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Mutual Adjustment of Glucose Uptake and Metabolism in *Trypanosoma brucei* Grown in a Chemostat

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The mutual adjustment of glucose uptake and metabolism in the insect stage of the protozoan parasite *Trypanosoma brucei* was studied. *T. brucei* was preadapted in the chemostat to conditions in which either glucose or proline served as the major carbon and energy source. Cells were grown and adapted to either energy or non-energy limitation at a low dilution rate (0.5 day^{-1}) or a high dilution rate (1 day^{-1}). The cells were then used in short- to medium-term uptake experiments with D-[^{14}C]glucose as a tracer. In time course experiments a steady state was reached after 15 min regardless of the preadaptation conditions. This steady-state level increased with increasing glucose availability during preadaptation. The rate of glucose uptake and the hexokinase activity were linearly correlated. In short-term (5- to 90-s) uptake experiments a high transport rate was measured with cultures grown in excess glucose, an intermediate rate was measured with proline-grown cultures, and a low rate was measured in organisms grown under glucose limitation. Glucose metabolism and proline metabolism did not affect each other during the 15-min incubations. Glucose uptake, as a function of the external glucose concentration, did not obey simple Michaelis-Menten kinetics but could be described by a two-step mechanism: (i) transport of glucose by facilitated diffusion and (ii) subsequent metabolism of glucose. The respective rates of the two steps were adjusted to each other. It is concluded that *T. brucei* is capable of adjusting the different metabolic processes in a way that gives maximum energy efficiency at the cost of short-term flexibility.

For optimal adaptation to its environment, an organism needs to obtain the maximum yield from the available energy source. This requires that the individual steps in the metabolic pathway be adjusted to each other in a way that prevents overcapacity in one step relative to the others. Bloodstream trypomastigotes of the protozoan parasite *Trypanosoma brucei* thrive in the blood and body fluids of their mammalian host and enjoy a stable environment. The uptake and subsequent metabolism of glucose in this organism are an example of an adjustment leading to maximum energy efficiency (26). However, this adjustment should not be considered the general rule in the Trypanosomatidae, because the insect stage of a related species, *Leishmania donovani*, which in the sandfly midgut is confronted with widely varying conditions, strives for internal homeostasis even at the expense of energy (28). Thus these two very different metabolic strategies may represent two opposing trends: efficient adaptation at the expense of short-term flexibility on the one hand and the ability to rapidly adapt to environmental changes at the expense of energy on the other. Intermediate situations may be found in organisms that encounter only moderate fluctuations in their environmental conditions. An example of the latter type of organism is the procyclic insect stage of *T. brucei*, under study here, which resides in the midgut of the tsetse fly.

T. brucei bloodstream trypomastigotes, when ingested with the blood meal by the tsetse fly, transform in the midgut to the procyclic insect stage. Unlike bloodstream-stage *T. brucei*, organisms in the insect or procyclic stage are not exclusively dependent on glucose as the carbon and energy source. They are also capable of utilizing proline and other amino acids (7, 22). While the blood proteins are being digested by the fly, the parasite is exposed to variations in the concentrations of both glucose and amino acids. Parasite stages in insects may therefore represent an interesting model for the study of adaptations to the varying environments that may occur under natural conditions.

The best approach to the study of long-term adaptation in microorganisms is with continuous culture in chemostats, which allow the growth of cultures under constant conditions for long periods of time (31). Environmental parameters such as the nature and concentration of the carbon and energy source can be chosen according to the requirements of the experiment, and cells adapted to such conditions may then be harvested for short-term experiments or determination of cellular parameters.

In this study we present a series of short-term experiments in which we measured the time and concentration dependence of the uptake and subsequent metabolism of glucose by preadapted *T. brucei*. The results suggest that, contrary to what Parsons and Nielsen (16) and Munoz-Antonia et al. (13) claim and contrary to the situation encountered in *L. donovani*, insect-stage *T. brucei* organisms are not capable of actively transporting glucose and concentrating it inside. Insect-stage *T. brucei* takes up glucose by a facilitated diffusion carrier, as does bloodstream-stage *T. brucei* (5, 8, 9, 26). Moreover, we show that *T. brucei* adapts itself to achieve maximal energy efficiency at the expense of metabolic flexibility.

MATERIALS AND METHODS

T. brucei 427 procyclic trypomastigotes were grown in a single-stage flow-controlled chemostat with a working volume of 200 ml as described previously (27, 28). The medium

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TABLE 1. Experimental conditions and conditions during preincubation of *T. brucei* for the experiments reported in Fig. 1 through 4

Incubation			Preadaptation (in chemostats)			
Fig. no.	Incubation time (min)	Glc_{out} (mM)	C and energy source	D (day^{-1})	Glc_{out} (mM)	Symbol
1B	Variable	5.172	Glucose (7.5 mM)	0.549	0.068	○
1B	Variable	5.304	Glucose (20 mM)	0.542	4.099	●
1B	Variable	5.787	Glucose (7 mM) and proline (10 mM)	0.516	0.052	▲
1B	Variable	4.980	Proline (7.5 mM)	0.507	(0.12 mM proline)	△
1B	Variable	5.088	Proline (20 mM)	0.495	(8.31 mM proline)	■
1A	Variable	5.494	Glucose (7.5 mM)	0.552	0.058	○
1A	Variable	5.371	Glucose (20 mM)	0.562	5.671	●
1A	Variable	5.113	Proline (7.5 mM)	0.538	ND[a]	△
2A	15	Variable	Glucose (7.5 mM)	0.567	0.071	○
2A	15	Variable	Glucose (20 mM)	0.523	2.883	●
2A	15	Variable	Glucose (7 mM) and proline (10 mM)	0.516	0.048	▲
2A	15	Variable	Proline (7.5 mM)	0.528	(0.34 mM proline)	△
2B	15	Variable	Glucose (7.5 mM)	1.015	0.386	○
2B	15	Variable	Glucose (20 mM)	1.032	4.067	●

[a] ND, not detected.

used was filter-sterilized SDM 79 (1) with glucose or proline as the carbon and energy source. When glucose or proline was to be rate limiting, its concentration in the medium was approximately 7.5 mM with the addition of 10% (vol/vol) fetal calf serum. When excess glucose or proline was desired, a concentration of 20 mM was used and 7% fetal calf serum was added. The density of the culture was monitored daily by counting cells in a Petroff-Hauser cell to determine the establishment of a steady state. Steady states were assumed to occur when cell density had not changed for at least one doubling time and the growth conditions had been constant for at least five doubling times. At each steady state, samples were taken for the determination of the concentration of the carbon and energy source; then 150 ml of culture was withdrawn from the vessel, centrifuged at 500 × *g* for 8 min, and resuspended in low-glucose (0.3 mM) GLSH medium (11) for experiments.

Three types of experiments were performed. (i) The uptake of D-glucose at an external glucose concentration (Glc_{out}) of about 5 mM as a function of time was measured. The incubation times used were between 5 s and 30 min. (ii) Glucose uptake after 15 min as a function of Glc_{out} over a range of 0.3 to 10 mM was measured. (iii) Glucose uptake as a function of the external proline concentration (0 to 25 mM) and proline uptake as a function of Glc_{out} (0 to 12.5 mM) were measured. Glucose uptake was measured at a Glc_{out} of 5 mM and proline uptake was measured at an external proline concentration of 10 mM. For the latter experiment, *T. brucei* was grown in batch cultures in the same medium with an excess of either proline or glucose.

Glucose uptake was measured as the uptake of D-[^{14}C]glucose, rather than glucose analogs, in combination with the silicone-oil centrifugation technique (6, 26). Incubations lasting longer than 30 s were performed as reported earlier (26), with a slight modification: four 400-μl tubes containing 75 μl of a mixture of 3 parts 1-bromododecane and 1 part DC 200 silicone oil (Serva) were simultaneously filled with 250 μl of incubation medium and centrifuged for 7 s at 13,000 × *g* in a Beckman Microfuge E at the indicated time. Quenching was corrected for as previously described (26). It must be pointed out that this method, in contrast to methods employing nonmetabolizable glucose analogs, measures the accumulation of both glucose and its metabolites. This enables the study of the interaction between glucose uptake and metabolism as reported herein.

The activities of the glycolytic enzymes hexokinase and pyruvate kinase were determined on cells lysed in 0.1% Triton X-100 as described by Misset and Opperdoes (12) and Callens et al. (3), respectively. The protein content of corresponding samples was measured by the fluorescamine method (23).

The respective volumes of both glucose- and proline-grown cells were determined by using ^3H_2O and [^{14}C]carboxyinulin as described by Rottenberg (20). The internal cell volume (3.3 μl per 10^8 cells) appeared to be independent of culture conditions. The average protein content of *T. brucei* is 0.628 mg of protein per 10^8 cells, and the dry weight is 1.39 mg per 10^8 cells.

The glucose concentration in the incubation medium or in the chemostat was measured enzymatically. The proline concentration was determined by using the acid ninhydrin method measuring A_{440} (34) and a standard curve of proline dissolved in proline-free medium.

RESULTS

All experiments were carried out on procyclic trypomastigote insect-stage *T. brucei* grown in chemostats under the conditions shown in Table 1. Three types of experiments were carried out: time course experiments (Fig. 1), experiments in which the glucose concentration was varied (Fig. 2), and experiments in which the influence of proline and glucose metabolism on each other was examined (Fig. 3). The experiments were set up identically in each of the series. The only differences between the various incubations were the culture conditions of the organisms before the experiments (Table 1).

Short- and medium-term time course experiments were performed to distinguish between transport (first seconds) and subsequent metabolism (up to 15 min). Organisms grown on glucose took up approximately one-third of the maximum uptake reached after 15 min during the first 5 s (Fig. 1).

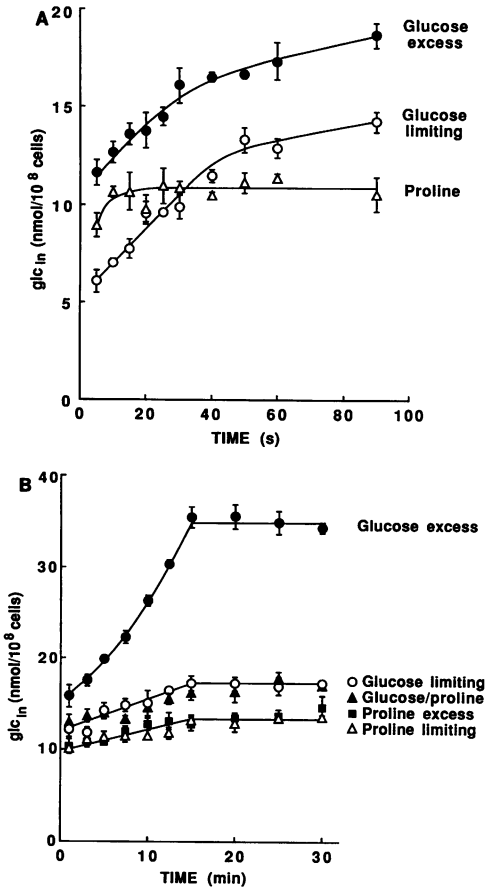

FIG. 1. Uptake of D-[^{14}C]glucose by *T. brucei* procyclic trypomastigotes as a function of time after different preincubations in chemostats. The exact culture conditions are given in Table 1. The dilution rate was approximately 0.5 day^{-1} in all cases. Symbols: ●, glucose, non-rate limiting; ○, glucose, rate limiting; ▲, mixture of glucose and proline; △, proline, rate limiting; ■, proline, non-rate limiting. (A) Short-term experiments (5 to 90 s); (B) medium-term experiments (1 to 30 min).

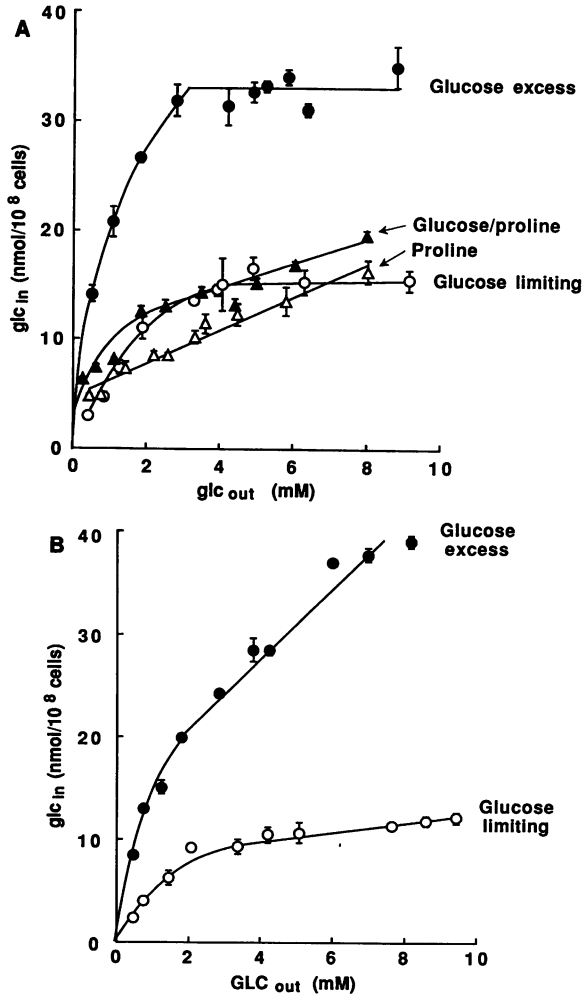

FIG. 2. (A) Uptake of D-[^{14}C]glucose by *T. brucei* grown at a low dilution rate (D = 0.5 day^{-1}) as a function of Glc_{out}. The incubation time was 15 min. The culture conditions are given in Table 1, and symbols are as in Fig. 1. (B) Similar experiment for *T. brucei* grown at a high dilution rate (D = 1 day^{-1}) under glucose-limited (○) and non-glucose-limited (●) conditions.

Organisms grown on proline showed very little additional uptake after the first 5 to 10 s (Fig. 1). This uptake during the first 5 s (Fig. 1A) represents a rapid inflow of glucose into the cells. Then a gradual increase caused by the conversion of glucose into its metabolites was observed. After 15 min a steady state was reached, when the inflow of glucose and the outflow of metabolic end products became equal (Fig. 1B). There were, however, important differences in the total amount of label incorporated, which represents the sum of both glucose and glucose metabolites. *T. brucei* grown on proline showed the lowest uptake of glucose and reached the maximum uptake of glucose in 5 to 10 s, after which very little additional uptake occurred, suggesting the absence of any significant further conversion of glucose into its metabolites. There was no difference between organisms grown under proline limitation and those grown in the presence of excess proline. Cells grown on glucose as a rate-limiting substrate had a 30% higher uptake. The mixture of proline and glucose, with almost all of glucose consumed in the culture vessel, gave similar results. Cells grown on excess glucose showed a much higher uptake, exceeding that of glucose-limited organisms by a factor of more than 2. All of these experiments were carried out on cultures grown at dilution rates (D) of 0.50 to 0.55 day^{-1}. Cells obtained from cultures grown at higher D (0.96 to 0.99 day^{-1}) yielded a time curve for glucose uptake that strongly resembled the curves obtained for organisms grown at low D (data not shown).

Measurements of glucose uptake and metabolism by *T. brucei* as a function of the Glc_{out} are shown in Fig. 2. None of the curves obtained obeyed Michaelis-Menten kinetics. The curve indicating glucose uptake by *T. brucei* grown on glucose at low D (Fig. 2A) resembled the curve described previously for bloodstream-form *T. brucei* (26). At a low Glc_{out}, Michaelis-Menten kinetics can be applied; at Glc_{out} above 3 to 4 mM, no further uptake occurred, because then the phosphorylation of glucose by hexokinase probably becomes the rate-limiting step (26) (see below). The K_m found for the enzymatic part of the curve, probably representing the kinetics of the carrier, was approximately 2 mM, the same value as that found for the bloodstream form (5, 8, 9, 26). The inflection point for non-glucose-limited cultures

was at a Glc_{out} of 3 mM, whereas the glucose concentration in the culture vessel was 2.88 mM. In glucose-limited cultures grown at a constant glucose concentration of 0.07 mM, an inflection point at a Glc_{out} of 4 mM was observed.

Cells grown on proline only (Fig. 2A) showed a curve for glucose uptake that is best described as a straight line intersecting the vertical axis above zero. Incubations for 0.5 min again yielded a straight line with an equal slope but crossing the y coordinate only slightly above zero (data not shown). This linear dependence of uptake on the external concentration suggests that diffusion is the rate-limiting step. Extrapolation of the line obtained after 15-min incubations to the y coordinate would suggest a component of approximately 4 nmol of glucose 10^8 cells^{-1} or its equivalent in metabolites to be added to the diffusion component. Free unphosphorylated glucose was not found intracellularly in *T. brucei* grown in chemostats under glucose limitation (28). This indicates that the relatively small additional uptake over a period of 15 min is in the form of metabolites. The size of this components did not depend on the external glucose concentration over the range measured (0.5 to 10 mM). The rate-limiting step for this component may very well be phosphorylation of glucose by hexokinase, because hexokinase would operate close to the V_{max} in this concentration range (K_m, 0.1 mM) (11a) and because in the insect form it has a low activity compared with those of the other glycolytic enzymes (10). Apparently a rapid equilibration of glucose occurs over the plasma membrane, suggesting that glucose entry never limits the rate of metabolism. This rapid equilibration suggests overcapacity of the transporter in relation to hexokinase activity.

T. brucei grown on a mixture of proline and glucose (Fig. 2A) and cultures grown on glucose at higher D values (Fig. 2B) had glucose uptake curves that can be described by assuming an enzymatic and a diffusion component in parallel according to the equation $V = Ve_{max}\,(S/[K_m + S]) + (K_d \times S)$ (14, 25), where V is the total uptake, Ve_{max} is the maximum enzyme mediated uptake, S is Glc_{out}, K_m is the value of Glc_{out} at which the Ve is 1/2 of the Ve_{max}, and K_d is the apparent cellular diffusion constant (which takes into account the distance over which diffusion occurs, the cell surface, and the biomass). The diffusion component ($K_d \times S$) can be estimated from the slope of the linear part of the curve.

The experiments reported in Fig. 1 and 2 demonstrate that glucose uptake and metabolism in the insect stage of *T. brucei* are strongly influenced by the culture conditions before the experiment. To examine whether there was only short-term influence of glucose and proline metabolism on each other, glucose uptake (at a Glc_{out} of 5.3 mM) was measured as a function of increasing proline concentrations (Fig. 3A) and proline uptake was measured as a function of increasing Glc_{out} (Fig. 3B). For these experiments *T. brucei* was grown in batch cultures with either glucose or proline as the carbon and energy source. The metabolism of glucose by glucose-grown organisms was not hampered by proline and was even slightly stimulated (Fig. 3A). The glucose metabolism of cells grown on proline was not influenced by the external proline concentration. The addition of glucose to the incubation medium did not influence proline uptake in *T. brucei* grown on glucose, but proline-grown cells showed a slight decrease at high Glc_{out} (Fig. 3B).

To examine regulation of glucose metabolism at the enzymatic level, the activities of hexokinase and pyruvate kinase, two enzymes that are believed to regulate the glycolytic flux (3, 30, 33), were measured on glucose-limited, non-glucose-limited, and proline-grown cultures. The activities of both enzymes were regulated (Table 2); hexokinase was regulated to a larger degree than was pyruvate kinase. The detected hexokinase activities are linearly correlated to the quantities of ^{14}C label measured in time course experiments (Fig. 4) on *T. brucei* preincubated under the same conditions.

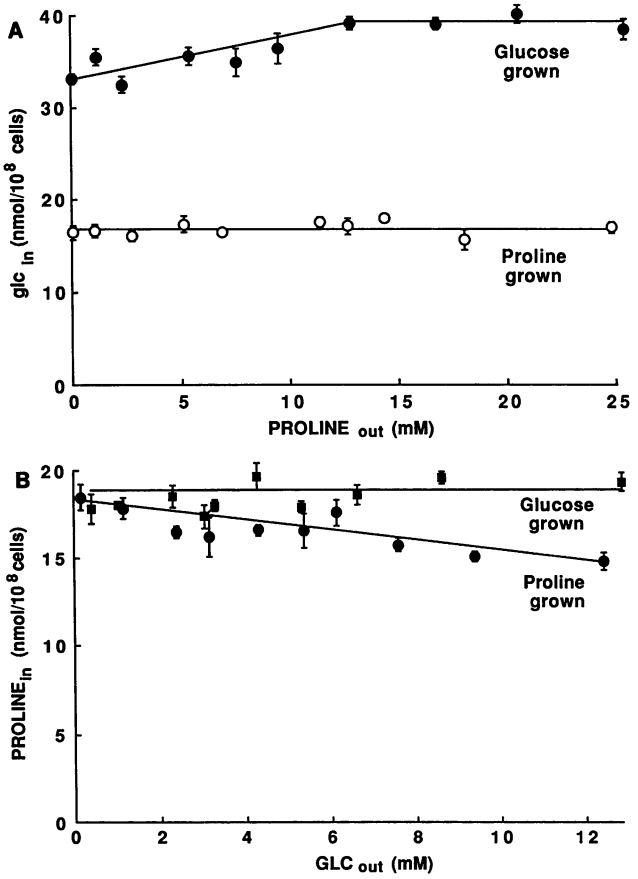

FIG. 3. (A) Influence of the external proline concentration on D-[^{14}C]glucose uptake by *T. brucei* grown on glucose (●) or proline (○) in batch cultures. The incubation time was 15 min. (B) Influence of the external glucose concentration on [^{14}C]proline uptake by *T. brucei* grown on glucose (■) or proline (●) in batch cultures.

DISCUSSION

Nature of the glucose carrier. In bloodstream-form *T. brucei*, glucose transport over the plasma membrane occurs by facilitated diffusion (5, 8, 9, 26). A similar mechanism is

TABLE 2. Activity of the glycolytic enzymes hexokinase and pyruvate kinase and of glucose transport under different culture conditions

Culture conditions	Activity (μmol produced min^{-1} mg of protein^{-1})		
	Hexokinase	Pyruvate kinase	Transport
Glucose			
Non-rate-limiting	0.0554	0.0609	0.139
Rate-limiting	0.0251	0.0423	0.073
Proline	0.0229	0.0399	0.107

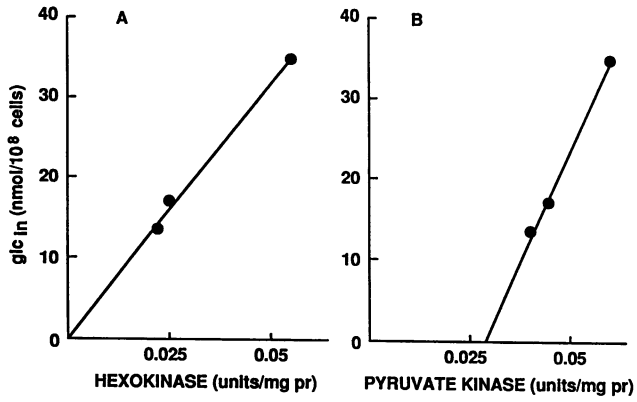

FIG. 4. Average [^{14}C]glucose uptake after 15 to 30 min as a function of the activities of hexokinase and pyruvate kinase. Note that the pyruvate kinase activity is higher than the hexokinase activity, suggesting that pyruvate kinase is involved not only in the glycolytic pathway but also in other pathways.

probably operational in the insect stage. We base this assumption on the following observations. First, in chemostat cultures the internal concentration of free (unphosphorylated) glucose was always less than the external concentration, whereas in parallel experiments *L. donovani* concentrated glucose inside by a factor of up to 600 (28), probably with a proton-glucose symporter (35). Second, in the experiments reported in this study the concentration of total label taken up was in some cases slightly higher than the external glucose concentration. However, if one takes into account that the majority of the label represented metabolites, then the internal concentration of free glucose was well below the external concentration. By contrast, Parsons and Nielsen (16) and Munoz-Antonia et al. (13) suggested that in insect-form *T. brucei* glucose is actively transported and concentrated. Their conclusions were based on the measurement of 2-deoxy-D-glucose rather than glucose itself. However, 2-deoxy-D glucose is phosphorylated by hexokinase, and the resulting 2-deoxy-D-glucose-6-phosphate is labile and easily hydrolyzed. We found that, in the presence of *T. brucei* cells dissolved in 5.5% perchloric acid, 22.6% of the 2-deoxy-D-glucose-6-phosphate initially present was hydrolyzed after 15 min. In the experiments of Parsons and Nielsen (16), 77% of the label taken up was recovered in the phosphorylated form, but no care was taken to demonstrate that the remaining 23% of unphosphorylated 2-deoxy-D-glucose could not have resulted from hydrolysis of the phosphorylated compound during and after the incubation, thus leading to a gross overestimation of the intracellular concentration of the unphosphorylated form. In similar experiments with D-glucose, there was hardly any incorporation (16). Moreover, when we calculate the internal concentration of free unphosphorylated 2-deoxy-D-glucose from the data of Munoz-Antonia et al. (13), it remains well below the external glucose concentration. In conclusion, we believe that we have demonstrated that insect-form *T. brucei*, like bloodstream-form *T. brucei*, takes up glucose by facilitated diffusion rather than by active transport.

Adaptation of the transport and enzyme activities. Bloodstream-form *T. brucei* adapts the uptake of glucose to the subsequent metabolic steps in such a way that maximum energy efficiency is obtained (26). We suggest that the organism synthesizes the exact number of transporters required to saturate the enzymes of the glycolytic pathway. As opposed to the bloodstream-form organisms, insect-stage *T. brucei* is confronted with widely varying glucose concentrations and is capable of switching to another carbon and energy source, such as proline. Adaptation to these varying situations requires extensive metabolic adjustments.

Regulation at the level of the transporter. The regulation of glucose transport activity over the plasma membrane is best demonstrated in Fig. 1A, from which the data of Table 2 are calculated. Low glucose transport activity is found in *T. brucei* grown at a low glucose concentration. High transport activity is found in cells cultured in the presence of excess glucose and in proline-grown cultures. The high transport capacity is needed in two cases: (i) when glucose is consumed at a high rate and (ii) at very low Glc_{out}, in order to scavenge the little glucose available. At intermediate Glc_{out}, such as that in glucose-limited continuous cultures, the transport activity can be adjusted to exactly saturate glycolysis. It will be noted that adaptation usually occurs in a reverse manner: organisms grown under glucose-limited conditions have higher glucose transport activity than do cells grown in excess glucose. We suggest that this is due to a metabolic strategy unique to *T. brucei*; in this strategy maximum energy efficiency takes precedence over constant internal conditions (see below).

Glucose transport in yeast species is subject to metabolic regulation (2, 17–19, 21). The mechanism for adaptation consists of two (sometimes even three) carriers, each with different properties and induced under various conditions (2, 17–19, 21) or by conversion of a facilitated diffusion carrier into an active glucose-proton symporter (32). High-affinity carriers are often subject to catabolite repression (2, 19, 21, 29). There is no evidence for the existence of more than one carrier in *T. brucei*; hence, adjustment of the transport capacity probably occurs by carrier recruitment.

Adaptation at the enzyme level. The levels of the key glycolytic enzymes hexokinase and pyruvate kinase are clearly adjusted according to glucose availability; the more glucose is present, the higher the enzyme activity (Table 2). A similar observation was made in the case of *T. cruzi* (4). The activity of hexokinase in *T. brucei* differs by a factor of 2.4 between proline-grown cells and organisms grown in the presence of excess glucose, whereas the relative hexokinase activity of *T. brucei* grown in a glucose-limited chemostat does not change as a function of the growth rate (28). This suggests that the degree of regulation in response to metabolic changes at the enzyme level by insect-form organisms is small relative to the change in hexokinase activity upon transformation from bloodstream to insect form (14-fold) (10).

Interaction between the glucose carrier and subsequent metabolism. Analysis of the relation between glucose uptake and the external glucose concentration (Fig. 2) shows three situations.

(i) Diffusion is the only rate-limiting step in organisms grown on proline when the products of transport step have a higher capacity than the subsequent metabolism. Glucose equilibrates over the membrane, and the internal and external glucose concentrations become equal (Fig. 2A). Transport is not rate limiting, and therefore zero-trans conditions do not occur. As a consequence, Michaelis-Menten kinetics is not observed (24).

(ii) The uptake of glucose by *T. brucei* grown at low *D* on glucose only yielded curves similar to those observed in the bloodstream form (Fig. 2A) (26). As we showed, these curves can be described by a mathematical model based on the assumption that transport is the rate-limiting step at low

Glc_{out}, whereas another step, probably phosphorylation by hexokinase, becomes rate limiting at Glc_{out} above 3 to 4 mM in cultures. Under these conditions the internal concentration of unphosphorylated glucose remains low. This agrees with the observation that in chemostat cultures grown under glucose limitation it is phosphorylation by hexokinase rather than the uptake step that limits the overall rate of metabolism (28). Hence, the number of transporters must be sufficient to saturate hexokinase at the very low Glc_{out} and low turnover rates in a glucose-limited chemostat at low *D*. Therefore zero-trans conditions exist at low Glc_{out}, and the initial part of the curve can be described by Michaelis-Menten kinetics.

(iii) Finally, an enzymatic component and a diffusion component are observed in parallel (Fig. 2B). This situation may be considered as intermediate between situations i and ii and is observed in glucose-grown cells at high growth rates and in glucose-proline-grown organisms (Fig. 2). It is caused by the occurrence of partial zero-trans conditions at low Glc_{out}, yielding Michaelis-Menten kinetics in the initial part of the curve. At higher Glc_{out}, equilibration of glucose over the membrane, like that observed in proline-grown cells, occurs.

Interaction of metabolic pathways with each other. In bloodstream-form *T. brucei*, glucose and glycerol share part of their respective catabolic pathways (15). The capacity of the enzymes involved in the metabolism of these compounds was regulated in such a way that glycerol did not hamper the metabolism of glucose, the preferred substrate, but that glucose did reduce glycerol metabolism (26). The metabolic pathways for glucose and proline do not have any steps in common. Therefore, only indirect interference would be possible. The data of Fig. 3 suggest that such interference is indeed minimal. Proline slightly stimulated glucose consumption in glucose-grown organisms, whereas glucose slightly reduced proline uptake in *T. brucei* cultured on proline. The conclusion from these experiments must be that *T. brucei* has no short-term mechanism for the regulation of its energy generation. Both pathways can metabolize at the same time at full capacity.

Coordination and regulation of cellular processes. Hexokinase and pyruvate kinase activities are regulated simply according to glucose availability. Glucose transport over the plasma membrane is subject to a more complex system of regulations and adjustments aimed at exactly saturating the capacity of the subsequent glucose-metabolizing system without creating unnecessary overcapacity. As an example of the regulation and coordination of intracellular systems relative to each other, the glucose transport and metabolism in the insect form of *T. brucei* follow the principle of maximum energy efficiency.

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