A Derepressible Active Transport System for Glucose in Neurospora crassa*

M. M. Neville, S. R. Suckind, and Saul Roseman

SUMMARY

Two glucose uptake systems have been detected in ungerminated conidia of wild type Neurospora crassa, strain 74A; the high affinity system has been characterized. Uptake and substrate competition experiments showed that the high affinity system was relatively specific for D-glucose but also transported the nonmetabolizable analogues 6-deoxy-D-glucose, 2-deoxy-D-glucose, and 3-O-methyl-D-glucose. The system exhibited a \( K_m \) of 0.07 mm and a \( V_{\text{max}} \) of 4 mmoles per mg, dry weight, per min for 3-O-methyl-D-glucose uptake. The nonmetabolizable analogues were accumulated against concentration gradients; chromatographic analysis showed that the material accumulated in each case was the free sugar. Uptake by the high affinity system was inhibited by azide and dinitrophenol. These results were consistent with an active transport mechanism for uptake. Two alternative mechanisms, group translocation and coupled transport, were considered unlikely because no transitory substrate derivative could be detected during uptake and because influx of 3-O-methyl-D-glucose was not coupled to efflux of any organic metabolite. The high affinity system was repressed when the conidia were germinated on glucose medium but was restored when the glucose was either depleted or removed from the medium. This restoration was inhibited by cycloheximide.

Two additional kinetic features were of importance in interpreting 3-O-methyl-D-glucose transport. First, previously accumulated substrate stimulated the subsequent influx of 3-O-methyl-D-glucose. The stimulation was proportional to the internal substrate concentration and was inhibited by azide. Second, efflux appeared to be a carrier-mediated process, but, in contrast to the high affinity uptake system, it was not inhibited by azide or by sulfhydryl reagents.

The uptake of sugars into various organisms has been investigated for many years, yet only recently has significant progress been made in understanding the molecular mechanism involved. Recent studies on sugar transport in bacterial systems have implicated a phosphotransferase system in the translocation of many hexoses across bacterial membranes (1). Since then, intensive genetic and biochemical analysis of bacterial sugar transport systems has resulted in some understanding of both the membrane proteins and the energy-coupling mechanisms involved.

By comparing the transport systems in bacteria with those of other organisms, mechanistic features common to these systems might be revealed; on the other hand, differences in the mechanisms might also illuminate the transport processes operative in each. To this end, Neurospora crassa is a valuable experimental organism for several reasons: large populations can be handled easily; procedures for mutant selection and genetic characterization are available; and both cell membrane and cell wall mutants have been described (2-5). Further, the energy conversion processes in N. crassa, unlike bacteria, are compartmentalized by typical eukaryotic mitochondria.

There have been few reports on the sugar transport systems in filamentous fungi. In N. crassa, the lactose (6) and L-sorbose (7-9) transport systems have been studied, and recently a system for glucose was described (10). The present report describes an active transport system in ungerminated conidia of N. crassa which was specific for glucose and its analogues. As shown by kinetic analysis and by repression-induction characterizations, this system was distinct from the previously reported glucose transport system (10).

**EXPERIMENTAL PROCEDURE**

Conidial Strains and Preparations

Ungerminated conidia of N. crassa wild type strain 74A from the Fungal Genetic Stock Center, Dartmouth (FGSC No. 262), were used for most of the work described. Other strains examined were wild type strains 5297a (FGSC No. 322) and SY7A (FGSC No. 622), and a tryptophan synthetase mutant td-la (FGSC No. 73).

Wild type conidia were obtained from cultures grown on Vogel's solid medium (11) for 5 to 10 days at 30° with continuous fluorescent illumination. In the case of strain td-la, D-trryptophan was added to a concentration of 0.78 mm. Conidial suspensions were prepared by adding distilled water to the growth

* This work was supported by Grant GM-15583 and Grant AM-09851 from the National Institutes of Health, and Grant P-544 from the American Cancer Society. One of us (M. N.) was supported by Grant GM-57; this report partially fulfills the requirements for the degree of Doctor of Philosophy. It is Contribution 101 from the McCollum-Pratt Institute.

**All monosaccharides are of the D conformation unless designated otherwise.**
flasks, shaking them vigorously, and filtering the resulting suspensions through several layers of gauze to remove hyphal fragments. The conidia, collected by filtration of the suspension through Millipore HA filters, were washed with distilled water and resuspended in the assay buffer. Lester and Hechter obtained a value of 2.5 ml internal volume per mg, dry weight (12); that value was used in the present studies. The weight of 107 ungerminated conidia of strain 74A was 0.38 mg.

When germination of the conidia was required, the following procedure was used. Ungerminated conidia were inoculated into 1-liter Erlenmeyer flasks, each containing 100 ml of the salt solution from Vogel’s medium (11), with 2.8 mM glucose instead of sucrose. The conidial concentration was adjusted to approximately 106 conidia per ml. The flasks were incubated overnight at 30° in a reciprocating shaker water bath, set at 90 rpm with a stroke length of 1 inch. Under these conditions, approximately 80% of the conidia germinated. Most of these grew to several hundred microns in length, there was little branching or clumping, and the conidia were derepressed for the uptake system under study. Before use, the germinated conidia were filtered, washed, and resuspended in the assay buffer.

Radioactive Isotopes
Radioactive sugars were obtained from the following sources: 3-O-14C-methyl-D-glucose (at least 95% pure radiochemically) and 2-deoxy-1-14C-glucose from New England Nuclear, and uniformly labeled 14C-glucose from the Cal.Atomic Division of Calbiochem. The 6-deoxy-6-3H-glucose was prepared by Dr. Milton Saier by reported procedures (13, 14). The 14C or 3H content of the conidia or the medium (or both) was monitored by a Nuclear-Chicago scintillation spectrometer. Three scintillation fluids were used: (a) toluene with 6.0 g of 2,5-diphenyloxazole (PPO) and 0.1 g of 1,4-bis[2-(5-phenyloxazolyl)]benzene (POPOP) per liter; (b) Triton X-100 and toluene (1:2, v/v) with 5.5 g of PPO and 0.125 g of dimethyl-POPOP per liter; and (c) dioxane with 100 g of naphthalene, 10 g of PPO, and 0.25 g of POPOP per liter.

Standard Uptake Assay
Unless otherwise stated, uptake of 3-O-methylglucose was measured by the following procedure. Ungerminated, washed conidia of strain 74A were suspended in 10 mM citrate phosphate buffer, pH 5.5, to a concentration between 107 and 2.5 × 107 conidia per ml, as determined by hemocytometer count, and stored in an ice bath. Before assay, each aliquot was equilibrated to 30° by a 10-min incubation in a reciprocal shaker water bath, shaking at 90 rpm. The 3-O-14C-methylglucose was then added to a final concentration of 1 mM; uptake was investigated. With citrate phosphate and phosphate buffers, the pH optimum for initial rate of 3-O-methylglucose uptake was 5.0 to 5.5, although dependence on pH was very slight between pH 4 and pH 7. At pH 5.5, initial rates of uptake were identical in 10 mM citrate phosphate, 25 mM Sorenson's phosphate, and 25 mM phthalate buffers, but assays done in 25 mM esculine, 50 mM acetate, and 25 mM cacodylate buffers (15, 16) yielded reduced rates. The rate in distilled water was approximately half that in 10 mM citrate phosphate buffer. A number of salts—MgCl₂, MnCl₂, NaCl, KCl, CaCl₂, Na₂HPO₄, and NaNO₃—were tested on suspensions of conidia in distilled water. At concentrations of 0.1 and 1 mM, none of these salts affected the initial rate of 3-O-methylglucose uptake. The initial rate of uptake was proportional to conidial concentration between 0.5 and 2.5 × 10⁶ conidia per ml. Several strains of N. crassa (5297a, SY7A, td-la) were surveyed; initial rates of 3-O-methylglucose uptake were similar to those obtained in 74A. Although the temperature optimum for initial rate of uptake was between 37° and 50°, assays were routinely conducted at 30°, the optimal growth temperature. Uptake was completely inhibited at 0°. When conidia were incubated in the citrate phosphate buffer at 30° for varying times (0 to 5 hours) before assay, the conidia exhibited an increase in the initial rate of uptake during the first 3 hours of prior incubation, reaching rates of 2 to 3 times the original rate, and then decreased again.
Fig. 2. Initial rate of 3-O-methylglucose uptake as a function of substrate concentration. Initial rates of uptake, measured as described under “Experimental Procedure” but with various 3-O-methylglucose concentrations, are expressed as millimicromoles of 3-O-methylglucose accumulated per mg, dry weight, per min. External substrate concentrations are expressed as millimolar 3-O-methylglucose.

**Table I**

Inhibition of 3-O-methylglucose uptake by various sugars

<table>
<thead>
<tr>
<th>Sugar</th>
<th>Concentrations (mM)</th>
<th>Initial rate of uptake (nmoles/mg, dry wt/min)</th>
</tr>
</thead>
<tbody>
<tr>
<td>None</td>
<td></td>
<td>2.36</td>
</tr>
<tr>
<td>Glucose</td>
<td>0.05</td>
<td>1.37</td>
</tr>
<tr>
<td></td>
<td>0.1</td>
<td>0.95</td>
</tr>
<tr>
<td></td>
<td>0.5</td>
<td>0.31</td>
</tr>
<tr>
<td>6-Deoxyglucose</td>
<td>0.05</td>
<td>1.52</td>
</tr>
<tr>
<td></td>
<td>0.1</td>
<td>0.67</td>
</tr>
<tr>
<td></td>
<td>0.5</td>
<td>0.51</td>
</tr>
<tr>
<td>2-Deoxyglucose</td>
<td>0.05</td>
<td>1.69</td>
</tr>
<tr>
<td></td>
<td>0.1</td>
<td>1.36</td>
</tr>
<tr>
<td></td>
<td>0.5</td>
<td>0.47</td>
</tr>
<tr>
<td>Xylose</td>
<td>0.5</td>
<td>1.90</td>
</tr>
<tr>
<td></td>
<td>1.0</td>
<td>1.61</td>
</tr>
<tr>
<td></td>
<td>5.0</td>
<td>0.63</td>
</tr>
<tr>
<td>Mannose</td>
<td>0.5</td>
<td>1.71</td>
</tr>
<tr>
<td></td>
<td>1.0</td>
<td>1.15</td>
</tr>
<tr>
<td></td>
<td>5.0</td>
<td>0.35</td>
</tr>
</tbody>
</table>

Conidia incubated at 0° did not show this change in initial rate of uptake.

Sugar Characterization

In order to characterize the sugar accumulated, 1-ml aliquots of the uptake incubation mixture were filtered through Millipore HA filters and washed with distilled water; the conidia were extracted for 10 min in 4 ml of 70% ethanol at 95°. Extracts were cooled and filtered through Millipore HA filters before analysis. The residual material on the filter was essentially free of radioactivity. Each ethanol extract was lyophilized and then resuspended in 1 drop of distilled water. The samples were characterized as described below.

Electrophoresis—The entire sample was applied to Whatman No. 3MM paper, 46 × 57 cm, which had been saturated with the electrophoresis buffer. Electrophoresis was conducted for 45 min in 50 mM pyridinium acetate buffer, pH 6.5, at 35 volts per cm. The sheets were dried and the sample strip was cut into 1-inch segments, which were counted in the toluene scintillation fluid.

Chromatography—The entire sample was applied to Whatman No. 1 paper and chromatographed overnight in a descending system, with one of the following solvent systems: 1-butanol-pyridine-water (10:3:3, v/v/v), and ethyl acetate-acetic acid-formic acid-water (18:3:1:4, v/v/v/v). The sheets were dried, cut, and counted as described in the electrophoretic method.

**RESULTS**

Kinetic Characterization—The initial rate of 3-O-methylglucose uptake in ungerminated conidia was studied as a function of external substrate concentration. The biphasic curve shown in Fig. 2 suggested the presence of two uptake systems for 3-O-methylglucose. The high affinity system exhibited a \( K_m \) of 0.07 ± 0.02 mM and a \( V_{max} \) of 4 ± 1 nmole of 3-O-methylglucose accumulated per mg, dry weight, per min. The low affinity uptake system exhibited a \( K_m \) of approximately 10 mM.

To study the sugar specificity of the high affinity uptake system, a number of substrate competition experiments were performed (Table I). Glucose, 2-deoxyglucose, and 6-deoxyglucose were potent inhibitors of 3-O-methylglucose uptake; mannose and xylose inhibited at high concentrations; the other sugars inhibited only slightly if at all. Glucose was a com-
Mechanism of Uptake for High Affinity System—All observations were consistent with the hypothesis that 3-O-methylglucose uptake occurred by a process of active transport. At the point of maximum net accumulation, the internal concentration of radioactive material was 90 times the external 3-O-methylglucose concentration (Fig. 3). The bulk of the accumulated material was indistinguishable from 3-O-methylglucose by electrophoretic analysis of ethanol extracts or by chromatography in two solvent systems, performed as described under “Experimental Procedure.” Similar results were obtained with 6-deoxyglucose as the substrate; the internal concentration reached 82 times the external, and, after 9 hours of incubation, at least 98% of the accumulated material cochromatographed with authentic 6-deoxyglucose.

That energy was required for 3-O-methylglucose uptake was indicated by the effects of metabolic inhibitors. The initial rate of uptake was inhibited by 0.1 mM azide or 0.1 mM dinitrophenol; other compounds were much less effective (Table II). Retention of 3-O-methylglucose against a concentration gradient was also inhibited by 0.1 mM azide. When the azide was added 30 min after a standard 3-O-methylglucose uptake assay had been initiated, the conidia rapidly lost the accumulated sugar until the internal and external concentrations were approximately equal.

One alternative to active transport as the mechanism of 3-O-methylglucose uptake would be group translocation coupled with a second reaction to convert the product back to 3-O-methylglucose, i.e., the formation of a transitory intermediate during the transport process. In order to test this, the incubation mixture was examined for possible intermediates at 1 and 2 min after the initiation of uptake of 14C-labeled 3-O-methylglucose. To look for derivatives, aliquots were pipetted directly into 95% ethanol (final concentration, 70%) and extracted for 10 min; the ethanol extracts were analyzed by electrophoresis, as described under “Experimental Procedure.” No 14C-labeled compound other than 3-O-methylglucose was detected at these times.

To determine the corresponding intracellular 3-O-methylglucose concentrations, an uptake assay was performed simultaneously from the same incubation mixture. Accordingly, less than 5% of the intracellular radioactive sugar at 1 or 2 min was negatively charged. Conidia did form a negatively charged material was isolated and treated with alkaline phosphatase. Further identification of the compounds was made possible by the electrophoretic mobility of glucose-6-P. When this was negatively charged, conidia did form a negatively charged compound other than 3-O-methylglucose. When this was negatively charged, conidia did form a negatively charged compound other than 3-O-methylglucose. When this was negatively charged, conidia did form a negatively charged compound other than 3-O-methylglucose.
Evidence that influx of 3-O-methylglucose does not require coordinate efflux

Conidia were germinated for 9 hours as described under "Experimental Procedure," except that citrate was deleted from the medium and uniformly 14C-labeled d-glucose (final concentration, 1.6 mM, 6 x 10^6 cpm per pmole) was added. The germinated conidia were filtered, washed, resuspended to a concentration of 0.34 mg, dry weight, per ml in 10 mM citrate phosphate buffer, pH 5.5, at 30°C, and divided into three batches, with (a) no additions, (b) 1 mM nonradioactive 3-O-methylglucose, and (c) 1 mM 14C-labeled 3-O-methylglucose (6 x 10^6 cpm per pmole). For efflux measurements, 1-ml aliquots were filtered from Batches a and b at the times shown; the total filtrates were mixed with the dioxane scintillation fluid ("Experimental Procedure") for counting. For influx measurements, 1-ml aliquots were filtered from Batch c at the times shown; the conidia were washed and extracted in 70% ethanol; and extracts were chromatographed in the 1-butanol-pyridine-water system (see "Experimental Procedure" for details). To determine the level of radioactivity in the 3-O-methylglucose region of the chromatograph due to the fact that the conidia were grown on 14C-labeled glucose, extracts prepared from Batches a and b were also chromatographed; radioactivity equivalent to 10 mpmoles of 3-O-methylglucose has been subtracted from the influx measurements to correct for this cell extract background. The values given for efflux and influx are averages of duplicates, which were generally within 5% of each other.

<table>
<thead>
<tr>
<th>Incubation time</th>
<th>Efflux</th>
<th>Sugar-dependent efflux</th>
<th>Influx</th>
</tr>
</thead>
<tbody>
<tr>
<td>Batch a (no additions)</td>
<td>Batch b, nonradioactive 3-O-methylglucose added to 1 mM</td>
<td>(Batch b - a)</td>
<td></td>
</tr>
<tr>
<td>hrs</td>
<td>mpmoles</td>
<td>mpmoles</td>
<td>mpmoles</td>
</tr>
<tr>
<td>1/2</td>
<td>22.8</td>
<td>-</td>
<td>25.1</td>
</tr>
<tr>
<td>1</td>
<td>27.8</td>
<td>-</td>
<td>71.3</td>
</tr>
<tr>
<td>1 1/2</td>
<td>30.7</td>
<td>-2.6</td>
<td>95.5</td>
</tr>
<tr>
<td>2</td>
<td>39.0</td>
<td>1.0</td>
<td>102.2</td>
</tr>
</tbody>
</table>

The rate of 3-O-methylglucose phosphorylation (Fig. 3). A second alternative to active transport for the mechanism of 3-O-methylglucose uptake would be 3-O-methylglucose flux as a coupled transport system.2 The possibility of cotransport was rejected because a suspension of conidia in distilled water could accumulate 3-O-methylglucose. To test one possibility of countertransport, that of 3-O-methylglucose influx coupled to the efflux of some organic metabolite, the experiment shown in Table IV was performed. Conidia were germinated in uniformly labeled 14C-glucose as the sole source of carbon under conditions which allowed the uptake system to be expressed. Aliquots of this preparation were used to study (a) influx in the absence of external sugar, (b) efflux in the presence of external 3-O-methylglucose, and (c) influx of 3-O-methylglucose. If 3-O-methylglucose uptake were due to countertransport, the sugar-dependent efflux, b minus a, should be stoichiometric with...
the 3-0-methylglucose influx (c). The data in Table IV show that the sugar-dependent efflux of 14C-labeled metabolites was less than 1% of the 3-0-methylglucose influx.

Trans-stimulation—When conidia were loaded either with nonradioactive 3-O-methylglucose or with 2-deoxyglucose, the subsequent initial influx of 3-0-14C-methylglucose was increased as much as 10-fold. This phenomenon has been called trans-stimulation (19); the flux of sugar from outside the cell (cis) to inside (trans) sugar. Influx, not the rate of net accumulation, showed trans-stimulation; influx and efflux increased simultaneously (Fig. 4). Trans-stimulation of influx was approximately proportional to the concentration of the intracellular sugar, and was not a function of loading time (Fig. 5). Table V shows that the trans-stimulation effect was inhibited by azide, but that it was somewhat less sensitive than initial influx into conidia that had not been previously loaded.

Efflux—If conidia were loaded with 3-0-methylglucose, washed, and resuspended in buffer, they rapidly lost the accumulated sugar (Fig. 6); the rate of efflux varied with internal 3-0-methylglucose concentration (Fig. 4). Efflux was not simply leakage by passive diffusion; it appeared to be mediated by a carrier because it was temperature-dependent and was completely inhibited at 0°, and because it was stimulated rather than inhibited by the presence of 1 mm extracellular 3-O-methylglucose (Fig. 6). Unlike influx, efflux was not inhibited by azide (Fig. 6).

Effect of Sulfhydryl Reagents—Sulfhydryl inhibitors inactivated the high affinity active transport system but did not inhibit the...
The effect of cycloheximide on restoration of $\text{S-O-methylglucose}$ uptake

Glucose concentration was $1 \text{ mM}$. Incubation as the initial rate at 20 min. External $\text{a-o-methylglucose}$ against a gradient (O—O) was measured from the same standard uptake assay. Ability to concentrate $3\text{-O-methylglucose}$ against a gradient (O—O) was measured from the same incubation as the initial rate at 20 min. External $3\text{-O-methylglucose}$ concentration was 1 mm.

**Table VI**

<table>
<thead>
<tr>
<th>Time of incubation</th>
<th>Initial uptake</th>
<th>Net accumulation after 20 min</th>
</tr>
</thead>
<tbody>
<tr>
<td>Before cycloheximide addition</td>
<td>After cycloheximide addition</td>
<td></td>
</tr>
<tr>
<td>0.5</td>
<td>0</td>
<td>0.9</td>
</tr>
<tr>
<td>3.5</td>
<td>0</td>
<td>5.2</td>
</tr>
<tr>
<td>4.5</td>
<td>0</td>
<td>3.7</td>
</tr>
<tr>
<td>5.5</td>
<td>0</td>
<td>5.4</td>
</tr>
<tr>
<td>0</td>
<td>0.5</td>
<td>0.5</td>
</tr>
<tr>
<td>0</td>
<td>3.5</td>
<td>0.5</td>
</tr>
<tr>
<td>0</td>
<td>5.5</td>
<td>5.5</td>
</tr>
<tr>
<td>4.5</td>
<td>0.08</td>
<td>4.5</td>
</tr>
<tr>
<td>4.5</td>
<td>1.0</td>
<td>5.2</td>
</tr>
</tbody>
</table>

efflux system. To test the high affinity uptake system, ungerminated conidia were incubated for various times with either 1 mm N-ethylmaleimide or 0.1 mm HgCl$_2$, washed free of excess inhibitor, and resuspended in citrate phosphate buffer. Subsequent initial influx measurements revealed that inactivation of the high affinity influx system was rapid in both cases and had reached its maximum of 75 and 90%, respectively, after 5 min of exposure. The presence of 10 mm 3-O-methylglucose during the incubation with the sulfhydryl reagents did not protect the influx system from inactivation by either inhibitor. To test the efflux system, ungerminated conidia were loaded with 3-O-$^{14}\text{C}$-methylglucose, washed, and resuspended in fresh buffer plus 1 mm N-ethylmaleimide or 0.1 mm HgCl$_2$. Efflux (measured by loss of $^{14}\text{C}$ from the conidia) was not inhibited in either case.

Glucose Repression—Both the initial influx via the high affinity uptake system and the ability to accumulate 3-O-methylglucose against a concentration gradient were repressed by glucose. Fig. 7 shows that both the initial rate of uptake and the accumulation were greatly diminished during germination in medium containing 2.8 mm glucose so long as glucose remained in the medium, but that both were restored after the glucose had been depleted. The restored uptake system, measured in conidia germinated for 8 hours in the 2.8 mm glucose medium, had a $K_a$ of 0.03 mm for 3-O-methylglucose. Table VI shows that conidia germinated overnight in media containing 55 mm glucose were repressed, but that, when the conidia were washed and resuspended in fresh germination media minus glucose, both the high affinity uptake system and the ability to accumulate 3-O-methylglucose against a gradient were restored. This restoration was inhibited by 4 $\mu\text{g}$ per ml of cycloheximide, an inhibitor of protein synthesis. Once the uptake system had been restored, however, cycloheximide did not inhibit its functioning. If the germinated conidia were resuspended in 10 mm citrate phosphate buffer, pH 5.5, rather than in germination media minus glucose, the high affinity uptake system and the accumulation were not restored during a 6-hour incubation at 30°C.

**Discussion**

Kinetic characterization of sugar uptake in ungerminated conidia of wild type N. crassa strain 74A revealed the presence of two uptake systems capable of transporting 3-O-methylglucose, one (the high affinity system) with a $K_a$ of 0.07 mm and one (the low affinity system) with a $K_a$ of 10 mm for 3-O-methylglucose. The low affinity system may be identical with the system described as a facilitated diffusion glucose uptake system found in conidia germinated on 55 mm glucose (10).

This report deals primarily with the high affinity system. Uptake and substrate competition experiments showed that this system was relatively specific for glucose but also transported the nonmetabolizable analogues 3-O-methylglucose, 2-deoxyglucose, and 6-deoxyglucose. All observations suggested that the mechanism was one of active transport: conidia were able to accumulate 3-O-methylglucose against a concentration gradient as the free sugar, and azide and dinitrophenol strongly inhibited both the influx and the retention of 3-O-methylglucose. Two other possible mechanisms for the high affinity uptake system, group translocation and coupled transport, were considered unlikely because no transitory substrate derivative was detected during uptake and because influx of 3-O-methylglucose was not coupled to efflux of any organic metabolite.
One of the more unexpected observations concerning 3-O-methylglucose transport was the trans-stimulation of influx; i.e., influx was stimulated by the presence of intracellular 3-O-methylglucose. The phenomenon has been previously reported for glycine uptake in Ehrlich ascites cells (21, 22) and for chloride transport across gastric mucosa of frog (23). It has been discussed in theoretical terms by Jacquez, who used kinetic analysis to predict the results of azide inhibition if a monovalent carrier involved in trans-stimulation was related to the energy-supplying process of the active transport system in different ways (24).

In a second theoretical analysis, Wong discussed trans-stimulation as a possible consequence of polyvalent carriers under certain kinetic conditions (19). One possibility for the mechanism of trans-stimulation would be a countertransport process in which the additional influx of 3-O-methylglucose would be coupled to efflux of the internal 3-O-methylglucose. Since the efflux would provide the driving force, the additional influx should not require metabolic energy (24). Azide inhibited the trans-stimulation effect; this suggested that energy was a requirement, either directly, in the mechanism of trans-stimulation, or indirectly. An equally possible alternative for the mechanism of trans-stimulation would be that the accumulated 3-O-methylglucose influenced either the regulation or the activity of one or both uptake systems.

Efflux was another important consideration in the interpretation of 3-O-methylglucose transport mechanisms. Two observations—that efflux was completely inhibited at 0°C, and was stimulated rather than inhibited by the presence of external substrate—indicated that the efflux was mediated by a carrier. The mechanism of efflux seemed to differ from that of the high affinity uptake system, however. First, efflux was not inhibited by azide, and therefore differed in the energy linkage requirements. Second, the uptake system was rapidly inhibited by N-ethylmaleimide and HgCl₂, but efflux was not affected by either of these sulphydryl reagents.

The high affinity active transport system (measured both by initial rates of uptake and by the ability to accumulate 3-O-methylglucose against a concentration gradient) was greatly reduced in conidia germinated on glucose medium, but was restored by depletion or removal of the glucose from the medium. This restoration could be prevented by cycloheximide, an inhibitor of protein synthesis. These results suggest that the high affinity active transport system is subject to regulation by glucose repression. The active transport system would be unnecessary and uneconomical when glucose is plentiful, since the low affinity system, which appears to be a constitutive facilitated diffusion system (10), would be sufficient. However, under conditions of limited glucose availability, the high affinity active transport system would be derepressed, enabling the conidia to obtain the sugar more effectively.

Acknowledgments—We would like to thank Doctors Judith Dorsey, Harry Schachter, Werner Kundig, and Milton Saier for their suggestions and discussions.

REFERENCES

* While this manuscript was in the final stages of preparation, a short paper appeared in which a sorbose uptake system for N. crassa was described (25). Since the system was repressed by glucose and had a Kᵢ for glucose of 0.01 mm, it appears to be the same system as the one characterized here.
A Derepressible Active Transport System for Glucose in *Neurospora crassa*
M. M. Neville, S. R. Subkind and Saul Roseman


Access the most updated version of this article at http://www.jbc.org/content/246/5/1294

Alerts:
- When this article is cited
- When a correction for this article is posted

Click here to choose from all of JBC's e-mail alerts

This article cites 0 references, 0 of which can be accessed free at http://www.jbc.org/content/246/5/1294.full.html#ref-list-1