Mannitol Uptake by *Saccharomyces cerevisiae*

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The uptake of mannitol, a nonmetabolized hexitol, by *Saccharomyces cerevisiae* was measured. Various characteristics examined include: effects of temperature on uptake, inhibition of uptake by uranyl nitrate, competition for uptake by glucose, counterflow of mannitol by glucose, and the affinity of mannitol for a carrier system as measured by a Michaelis constant. That energy is required for uptake was shown by a decreased uptake in the presence of energy inhibitors, by an increased uptake upon addition of energy sources, and by the absence of uptake under anaerobic conditions with no fermentable energy sources available. That mannitol is bound to some cellular constituent after it enters the cell was shown by its attachment to nondialyzable cell fragments and by the lack of an osmotic response, both of which are consistent with a minimal efflux.

Reports in the literature indicate that mannitol, a hexitol, does not enter cells of the yeast *Saccharomyces cerevisiae* (9). This conclusion is based primarily upon the observation that optical density changes are not detected when yeast spheroplasts are suspended in a solution of mannitol. Changes in cell volume can show whether a compound entering a cell is osmotically active, and measurements of the optical density of a spheroplast suspension can show changes in cell volume (9, 12). However, other results indicate that D-mannitol is taken up by *S. cerevisiae* (15, 16).

Although several types of yeasts can utilize mannitol as a carbon and energy source, *Saccharomyces cerevisiae* does not metabolize this compound (17). Transport of various nonmetabolized sugars, e.g., sorbose, has been examined in studying mechanisms of sugar transport (3–5). Complicating metabolic reactions are eliminated with nonmetabolized compounds and thus allow the transport system to be studied independently. For this reason, studies with mannitol could prove useful, particularly because this compound, despite its acyclic structure, evidently is transported by the glucose transport system (W. A. Maxwell and E. Sporl, Bacteriol. Proc., p. 145, 1970). Conceivably, a better understanding of transport mechanisms will be derived from a knowledge of the interrelationship of mannitol and the glucose system.

Various criteria of transport have been examined with mannitol and are described below. The measurements include kinetic and inhibition studies, temperature and energy relationships, and mannitol binding affinities. The significance of such measurements has been described in several instances (3, 18).

**MATERIALS AND METHODS**

**Organism and handling.** *S. cerevisiae* was grown aerobically as previously described (15). Unless otherwise stated, cells were harvested by centrifugation during the stationary phase of growth at a count of 2 × 10⁹/ml and washed twice with distilled water. They were suspended at a concentration of 4 × 10⁷/ml in 0.02 M potassium phosphate buffer for experimental measurements, unless otherwise noted.

**Uptake measurements.** Cells were suspended in buffer (pH 7.0) with appropriate concentrations of D-mannitol-1⁴C (Amersham-Searle) and incubated at 30 C. Samples were collected on membrane filters (Millipore Corp.) as described previously (15). Cells and filter were placed in Bray's scintillator solution (2), and radioactivity was counted in a scintillation spectrometer. Chemicals to be examined for their effects on mannitol uptake were included in the uptake solution at 0 min. Suspensions with uranyl nitrate were adjusted to pH 3.5 with HCl; buffer was not used. To produce anaerobic conditions, nitrogen gas was bubbled through cells suspended in buffer. The gas was then evacuated, and the procedure was repeated. A constant flow of nitrogen through cell suspensions was maintained during anaerobic measurements.

Although commercial ¹⁴C-mannitol is described as chromatographically pure, it was necessary to remove trace amounts of a labeled contaminant. This contaminant may have been mannose which is formed at one stage in the preparation of ¹⁴C-mannitol. Mannose has a high affinity for the yeast carrier, allowing it to enter the cells readily, as did the contaminant. The ¹⁴C-mann-
nitol solution, consisting of labeled source plus unla-
beled reagent grade mannitol in buffer solution, was
purified by incubating it with yeast cells (4 × 10⁷/ml)
for 2 hr and then discarding the cells. Two such treat-
ments were used to insure removal of the contaminant.

Counterflow measurements. Cells were loaded with
mannitol by aerobic incubation for 120 min at 30 C in
buffer solutions of 0.1 M ¹⁴C-mannitol. At 0 min, 0.4 ml
of buffer-glucose solution was added to 6.6 ml of the
cell-mannitol suspension, and samples were collected at
appropriate intervals.

Sonic treatment and dialysis of cell material. Five-
milliliter portions of a suspension of cells in buffer were
sonically treated at 0 C for 1 hr in a Bronwill Biosonic
model 020 sonic oscillator. One-milliliter samples of the
sonically treated material were placed in sacks made
from ¹/₄-inch (∼0.64 cm) cellophane dialysis tubing and
suspended in 1 liter of distilled water at 7 C. The water
was stirred continuously and changed three times
during a 48-hr period. After dialysis, the sample was
added to 10 ml of Bray’s solution for radioactivity
counts. Cells loaded with mannitol had been incubated
in 0.1 M ¹⁴C-mannitol in buffer for 90 min at 30 C be-
fore sonic oscillation.

RESULTS

Uptake; temperature effects. Figure 1 shows
¹⁴C-mannitol uptake by yeast at several different
temperatures. As the temperature of incubation
decreased, the rate of uptake also decreased. The
Qₐ for the temperature response was calculated to be
greater than 3.0. Mannitol uptake will con-
tinue beyond 150 min. Internal concentrations
which exceed external concentrations by three- to
tfourfold were obtained with added energy sources
and with lower external concentrations of man-
nitol.

Inhibition by uranyl nitrate. Uranyl nitrate, a
compound commonly used to block hexose trans-
port in yeast (6), inhibited mannitol uptake. Con-
centrations as low as 2.5 × 10⁻⁵ M reduced up-
take by 85%. Higher concentrations inhibited
uptake to an even greater extent. Because uranyl
nitrate apparently interacts with cell membranes
by selectively affecting transport systems (7, 13),
these results indicate that mannitol uptake in-
volves a transport system.

Affinity of mannitol for a carrier system. Figure
2 is a Lineweaver-Burk plot of the initial velocity
of mannitol uptake. The process conforms to
Michaelis-Menten kinetics, indicating that man-
nitol enters the cells via a transport system rather
than by simple diffusion. A Kₘ value of 0.6 M is
obtained.

Mannitol uptake and glucose transport. Figure
2 includes plots of mannitol uptake in the pres-
ence of glucose; interactions between these com-
ounds could indicate a common transport sys-
tem, an energy requirement, or other relation-
ships. At a 0.01 M concentration, glucose inhib-
ited mannitol uptake; the increased Kₘ and the
unchanged Vₘₐₓ indicate a competitive inhibition.
At a 0.001 M concentration, glucose increased
mannitol uptake; the Kₘ remained essentially
unchanged but the Vₘₐₓ increased. Glucose at
concentrations as low as 0.005 M inhibited the
uptake of 0.1 M mannitol, whereas lower concen-
trations stimulated uptake (Fig. 3).

![Figure 1](http://example.com/fig1.jpg)

**FIG. 1.** Uptake of 0.1 M ¹⁴C-mannitol by yeast cells incubated at different temperatures.

![Figure 2](http://example.com/fig2.jpg)

**FIG. 2.** A Lineweaver-Burk plot of ¹⁴C-mannitol uptake. Rates were measured for the initial 10 min of uptake.
Mannitol uptake by S. cerevisiae

Counterflow, in which transport of one compound into a cell causes another compound utilizing the same carrier to leave the cell against a concentration gradient, provides a well known test for a common carrier. Mannitol counterflow occurred at glucose concentrations as low as 0.003 M (Fig. 4). The concentration of mannitol in the cell solution, based upon the total content of the cells of Fig. 4 and using a value of 65% as the sugar space (4, 16), is slightly above the 0.094 M external concentration. However, because some of the mannitol is bound the concentration actually is lower; a high retention even when counterflow was obtained with 0.1 or 0.4 M glucose substantiates other evidence for binding.

Energy involvement. From the data of the previous section, it appears that glucose at different concentrations can inhibit and stimulate mannitol uptake. Stimulation indicates that metabolic energy may be required for uptake.

Table 1 lists data showing that compounds which inhibit energy metabolism decrease mannitol uptake. Iodoacetic acid, sodium azide, and dinitrophenol are well known inhibitors of energy production in cells.

Fig. 3. Uptake of 0.1 M ^14C-mannitol by yeast cells incubated in the presence of different concentrations of glucose.

Fig. 4. Counterflow of mannitol from yeast suspended in ^14C-mannitol solution. Glucose was added at 0 min.
Mannitol uptake per cell also decreased when suspensions were employed which contained a larger number of cells than usual (4 x 10^9/ml). Addition of energy sources and oxygen to these suspensions (2 x 10^9 cells/ml) increased uptake (Fig. 5). Thus, at high cell concentrations an oxygen lack limited energy production and accounted for a decreased uptake. Addition of 0.1 M glucose produced an inhibition of uptake which was followed by an increased uptake. This result may be explained by assuming that after glucose was metabolized to a level at which competitive inhibition of mannitol transport no longer occurred, energy from the metabolism of glucose stimulated mannitol uptake.

Under anaerobic conditions, when endogenous reserves are largely unavailable to yeast (14), mannitol uptake was greatly depressed (Fig. 6). Addition of glucose, a fermentable energy source, produced uptake equivalent to that of aerobically incubated cells. On the other hand, ethanol, which is metabolized through the terminal respiratory system, was ineffective in producing uptake anaerobically.

The preceding results were obtained with mature cells harvested in the stationary phase of growth. Additional experiments were carried out with exponential phase cells, which have lower levels of energy reserves. Mannitol uptake by these cells was much less. Addition of energy sources, 0.001 M glucose or 0.1 M or 0.05 M ethanol, increased uptake three to four times.

Mannitol binding. Many nonmetabolized sugars exit readily from the yeast cell (15, 16). However, mannitol efflux is minimal, with as much as 68% of the cell content being retained (16; W. A. Maxwell and E. Spoerl, Bacteriol. Proc. p. 145, 1970). Such results indicate a highly differential membrane permeability, a compartmentation, or that mannitol is bound.

That binding occurred was shown with cells which were sonically oscillated and dialyzed after taking up 14C-mannitol. The data of Table 2 show that mannitol readily passed through the dialyzing membrane. However, up to 30% of the mannitol of whole cells was not removed from cell fragments by dialysis. The 100% value assigned in Table 2 to whole cells dialyzed after mannitol uptake is 60% of the cell content as measured in usual uptake experiments. Similar experiments with cells which had taken up sorbose showed that no sorbose was retained by the cell fragments. Binding appears to require cell

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**Table 1. Effect of different inhibitors of energy production on the uptake of 14C-mannitol**

<table>
<thead>
<tr>
<th>Inhibitor</th>
<th>Conc (mM)</th>
<th>Inhibition of uptake at 150 min (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Iodoacetic acid</td>
<td>0.1</td>
<td>50</td>
</tr>
<tr>
<td></td>
<td>1</td>
<td>100</td>
</tr>
<tr>
<td></td>
<td>10</td>
<td>100</td>
</tr>
<tr>
<td>Dinitrophenol</td>
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<td>75</td>
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<tr>
<td></td>
<td>5</td>
<td>100</td>
</tr>
<tr>
<td></td>
<td>10</td>
<td>82</td>
</tr>
<tr>
<td>Sodium azide</td>
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<td>45</td>
</tr>
<tr>
<td></td>
<td>4</td>
<td>69</td>
</tr>
<tr>
<td></td>
<td>10</td>
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</tbody>
</table>

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**Fig. 5.** Uptake of 0.1 M 14C-mannitol by yeast cells at a high concentration (10^9 cells/ml). Effect of energy sources and oxygen is shown.

**Fig. 6.** Uptake of 0.1 M 14C-mannitol by yeast cells. Effects of aerobicism, anaerobiosis, and several energy sources are shown.
integrity; when mannitol was added to disrupted cells and the mixture was incubated for 90 min before dialysis, only 6% of the mannitol remained with the cell fragments after dialysis.

Measurements of viability and of the loss of 260-nm absorbing materials after sonic oscillation indicate that any mannitol free within the cytoplasm should have been lost from the cells. After 1 hr of sonic treatment, 98% of the cells were nonviable. Because death from sonic oscillation results primarily from cell disruption (10), a free flow of cytoplasmic solutes should have been possible. Also, more than 95% of the total amount of ultraviolet absorbing material which could be released from the cell was released by the sonic oscillation.

Osmotic responses provide additional evidence that mannitol is bound within the cell. When the osmolality of the medium was increased by adding glycine (to 0.5 M), sorbose was lost from suspended cells (Fig. 7). In such an experiment, it is assumed that a loss of cell water increases the internal concentration of sorbose relative to the external concentration, resulting in an efflux of sorbose from the cell. However, the cellular content of mannitol was not affected by a similar increase in external osmolality. Such a result would occur if mannitol was bound internally and was not osmotically active. Similar results were obtained when NaCl was used to increase osmotic pressure.

**DISCUSSION**

The experiments described show that mannitol is taken up by *S. cerevisiae*. Whether transport into the cell is active in a usual sense is not entirely clear. Energy is required and accumulation above external concentrations occurs. However, because some mannitol is bound, internal solution concentrations may not exceed external so that accumulation against a concentration gradient may not occur, and this criterion of active transport would not be met. Energy input, with binding an important factor, also could be at a site other than the transport system. The high $Q_{10}$ for uptake confirms the involvement of an enzymatic-like process, but does not distinguish between an energy producing system and a transport system. The minimal uptake at 0 C shows that uptake is not merely nonspecific surface binding.

Inhibition by low concentrations of uranyl nitrate (13) and conformity with Michaelis-Menten kinetics are evidence that a transport system is involved in mannitol uptake. The relatively high apparent $K_m$ (0.6 M), i.e., the low affinity for a carrier system, causes some difficulties in characterizing the system by measurements of competitive inhibition. The high concentrations which must be used of both substrate and possible inhibitors such as sorbitol can have osmotic or other effects on the cell. However, glucose and mannose with low $K_m$ values competed strongly. Counterflow of mannitol by glucose provided additional evidence that a common carrier is utilized. Because specific structural requirements have been shown to exist for effective transport by the glucose carrier (5, 9, 11), a difficulty remains in understanding why mannitol, being acyclic, should be carried.

The experimental data suggest that energy is required for mannitol uptake, but do not define the locus of involvement. Energy may be required.
by some facet of the transport system, but it could also be required directly for binding mannitol, or even for altering the mannitol molecule. One could speculate that mannitol is converted to an isomeric form closely related to glucose which then competes in the glucose transport system. Although labeled material extracted from cells after uptake of 14C-mannitol has the same Rf in radiochromatograms as labeled mannitol (16), it is possible that this assay is inadequate because several related sugars and polyols have similar chromatographic characteristics (1).

That the optical density of a suspension of spheroplasts in a solution of mannitol does not change (9) is consistent with mannitol being bound by yeast cells. No change in volume would occur if mannitol did not enter the cells, but on the other hand no change would occur if mannitol was bound and, therefore, did not exert an osmotic effect. Moreover, binding is not inconsistent with a tendency by mannitol to form hydrogen bonds with various types of proteins (8). The nature and location of the binding, as well as changes which may occur in mannitol itself, are important matters for further study in this regard. Uptake and binding of mannitol suggest that consideration be given to possible internal effects of this compound when spheroplasts are suspended in solutions of it for biochemical or physiological measurements. Reduced respiratory capabilities of yeast incubated in mannitol solutions have been observed, presumably due to such effects of mannitol (17).

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LITERATURE CITED