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Efflux of 6-deoxy-D-glucose from *Plasmodium falciparum*-infected erythrocytes via two saturable carriers

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Abstract

Glucose transport in human erythrocytes infected with the malaria parasite, *Plasmodium falciparum*, has been studied using 6-deoxy-D-glucose (6DOG) as a non-metabolised glucose analogue. Inhibition studies using cytochalasin B, a powerful inhibitor of the erythrocyte glucose transporter, GLUT1, indicate that in the infected red blood cell (IRBC), glucose is transported via a saturable carrier. However, inhibition is not as complete as in the uninfected erythrocyte. The synergistic inhibition effect of 6DOG entry by niflumic acid, an inhibitor of the non-specific malaria-induced pore, in the presence of cytochalasin B suggests that some glucose may also enter the infected erythrocytes through the pore, if entry via the carrier is blocked. The time course of 6DOG efflux from infected erythrocytes in the presence of cytochalasin B did not follow simple first-order kinetics. To elucidate the kinetic mechanism of 6DOG efflux from the infected erythrocytes, the concentration dependence of efflux was determined. Eight two-compartment kinetic models were simulated, involving first-order pore diffusion and carrier-mediated saturable diffusion in two systems, one ductless and one assuming the existence of a parasitophorous duct. The only two models showing reasonable fits to the efflux data each involve two saturable carriers. It is likely that one of the saturable carriers is associated with the parasite itself. Evidence that the parasite carrier has different inhibitor sensitivities from that of GLUT1 is presented. © 1997 Elsevier Science B.V.

Keywords: Glucose; Transport; Malaria; *Plasmodium falciparum*; Erythrocyte

Abbreviations: RBC, red blood cell; IRBC, infected red blood cell; PVM, parasitophorous vacuolar membrane; 2DOG, 2-deoxy-D-glucose; 6DOG, 6-deoxy-D-glucose; SSR, sum of squares residual.

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1. Introduction

Glucose is the major metabolic energy source in the intraerythrocytic stages of the life cycle of *Plasmodium falciparum* [1,2]. The 'classical' view of the *Plasmodium*-infected red blood cell (IRBC) promulgates that three membranes—the erythrocyte's plasma membrane, the parasitophorous vacuole membrane (PVM) enveloping the parasite, and the parasite's own plasma membrane—separate the parasite from its nutrient source in the bloodstream. More recently the existence of an alternative route, involving a duct connecting the parasite to the external environment, has been proposed [3,4].

Previous studies of glucose transport in plasmodium have utilised 2-deoxy-D-glucose (2DOG) as a transportable analogue of D-glucose. Work using 2DOG suggested that sugars were actively transported into the parasite via a unidirectional glucose/H+ symporter [5,6]. However, most hexokinases, including that of the erythrocyte, readily catalyse the phosphorylation of 2DOG by ATP [7,8]. Thus the contribution of backflux to the transport process cannot be assessed, as the efflux of phosphorylated 2DOG (if it occurs at all) is unlikely to take place on the same carrier that transports glucose by facilitative diffusion. Even if efflux of phosphorylated hexose occurred, the kinetic parameters for 2DOG-6-phosphate would be highly unlikely to approximate to those of 2DOG itself. Additionally, 2DOG may deplete intracellular ATP. Consequently, the use of 2DOG complicates the interpretation of the results of transport studies, and especially efflux experiments.

Studies on hexose transport in *Trypanosoma brucei* [9], another rapidly glycolysing parasite, have utilised 6-deoxy-D-glucose (6DOG), which is not a substrate for hexokinase, but can act as a glucose analogue in transport studies. We now report efflux studies using 6DOG in plasmodium-infected erythrocytes. The efflux of 6DOG is contrasted with the efflux of another non-metabolisable sugar, L-glucose, which is transported very slowly by the human red blood cell (RBC) [10].

2. Materials and methods

2.1. Materials

Cytochalasin B, phloretin, phlorizin and niflumic acid were purchased from Sigma. These compounds were dissolved in ethanol before use at concentrations at least 200 × those used in transport studies. Control experiments showed that the ethanol introduced had no significant effect. Unlabelled 6DOG and L-glucose were purchased from Sigma. Radiolabelled [1-^{14}C]-L-glucose was purchased from Amersham. The synthesis of [C6-^{3}H]-6DOG has been described previously [9]. Briefly, 6-iodo-6-deoxy-β-methyl-D-glucoside, which was synthesised from the corresponding chloro compound, was reduced by tritium gas to yield [C6-^{3}H]-6-deoxy-β-methyl-D-glucoside. Hydrolysis of the methyl group gave tritiated 6-DOG, which was purified using paper chromatography.

2.2. Maintenance and preparation of parasites

The asexual stages of *Plasmodium falciparum* (strain IT04, kindly donated by Dr C.I. Newbold) were maintained in RPMI-1640 medium, containing 10% human serum and other supplements using an adaptation of the method of Trager and Jensen [11]. The cells were synchronised using 5% sorbitol [12], and erythrocytes infected with trophozoite stage parasites were separated from uninfected cells using gelatine [13]. Parasitaemias greater than 80% were obtained. Cytocrits were obtained by centrifugation of a small volume of the purified cells in a capillary tube. The enriched cells were kept at 37°C in RPMI-1640 medium prior to the experiments and used within 5 h. During storage, sufficient culture medium was used to ensure that the parasites did not metabolise all the glucose in the medium. The pH of the medium did not change during this time. After 5 h storage under these conditions, cells that were diluted with uninfected RBCs and returned to culture behaved in a way indistinguishable from cells that had not been purified. Uninfected cells, which were used as controls, were of the same age and source as infected cells and also stored in RPMI-1640 medium before use.

2.3. Zero trans influx into parasitised cells

All reactions were performed in 3 ml reaction tubes (NUNC) and all centrifugations were carried out in an Ole Dich refrigerated centrifuge at $10\,000 \times g$ at 4°C. The zero-trans influx studies were performed using either 35 μM L-glucose containing 0.2 μCi (7.4 kBq) of ^{14}C label or 5 mM or 20 mM 6DOG containing 1 μCi (37 kBq) of ^{3}H label. Gelatine-concentrated *P. falciparum* infected erythrocytes ($\sim 2 \times 10^7$ cells) were washed three times with 3 ml prewarmed (37°C) glucose-free phosphate-buffered saline (PBS) (137 mM NaCl, 2.7 mM KCl, 4.3 mM Na_2HPO_4, 1.4 mM KH_2PO_4, pH 7.4). For inhibitor studies a fourth wash contained the inhibitor at the final concentration used. The cells were then resuspended in 100 μl of the sugar solution ($\pm$ inhibitor). After the required time, the reactions were stopped by the addition of 3 ml of stop solution (ice-cold PBS containing 320 μM phloretin and 200 μM phlorizin). The samples were then washed twice with 3 ml of stop solution. The time taken to wash the samples was controlled so that there was no variation from sample to sample. Control experiments determined that during the stopping and washing procedure less than 10% of the label escaped from the cells.

The washed cell pellets were lysed with 500 μl of 0.5% Triton X-100, and the proteins precipitated with 500 μl of 5% trichloroacetic acid. After incubation at 4°C for 30 min the samples were centrifuged for 2 min at $10\,000 \times g$ to pellet the proteins. Supernatant (900 μl) was counted with 5 ml of scintillation fluid (Optiphase safe) using a β-counter (LKB). Non-specific uptake, or background values, were obtained by resuspending the cells in stop buffer before the addition of the radiolabelled sugar substrate. All experiments were performed in duplicate.

Control experiments were performed to find the time required for cells to equilibrate with L-glucose and 6DOG. At 20 mM, 6DOG ceased to accumulate in 60 s, whereas 35 μM L-glucose required 10 min to reach equilibrium. The volume accessible to L-glucose and 6DOG was the same in infected and uninfected erythrocytes indicating that 6DOG equilibrated across all compartments in an infected erythrocyte.

2.4. Zero trans efflux from parasitised cells

All experiments were conducted in 3 ml reaction tubes (NUNC); centrifugations were at $10\,000 \times g$ and 4°C. Efflux of L-glucose (35 μM) and 6DOG (5–30 mM) was measured under a variety of conditions. The cells were washed three times using glucose-free PBS and then were incubated in 100 μl PBS containing the sugar analogue. As with the influx experiments 1 μCi (37 kBq) of radiolabel was used for the 6DOG experiments and 0.2 μCi (7.4 kBq) of radiolabel for the L-glucose experiments. The cells were incubated in the sugar solution for sufficient time to ensure that the sugar reached equilibrium across all cell compartments. For inhibition experiments, the cells were incubated for a further 5 min in 100 μl of the inhibitor, dissolved in PBS containing either L-glucose or 6DOG as required. Efflux was followed after rapid dilution of the cells to 1 ml with PBS, and terminated by addition of 2 ml stop buffer. The cells were washed three times with 3 ml stop buffer, spun, and the pellet was then lysed, the proteins pelleted and the samples counted as described above. Non-specific uptake, or background values, were obtained by adding stopping buffer to the cells before adding labelled substrate. Efflux studies using L-glucose were performed at 37°C; studies using 6DOG were performed at room temperature due to the rapid efflux of 6DOG at 37°C. All experiments were performed in duplicate.

The efflux data were analysed using QPRO V5.0 for DOS (Borland) and the fractional filling calculated. The fractional filling F was defined as:

$$F = (C_t - C_\infty)/(C_0 - C_\infty)$$

where C_0 is the amount of label present at equilibrium, C_t is the amount of label present after time t and C_∞ is the amount of label present at infinite time.

3. Results and discussion

3.1. The human glucose transporter is the major transporter of 6DOG in parasitised erythrocytes

Phloretin is a competitive inhibitor of D-glucose influx in human erythrocytes. Phlorizin is not a specific inhibitor of glucose transport but both phloretin and phlorizin non-specifically inhibit many transport processes at concentrations greater than 0.1 mM. The effects of 320 μM phloretin and 200 μM phlorizin on the efflux of 20 mM 6DOG and 35 μM L-glucose from IRBC are shown in Fig. 1. Significant differences were found in the effects of the inhibitors on the efflux rates. 6DOG efflux was inhibited partially by 200 μM phlorizin and almost completely by 320 μM phloretin (Fig. 1a). However, the efflux of L-glucose was inhibited to the same extent by both inhibitors when used at these concentrations (Fig. 1b).

Previous work has suggested that L-glucose transport into IRBC is almost completely via a non-saturable, high-capacity pore that has been inserted into the red blood membrane by the parasite [10,14]. Consistent with these reports is the observation made by many previous workers that

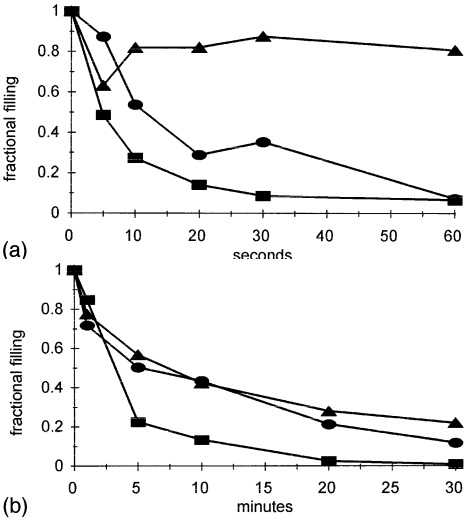


Fig. 1. The effects of 320 μM phloretin (▲), 200 μM phlorizin (●) or no inhibitor (■) on the zero-trans efflux of (a) 20 mM 6DOG or (b) 35 μM L-glucose from malaria-infected red blood cells.

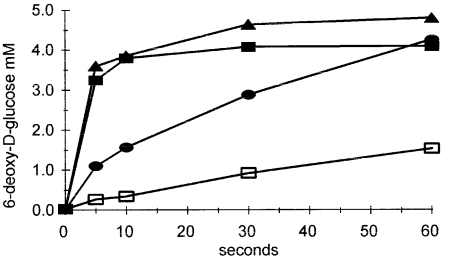


Fig. 2. Zero-trans uptake of 5 mM 6DOG into IRBC in the presence of no inhibitor (■), 500 μM niflumic acid (▲), 50 μM cytochalasin B (●), or 500 μM niflumic acid and 50 μM cytochalasin B (□).

phlorizin is an inhibitor of this pathway [15,16]. It has recently been shown that this non-specific pathway is inhibited not only by phlorizin, but also by other anion transport inhibitors. In particular the pathway is inhibited by NPPB, niflumic acid and furosemide—all potent inhibitors of chloride channels [17]. This pathway has a preference for anions over cations and for hydrophobic molecules over hydrophilic ones. The neutral and hydrophilic sugars, although transported by this pathway, are transported only slowly. Our data are entirely consistent with L-glucose leaving the parasitised cells through this pore pathway.

However, the nature of the inhibition of efflux shown in Fig. 1 suggests that 6DOG leaves the parasite via a different pathway, which is capable of transporting D-glucose and its analogues much more rapidly than is possible for a hydrophilic monosaccharide via the pore. The 6DOG pathway is only mildly sensitive to phlorizin, but very sensitive to phloretin. This is consistent with 6DOG leaving the IRBC through the human glucose transporter GLUT1 in the erythrocyte membrane.

Cytochalasin B is known to be a strong and specific competitive inhibitor of glucose efflux in uninfected erythrocytes; 50 μM cytochalasin B will completely block transport of sugar through the host's transporter in RBCs [18]. It has also been shown that 500 μM niflumic acid blocks all transport through the malaria induced pore [17]. The influx of 6DOG into malaria infected cells in the presence or absence of 50 μM cytochalasin B or 500 μM niflumic acid was investigated. The

data of Fig. 2 show that niflumic acid alone is unable to inhibit transport into malaria infected cells whereas cytochalasin B significantly inhibits uptake. When the two inhibitors are used in conjunction they have a synergistic effect, suggesting that although not the main pathway, the pore allows some entry of 6DOG. Also significant is the incomplete inhibition observed when using both cytochalasin B and niflumic acid. At the inhibitor concentrations used, complete inhibition might be expected as both the pore and carrier pathways should be blocked. The incomplete inhibition observed might be consistent with the presence of a parasitophorous duct and a less cytochalasin B sensitive glucose carrier on the parasite surface.

3.2. Efflux of 6DOG from parasitised erythrocytes is biphasic

The rate of efflux of 6DOG from malaria infected and uninfected erythrocytes was investigated in the presence and absence of cytochalasin B. Fig. 3 shows that cytochalasin B inhibited 6DOG efflux from both infected and uninfected erythrocytes. Although the rate of efflux of 20 mM 6DOG is slower from infected than uninfected cells, both rates are severely reduced in the presence of 50 μM cytochalasin B. This indicates that 6DOG exits primarily through a cytochalasin B sensitive pathway. The linearity of the semilogarithmic plots (Fig. 3) of the uninhibited efflux of 6DOG from the uninfected cell indicates first order kinetics. The corresponding efflux from the parasitised cell is of a more complex nature. This is consistent with a multicompartmental system, at least two components of which might contain a transport system for 6DOG with differing permeabilities. As stated in Section 2, identical levels of uptake were observed in infected and uninfected cells suggesting that 6DOG enters both the parasite and erythrocyte compartments. The data shown in Fig. 3 support this conclusion.

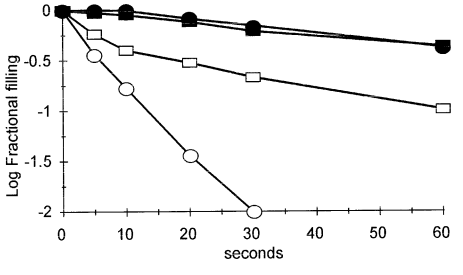


Fig. 3. The effect of 50 μM cytochalasin B on the efflux of 20 mM 6DOG from IRBC and uninfected erythrocytes. Efflux of 6DOG was followed in the presence (■, ●), and absence (□, ○) of 50 μM cytochalasin B. The square symbols refer to IRBC; the circles, to uninfected RBCs.

3.3. Evidence for the involvement of two saturable carriers in the Plasmodium-infected erythrocyte

In order to elucidate further the possibility of a multicompartmental system for glucose transport, the efflux of 6DOG from IRBC was studied at a range of concentrations (Fig. 4a). Based on the biphasic nature of 6DOG efflux (Fig. 3), the data were first fitted to a biexponential Eq. (1)

$$S_i = \boldsymbol{a}\, e^{-k1t} + \boldsymbol{b}\, e^{-k2t} \quad (1)$$

where S_i is the amount of sugar inside the cell at time t; $\boldsymbol{a}$, and $\boldsymbol{b}$ are constants that depend on the substrate concentration, and k_1 and k_2 are the rate constants corresponding to two transport processes. When the data are expressed as fractional filling, $\boldsymbol{a}$ and $\boldsymbol{b}$ are the relative volumes of the two compartments. At $t = 0$, S_i will be 1 and so $\boldsymbol{a} + \boldsymbol{b} = 1$. Fig. 4a shows the data fitted using estimates of 0.6 and 0.4 for $\boldsymbol{a}$ and $\boldsymbol{b}$ respectively. Estimates of k_1 and k_2 were obtained at each substrate concentration. If a saturable carrier is involved in efflux, these observed rate constants, k_{obs}, will depend on the 6DOG concentration according to Eq. (2), which is obtained from the Michaelis equation for competitive inhibition at low concentrations of substrate relative to the apparent K_M.

$$\frac{1}{k_{obs}} = \frac{K_M}{V_{max}} + \frac{K_M}{V_{max} K_i}[I] \quad (2)$$

where K_M and K_i are the Michaelis constants for labelled and unlabelled 6DOG respectively, V_{max} is the maximum velocity, and $[I]$ is the concentration of unlabelled 6DOG. Plots of $1/k_1$ and $1/k_2$ against [6DOG] are linear (Fig. 4b) with X-axis intercepts ($-K_i$ apparent) of 3.85 ± 0.61 mM and

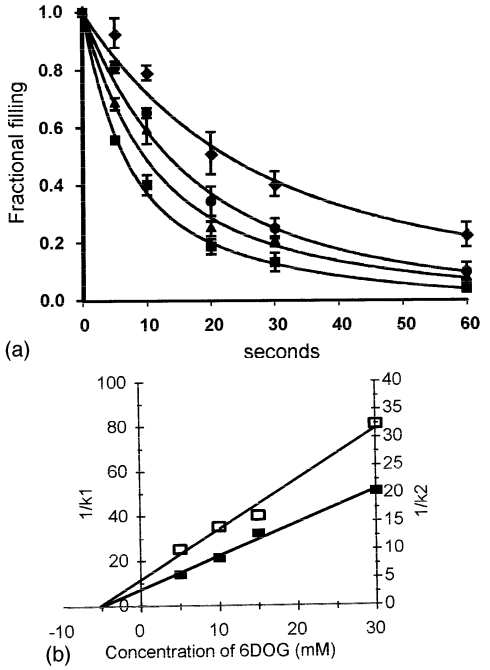
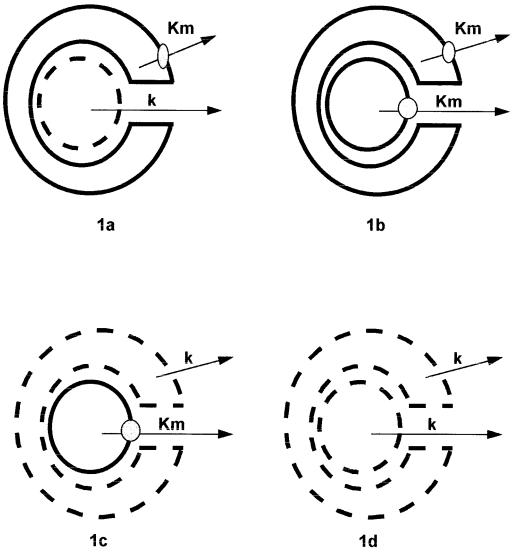


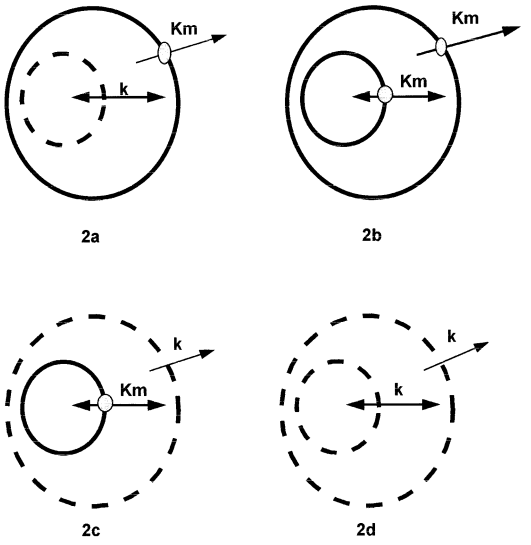
Fig. 4. (a) Zero-trans efflux of 6DOG from IRBC preloaded to concentrations of 5 mM (■), 10 mM (▲), 15 mM (●) and 30 mM (◆). The cells were incubated in 6DOG at the required concentration for 10 min and the efflux followed as described in the Methods. The lines were generated using Eq. (1) and estimates of 0.6 and 0.4 for a and b respectively. The results are the means of three experiments, each performed in duplicate. Error bars represent the S.E.M. (b) A plot of the reciprocals of the constants k_1 (□) and k_2 (■) against the initially loaded 6DOG concentration.

5.41 ± 0.88 mM for the corresponding K_i values. The linear relationship between the reciprocals of the exponential coefficients (k_1 and k_2) of the fitted biexponential equation and the loaded concentrations of 6DOG is highly suggestive of a mechanism for efflux involving a system containing two saturable carriers. However, biexponential equations are notorious for their ability to fit many functions [19,20]. This ill-conditioned character also means that more than one set of parameter values can give highly reasonable fits to a set of data. Indeed the linear relationship of $1/k$ with 6DOG was also obtained using other estimates of the linear coefficients, ***a*** and ***b***, in the range 0.3–0.7 for each presumed compartmental volume. Thus the fit to a biexponential equation is, strictly speaking, an empirical relationship only, and the numerical values of the parameters do not necessarily have physical significance.

We therefore considered reasonable models that might account for the observed biphasic efflux kinetics in the plasmodium-infected red cell. Four possibilities were examined: (a) first-order diffusional efflux (i.e. via a pore) from the parasite, and facilitative diffusional efflux (i.e. via a carrier) from the red cell; (b) carrier-mediated efflux from both compartments; (c) diffusional efflux from the red cell and carrier-mediated efflux from the parasite; (d) diffusional efflux from both compartments. These four possibilities can be accommodated in a ducted system and in a ductless system, giving eight models in all to be examined. These are illustrated in Schemes 1 and 2, where the ducted models are termed models 1a–1d, and the corresponding ductless models 2a–2d, respectively. In the ducted system (Scheme 1)



Scheme 1. Models for efflux from parasitised erythrocytes in a ducted system. The dashed lines represent a pore-containing membrane; the presence of pores is indicated by a simple rate constant, k. The solid lines represent a membrane containing saturable carriers, indicated by K_m. (a) Non-saturable (pore) diffusion from the parasite and carrier-mediated transport from the red blood cell, (b) carrier-mediated transport from both compartments, (c) carrier-mediated efflux from the parasite and pore diffusion from the RBC, (d) pore diffusional efflux, from both compartments.



Scheme 2. Models for efflux from parasitised erythrocytes in a ductless system; symbols as in Scheme 1. (a) non-saturable diffusion from the parasite and irreversible carrier mediated transport from the red blood cell, (b) carrier-mediated transport from both compartments, (c) carrier-mediated efflux from the parasite and pore diffusion from the RBC, (d) pore diffusional efflux from both compartments.

6DOG in both compartments is considered to have direct and unrestricted access to the external environment via a pore or a carrier, depending on the model. Under the experimental conditions (i.e. zero-trans efflux), efflux from these compartments will be essentially irreversible. In the ductless model, efflux from the red cell compartment is also irreversible, but efflux from the parasite into the red cell is reversible. Kinetically, the models of Scheme 1 (ducted models) represent two effectively irreversible processes in parallel, while those of Scheme 2 (ductless models) represent two processes in sequence—the first one reversible and the second effectively irreversible.

For the ducted system (Scheme 1), efflux by simple diffusion was modelled by an irreversible first-order rate equation (3); carrier-mediated (saturable) diffusion was modelled by an equation of the form of the Michaelis equation (4);

$$-\frac{d[S]}{dt} = k[S] \tag{3}$$

$$-\frac{d[S]}{dt} = \frac{V[S]}{K+[S]} \tag{4}$$

where K is the Michaelis constant for the transporter, V the maximum velocity, k the first-order rate constant, and $[S]$ the 6-DOG concentration in the compartment.

For the ductless model (Scheme 2), efflux from the red cell is effectively irreversible due to the zero-trans conditions of the efflux experiment, and was modelled using Eqs. (3) and (4), as appropriate. Efflux from the parasite compartment must however be considered as a reversible process. For models 2a and 2d parasite efflux was modelled by a reversible first-order equation (5) for simple diffusion. For models 2b and 2c carrier-mediated transport was simulated using a minimal facilitative diffusion equation (6), which will produce reversible saturable kinetics. Eq. (6) also assumes a symmetrical carrier.

$$-\frac{d[S_p]}{dt} = k([S_p]-[S_r]) \tag{5}$$

$$-\frac{d(S_p)}{dt} = \frac{V/K([S_p]-[S_r])}{1+[S_p]/K+[S_r]/K+[S_p][S_r]/K^2} \tag{6}$$

where $[S_p]$ and $[S_r]$ are the concentrations of 6DOG in the parasite and red cell compartment, respectively.

Simulation of the concentration dependence of the time courses of efflux for all eight models, as expressed in terms of fractional filling, was carried out using values of unity for all parameters and default values of 0.4 and 0.6 for the fractional volumes of the parasite and red cell. The loading concentrations were varied over a 100-fold range (0.1–10). The simulations are shown in Fig. 5. Models 1d and 2d showed no concentration dependence; i.e. identical progress curves were obtained at all loaded concentrations. This is to be expected, as both these models involve two first-order processes only. Model 2c displayed a fan-shaped dependence of progress curves, with no discernible concentration dependence of the efflux curves at the early stages of the time course. This behaviour was seen over a wide range of simulated parameter values. It was thus possible to eliminate these three models from consideration.

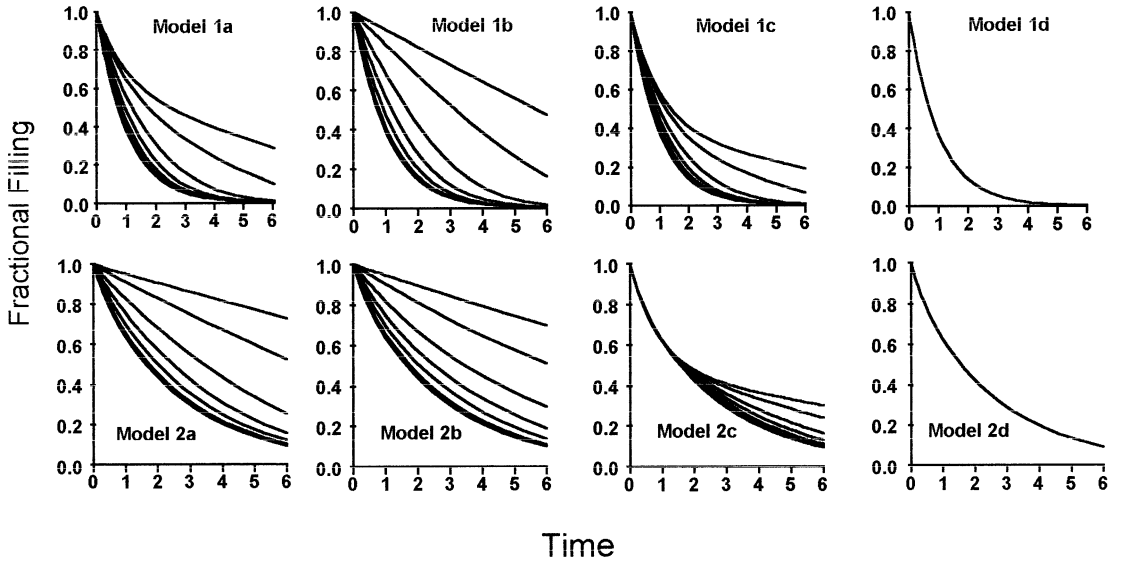


Fig. 5. Simulated efflux time courses for the models shown in Schemes 1 and 2. Parameters ***a*** and ***b*** were assigned values of 0.4 and 0.6. All other parameters were defined as unity. The initial loaded concentration of substrate varied from 0.1 to 10. Time is in arbitary units.

The five remaining models were fitted to the efflux data using as floating parameters the K_M and V_{max} values of the putative carriers, or the first-order diffusional rate constant, k, for the parasite and red cell compartment, as appropriate for the particular model. The relative parasite volume was also used as a floating parameter. The fits were performed using Scientist (Micromath) software, employing a non-linear least squares minimisation method of the numerically integrated rate equations utilising Powell's algorithm. The results for the ductless models 2a and 2b are shown in Fig. 6a. Visual inspection indicates that model 2b gives a somewhat better fit to the data than model 2a, the ratios of the sum of squares of the residuals (SSR) being 1.5. However, as model 2b contains one additional parameter, (the K_M of the parasite carrier), an improvement in fit is expected. Models 2a and 2b can be regarded as hierarchical, 2a being a restricted case of 2b in which K_M for the parasite compartment is infinite. It is therefore possible to compare the models and to test whether the improvement in fit results simply from incorporation of an additional parameter (the Null Hypothesis), or from the model itself. Analysis of the residuals gave a variance ratio of 6.97. Reference to the Table of F-values at the appropriate degrees of freedom shows that the improvement due to the model is significant at a P value of < 0.025, indicating a strong likelihood that the improved fit given by model 2b is due to the model itself and not simply to the incorporation of an additional parameter. As the additional parameter of model 2b is the K_M of the parasite transporter, the results are consistent with a system containing two saturable carriers for 6DOG.

The fits to the corresponding ducted models 1a and 1b are shown in Fig. 6b. Here the ratio of the SSR is 3.79. Models 1a and 1b have the same hierarchical relationship to each other as models 2a and 2b. Applying similar analysis to these fits gives an F-value of 39 corresponding to a P-value of less than 0.005. Thus the improved fit given by the two-carrier model in the ducted system is also due to the model itself rather than to chance, and model 1a can be eliminated. Models 1a and 1c are kinetically identical and complementary, the only difference being in the relative volumes of the red cell and parasite compartments. Thus model 1c can likewise be discarded.

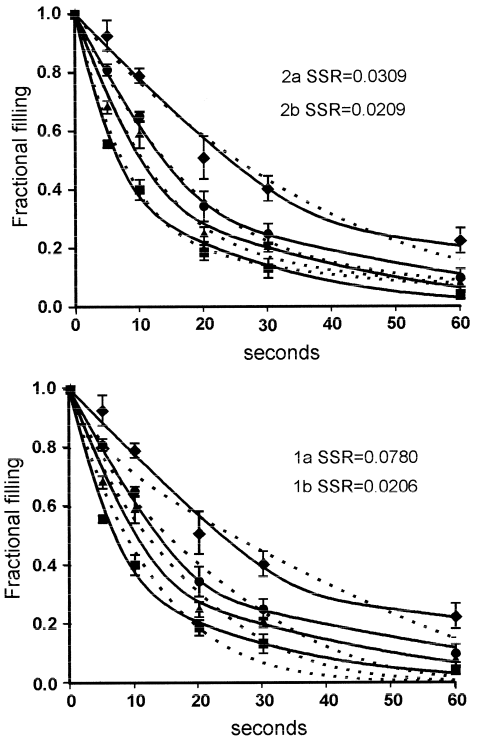


Fig. 6. (a) Zero trans efflux of 6DOG at 5 mM (■), 10 mM (▲), 15 mM (●) and 30 mM (◆). The data are fitted to models 2a (dashed line) and model 2b (solid line) as shown in Scheme 2. (b) The data are fitted to models 1a (dashed line) and 1b (solid line) as shown in Scheme 1.

The 6DOG efflux data are consistent only with models comprising two carrier-mediated components. The parameter values from these models are given in Table 1. The values for fractional parasite volume are consistent with those of the plasmodium-infected red cell in its trophozoite stage. The K_M estimates for the parasite and red cell transporters are similar for the two models and have values compatible with glucose concentrations likely to be encountered in vivo. The K_M for the erythrocyte compartment carrier may also be compared with the K_M obtained for 6DOG efflux from uninfected erythrocytes ($K_M = 2.28 \pm 0.36$ mM; $V_{max} = 0.66 \pm 0.03$ mM/s (data not shown)).

It should be noted that if the ductless model is operative, the putative parasite glucose carrier might be present in the parasite membrane or in the PVM (or both). But if the ducted model is applicable, the parasite K_M would refer to a carrier in the parasite plasma membrane only. The efflux data do not allow discrimination between the ducted and ductless systems, although the incomplete inhibition of 6DOG influx by niflumic acid and cytochalasin B might tend to favour the ducted system.

The non-specific pore or chloride channel was thought to be required to meet the parasite's high demand for nutrients [21,22] and to remove the large amount of lactate that the parasite produces [23,24]. Since the description of the parasitophorous duct [3], this hypothesis has been called into question [17]. The evidence presented here eliminates a pore-diffusion mechanism as a major contributor to the transport of glucose in the malaria-infected erythrocyte. It is significant that the models providing a reasonable fit to the efflux data involve a transporter in the erythrocyte membrane, presumably GLUT1. The kinetics suggest that there is, in addition, a saturable carrier for glucose associated with the parasite compartment, although the model does not distinguish between the parasite's plasma membrane and the PVM, in the case of the ductless model. (In the ducted model, the PVM is assumed to play no role in the transport process).

The incomplete inhibition of 6DOG uptake by a combination of niflumic acid and cytochalasin B (Fig. 2) suggests that the specificities of the host

Table 1
K_m, V_{max} and relative volume estimates obtained from models 1b and 2b

Model	K_M (parasite) (mM)	K_M (RBC) (mM)	V_{max} (parasite) (mM/s)	V_{max} (RBC) (mM/s)	Relative parasite volume
1b	4.01 ± 0.12	3.45 ± 0.06	0.26 ± 0.01	1.07 ± 0.01	0.39 ± 0.01
2b	6.24 ± 0.35	2.59 ± 0.03	0.51 ± 0.03	0.98 ± 0.01	0.26 ± 0.01

and parasite glucose transporters are different. It is interesting to note that differences in transporter specificities between host and parasite have also been observed in *T. brucei* [25]. Glucose transport in IRBC may therefore provide an attractive target for the development of antimalarial drugs.

4. Note added in proof

Since the submission of this manuscript, a paper has appeared in this Journal (Kirk et al., (1996) 82, 195–205) on glucose uptake in *Plasmodium falciparum*-infected erythrocytes. The conclusions of Kirk et al. are entirely compatible with those of this paper.

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