Adaptation of the carbon metabolism of *Trichomonas vaginalis* to the nature and availability of the carbon source

Benno H. ter Kuile

The Rockefeller University, 1230 York Avenue, New York, NY 10021, USA

The anaerobic parasitic protist *Trichomonas vaginalis* was adapted in chemostats to eight different conditions defined by different growth rates and carbon regimes. Glucose or maltose was used as carbon and energy source. Cells cultured under well-defined steady states were tested in short-term experiments. The kinetics of glucose and maltose uptake were determined and their glucokinase and α-glucosidase activities were measured. Uptake in 20 min was measured with radiolabelled glucose and maltose, rather than analogues, using the silicone oil centrifugation technique. Hence, the accumulated label represents both transport and metabolic activity. The total uptake of glucose was highest in organisms that had been starved for glucose during growth. The kinetics of glucose uptake can be understood by assuming rate-limitation by transport across the plasma membrane at low external concentrations and by the subsequent metabolism at concentrations exceeding a cross-over value. The specific glucokinase activity correlated in only four out of eight cases with the saturation uptake. The kinetics of maltose uptake indicated rate-limitation at low maltose concentrations by a diffusion-limited step and at higher levels by metabolic steps. The uptake of maltose was primarily affected by the growth rate during culture, the highest growth rates resulting in most uptake. Maltose uptake was determined only partially by the cellular α-glucosidase activity. The activities of both transport and metabolic enzymes changed due to the culture conditions suggesting that the control over glucose and maltose metabolism is shared by several steps in the pathway.

**Keywords**: *Trichomonas vaginalis*, carbohydrate metabolism, metabolic adaptation, chemostats, energy metabolism

**INTRODUCTION**

The hydrogenosome-containing parasitic protist *Trichomonas vaginalis* can utilize both glucose and maltose as carbon and energy source. The metabolism is fermentative and a variety of end-products is formed including glycerol, lactate, acetate, CO₂ and, under anaerobic conditions, molecular hydrogen (Müller, 1991). The mechanism for glucose transport across the plasma membrane in *T. vaginalis* is facilitated diffusion (Ter Kuile & Müller, 1993). Maltose is hydrolysed extracellularly by an α-glucosidase and the glucose formed is then taken up (Ter Kuile & Müller, 1994). In culture *T. vaginalis* can reach division times of 4 h, but in the urogenital tract, growth is estimated to be much slower. Chemostat cultures remain viable over a wide range of growth rates (Lehker & Alderete, 1990; Ter Kuile, 1994), indicating considerable metabolic flexibility. In the accompanying study on the carbon metabolism of *T. vaginalis*, it was concluded that this organism may not be optimally energy efficient (Ter Kuile, 1994). It does, however, have a flexible metabolism that is able to adapt itself to the nature and the availability of its carbon source. This study investigates how metabolic adaptation in *T. vaginalis* occurs by preadapting cells for multiple generation times in the chemostat and then testing the cells in short-term uptake experiments. Cells were adapted to growth on either glucose or maltose, under rate-limiting or non-rate-limiting conditions and at high or low growth rate. The results of the uptake experiments are discussed in terms of the effects of changes in the activities of the transport and metabolic processes.

Changes in metabolism due to changes in the growth conditions are known to occur widely. Two extensively studied examples are the induction of different sugar...
transporters according to sugar availability in yeasts (reviewed by Bisson et al., 1993) and the induction of CO₂-concentrating mechanisms in algae and cyanobacteria (Kaplan et al., 1980). The less extensive data on metabolic adaptation in parasitic protists suggested that induction of different types of uptake mechanisms does not occur. Therefore the data in this report will be compared primarily to the findings in other parasitic protists. Adaptation of the carbon metabolism to the culture conditions has been demonstrated in the glycosome-containing protists Trypanosoma brucei and Leishmania donovani. The metabolism of Tryp. brucei adapts to the availability of glucose in the most energy-efficient manner, increasing its capacity when growing at higher glucose levels (Ter Kuile & Oppendoes, 1992c). Due to such adaptive changes, wide fluctuations in the metabolic rate can be expected when the availability of the carbon source varies. In addition, the degree of oxidation varies at different growth rates and glucose availability. In contrast to this, L. donovani takes up only slightly more glucose when it has been starved for glucose as compared to organisms grown on excess glucose (Ter Kuile & Oppendoes, 1993), while proline metabolism shows no adaptation (Ter Kuile & Oppendoes, 1992b). The metabolic properties of L. donovani are thus kept constant.

The key regulatory enzyme of the glycolytic flux in Tryp. brucei is hexokinase (Oppendoes, 1987). In contrast to mammalian hexokinases, which show product inhibition (Colowick, 1973), hexokinase of Tryp. brucei is not regulated (Oppendoes, 1987). Instead of a hexokinase with broad specificity, T. vaginalis contains a glucokinase, specific for glucose only (Mertens & Muller, 1990). The step catalysed by hexokinase or glucokinase, phosphorylation of glucose to glucose 6-phosphate, is irreversible. The uptake of glucose by the insect stage of Tryp. brucei was strongly correlated to the hexokinase activity (Ter Kuile & Oppendoes, 1992c). It is thus of interest to determine to what extent glucose metabolism of T. vaginalis is controlled by glucokinase, and, in the case of maltose metabolism, whether the α-glucosidase that hydrolyses maltose extracellularly to form glucose (Ter Kuile & Muller, 1994) exerts most flux control.

The results of the present study suggest that T. vaginalis adapts its metabolism to maintain functional homeostasis in the changing environment of the human vagina. This strategy may enable T. vaginalis to survive for prolonged periods in an environment that does not support continuous growth.

METHODS

Culture conditions. Trichomonas vaginalis (strain NIH C1, ATCC 30001) was grown in single-stage, flow-controlled chemostats with pH regulation and nitrogen as driving gas, as described in the accompanying paper (Ter Kuile, 1994). The medium was phosphate-buffered tryptone/yeast extract medium (Diamond, 1957) supplemented with horse serum and having either glucose or maltose as carbon source, rate-limiting (total concentration 5 and 3.5 mM, respectively) or in excess (45 and 30 mM).

Determination of metabolic parameters. The density of the culture was monitored by counting cells daily and once a steady state was achieved, samples were taken for measurement of cell density (culture liquid), cellular protein and carbohydrate content and enzyme activities (sedimented cells) and the residual substrate concentration (rapidly filtered culture fluid). For the uptake experiments reported in the present study approximately 60 ml of culture fluid was withdrawn from the chemostat, centrifuged at 700 g for 10 min and the pelleted cells were resuspended in phosphate/saline buffer.

The uptake of glucose and maltose was measured with radiotracers techniques using D-[6-3H]glucose and [U-14C]maltose as tracers, rather than non-metabolized analogues. The silicone oil centrifugation technique was employed in all experiments, using four 400 μl microfuge tubes with 75 μl 1-bromododecane on the bottom as described earlier (Ter Kuile & Oppendoes, 1991, 1992c; Ter Kuile & Muller, 1993). The tracer accumulated represents both glucose or maltose and the metabolites derived from it, but any end-products excreted by the cell are not included in the measurement. Hence the reported uptake represents net accumulation of label in glucose or maltose equivalents and is affected by both the transport over the plasma membrane and the subsequent metabolic steps. The term 'uptake' is used without any implication of its chemical nature. Since the same incubation time (20 min) was used in all experiments and this time is in the linear range of the time-course for uptake experiments (Ter Kuile & Muller, 1993) the comparison between the different experiments remains valid.

The uptake of glucose or maltose was measured as a function of concentration, the only variable between experiments being the culture conditions of the cell. For each experiment 10 test tubes containing 2 ml phosphate/saline buffer with substrate concentrations increasing from 1.5 to 45 mM were prepared. These were mixed with 1 ml of a suspension of washed cells free of substrate, giving final concentrations in the range 1-30 mM. After a preincubation of 5-10 min, the radiolabel was added while mixing vigorously. Four 250 μl samples were transferred to microfuge tubes 30 s and 20 min after adding radiolabel and centrifuged for 7 s at 13000 g. The pellet was frozen, cut off and the accumulated radioactivity measured by liquid scintillation counting. At the end of the experiment the densities of the incubations were determined by counting cells. Duplicate 25 μl samples were taken from each test tube for radioactive counts and centrifuged (18 s at 13000 g). Samples of the incubation medium of each tube were stored at −20 °C for later determination of the substrate concentration.

Glucose and maltose concentrations were determined enzymically as described earlier (Ter Kuile & Muller, 1993; Ter Kuile, 1994). Glucokinase activity was measured according to the method for hexokinase (Bergmeyer, 1974). The activity of α-glucosidase was measured by monitoring the liberation of glucose from maltose (Ter Kuile & Muller, 1994; Ter Kuile, 1994).

RESULTS

The uptake of glucose or maltose by T. vaginalis was measured as a function of the external concentration, using cells preadapted in the chemostat to eight different carbon regimes. The purpose of these experiments was to examine the adaptation of the metabolism to the changing nature and availability of the carbon and energy source. Radiolabelled glucose and maltose were used instead of non-metabolized analogues to study the combined effect of the transport step and the subsequent metabolism. A total of 16 uptake experiments were performed, eight each
for glucose and maltose. Within each group of eight the only variable was the culture conditions of the cells prior to the experiment: the organisms were grown in chemostats at high or low growth rates on either maltose or glucose, which was either rate-limiting or non-rate-limiting. The observed differences in the results of the individual experiments are due to long-term adaptation, because the growth conditions were the only variable. The organisms were harvested for uptake experiments when the culture was at steady state. Samples were taken for measurements of the substrate concentration in the culture fluid and for cell density, cellular protein and carbohydrate content and glucokinase activity. The dilution rate \( (D) \), which equals the specific growth rate, and the steady-state substrate concentration are indicated in the figure legends.

**Glucose uptake**

The uptake of glucose by cells grown with glucose is presented in Fig. 1. The data can be described by two types of curves: the first was a biphasic curve obeying Michaelis–Menten kinetics at low external glucose concentrations, \([Glc_{\text{out}}]\), reaching an apparent saturation level with no further increase above a certain cross-over concentration. This type of curve fitted the data on glucose uptake of cells grown at low \( D \). The second type of curve, found in the case of cells cultured at high \( D \), also had Michaelis–Menten kinetics at low \([Glc_{\text{out}}]\), but uptake continued to increase linearly at higher \([Glc_{\text{out}}]\). The first type of curve obeys an equation that was derived by combining the equation for facilitated diffusion and a subsequent enzymic step (Ter Kuile & Cook, 1994):

\[
V = \frac{1}{2} \left( \frac{V_{\text{max}}(KR_{00} + R_{12}S) + S + K_m}{KR_{00} + R_{12}S - K_m(R_{21} + \frac{R_{ee}S}{K})} \right)
\]

Modelling based on this equation demonstrated that while the transport step is limiting the overall reaction rate at low \([Glc_{\text{out}}]\), the enzymic step following it becomes rate-limiting at higher substrate levels. The rates of both the transport and the metabolic steps are much higher in glucose-limited cells than in those grown on excess substrate.

The above model is based on the assumption that the net flow of radiolabel across the plasma membrane equals the overall metabolic rate. This assumption apparently does not hold for the organisms cultured at high growth rates, probably due to equilibration of the label across the plasma membrane at rates exceeding phosphorylation of glucose by glucokinase. In this case the data are best described by a second type of curve that can be modelled to the equation that gives the rate of an enzymic reaction in parallel with a diffusion component (Ter Kuile & Opperdoes, 1992c). The enzymic component represents again the kinetics of the transporter; the ‘diffusion’ component consists of label equilibrated with the cells’ volume, but not (yet) converted into metabolites. The size of this component can be estimated from the slope of the linear part of the curve, and represents equilibration of about 65% of the cells’ volume (based on a cell volume of 0.60 \( \mu \)l per 10^6 cells; Ter Kuile & Muller, 1993) with the label present externally.

The concentration dependence of glucose uptake by *T. vaginalis* grown with maltose as carbon and energy source is shown in Fig. 2. The same relationship described by the biphasic curve discussed above for cells grown with glucose at low \( D \) fitted the data in three cases, but not for organisms grown on excess maltose at high \( D \). The kinetic parameters at low \([Glc_{\text{out}}]\), which are controlled primarily by the transporter, were the same as those for cells grown at low \( D \) under glucose limitation. The saturation level of glucose uptake for organisms growing at high \( D \) under maltose limitation or at low \( D \) on excess maltose was similar to those of glucose-limited cells at low \( D \). A slightly higher uptake was measured in cells grown at low \( D \) under maltose limitation. This small additional uptake may be caused by replenishing of the storage carbohydrate, which is lower in maltose- than in glucose-limited cells (Ter Kuile, 1994). The concentration dependence of glucose uptake of cells that were grown at
In C.-500-2 n
wl wr C
300-

Fig. 2. Uptake of glucose, as in Fig. 1, of *T. vaginalis* cultured on maltose as carbon and energy source in the chemostat at the following glucose and maltose concentrations, respectively, and dilution rates: 0.241 mM, 0.273 mM, 1.71 d⁻¹ (○); 0.471 mM, 1.064 mM, 5.05 d⁻¹ (■); 3.650 mM, 5.368 mM, 1.49 d⁻¹ (△); 0.446 mM, 20.61 mM, 4.99 d⁻¹ (▼).

high *D* on excess maltose can be explained by the combination of metabolism and equilibration of the label, as for cells grown at high *D* on glucose (Fig. 1).

In order to examine whether the specific glucokinase activity of *T. vaginalis* can be correlated to its specific glucose consumption, it was measured on cells from chemostat cultures grown at a wide range of growth rates under the four carbon regimes applied throughout the study (Fig. 3a). The nature of the carbon source strongly influenced the relationship between the glucokinase activity and the growth rate. When glucose was the rate-limiting substrate, the glucokinase activity remained constant around 400 nmol min⁻¹ (mg protein)⁻¹. When glucose was not rate-limiting, it decreased from almost 600 nmol min⁻¹ (mg protein)⁻¹ at a *D* value of less than 1 d⁻¹ to less than 200 nmol min⁻¹ (mg protein)⁻¹ at a *D* value of 5 d⁻¹. Maltose-grown organisms almost always had lower glucokinase activity than those grown on glucose. Cells growing at high *D* on excess maltose, which resemble most the batch cultures used in a study on glucokinase of *T. vaginalis* (Mertens & Müller, 1990), had a specific activity of 100 nmol min⁻¹ (mg protein)⁻¹, the same value as reported in that study. In none of these four cases could the glucokinase activity be correlated to the specific substrate consumption during growth in the chemostat. A plot of the saturation level of glucose uptake as a function of the glucokinase activity measured on the same batch of cells showed the expected correlation in only four out of eight cases (*R²* = 0.994 for the selected cases; *R²* = 0.26 for all eight cases) (Fig. 3b). In two cases saturation was not reached and these can thus be expected to show lower glucokinase activity than would be expected from the glucokinase activity. A simple direct relationship between glucokinase and overall metabolic activity therefore does not exist.

**Maltose uptake**

The relationship between the external maltose concentration, [Maltₜₜₜₜ], and its uptake by *T. vaginalis* (Figs 4 and 5) was distinctly different from that for glucose. Initially the uptake increased linearly with the concentration, until a breakpoint, after which no additional uptake occurred. This type of curve can be understood as rate limitation at low concentrations by a diffusion step, followed by an enzymic reaction that becomes limiting at higher levels, according to:

\[
V = \frac{1}{2}(K_m P_u + s P_u + V_m) - \left(\frac{(K_m P_u + s P_u + V_m)^2}{4 s P_u V_m}\right)^{\frac{1}{2}}
\]

Fig. 3. (a) Specific activity of glucokinase as a function of the dilution rate (*D*) of chemostat cultures of *T. vaginalis* grown under the following conditions: •, glucose rate-limiting; ▲, maltose rate-limiting; ◼, glucose non-rate-limiting; ◐, maltose non-rate-limiting. (b) Saturation values of glucose uptake (see Figs 1 and 2) as a function of specific hexokinase activity. A good correlation exists in only four of the six cases where saturation is reached.
Metabolic adaptation in T. vaginalis

**Fig. 4.** Uptake of maltose as a function of the external maltose concentration by T. vaginalis cultured using glucose as carbon and energy source. Cultures were grown at the following steady-state glucose concentrations and dilution rates: \(\nabla\), 35.55 mM and 1.72 \(\text{d}^{-1}\); \(\bigcirc\), 0.161 mM and 1.70 \(\text{d}^{-1}\); \(\triangle\), 27.58 mM and 1.70 \(\text{d}^{-1}\); \(\blacksquare\), 1.942 mM and 5.06 \(\text{d}^{-1}\).

**Fig. 5.** Uptake of maltose by T. vaginalis grown in chemostats on maltose at the following steady-state glucose and maltose concentrations, respectively, and dilution rates: 0.466 mM, 20.51 mM, 4.99 \(\text{d}^{-1}\) (\(\nabla\)); 0.466 mM, 1.241 mM, 5.10 \(\text{d}^{-1}\) (\(\blacksquare\)); 1.942 mM, 14.42 mM, 1.74 \(\text{d}^{-1}\) (\(\triangle\)); 0.064 mM, 0.289 mM, 1.63 \(\text{d}^{-1}\) (\(\bigcirc\)).

where \(V\) equals the reaction rate at substrate concentration \(S\), \(K_m\) is the affinity constant of the enzymic reaction, \(V_m\) is the maximum rate of the enzyme reaction and \(P_d\) is the diffusion constant divided by the distance over which diffusion occurs (Hill & Whittingham, 1955; Thomson & Dietschy, 1984). The initial slope is determined by the diffusion component and the saturation level by the maximum rate of the enzymic reaction. The slope for the glucose-grown cells was similar except for those grown at high \(D\) under glucose limitation, which had both a lower slope and a lower saturation level (Fig. 4). Maltose uptake of slow-growing organisms saturated at a level of 4 nmol maltose per 10^6 cells, which, in carbon equivalents, corresponds to 8 nmol glucose, considerably lower than the uptake of glucose. Saturation was not reached in the case of cells cultured at high \(D\) on excess glucose.

Maltose uptake by maltose-grown cells showed similar kinetics, governed by equation (2) as well. The uptake at low \([\text{Malt}_\text{out}]\) was either higher, for cells grown at high \(D\), or lower, in the case of cells growing at low \(D\), than the uptake measured in glucose-grown cells (Fig. 5). Organisms grown at a low \(D\) did not reach saturation in the range of concentrations used in these experiments. Cells cultured at high \(D\) took up around 9 nmol per 10^6 cells at saturating \([\text{Malt}_\text{out}]\), double the maximum value of maltose uptake by glucose-grown cells, but, in carbon
equivalents, half of the glucose uptake measured in most cases.

The first step in the uptake and metabolism of maltose is its hydrolysis to form two molecules of glucose, a reaction catalysed by an ectoenzyme located at the cell surface (Ter Kuile & Müller, 1994). The glucose thus formed can then no longer be distinguished from glucose already present in the medium. The cell-bound α-glucosidase activity was measured in a separate experiment on cells grown in chemostats under the same eight conditions as used for uptake experiments: glucose or maltose as carbon source, high or low D, limited by the carbon source, or the C source present in excess. The maltose uptake at saturating [Malt\textsubscript{eq}] was then plotted as a function of the α-glucosidase activity in the cells of the similar incubation. There was only weak correlation between the saturation level and the α-glucosidase activity ($R^2 = 0.69$) (Fig. 6a). A much better, but still far from ideal, correlation exists between the initial slope of uptake as a function of [Malt\textsubscript{eq}] and the α-glucosidase activity ($R^2 = 0.85$) (Fig. 6b). This is to be expected, because the diffusion component most likely represents maltose diffusing towards the α-glucosidase attached to the cell surface. The more α-glucosidase that is present, the greater the diffusion component. The weak correlation, however, indicates that the α-glucosidase activity is not the sole factor regulating maltose metabolic rates.

**DISCUSSION**

**Adaptation to substrate availability**

The concentration dependence of glucose and maltose uptake, measured in experiments that were identical except for the culture conditions of the cells, showed that the carbon metabolism of *T. vaginalis* was extensively adapted to the culture conditions. Generally, when the carbon and energy source was scarce, the capacities of the transporter and the subsequent metabolic pathway were higher. This indicates that *T. vaginalis* increased its metabolic capacity in reaction to carbon depletion, reducing the effects of the low carbon availability as much as possible.

Uptake of glucose was highest in *T. vaginalis* starved of the carbon and energy source, grown either at low rates under glucose starvation or grown under maltose limitation. Such higher uptake could have been caused by induction of a concentrating mechanism, as has been suggested to occur in yeasts (Gasnier, 1987; Peinado et al., 1988; Postma et al., 1988). Alternatively, an increased capacity of the metabolic pathway in combination with an increase in the number of the facilitated diffusion transporters can also cause higher uptake, as was demonstrated for *Tryp. brucei* (Ter Kuile & Opperdoes, 1992c). The biphasic uptake kinetics of the type observed for glucose uptake by *T. vaginalis* clearly indicate facilitated diffusion as transport mechanism followed by enzymic conversion of the substrate (Ter Kuile & Cook, 1994) and thus induction of an active carrier can be ruled out. The facilitated diffusion carrier cannot concentrate glucose against the gradient (Stein, 1986), but an increase in the number of carriers will lead to increased uptake if the capacity of the subsequent enzymatic pathway is also increased. Analysis of the relationship between [Glc\textsubscript{eq}] and glucose uptake suggests that the activity of the transporter was unchanged in four out of eight experiments. Organisms grown at low D on excess glucose had both the transport and the metabolic activity reduced, while the other three incubations, of cells grown at higher D on glucose and excess maltose, had an overcapacity of the transporter in comparison to the activity of the metabolic pathway. These latter had relatively high uptake after 30 s incubations (data not shown), indicating that the labelled substrate equilibrated rapidly and thus confirming a higher transport activity than observed in cells grown at low rates on excess glucose.

The glucokinase activity of *T. vaginalis* cells from chemostat cultures did not show a direct correlation to glucose availability, even though the specific activity changed. Saturation levels of glucose uptake could in some but not all cases be correlated to the glucokinase activity. The hexokinase activity and the saturation glucokinase uptake of the insect form of *Tryp. brucei* on the one hand and the specific glucose consumption and the specific hexokinase activity of *L. donovani* on the other were correlated (Ter Kuile & Opperdoes, 1992a, c). This suggests that phosphorylation of glucose by hexokinase is the primary rate-controlling step in the metabolic pathway of these two species. The partial correlation between glucokinase activity and glucose uptake in *T. vaginalis* indicates that glucokinase may be the primary flux-controlling step for its glucose metabolism grown under some conditions, but not all. This change of primary flux-controlling step is predicted by the flux control theory, which states that the control over metabolic fluxes can be shared between several steps depending on the activities of other enzymes in the pathway (e.g. Heinrich & Rapoport, 1977; Kacser & Porteous, 1987; Petterson & Petterson, 1990).

There should be no difference between glucose and maltose metabolism once maltose is hydrolysed to glucose. Therefore, the differences between maltose and glucose metabolism, the role of the hydrolysis step preceding the common metabolism must be examined. It is likely that the uptake rates at low [Malt\textsubscript{eq}] are determined by the α-glucosidase activity, because the initial slope and the α-glucosidase activities could be correlated (Fig. 6b). However, this correlation is only partial, hence additional factors must be in play. Indeed, the two incubations having lower uptake rates than predicted from the α-glucosidase activity also have lower activities of the glucose transporter, as estimated from the parallel glucose uptake experiments. In those two cases, the flux control may be shared between the hydrolysis and the transport step. In glucose equivalents, the saturation levels of maltose uptake are much lower than those for glucose by cells grown under similar conditions and the glucokinase activity is thus, most likely, not rate-limiting. Indeed, there is no correlation between the glucokinase activity and the saturation level of maltose uptake.
Metabolic strategies

As discussed above, the carbon metabolism of *T. vaginalis* was extensively adapted to the availability of the carbon source in a manner that counteracted the fluctuations in the environment. This is the mode of adaptation most often encountered, for example in yeasts adapting to sugar availability (Bisson *et al.*, 1993) and algae and cyanobacteria adapting to changes in inorganic carbon concentration (Kaplan *et al.*, 1980). An opposite but similarly strong influence of the growth conditions on the glucose metabolism was found for the procyclic trypanomastigote, or insect, stage of *Tryp. brucei* (Ter Kuile & Opperdoes, 1992c). At higher glucose levels both the transport and the metabolic activities were increased. It was suggested that *Tryp. brucei* adapted the activities of the glucose transporter and the metabolic enzymes to glucose availability and to each other in such a manner that maximum energy efficiency is obtained. In contrast to *Tryp. brucei*, but similar to *T. vaginalis*, *L. donovani* increased the uptake of glucose after growth at low carbon availability (Ter Kuile & Opperdoes, 1993), but showed no adaptation at all of its proline utilization (Ter Kuile & Opperdoes, 1992b).

The metabolic strategy of *L. donovani*, not only considering carbon metabolism, was characterized as striving to keep the internal conditions constant (Ter Kuile & Opperdoes, 1992a; Ter Kuile, 1993). Generally, organisms spend the minimum maintenance energy needed to maintain functional homeostasis of their internal conditions (Hochachka & Somero, 1984). The metabolic strategies of *L. donovani* and *Tryp. brucei* can be understood as responses to the different ecological challenges that their respective environments pose to them (Ter Kuile, 1993). The interaction between glucose uptake and metabolism suggests that *T. vaginalis* counters the effects of low glucose availability more strongly than *L. donovani*. It forms a storage carbohydrate, which can be considered to be part of a strategy to survive prolonged periods of low carbon availability. The less than perfect correlation between glucokinase and α-glucosidase activities and glucose and maltose uptake respectively, suggests, however, that the metabolic strategy of *T. vaginalis* is much less aimed at energy efficiency than those of the other two species discussed. The accompanying study showed that *T. vaginalis* has a very high maintenance energy. The selective pressures exerted on *T. vaginalis*, a parasite of the human vagina, are likely to be very different than those that *Tryp. brucei* and *L. donovani*, residing in insect guts, are exposed to. Thus its carbohydrate metabolism faces different challenges than that of the other two species. Therefore, the fine metabolic adaptations observed in *T. brucei* and *L. donovani*, may not occur in *T. vaginalis*, but on the other hand, *T. vaginalis* is able to survive prolonged periods of near zero growth.

ACKNOWLEDGEMENTS

This study was supported by NIH grants AI 11942 and RR 07067. I would like to thank Dr M. Müller for extensive discussions and comments on an earlier version of this manuscript. The author was the recipient of a fellowship from the Norman and Rosita Winston Foundation. However, the statements made and the views expressed are solely the responsibility of the author.

REFERENCES


diffusion followed by enzymatic conversion of the substrate. Biochim Biophys Acta (in press).


Received 25 February 1994; revised 3 May 1994; accepted 11 May 1994.