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Reversible Permeability Changes in the Membrane of a Yeast Cell Sugar Compartment

Edward Spoerl*, S. H. Benedict, S. N. Lowery, John P. Williams, and J. P. Zahand

Army Medical Research Laboratory, Fort Knox, Kentucky 40121

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Summary. Sorbose uptake by *Saccharomyces cerevisiae* was increased 40 to 60% by glucose and other metabolizable sugars. Neither growth nor binding accounted for the increased uptake. However, accessibility of a restrictive intracellular compartment was increased as shown by counterflow and efflux measurements. Efflux from the compartment was more than doubled by glucose. This effect was reversed by washing and was prevented by iodoacetic acid and other inhibitors, but not by cycloheximide. No evidence was found for a facilitated transport system in the compartment membrane such as exists in the external cell membrane. It was concluded that sorbose crosses the compartment membrane by simple diffusion and that a reaction requiring the metabolism of sugars increases the permeability of the membrane. Arabinose and fucose entered and were lost from the compartment like sorbose, whereas dimethylsulfoxide was unaffected by the compartment. All three of these latter compounds were bound by the cells when glucose was available in uptake suspensions. Binding was prevented by iodoacetic acid, but not by cycloheximide.

Reports by several authors [15, 18, 25] indicate that sugars are compartmented within yeast cells, and evidence exists that the vacuole functions as a sugar compartment [23, 25]. Intracellular compartments which restrict solute distribution or metabolism may affect various aspects of cell function including transport [7, 8, 14]. Too little is known of the nature and function of these structural or metabolic restrictions and of the processes they may affect or participate in.

Efflux measurements with nonmetabolized compounds, which avoid complications of incorporation, effects on transport [28], etc., are highly useful in compartment studies; two or more efflux components directly indicate that intracellular solute distributions probably involve compart-

* *Present address;* Army Foreign Science and Technology Center, 220 7th St., NE, Charlottesville, Virginia 22901.

ments [7, 15, 25]; insignificant solute return flows are readily arranged [28]; and a variety of experimental conditions can be employed [7]. Uptake measurements generally do not reveal compartments as directly, although those reported below provide additional evidence for a yeast cell sugar compartment.

Kotyk and Haskovec [15] have previously reported an increased uptake and transport of several sugars by yeast as the consequence of a specific induction by D-galactose. These authors suggested that a sugar carrier was formed in the membrane of a compartment normally inaccessible to the sugars. In the studies reported below, it is concluded that sorbose, a non-metabolized sugar [26], normally penetrates an intracellular compartment membrane by simple diffusion, and that an increased rate of penetration, brought about by metabolized sugars, is the result of an increase in membrane permeability rather than a stimulation or initiation of a transport process.

Materials and Methods

Organism and Handling

Saccharomyces cerevisiae ATCC 24297 was grown aerobically as previously described [25]. Cells were harvested during exponential growth at a count of 2×10^7/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 additions as specified for the various measurements. Aliquots of washed cells taken during harvesting were dried overnight at 105 °C for weight measurements. Cells for experimental treatments and measurements were suspended in small Erlenmeyer flasks and shaken continuously in a water bath at 30 °C. Flasks were covered loosely with aluminum foil during long incubations, or a correction for evaporation was applied. When lower temperatures were used, cell suspensions were maintained similarly in constant temperature water baths. Pretreatment with iodoacetic acid (IAA) was accomplished by incubating cells (4×10^7/ml) for 40 min in buffer-IAA solutions and then washing the cells twice with water. Cells were boiled for 5 min by immersing the centrifuge tube containing them in a boiling water bath, and binding was determined by a usual sorbose uptake measurement.

Growth and Volume Measurements

Cells were resuspended at a concentration of 4×10^7/ml. Dry weight was determined as above. Optical density (OD) measurements were made in a DU spectrophotometer at a wavelength of 610 μm. Cells were counted microscopically in a hemocytometer; unit counts are the sum of single and budded (or paired) cells; total counts are the sum of single cells, mother cells, and buds larger in diameter than half the diameter of the mother cell. Cell volume was determined from size distribution plots obtained with a Coulter counter, Model B.

Water Space

Cells were packed by centrifugation (30 min, approximately $1200 \times g$) in Bauer-Schenk tubes and their volume read (0.3 to 0.5 ml). Supernatant buffer was decanted

and, after the wet weight of the cells was determined, replaced with 0.3 ml of [^{14}C]-inulin of known radioactivity (cpm). The cells were stirred and centrifuged, and after 0.1 ml aliquots of the supernatant were taken for measurements of radioactivity, were dried at 105 °C. Intercellular space was calculated from the inulin dilution, and it and the dry weight were subtracted from the wet weight to give the water content of the cells. Alternatively, additional aliquots of cells were mixed with tritium-labeled water, and water space was determined by subtracting inulin determined intercellular space from the total water space obtained from the tritium-water dilution.

Uptake, Efflux, and Radioactivity Measurements

For uptake measurements aliquots of cells (4×10^7/ml) suspended in solutions of radioactive solute were sampled at regular intervals. In counterflow experiments glucose was added after 90 or 330 min of incubation, and then the suspensions were sampled at regular intervals. For efflux measurements cells (1×10^8/ml) were preloaded in solutions of radioactive solute (generally for 90 min; other time intervals are specified in the text), removed from the preloading suspension by centrifugation, washed twice with ice-cold distilled water by centrifugation, resuspended (4×10^7/ml) in buffer with specified additions and sampled at regular intervals. All sampled aliquots (0.5 ml) were added to 10 volumes of ice-cold water above a membrane filter (RAWP 025, Millipore Corp., Bedford, Mass.) and filtered; the cells were washed on the filter two times with 10 volume portions of ice-cold water. Washed cells and filter were placed in 10 ml of Bray's solution [3] in a counting vial and counted in a Packard Tri-Carb instrument to measure contained radioactivity. Aliquots contained approximately 0.44 mg (dry weight) of cells. Assays of radioactive solutions were made by adding 0.1 ml of a 1:100 dilution directly to Bray's solution in a counting vial. Retention of radioactivity by the filters was measured by filtering aliquots of solutions without cells and washing and counting as above. All samples were counted to a statistical error of less than 5%, usually much less, and replicated to assure reproducibility.

Chemicals

L-[U-^{14}C]Sorbose (3.0 mC/mmole), D-[1-^{14}C]arabinose (2.5 mC/mmole) and L-[1-^{3}H]-fucose (1.0 C/mmole) were purchased from Amersham Searle Corp., Arlington Heights, Ill.; [^{14}C]dimethylsulfoxide (DMSO) (1.5 mC/mmole), inulin-[^{14}C]carboxyl (2.7 mC/mg) and tritium-labeled water (0.1 mC/mg), from New England Nuclear Corp., Boston, Mass. Ten mM solutions of sorbose, used most frequently, were labeled at approximately 40 cpm/μmole (solutions were individually assayed [25] to allow specific calculations of cell contents). Solutions of different molarity were labeled with the same amount of stock radioactive solution added to different amounts of carrier. After diluting radioactive stock with carrier in buffer, sorbose solutions were purified (scavenged) [22], except where it is noted in the text that unscavenged sorbose was used, by incubating them with stationary phase yeast cells (4×10^7/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. Paper chromatographs of cell extracts showed only one radioactive spot which paralleled that of the original sorbose [27]. Arabinose, fucose and DMSO solutions, used at 10 mM concentrations, were labeled at approximately 40 cpm/μmole. Radioactive arabinose solutions were scavenged like sorbose solutions. Inulin and water were labeled in an amount to provide convenient counting. Other chemicals were the purest available from standard sources (Calbiochem; Schwarz-Mann; and Fisher Scientific).

Results

Stimulated Uptake

Sorbose uptake from scavenged solutions by these yeast cells was increased 40 to 60% in total amount upon addition of a metabolizable sugar such as D-fructose, D-mannose or D-glucose (Figs. 1 and 2). Initially the rate of sorbose uptake was reduced because of competition with the added sugar for the carrier sites which all of these sugars utilize [4]. The competitive effect was large with 10 mM glucose, but was minimized at lower concentrations. Sorbose uptake with 4 mM D-arabinose, another non-metabolized sugar [26], or with 100 mM glycine did not differ from the control (Fig. 1). A high concentration of arabinose (300 mM) produced an apparent osmotic effect, and 10 mM 2-deoxy-D-glucose (DOG) reduced the initial rate of uptake by competition with sorbose for transport [14].

An increased total uptake was first observed in comparisons of scavenged and unscavenged solutions of [^{14}C]sorbose, in which unscavenged solutions gave cell radioactivity measurements 30% higher than scavenged solutions. Although both the carrier and the radioactive sorbose were purchased as

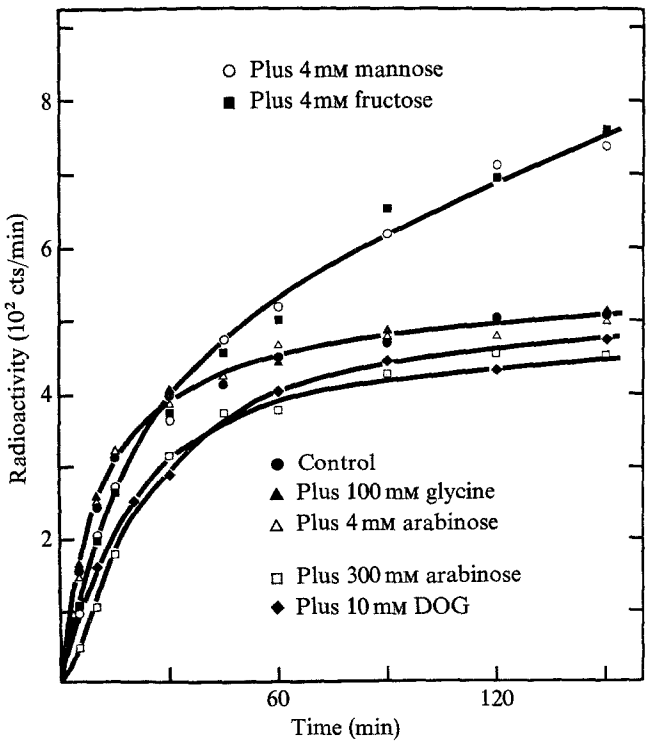

Fig. 1. Effects of several sugars and glycine on sorbose (10 mM) uptake

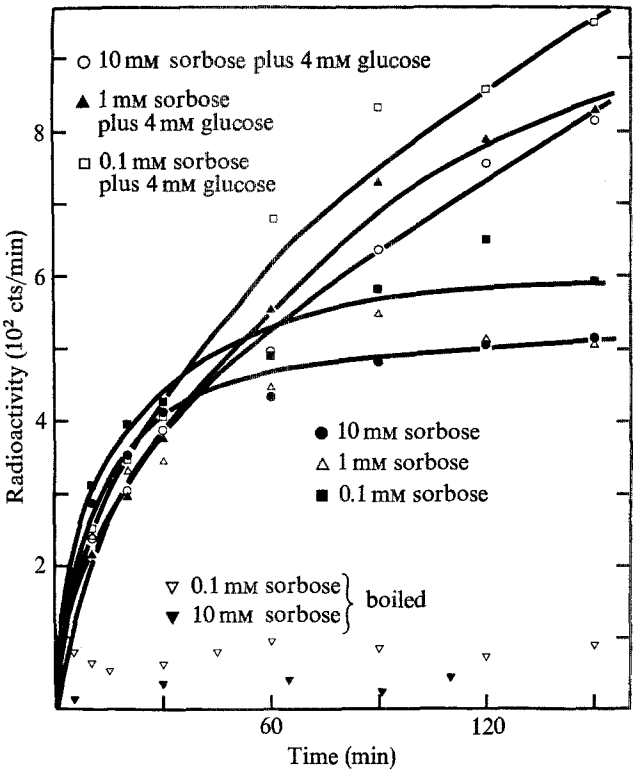

Fig. 2. Uptake of 10, 1, and 0.1 mM sorbose with and without glucose, and 10 and 0.1 mM sorbose after cells were boiled. The same amount of stock radioactive sorbose was included in each solution; molarities differ depending upon the amount of unlabeled carrier sorbose included

chromatographically pure, this difference probably involved a radioactive contaminant (*see also* Ref. [22]). Further examinations with scavenged sorbose uncovered the stimulating effect of metabolizable sugars.

Possible explanations for an increase in sorbose uptake appeared to include an active uptake process, an increased binding of sorbose accompanying sugar metabolism, cell growth, and an increased accessibility of a compartment normally restricting sorbose entry. If uptake was active, the mechanism obviously was much less efficient than that for glycine [11] or maltose [12], for example, and because sorbose uptake has repeatedly been described as a facilitated diffusion only [4, 33], the possibility of active uptake was set aside to first examine the other possibilities.

Sorbose Binding

Fig. 2 shows that relative uptake differed little throughout a wide range of sorbose concentrations, though the 0.1 mM concentration was taken up

to the greatest extent. (Because identical amounts of radioactively labeled stock sorbose were included at each sorbose concentration, cpm in the cells would be alike if uptake were proportional at the different molarities.) Addition of glucose resulted in an increased uptake in each case (increases of 48, 53, 53, and 65%, each a mean of 3 values, occurred, respectively, at 100, 10, 1, and 0.1 mM concentrations). The relatively greater uptake at the 0.1 mM concentration indicates that some sorbose was bound. Boiled cells bound about 12% of the amount normally taken up from a 0.1 mM solution (Fig. 2); a smaller portion was bound at higher sorbose concentrations; cells maintained at zero °C bound similar amounts (variables in these measurements were not evaluated completely because of the small amount of binding). These small amounts and the lack of relative differences in sorbose uptake over a wide concentration range, with and without glucose, indicate that binding is an insignificant factor in the larger increases in uptake under examination. Binding has been reported to occur with other sugars at low concentrations [17], and arabinose and L-fucose are bound in significant amounts when glucose is available *(see below)*.

Cell Growth

The dry weight of yeast in sorbose-buffer solutions increased 14% in 150 min in suspensions supplemented with 4 mM glucose (Table 1). The total number of cells (which includes changes due to increases in bud size) and the number of cell units (which increases as buds separate) increased about this same amount. Insignificant changes in number occurred in buffer or sorbose solutions without glucose. Optical density measurements of cells in 10 mM sorbose solutions showed small percentage increases in absorption, probably the result of an increase in cell mass. Osmotic swelling, indicated by a decrease in OD [10], occurred only at a higher (1.0 M) sorbose concentration (OD responses of cells in 100 mM sorbose solutions were similar to those in 10 mM solutions). Thus, increases in average volume presumably reflect bud or cell growth rather than swelling. Together, these various measures indicate that cells grew only 10 to 20% in 150 min.

Another means for examining growth as a factor in increased uptake is to inhibit it. Cycloheximide, an inhibitor of cytoplasmic protein synthesis [24], effectively stopped increases in cell number, volume, and dry weight (Table 1). However, it did not inhibit sorbose entry into control cells, and total uptake with both cycloheximide and glucose present was increased (Fig. 3), though the cells filled more slowly because a stimulated rate of transport was prevented [28].

Table 1. Cell growth

Measurement	Suspending solution [a]			% of initial value [b]	
	Sorbose (mM)	Glucose (mM)	Cyclo-heximide (mg/ml)	90 min	150 min
Dry weight	10	4	0		114
	10	4	10		103
	100	1	0		100
	100	4	0		112
	100	10	0		122
	100	100	0		131
	100	10	10		100
Cell number [c]	10	4	0	106	112
	10	4	10	96	98
	100	4	0	110	114
	100	10	0	113	118
	0	0	0	103	102
Cell units [c]	10	4	0	106	113
	100	4	0	110	115
Optical density	10	0	0	106	108
	10	4	0	109	114
	10	4	10	104	106
	1000	0	0	84 [d]	84
	1000	4	0	86 [d]	89
	1000	4	10	86	88
Cell volume [e]	10	4	0		110
	10	4	10		100
	100	4	0		106

[a] Cells were suspended in buffer solution plus the indicated compounds and shaken in a water bath at 30 °C.
[b] Suspensions contained 4×10^7 cells/ml at 0 min; cells averged 113 μm^3 in volume and 0.88 mg/ml in dry weight. Values are means of 3 to 6 separate measurements.
[c] Hemocytometer counts.
[d] At 30 min OD was 80% of initial value.
[e] Coulter counter measurements.

A Restrictive Compartment

Biphasic efflux curves show that sorbose exit from yeast is slowed by an intracellular compartment [25]. In the present experiments, efflux measurements after preloading cells for 15, 30, 60, 120, and 240 min allowed both total sorbose uptake (zero minute content) and compartment uptake to be plotted (not shown). Ordinate intersections by straight line extrapolations of the terminal portion (slow component) of the biphasic curves

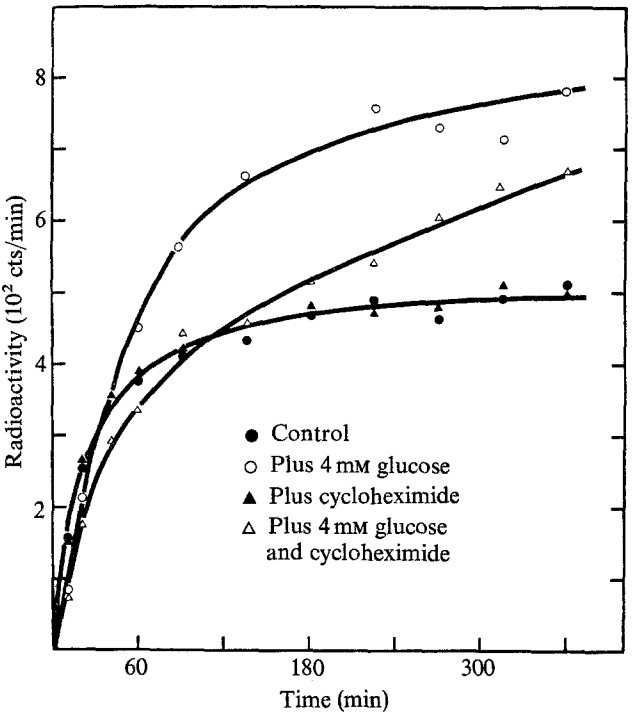

Fig. 3. Effect of cycloheximide on uptake of sorbose (10 mM) with and without glucose

indicated compartment contents. Initial rates estimated from the uptake curves showed the compartment rate to be markedly less than that for total uptake, demonstrating in this way also that sorbose enters part of the cell slowly. The time at which compartment contents reach a constant portion of total cell sorbose is beyond 240 min.

The compartment barrier which restricts sorbose efflux is eliminated by appropriate concentrations of IAA [23, 25]. Its removal should allow an increased total uptake. In fact, cells pretreated with 5 mM IAA took up, at a slower rate, 15–20% more sorbose in 150 min than control cells (not shown; the curve is similar in type to the one in Fig. 3 describing uptake with glucose and cycloheximide). Though not large, this effect was consistent (*see also* Ref. [25]). Pretreatment with N-ethylmaleimide (120 mM), which like IAA eliminates the barrier to efflux [25], resulted in a 20–30% increase in sorbose uptake. These increases in uptake provide independent evidence that a sorbose restrictive compartment exists.

The conclusion that metabolizable sugars act to make this compartment accessible is supported by the counterflow responses, sugar space measurements, and effects on sorbose efflux described below.

Counterflow

Because sorbose and glucose compete for external membrane carrier sites, they may induce counterflows of one another [29]. Cells supplemented with glucose (4 mM) during uptake contained more sorbose than control cells (Fig. 4) and more sorbose was caused to counterflow from these cells, evident in the fact that residual contents of treated and control cells were brought to the same level by additions of glucose (to a 100 mM concentration) at both 90 and 330 min. This result is consistent with a normally restricted compartment becoming more accessible as a result of the glucose supplement and allowing a ready exchange between compartment and other cell sorbose. A higher sorbose content as a result of cell growth, for example, might be expected to counterflow in an amount proportionate to that of control cells.

Reentry of sorbose to a cell content higher than that of control cells after a counterflow induced by 8 mM glucose (Fig. 4) provides added evidence for an increase in compartment accessibility. That is, because of such an increase, the compartment filled to a greater extent than in control

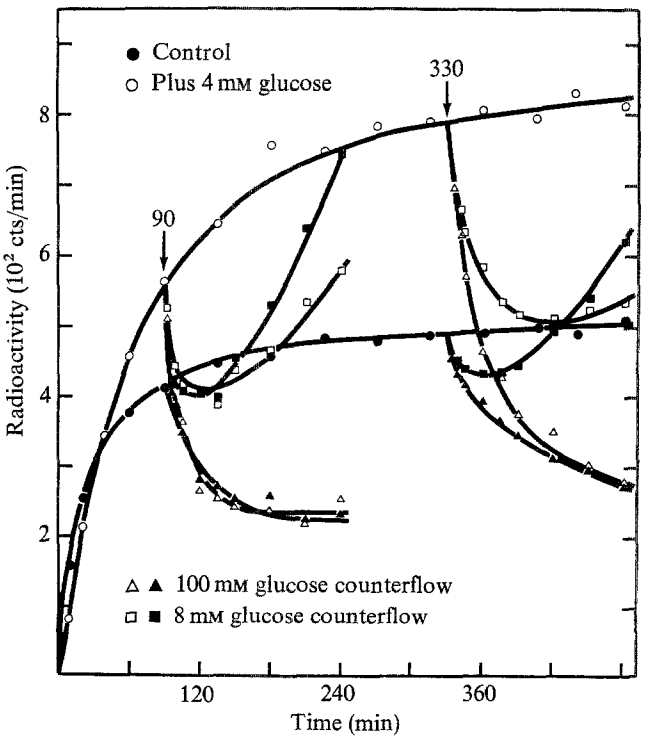

Fig. 4. Uptake of sorbose (10 mM) with and without glucose during an 8-hr period and counterflow of sorbose induced by glucose added at 90 and 330 min

cells. A higher sorbose content would not be expected for cells in which accessibility had already been increased by a glucose supplement during uptake, and it did not occur.

Sugar Space

Careful measurements of intracellular water space gave a value for control cells of $76 \pm 1.5\%$ (mean of 7 separate measurements). The water space of cells suspended for 150 min in 10 mM sorbose plus 4 mM glucose was $78 \pm 0.6\%$ (mean of 6 measurements). Other measurements using tritium-labeled water for total space and inulin for intercellular space consistently gave values 4 to 6% higher, apparently due to some procedural imprecision in one method or the other. Values in the literature tend to be smaller [1, 5, 34], although one of 78% has been reported for *S. carlsbergensis* [11], and variability has been noted depending upon conditions of growth and handling [9, 16, 34].

Calculations based on a water space of 78% showed internal sorbose concentrations of control cells to be only 65–75% of external concentrations after 150 min. Similar calculations for glucose-supplemented cells showed that internal concentrations approximated external concentrations. Thus, adequate cell space is available for the additional sorbose taken up when a metabolizable sugar is provided, and active accumulation, binding, and cell growth need not occur. (A previous report [27] indicating that internal sorbose concentrations of starved cells approached external concentrations was based on a low value for water space (not properly adjusted for cell volume).)

An additional measurement provides excellent support for the above conclusion. DMSO enters yeast passively [9] and is not affected by an increase in the rate of sugar transport [28]. Conceivably, then, it would not be restricted by a sugar compartment. Uptake measurements showed that DMSO filled the cell water space (*see also* Ref. [9]) calculated on a 78% basis, indicating that this value is appropriate.

Effects of Metabolizable Sugars on Efflux

Increased compartment accessibility should be directly apparent in a faster rate (steeper slope) for the slow component of sorbose efflux, a rate controlled by the compartment membrane [25]. Additions of glucose (Fig. 5*A*), fructose or mannose (not shown) to suspensions of preloaded cells increased this efflux more than twofold. Curves of the same type were obtained from cells preloaded from solutions of 100, 10, and 1 mM sorbose,

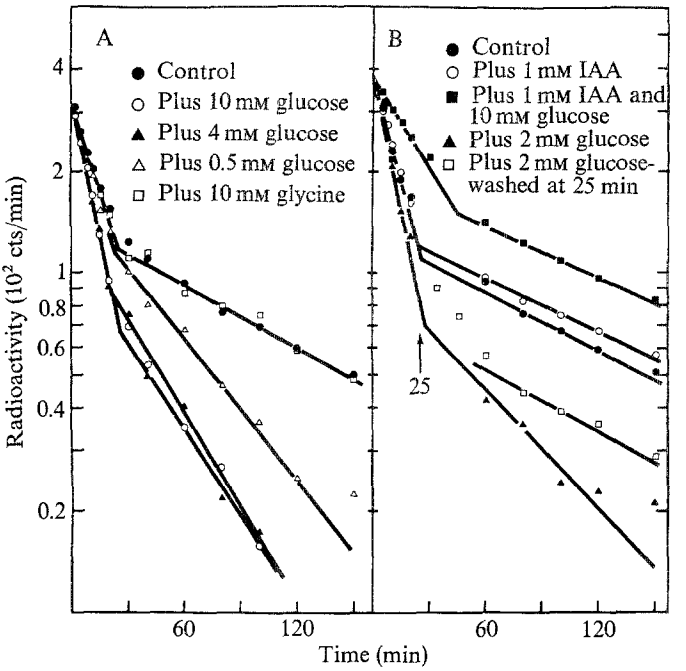

Fig. 5. Efflux from cells preloaded in 10 mM sorbose solution. (*A*) Effects of glucose and glycine included at 0 min. (*B*) Effects of IAA and IAA plus glucose included at 0 min and of washing glucose from the cells after 25 min

and metabolizable sugars increased the rate of loss from all these cells. ($T_{1/2}$ was 41 min (mean of 7) for cells preloaded in 10 mM sorbose solution and with 4 mM glucose in the efflux suspension.) The rate was increased about the same by glucose concentrations as disparate as 0.5 and 10 mM, though the higher concentration also increased initial loss by speeding transport across the external membrane (28; $T_{1/2}$, 4 to 5 versus 11 min). Compounds such as glycine and arabinose did not increase efflux.

Reversal and Inhibition of the Change in Compartment Accessibility

When cells were removed from an efflux suspension containing glucose and washed and resuspended in buffer solution without glucose, the slower efflux characteristic of control cells ($T_{1/2}$, 96 min) was resumed. Although detailed measurements were not made, the time required for the compartment to resume its usual efflux could be measured from curves such as those of Fig. 5*B*.

Similarly, accessibility, increased by including glucose in preloading suspensions, resumed its usual state when cells were washed for efflux

Table 2. Effect of inhibitors included in efflux suspensions at 0 min on the rate of the slow component of sorbose efflux [a]

Inhibitor (mM)	% of Control [b]
20 Na_2HAsO_4	101
20 $NaAsO_2$	73
10 NaN_3	70
20 NaF	42
0.5 Iodoacetic acid	35
5 N-ethylmaleimide	41
10 Cycloheximide	94

[a] Cells were preloaded in 10 mM sorbose solution and 4 mM glucose was included in all efflux suspensions.
[b] The control rate, the $T_{1/2}$ of the slow component of efflux from control cells, was 41 min. Values are means of 3 to 6 measurements.

measurements, and sorbose exited from the compartment at its usual rate (not shown). It was also observed in repeated experiments that compartment sorbose contents were increased in amount, as seen in efflux curves, both by the inclusion of cycloheximide with glucose during preloading, which slowed initial loss from the cells, and by the removal of glucose before preloading was completed, which insured adequate time for the resumption of usual compartment accessibility. When glucose was included during efflux measurements sorbose was again released rapidly from any cells which had resumed normal accessibility, showing that accessibility can be repeatedly reversed.

IAA prevented the increase in efflux initiated by glucose (Fig. 5*B*); increased accessibility evidently was prevented when glucose metabolism was blocked. IAA without glucose reduced the rate of sorbose loss by only 13% (mean of six measurements). Other metabolic inhibitors affected the glucose response to different degrees (*see* Table 2). Na arsenate was ineffective, though it inhibits a glucose-stimulated increase in the rate of sorbose transport [28]. Cycloheximide did not inhibit the increase in efflux, confirming its lack of effect on uptake and indicating that the compartment membrane is altered without a synthesis of protein.

Uptake, Efflux and Binding of Other Solutes

In control cells, uptake and efflux of arabinose was much like that of sorbose. Added glucose increased total uptake and IAA blocked the glucose effect; cycloheximide alone did not affect uptake and with glucose it reduced

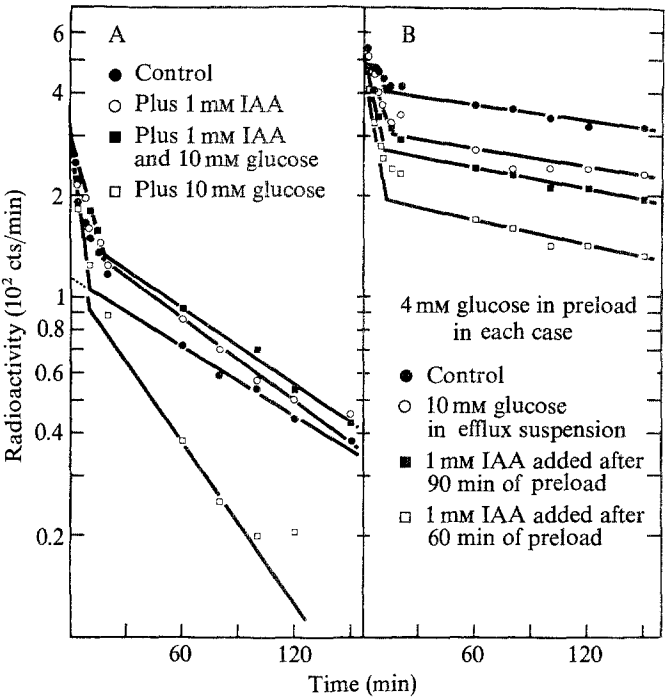

Fig. 6. Efflux of arabinose. (*A*) The effects of glucose, IAA and glucose plus IAA on efflux. (*B*) Efflux from cells preloaded with glucose present and with IAA added at different times during the preloading. Cells were preloaded for 150 min

total uptake only slightly; added glucose speeded efflux from the compartment (i.e., the slow component of efflux; $T_{1/2}$ was reduced from 78 to 42 min (mean of five each)) but did not compete and slow efflux when IAA was also included. However, after 150 min of uptake with 4 mM glucose present, the calculated (78% water space) internal arabinose concentration was 148% of external (mean of eight measurements), whereas that of control cells was only 69% (mean of 14). Moreover, efflux was markedly reduced after cells were preloaded with glucose present; and it was increased only a small amount by adding glucose to efflux suspensions of such cells (Fig. 6*B*). Additions of IAA at various times during preloading to inhibit glucose metabolism (0 min, Fig. 6*A*; 60 and 90 min, Fig. 6*B*) resulted in proportionate reductions in the amount of retained arabinose. Thus, when glucose was available and metabolized, arabinose was bound or metabolized by a reaction unaffected by cycloheximide.

A counterflow of arabinose induced by glucose showed that binding occurred independently of the sugar compartment. Addition of glucose (to a 100 mM concentration) caused more arabinose to counterflow from

cells preloaded with glucose present than from control cells, indicating, as in the sorbose measurements, a more complete exchange between compartment and other intracellular arabinose as the result of an increase in compartment accessibility. Because some was bound, counterflow did not deplete the arabinose content of these cells to a level as low as that of control cells. Recovery of control cells from a counterflow induced by 10 mM glucose was followed by the uptake of additional arabinose so that apparent internal concentrations at 150 min reached 162% of external (mean of 11).

The addition of glucose to uptake suspensions of DMSO and fucose also resulted in apparent internal concentrations markedly higher than external concentrations (167 and 182%, respectively). The extra amounts taken up did not leave the cells during efflux measurements, showing that these compounds, like arabinose, were bound. Fucose filled only 65 to 75% of the water space of control cells, indicating that its entry into the sugar compartment was restricted. Efflux of DMSO from control cells was rapid and complete; that of fucose was similar to sorbose and arabinose, a slow component ($T_{1/2}$, 53 min (mean of 4)) demonstrating that fucose had entered the sugar compartment.

The Nature of the Change in Accessibility
Sugar Competition Measurements

Possible explanations for the change in accessibility appeared to include activation of an existing transport system, induced synthesis of such a system, and an increase in the permeability of the compartment membrane.

When glucose, and enough IAA (1 mM) to block glucose metabolism, was included in efflux suspensions initial sorbose loss was slowed, whereas efflux of the slow component was unaffected (*see* Fig. 5*B*). DOG, at a concentration approximating its K_m of transport (4 mM, *see* Ref. [6]) had a similar effect on sorbose efflux (Fig. 7*A*). Although these sugars are known to compete effectively for external membrane carriers which transport sorbose [6], they evidently do not compete at the compartment membrane, indicating that this membrane lacks a glucose-sorbose transport system.

Glucose and DOG also slowed sorbose efflux when a higher concentration of IAA, 4 mM, was included in efflux suspensions (Fig. 7*B*). At this concentration IAA inactivates or injures sugar carriers – initial sorbose efflux was reduced to a $T_{1/2}$ of 36 min (mean of 14 measurements) – and eliminates the sugar compartment in 40 min or less [23, 25]. Because the compartment is eliminated, the persisting inhibitions of sorbose efflux by glucose and

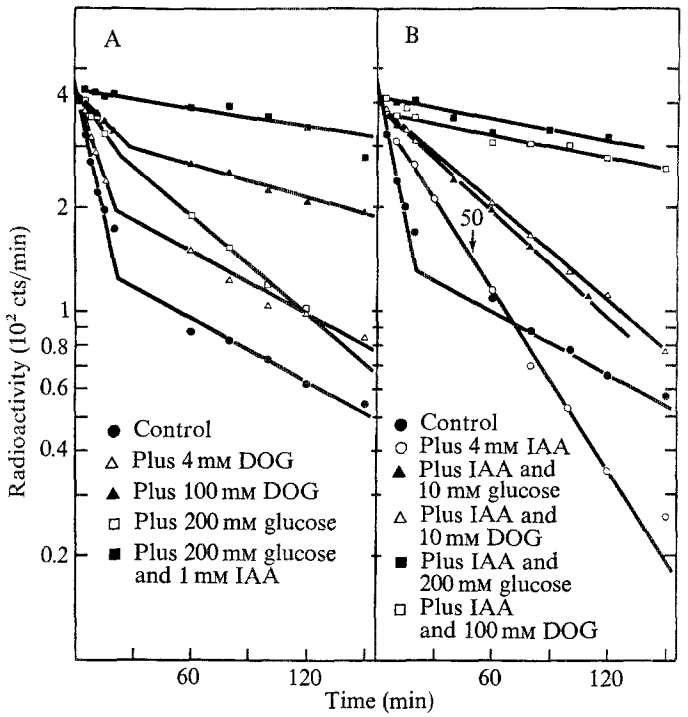

Fig. 7. Effects on sorbose efflux of sugars and sugars plus inhibitors included in efflux suspensions of cells preloaded in 10 mM sorbose solutions. (*A*) DOG and high concentrations of glucose were included at 0 min. (*B*) IAA, 4 mM, and IAA plus glucose and DOG were included at 0 min

DOG verify that the competitive effects of these sugars on sorbose transport occurred at the external cell membrane. Thus, a reduced terminal rate of sorbose efflux (normally the slow component) produced by high concentrations of glucose (plus IAA) and DOG (Fig. 7*A*) actually was due to slowed transport across the external membrane; the high concentrations tied-up external membrane carriers to such an extent that efflux through this membrane was less than that at which sorbose normally leaves the compartment. When glucose metabolism was not blocked by IAA, sorbose efflux was the resultant of a combination of competitive(slowing) and metabolic(speeding) effects (Fig. 7*A*, plus 200 mM glucose).

Normal sorbose efflux after 50 min (i.e., the slow component) is controlled by the compartment membrane and not by the external membrane [25]. Therefore, sugars or inhibitors added at 50 min can show directly their effects on sorbose loss or on the compartment membrane. Glucose and mannose added at 50 min immediately speeded efflux, again to a $T_{1/2}$

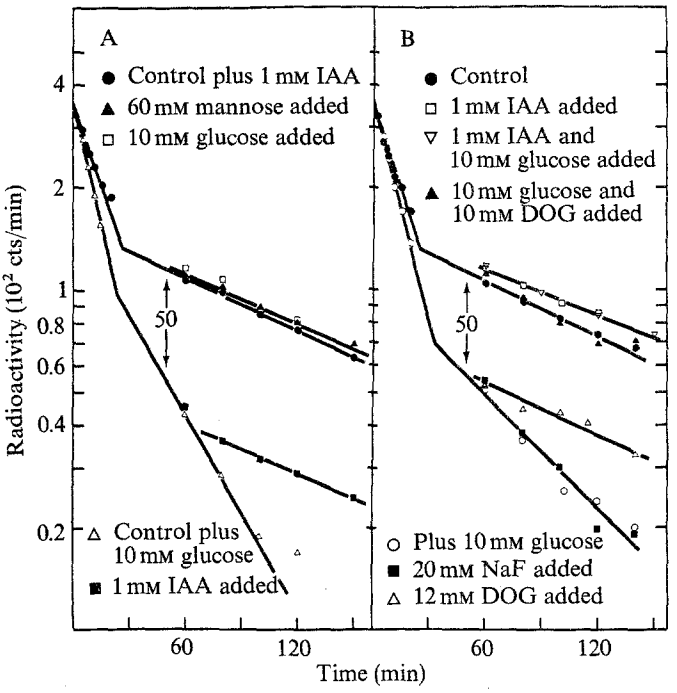

Fig. 8. Effects on sorbose efflux of sugars, IAA, and NaF added to efflux suspensions 50 min after efflux was initiated with cells preloaded in 10 mM sorbose solution. (*A*) Glucose and mannose were added to cells in IAA solution and IAA was added to cells in 10 mM glucose solution. (*B*) IAA, IAA and glucose, and glucose and DOG were added to control cells, and NaF and DOG were added to cells in 10 mM glucose solution

of about 41 min (not shown). When these sugars were added to cells in IAA solution (1 mM) sorbose efflux was slowed only slightly (Fig. 8*A*), similar to the slowing caused by the addition of these sugars with IAA to cells in buffer (Fig. 8*B*; 18% by 5 or 10 mM glucose and IAA (mean of 3 at each concentration), 15% by 60 mM mannose and IAA (mean of 3)), or by the addition of IAA alone (Fig. 8*B*). The K_m concentrations [6] of the added sugars should have half-saturated the sugar carriers, if such carriers exist in the compartment membrane, and approximately halved sorbose efflux. High concentrations of glucose and DOG (as for Fig. 7*A*) added at 50 min reduced efflux by competing at the external membrane as described above.

When glucose (10 mM) was included in cell suspensions at 0 min and IAA was added at 50 min (Fig. 8*A*), efflux was rapid until the IAA was added, after which it slowed to approximately parallel that of control cells (76% of the control (mean of 4)). Evidently the compartment membrane resumed its original permeability when glucose metabolism was blocked by IAA, as it did when glucose was washed from the cells (*see* Fig. 5*B*).

To insure that IAA was not affecting the compartment membrane to conceal competitive sugar interactions, measurements were also made with NaF as the metabolic inhibitor. When 20 mM NaF was included at 0 min and 10 mM glucose was added later, the effect, similar to that with IAA, was minimal (sorbose loss was slowed 16% (mean of 3); not shown). Sorbose efflux remained at 70% (mean of 3) of the control cell rate when 20 mM NaF and 10 mM glucose were added at 50 min, and at 81% (mean of 3) of the control when NaF was added alone at 50 min. However, addition of NaF at 50 min to a cell suspension in which glucose had been included at 0 min did not slow compartment efflux (Fig. 8*B*). This result may indicate simply a less effective metabolic inhibition by NaF than by IAA, or that effects on the membrane are different.

Temperature Effects

The relatively small change in rate which occurred as temperatures were lowered (Fig. 9) provides independent and supporting evidence that passage

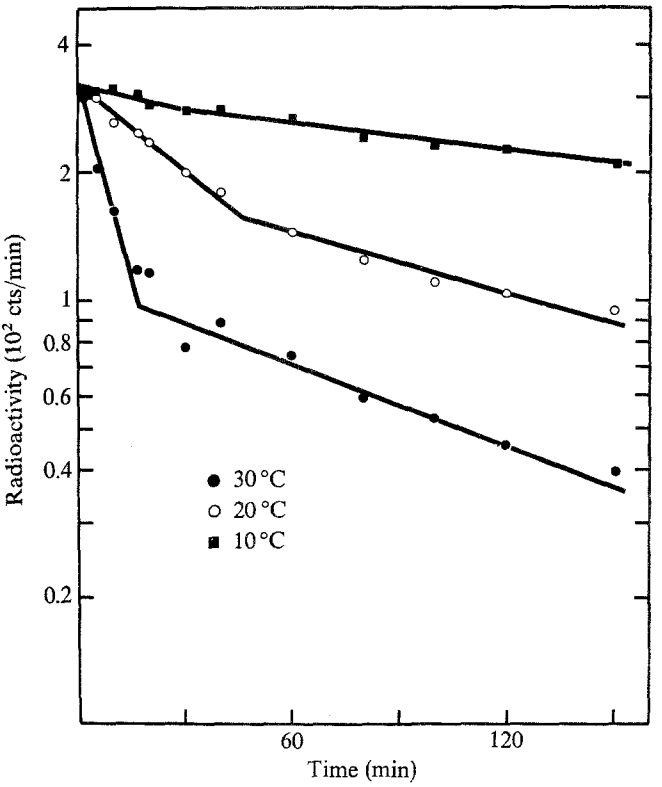

Fig. 9. Efflux at 10, 20, and 30 °C from cells preloaded in 10 mM sorbose solution

across the compartment membrane is not a facilitated process. Facilitated transport through the external membrane was markedly slowed by reduced temperatures ($T_{1/2}$ at 10 °C was about ten times that at 30 °C). In contrast, the change in the rate of compartment loss was severalfold less ($T_{1/2}$ at 10 °C was only about three times that at 30 °C).

Because a facilitated transport system evidently does not preexist in the compartment membrane, it cannot be activated by metabolized sugars to speed sorbose efflux. Whether or not a transport system is newly synthesized and accounts for the increased efflux cannot be tested for by competition measurements with metabolizable sugars because these sugars initiate the increased efflux. However, tests with DOG (not metabolized) showed that this sugar reversed the glucose effect (Fig. 8*B*) so that the rate of sorbose efflux slowed to parallel that of control cells (88% of control (mean of 3)). When DOG and glucose together were added to control cells at 50 min, compartment efflux was unaffected. In both of these tests cells contained concentrations of highly competitive sugars (glucose and DOG) more than adequate to severely inhibit sorbose efflux, at least through a glucose-sorbose facilitated transport system such as that of the external membrane. These results indicate that DOG inhibits the glucose reaction rather than competing with sorbose for a newly induced compartment transport system which evidently is not formed.

Discussion

The increases in total sorbose uptake and in sorbose efflux caused by metabolized sugars are best accounted for by an increase in the permeability of the membrane of an intracellular compartment. The permeability change was reversed when glucose was washed from the cells, indicating that a direct metabolic involvement occurs rather than the synthesis of a compartment membrane component or transport process. The inability of cycloheximide to prevent the glucose effect and the apparent lack of a time requirement for synthesis make this conclusion unavoidable. It is also obvious that the increase in sorbose uptake and the elimination of the slow component of efflux achieved by pretreatment with IAA do not involve a synthesis. Temperature responses and the lack of competitive effects by other sugars demonstrated that the compartment does not possess a sorbose transport system. By eliminating both the existence of a constituent transport system and the possibility of a synthesis of an induced system it is apparent that the changes occurred as the result of an increase in compartment permeability.

Metabolized sugars and other energy sources also stimulate an increase in the rate of sorbose transport across the external cell membrane [28]. This effect on transport and the increase in compartment permeability are confounded in sorbose uptake measurements where both the rate of uptake and total uptake are increased. However, the two are distinguishable in efflux measurements. Thus, when glucose is included during preloading, initial sorbose efflux through the external membrane is speeded whereas efflux from the compartment occurs at a usual rate. The reversibility of the increase in permeability and the absence of an effect on it by cycloheximide also distinguish it from the increase in transport rate, which does not reverse and is inhibited by cycloheximide [28].

These results differ from studies which report that sorbose distributes throughout yeast cells. The measurements which show transport control to exist at the external membrane demonstrate also that the compartment under study does not have direct access to the exterior of the cell as had been considered a possibility for a yeast cell sugar compartment [15]. There is evidence that the cell vacuole functions as such a compartment [23, 25]. Increasing knowledge of this organelle indicates that it has a substantial membrane [2, 13] which could respond in the manner described here. Because the increase in total sorbose uptake after pretreatment with 5 mM IAA might be considered small to represent compartment space, it should be recalled that IAA also inactivates sugar carriers (Fig. 7*B*), and it injures some cells [23]. The increased uptakes after pretreatment with N-ethylmaleimide and with cycloheximide present indicate a larger compartment, more like that observed in efflux measurements. The increase in the amount of sorbose accounted for by the slow component of efflux, observed with increasing preloading times, confirms further that this component characterizes a compartment. And the repeated reversibility in the rate of efflux is consistent both with the existence of a compartment and with the fact that metabolized sugars increase efflux from cells in buffer suspensions even though the cells were grown in medium with a high glucose content (0.22 M). Some of the above observations might be accounted for by a compartment separate from the vacuolar compartment normally noted in efflux measurements [25], but the results as a whole indicate that the occurrence of such a second compartment is unlikely. Similarly, it is unlikely that glucose causes sorbose to enter some cells normally impermeable to it, an explanation which avoids a compartment entirely, because, among other reasons, such an effect would not account for the increased efflux also caused by glucose.

The selective capacity of several common energy inhibitors to prevent the increase in permeability, though possibly due simply to different effects

on glycolysis or phosphorylation, may offer a means to study the mechanism involved. The inhibitor effects are similar to those reported in studies of phosphate loss from *S. carlsbergensis* [30]. Moreover, the general loss of cell materials by shock excretion [20, 21] may be similar in nature to the sorbose loss, although another site for glucose action has been invoked.

Because IAA affects yeast cell membranes [23, 24], NaF affects sorbose efflux, and DOG may affect several yeast cell processes [19], possible complicating effects of these metabolic inhibitors in the competition measurements must be evaluated. Although a small fraction is phosphorylated [32], glucose, with IAA present, exists in the cell largely as the free sugar [5], able, therefore, to compete with sorbose for transport at the compartment membrane. DOG exists in both phosphorylated and free form; at a 10 mM external concentration, enough free sugar should be present inside the cell [31] to compete effectively with sorbose. DOG does not eliminate the slow component of efflux (*see* Fig. 7*A*) as IAA does [25]. Therefore, if IAA affects the compartment membrane to interfere with its normal transport functions, though such an effect does not occur at the external membrane [28], the utilization of DOG as a transport competitor should avoid this complication. The minimal slowing of efflux observed either with IAA, or NaF, and a sugar or with DOG justifies the conclusion that competition does not occur as it does at the external membrane.

Although the results with arabinose and fucose substantiate the conclusions reached with sorbose, it is apparent that the binding of these sugars would have greatly confused interpretation of compartment responses if only they had been used for measurements. The binding reactions have not been specified, but it is also apparent because cycloheximide had no inhibitory effect that any enzymes involved are available in the cells.

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