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INCREASED RATES OF SUGAR TRANSPORT IN *SACCHAROMYCES CEREVISIAE* A RESULT OF SUGAR METABOLISM

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SUMMARY

Preincubation of yeast cells with glucose or other metabolic energy sources increased the rate of sorbose efflux 2- to 3-fold. Stimulated rates persisted for several h, decreasing slowly. They were approximately halved by including K_m concentrations of highly competitive sugars such as deoxyglucose, glucose, fructose and mannose in sorbose efflux suspensions, and were greatly slowed at reduced temperatures. Inhibitors of energy metabolism blocked the rate stimulation, as did cycloheximide; added nitrogen sources increased the rate additionally. The rate of sorbose uptake was also increased, whereas that of dimethylsulfoxide, which enters the cell by simple diffusion, was not changed. Transport of arabinose and fucose also occurred at increased rates. The data indicate a change in the sorbose transport system rather than in membrane permeability. The change, apparently the synthesis of a transport system component, requires metabolic energy and involves protein synthesis.

INTRODUCTION

Lewis and Stephanopoulos[1] have reported experiments from which they conclude that glucose increased the permeability of the external membrane of yeast cells, and Indge[2] has reported a metabolic lysis of yeast protoplasts evidently also the result of an effect on the membrane by glucose. Yeast spheroplasts swell when glucose is added (Spoerl, E., unpublished) — a response apparently similar to that of *Staphylococcus* protoplasts reported earlier by Abrams[3]. Swelling could result from increased permeability and a consequent increased solute intake; however, glucose may function in other ways. It could supply energy for an active uptake process, though this would be unusual for uptake of sorbose, a non-metabolized sugar repeatedly described as entering yeast cells by facilitated diffusion[4,5]. Energy could also be used for the synthesis of transport system components, such as "carrier" or "permease", so that solute intake would be increased. An activation resulting in a speeded rate of transfer but not of solute accumulation might also be conjectured. The many cellular changes possible as a result of energy availability complicate measurements of the particular role of a compound such as glucose. For example, though glucose and galactose transport in yeast have received consid-

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erable attention, it is still not entirely clear when transport is active[6] and when it is a simpler facilitated diffusion[7].

In this paper we describe increased rates of sugar transport across the external membrane of yeast cells as a consequence of glucose metabolism. Non-metabolized sugars were used to measure the change brought about by metabolized sugars. Inhibition of the stimulation in rate by energy inhibitors and cycloheximide, and a reduction in the stimulated rate by low temperatures and competing sugars, indicate a change in the transport system rather than in membrane permeability.

MATERIALS AND METHODS

Organism and handling

Saccharomyces cerevisiae, a strain originally isolated from a cake of Fleishmanns baker's yeast and maintained over many years, was grown aerobically as previously described[8]. Cells were harvested during exponential growth at a count of $2 \cdot 10^7$ per ml, washed twice by centrifugation with distilled water and resuspended in 0.02 M potassium phosphate buffer, pH 4.5, containing 2 mM $MgCl_2$, with other additions as specified, for preincubation, preloading or uptake measurements. Preincubations were utilized to expose cells ($4 \cdot 10^7$ per ml) to indicated compounds before uptake measurements or preloading with radioactive solutes. Preincubation, preloading, uptake and efflux suspensions were shaken continuously in a water bath at 30 °C (or at other temperatures specified in the text) in small erlenmeyer flasks. Cells were washed twice by centrifugation with distilled water at room temperature after preincubations, and twice with ice-cold water after preloading for efflux measurements, unless otherwise noted. Aliquots of washed cells taken during harvesting were dried overnight at 105 °C for weight measurements.

Transport and radioactivity measurements

Cells, 10^8 per ml, were incubated for 70 or 90 min to preload them with radioactive solute after which they were washed and resuspended, $4 \cdot 10^7$ per ml, in buffer solution, with specified additions, for efflux measurements. Aliquots, 0.5 ml, were removed from the suspension at regular intervals, usually 1 min, and added to 10 vol. of ice-cold water above a membrane filter (RAWP 025, Millipore Corp., Bedford, Mass.), filtered and washed 2 times with 10 volume portions of ice-cold water. Measurements of solute uptake were made similarly by sampling cells ($4 \cdot 10^7$ per ml) from suspensions containing a radioactive solute. Sampled aliquots in both types of measurements contained approximately 0.44 mg (dry weight) of cells. Washed filters with cells were placed in 10 ml of Bray's solution[10] in a counting vial and counted in a Packard Tri-Carb instrument to measure contained radioactivity. Assays of radioactive solutes were made by adding 0.1 ml of a 1:100 dilution directly to Bray's solution in a counting vial.

Chemicals

L-[U-^{14}C]Sorbose, D-[1-^{14}C]arabinose and L-[1-^{3}H]fucose were purchased from Amersham-Searle, Corp., Arlington Heights, Ill.; [^{14}C]dimethylsulfoxide, from New England Nuclear Corp., Boston, Mass. These compounds were used in uptake and preloading solutions in 10 mM concentrations labeled at approxi-

mately 40 cpm/μmole. Solutions of radioactive sorbose and arabinose, after diluting radioactive stock with carrier in buffer, were purified (scavenged) by incubating them with stationary phase yeast cells ($4 \cdot 10^7$ per ml) in a shaker bath at 30 °C for 60 min and a second time for 90 min, the yeast being discarded after each incubation. Other chemicals were the purest available from standard sources (Calbiochem; Schwarz-Mann; and Fisher Scientific).

RESULTS

Efflux

Fig. 1 shows that an appropriate addition of D-glucose speeded sorbose efflux from these cells. A high concentration of glucose slowed efflux. Because sorbose, a non-metabolized sugar, is transported by a facilitated diffusion system which also transports glucose[4], these differing effects presumably involve both accelerated exchange[11], due to a continuing glucose influx for metabolic use, and competition. The data below demonstrate that when glucose is metabolized a further increase in efflux rate occurs. (Semi-logarithmic plots of sorbose efflux generally show two or more rates[8,9], only the first of which is plotted in Fig. 1. These curves, and others below, have not been corrected by subtracting succeeding slower-rate efflux components, so that slopes and $t_{\frac{1}{2}}$ values, though they provide data entirely adequate for the comparisons made, do not indicate maximum rates of passage across the external cell membrane.)

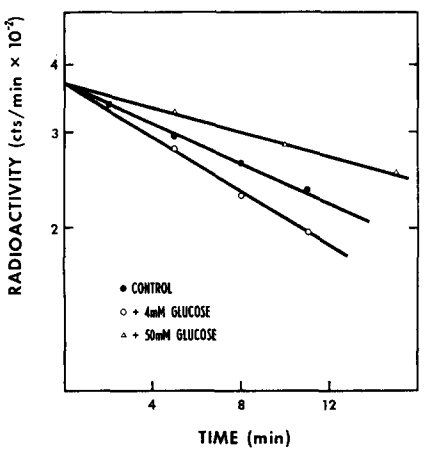

Fig. 1. Efflux of sorbose from yeast cells which had been preloaded in 10 mM sorbose solution.

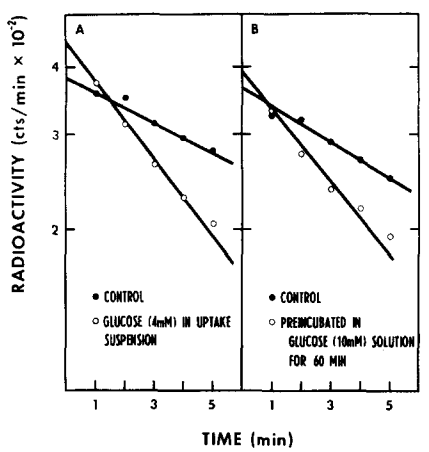

Fig. 2. Efflux of sorbose from control cells and from cells which had glucose available during sorbose preloading (A) or during preincubation (B).

Fig. 2 shows that sorbose efflux from cells exposed to glucose before efflux was measured was at a rate more than double that from control cells. Because sorbose alone occupied the transport system during efflux, *i.e.* glucose was not present, it is apparent that the glucose treatment resulted in either a speeded transport or an increased permeability of the cell membrane. Glucose was supplied

during sorbose preloading or in preincubations; in the latter case, the rate stimulation persisted during the 70-min period of sorbose preloading with no glucose available. Arabinose and fucose also were lost more rapidly from glucose-treated than from control cells. Relative rates, as well as data related to other figures, are summarized in Table I.

TABLE I

EFFECT OF VARIOUS COMPOUNDS INCLUDED IN PREINCUBATIONS OR IN PRELOADING SUSPENSIONS ON THE SUBSEQUENT RATE OF EFFLUX OR UPTAKE

The $t_{\frac{1}{2}}$ of efflux, usually measured over the first 3 min, for control cells was 11.4 min (mean of 14) for sorbose, 9.95 min (mean of 8) for arabinose and 17.7 min (mean of 12) for fucose. Cells were preloaded in 10-mM solutions of the transported solute. Values listed in the table are means of 3 or more separate measurements.

Method	*Additive*	*Solute transported*	*Rate measured*	*% of control*
In uptake	Glucose, 4 mM	Sorbose	Efflux	264
	Ethanol, 100 mM	Sorbose	Efflux	178
	Cycloheximide*	Sorbose	Efflux	81
Preincubation, 20 min	Glucose, 5 mM	Sorbose	Efflux	147
	Glucose, 1 mM	Sorbose	Efflux	124
	Fructose, 10 mM	Sorbose	Efflux	222
	Mannose, 10 mM	Sorbose	Efflux	174
	Glucose, 4 mM	Sorbose	Uptake	165
Preincubation, 30 min	Glucose, 10 mM	Arabinose	Uptake	158
	Glucose, 4 mM	Dimethylsulfoxide	Uptake	96
	Glucose, 10 mM, *plus* cycloheximide	Sorbose	Efflux	88
	Glucose, 10 mM, *plus* asparagine, 10 mM, *plus* cycloheximide	Sorbose	Efflux	97
	Ethanol, 100 mM	Sorbose	Efflux	142
Preincubation, 60 min	Glucose, 10 mM	Arabinose**	Efflux	122
	Glucose, 10 mM	Fucose**	Efflux	153
	Ethanol, 100 mM	Sorbose	Efflux	170
	Ethanol, 100 mM, *plus* cycloheximide	Sorbose	Efflux	100
	Ethanol, 100 mM, *plus* sorbose, 100 mM	Sorbose	Efflux	169

* 10 μg/ml in all cases.

** After preincubation cells were treated for 20 min with 1 mM iodoacetic acid before preloading, and this inhibitor was included in preloading and efflux suspensions.

It may be noted that efflux of 10 mM sorbose from these cells, because of the high K_m of sorbose transport (>1 M; see ref. 12), should normally occur within the usual limits of measurement at a constant rate approaching simple diffusion[11]. Moreover, the buffer solutions into which efflux occurred provided for more than a 200-fold dilution, so that measured rates were not complicated by return fluxes.

These rates also are not influenced by changes in cell number or size, as measurements of uptake rates might be.

Attempts to measure increased rates of D-mannose and 2-deoxy-D-glucose efflux were unsuccessful, evidently because metabolic reactions could not be controlled. Although cells were pretreated with 1 mM iodoacetic acid and the inhibitor was included in preloading and efflux suspensions (1mM NaN_3 was included with iodoacetic acid in some cases), total sugar uptake was greater in preincubated than in control cells and efflux was slower. The simpler metabolic situation in the case of 2-deoxy-D-glucose[13], allows the suggestion that this sugar was more extensively phosphorylated in treated cells with energy stored during preincubations, and as a consequence efflux was reduced compared to the control.

Uptake

An increased rate of passage across the cell membrane evident in the loss of a solute might be expected to be observable also in solute entry. Cells, washed after incubation in glucose solution and resuspended for uptake measurements without glucose, showed increased initial rates of sorbose uptake (Fig. 3). Arabinose was also taken up by treated cells at an increased rate, whereas dimethylsulfoxide, which enters the yeast cell by diffusion[14], was taken up by treated and control cells at the same rate, indicating that membrane permeability had not been altered.

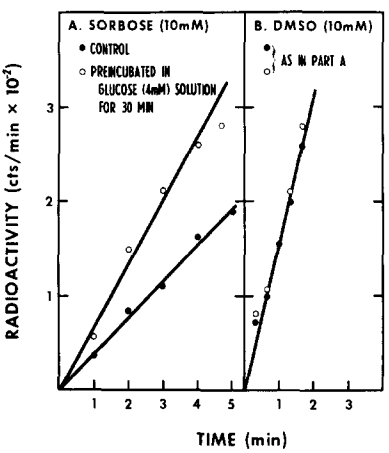

Fig. 3. Uptake of sorbose and dimethylsulfoxide (DMSO) by control cells and by cells preincubated in glucose solution.

Competition

Glucose, 2-deoxy-D-glucose, mannose and D-fructose, highly effective competitors of sorbose transport, blocked efflux according to expectations for a carrier mediated process and the relative affinities involved, *i.e.* sorbose efflux rates were halved by approximate K_m concentrations[12] of these sugars (Table II). High concentrations reduced sorbose efflux markedly. These results support the conclusion that accelerated efflux occurs *via* the glucose–sorbose-facilitated transport system and is not due to increased permeability of the external membrane. The decrease in efflux rate which accompanied a decrease in temperature also is consistent with

TABLE II

EFFECT OF COMPETING SUGARS ON SORBOSE EFFLUX RATES

Iodoacetic acid, 0.5 mM, was included in suspensions with glucose, fructose and mannose to inhibit metabolism. Efflux was unaffected by 1 mM iodoacetic acid (99% (mean of 7) of the accelerated rate without iodoacetic acid). Values listed are means of 3 or more separate measurements.

Cell pretreatment	*Competing sugar*	$t_{\frac{1}{2}}$ (*min*)
Glucose, 4 mM, in preloading suspension	None	4.8
	2-Deoxy-D-glucose, 4 mM	11.5
	2-Deoxy-D-glucose, 100 mM	46.0
	Fructose, 25 mM	11.5
	Glucose, 5 mM	11.6
	Mannose, 60 mM	10.5
Ethanol, 100 mM in preloading suspension	None	6.6
	2-Deoxy-D-glucose, 4 mM	14.6
	Fructose, 25 mM	14.5

this conclusion. Measured Q_{10} values of 1.9 and 2.9 (20–30 °C and 10–20 °C, respectively) for control cells and 2.1 and 2.7 for treated cells are of a magnitude which most often indicates a process other than simple diffusion. The doubling of sorbose efflux time when the above competing sugars were included at K_m concentrations shows half-saturation of the membrane carriers and, thus, unchanged K_m values.

Treatment variables

Incubations of cells for 30, 60 (pretreatment) and 70 min (inclusion in preloading suspensions) in glucose solutions were effective in increasing sorbose uptake and efflux rates. The data of Fig. 4 show that efflux rates increased rapidly with

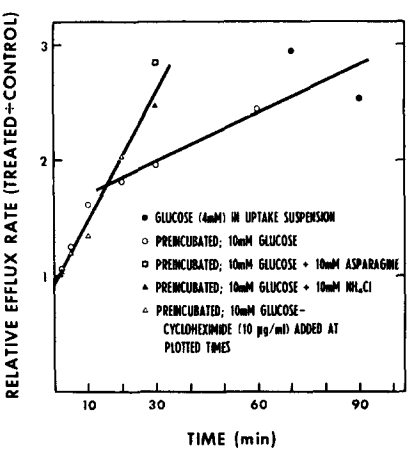

Fig. 4. Increase in the rate of sorbose efflux in relation to the time of glucose availability either during preincubation or during preloading, the time of addition of cycloheximide (10 µg/ml) during preincubation, and the effect of added asparagine and NH_4Cl in preincubations. Points are means of 3 or more separate measurements.

the time of incubation in 10 mM glucose solutions for 15 to 20 min, after which the rate of increase was slower. 5 mM glucose was less effective in 20 min than 10 mM, and 1 mM was still less effective (Table I) Incubation of cells in mannose and fructose solutions also increased rates of sorbose loss.

Good energy sources for these cells other than sugars are not numerous. Preincubation with ethanol (100 mM), or its inclusion in preloading solutions, increased efflux rates (Table I), though not as effectively as glucose, and the rate of efflux was approximately halved by K_m concentrations of 2-deoxy-D-glucose and fructose (Table II). Potassium lactate (10 mM) was ineffective or had a minimal effect in stimulating efflux rate, probably because of its relatively poor performance as an energy source. Sorbose (100 mM) or arabinose included in preincubations with ethanol or lactate did not increase efflux rates further (Table I).

Energy inhibitors

Several common inhibitors of energy metabolism included with metabolized sugars in preloading suspensions prevented the increase in efflux rate (Table III). These inhibitions and the effectiveness of different fermentable sugars and ethanol establish that metabolic energy is a requirement for the increase in rate. Incubation with a non-metabolized sugar (*i.e.* the period during which control cells were preloaded with sorbose for efflux measurements) provided only the base line, *i.e.* the control cell efflux rate.

TABLE III

EFFECT ON SUBSEQUENT EFFLUX RATES OF INHIBITORS INCLUDED WITH THE ENERGY SOURCE IN SORBOSE PRELOADING SUSPENSIONS

The control rate is that of cells exposed to the energy source alone. Values listed are means of 2–4 separate measurements.

Energy source	*Inhibitor*	*% of control*
Glucose, 4 mM	Na_2HAsO_4, 10 mM	48
	NaF, 10 mM	47
	NaN_3, 1 mM	34
	NaN_3, 3.3 mM	30
	2,4-Dinitrophenol, 1 mM	64
	N-Ethylmaleimide, 5 mM	23
	Iodoacetic acid, 0.5 mM	25
	Iodoacetic acid, 1.0 mM	23
Fructose, 4 mM	NaN_3, 3.3 mM	40
	Iodoacetic acid, 0.5 mM	28
Ethanol, 100 mM	NaN_3, 3.3 mM	46
	Iodoacetic acid, 0.5 mM	45
None	None	35*

* Based on rate of glucose-treated cells.

Protein synthesis

Cycloheximide, an inhibitor of cytoplasmic protein synthesis[15], included with glucose also prevented the increase in efflux rate (Table I and Fig. 4). The correlation between the amount of increase and the time of cycloheximide addition is apparent in Fig. 4. Fluorophenylalanine at high concentrations partly prevented the rate increase (44% reduction at a concentration of 10 mg/ml); chloramphenicol (2 mg/ml) had no effect.

If protein synthesis was involved in producing faster efflux, cell pools must have provided nitrogen, and probably limited the amount of increase. A nitrogen source, asparagine (a medium constituent) or NH_4Cl, added with glucose increased efflux rates additionally (Fig. 4), and cycloheximide also blocked these increases (Table I).

Stability

The increased capacity for transport decayed slowly with time (Fig. 5A). Decay and synthesis from cell nitrogen reserves, suggest that a continuous turnover of transport system components may take place. Incubation with either cycloheximide or iodoacetic acid alone resulted in lower rates of efflux (Table I and Fig. 5B), possibly because syntheses from reserve materials were inhibited. However, such turnover is limited; transport occurs effectively after overnight starvation[16], a period of time during which energy reserves must be greatly reduced. Efflux after cells had been incubated in buffer for 4 h was faster than from control cells, whereas after only 1 h it was slower (Fig. 5B). This late increase in rate indicated either a delayed formation of transport capacity from cell reserves or leakage of sorbose

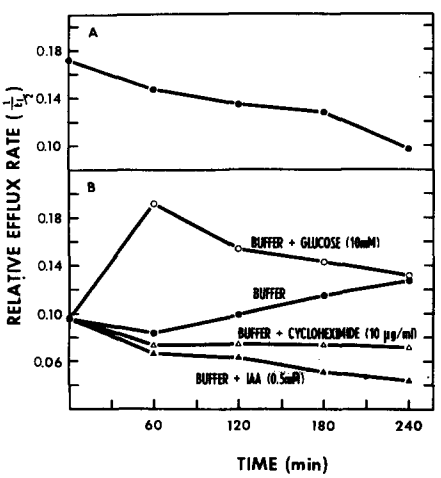

Fig. 5. Changes in sorbose efflux rate with time of incubation under different conditions. (A) Decrease in the stimulated rate of efflux. Glucose (10 mM) was added to preloading suspensions after 90 min and then washed from the cells 30 min later by centrifugation with cold unlabeled sorbose solution; cells were resuspended in [^{14}C]sorbose solution, sampled at indicated times, washed again and resuspended for efflux measurements. (B) Cells were preincubated in buffer or buffer *plus* indicated compounds for the plotted number of min; they were then washed by centrifugation, resuspended for sorbose preloading (70 min), sampled, washed again and resuspended for efflux measurements. IAA, iodoacetic acid.

through the cell membrane. Tests of competition for transport with 2-deoxy-D-glucose were inconsistent; however, 2-deoxy-D-glucose competition was less effective with cells incubated in water for 21 h (unpublished data) than with fresh cells, indicating that changes in membrane permeability had occurred during this period of time. Efflux from cells incubated in glucose (10 mM) solution for 4 h was slower than from cells incubated for 60 min. The reason is not evident, though as emphasized above, glucose may initiate many cellular changes. In addition to preventing stimulation by glucose, iodoacetic acid (0.5 mM) injured the cells as indicated by a decreasing transport rate as the time of exposure increased.

DISCUSSION

Sorbose transport by these cells was increased as a result of sugar metabolism. Competition for transport, the response to changes in temperature, and the lack of an effect on dimethylsulfoxide passage provide the basis for this conclusion and eliminate a general permeability change as an explanation for the increased sorbose flux.

The ordered effectiveness of different amounts of glucose (Table I) and the time sequence of rate stimulation (Fig. 4) indicate a stoichiometric relation between energy availability and the transport effect. A direct energy input into the transport process could be responsible only if energy were stored during preincubation and retained during preloading, and its transfer or coupling was not blocked by iodoacetic acid. No evidence has been reported in the literature for an active uptake of sorbose of the usual type in which an accumulation at least several times the external concentration occurs. Moreover, an unusual mechanism would be required to explain an energy coupling which speeds the loss of solute from the cell into empty solute space, as in these experiments. Though energy involvement in downhill galactoside transport in *Escherichia coli* has been reported[17], such an explanation in the present case can only be conjectural.

On the other hand, the slow decrease in the stimulated rate — an energy supply might be expected to dissipate more rapidly — marks a stability more consistent with the synthesis of a component of the sorbose transport system. Inhibition by cycloheximide and enhancement by an added nitrogen source provide evidence that the energy is utilized for the synthesis of a protein constituent. Although cycloheximide has been reported to inhibit ion uptake and glucose metabolism[18], it evidently may affect protein synthesis without affecting transport or energy availability[19,20]. It did not affect sorbose uptake or efflux at the concentration used above (unpublished).

Ethanol also stimulated the rate of sorbose transport, and its effect was blocked by energy inhibitors and cycloheximide. However, the rate was not further increased by adding a carried sugar, *i.e.* sorbose or arabinose, with the alcohol. Thus, the protein synthesis which occurs is not obviously the result of a specific induction. The increase was selective in that the transport rate doubled during a period of time (20 min) in which non-specific protein syntheses involved in growth could not have occurred to anywhere near the same extent. A generally non-growth environment, the cells being in buffer without a nitrogen source, seemingly

stimulated selective formation of a potentially highly useful increase in transport capacity.

If a permease which catalyzes sugar attachment to a carrier[6] had been synthesized, a K_m change might have been evident, sorbose and arabinose might have functioned as inducers, and the cells might have been able to accumulate intracellular concentrations of sorbose exceeding external. Accumulation did not occur (Spoerl, E., unpublished) nor do the competition measurements indicate a K_m change. Thus, such a synthesis apparently is not involved here, although it might have explained differences in the degree of stimulation of arabinose, fucose and sorbose transport[21].

Synthesis of a carrier or carrier component, on the other hand, may have involved an endogenous inducer or an altered metabolic control. Such a change might be expected to affect the transport of different sugars to the same degree. Because iodoacetic acid was necessary in efflux measurements with arabinose and fucose, this inhibitor, or a metabolic reaction such as the phosphorylation cited above for 2-deoxy-D-glucose, may have affected the measurements so that the full rate increase was not apparent. A lesser increase in the rate of arabinose transport also suggests the possibility that glucose stimulation is less significant for sugars with a low than with a high K_m.

An increase in carrier affinity for sorbose might also be considered as the means by which efflux is speeded. For example, a K_m change, conceivably an allosteric alteration caused by a metabolic intermediate of glucose metabolism, evidently accounts for differences in glucose uptake under aerobic and anaerobic conditions[22]. Although the possibility of an increased affinity cannot be disregarded, the long period of time after pretreatment with glucose (70 min preloading) argues against involvement of a glucose intermediate in the experiments reported here. Inhibition by cycloheximide and stimulation by a nitrogen source also indicate more than a change in existing molecular structure. Furthermore, competition measurements with several sugars did not indicate a significant K_m change. It might be considered that an increased affinity was not observed in the competition measurements because it was counterbalanced by an increase in membrane permeability. This possibility complicates rather than clarifies the experimental results, and the imprecision of available techniques for measuring small differences in K_m and in permeability make evaluation difficult. On the other hand, if the cytoplasmic membrane has only limited space for transport-system components, as appears to be the case for constitutive amino acid permeases[23], a change in K_m could more readily account for speeded sorbose transport. The possible difficulty here in an interpretation which specifies added transport system components cannot be resolved at present and probably will not be clarified until both the amino acid and the sugar transport systems are more fully characterized in yeast, and their components and affecting metabolic processes and products specified. The observed stimulation of sorbose transport offers a means for further exploration of the nature of the sugar-transport system.

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