 481–490
6. Manyai, S. & Szekely, M. (1954) *Acta Physiol. Acad. Sci. Hung.* **5**, 7–18
7. Magnani, M., Rossi, L., Cucchiari, L., Stocchi, V. & Fornaini, G. (1988) *Cell Biochem. Function* **6**, 175–182
8. Widdas, W. F. (1954) *J. Physiol. (London)* **125**, 163
9. Arese, P., Bosia, A. & Pescarmona, G. P. (1975) VII. Internationales Symposium über Struktur und Funktion der Erythrozyten (Rapport, S. & Jung, F., eds.), pp. 91–102, Akademie Verlag, Berlin

The error in the Michaelis–Menten equation when substrate depletion by binding to the enzyme is not taken into account

In deriving equations for interpreting equilibrium binding or enzymic assays it is convenient and often justified to neglect depletion of the substrate by binding to the enzyme. This leads to considerable simplification of the final formulae. The present letter provides a handy rule for deciding whether the approximation is justified. Consider enzymic mono-substrate conversion according to the mechanism:

where A, E, Q, EA and EQ are the substrate, enzyme, product, enzyme–substrate and enzyme–product complex, respectively. In the absence of product, the observed relative reaction rate r is given by:

$$r = v/V = u/(K_m + u) \quad (2)$$

where v and V are the initial and maximum reaction rate, respectively, u is the concentration of unbound substrate and K_m the Michaelis constant. The substrate-concentration balance is:

$$A_t = u + E_t \cdot u/(K_m + u) \quad (3)$$

where the terms on the right-hand side represent the unbound and enzyme-bound substrate, respectively, and A_t and E_t are the total concentrations of substrate and enzyme, respectively. Methods are available for determining the system parameters when the term for enzyme-bound substrate cannot be neglected in the concentration balance [1, 2]. The kinetic equation has been analysed in some detail by Reiner [3].

Neglect of the enzyme-bound substrate in eqn. 3 implies interpretation of the observed reaction rates r in terms of the model:

$$r_{\text{approx.}} = \tilde{u}/(K_m + \tilde{u}) \quad (4)$$

$$\tilde{u} = A_t \quad (5)$$

where $\tilde{u}$ is an approximate value for the total concentration of unbound substrate u . The relative error:

$$\epsilon \equiv (r_{\text{approx.}} - r)/r \quad (6)$$

has been discussed by Cha [4] in terms of a square-root function of the substrate concentration A_t . It can easily be shown that ϵ can either be expressed as a unique function of A_t/K_m and E_t/K_m :

$$\epsilon = F_1(A_t/K_m; E_t/K_m) \quad \text{square-root function} \quad (7)$$

or, equivalently, as:

$$\epsilon = F_2(r; E_t/K_m) \quad (8)$$

For deciding whether assays performed over a suitable range of r values (e.g. $0.1 \leq r \leq 0.9$) can be interpreted by the approximate model or not, the second choice (eqn. 8) is the more practical. The explicit form of eqn. 8 is mathematically simpler than that of eqn. 7. Further, the values of r being available, only an estimate of the ratio E_t/K_m , not of the individual values of E_t and K_m , is needed for evaluating the error.

The function F_2 is obtained as follows: $u = K_m \cdot r/(1-r)$, obtained from eqn. 2, is substituted into eqn. 3 yielding A_t as a function of r . Further making use of eqns. 4 and 5 one obtains $r_{\text{approx.}}$ and, finally, from eqn. 6:

$$\epsilon = (E_t/K_m) \cdot \frac{(1-r)^2}{1 + (E_t/K_m) \cdot (1-r) \cdot r} \quad (9)$$

The error decreases uniformly with increasing r ($d\epsilon/dr < 0$; $d^2\epsilon/dr^2 > 0$). It is maximum and equal to E_t/K_m at the origin of the saturation curves ($r = 0$) and equal to zero at saturation ($r = 1$). To an accuracy of 99%, the error formula can be simplified to:

$$\epsilon \approx (E_t/K_m) \cdot (1-r)^2$$

if: $(E_t/K_m) \cdot (1-r) \cdot r \leq 0.01$ (10)

Since the error decreases uniformly with increasing r , it is sufficient to determine its behaviour at some chosen, suitably low, value of r , say $r = 0.1$. At this reaction rate, eqn. 10 remains valid up to $E_t/K_m \leq 0.11$ which corresponds to an intolerably high percentage error $100 \cdot \epsilon \geq 8.9\%$. Maximum percentage errors of 1, 2.5 or 5% in r require E_t/K_m to be less than 0.012, 0.031 or 0.062, respectively, which is well within the range of application of eqn. 10. For discussing the error applicable to enzymic reactions in membrane-containing systems, K_m has to be replaced by the apparent Michaelis constant, K_m^{app} . [5].

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1. Smith, G. D., Eisenthal, R. & Harrison, R. (1977) *Anal. Biochem.* **79**, 643–647
2. Gains, N. (1979) *Biochem. J.* **179**, 697–700
3. Reiner, J. M. (1969) *Behavior of Enzyme Systems*, 2nd edn., Van Nostrand Co., New York, Cincinnati, Toronto, London, Melbourne
4. Cha, S. (1970) *J. Biol. Chem.* **245**, 4814–4818
5. Heirwegh, K. P. M., Meuwissen, J. A. T., Vermeir, M. & De Smedt, H. (1988) *Biochem. J.* **254**, 101–108

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