JOURNAL OF BACTERIOLOGY, Dec. 1979, p. 971-979
0021-9193/79/12-0971/09$02.00/0

Vol. 140, No. 3

Allantoate Transport in *Saccharomyces cerevisiae*

VANESSA TUROSCY AND TERRANCE G. COOPER*

Department of Biological Sciences, University of Pittsburgh, Pittsburgh, Pennsylvania 15260

Received for publication 28 September 1979

Allantoate uptake appears to be mediated by an energy-dependent active transport system with an apparent Michaelis constant of about 50 μM. Cells were able to accumulate allantoate to greater than 3,000 times the extracellular concentration. The rate of accumulation was maximum at pH 5.7 to 5.8. The energy source for allantoate uptake is probably different from that for uptake of the other allantoin pathway intermediates. The latter systems are inhibited by arsenate, fluoride, dinitrophenol, and carboxyl cyanide-*m*-chlorophenyl hydrazone, whereas allantoate accumulation was sensitive to only dinitrophenol and carboxyl cyanide-*m*-chlorophenyl hydrazone. Efflux of preloaded allantoate did not occur at detectable levels. However, exchange of intra- and extracellular allantoate was found to occur very slowly. The latter two characteristics are shared with the allantoin uptake system and may result from the sequestering of intracellular allantoate within the cell vacuole. During the course of these studies, we found that, contrary to earlier reports, the reaction catalyzed by allantoinase is freely reversible.

We have identified many of the structural components associated with the allantoin degradative pathway in *Saccharomyces cerevisiae* and are beginning to understand how they are integrated into a functional unit. One aspect of this work concerns the means by which allantoin pathway intermediates are taken into the cell. Thus far we have found three transport systems which participate in the uptake of urea and allantoin. Urea uptake occurs by two routes (3, 10). The first is a low-K_m, inducible, and repressible active transport system that appears to be driven by cytoplasmically generated ATP, perhaps in association with a proton-motive force. Urea accumulated by this system equilibrates rapidly with extracellular urea and is quickly lost from the cell upon addition of dinitrophenol. The second mode of urea uptake occurs via an apparently constitutive, energy-independent facilitated diffusion system. Allantoin uptake is mediated by an inducible and repressible, energy-dependent, low-K_m active transport system (8, 11). In contrast to the urea transport system, efflux and exchange of preaccumulated allantoin occurs very slowly. This contrasting behavior of the two transport systems probably reflects the differing intracellular distributions of urea and allantoin. Urea was found predominantly in the cytosol, whereas allantoin was shown to be efficiently sequestered in a cellular organelle (9, 15).

The present report extends our previous characterization studies to include the allantoate uptake system. The data obtained also explain why past attempts, in several laboratories, to isolate allantoate transport-defective mutants have been unsuccessful. Preliminary accounts of this work have already appeared (V. Turosky, J. McKelvey, R. Sumrada, and T. Cooper, Abstr. Annu. Meet. Am. Soc. Microbiol. 1979, K12, p. 147; V. Turoscy and T. G. Cooper, Abstr. Cold Spring Harbor Meet. Biol. Yeast, p. 107, 1979).

MATERIALS AND METHODS

Strains. The strains used in this work are listed in Table 1 along with their genotypes and biochemical phenotypes.

Culture conditions. The medium used throughout these experiments was Wickerham medium (14) that had been buffered at pH 6.0 by addition of 1% sodium citrate (pH was adjusted with HCl). Glucose (0.6%) and ammonium sulfate (0.1%) were added as sole sources of carbon and nitrogen, respectively.

Cell density measurements were made with a Klett-Summerson colorimeter (500- to 570-nm band-pass filter). One hundred Klett units is equivalent to approximately 3×10^7 cells per ml of culture.

Resting cell cultures were prepared as follows. Cells were grown overnight in buffered glucose-ammonia medium to a cell density of 40 to 60 Klett units. They were then harvested by filtration, washed with several volumes of nitrogen-free medium, and resuspended in one-half the original volume of prewarmed, preaerated buffered medium devoid of any nitrogen source. After incubation at 30°C for 18 to 26 h, the culture was ready for use in the allantoate transport assays. The cell density at the time of assay was approximately 250 to 310 Klett units. The prolonged incubation was needed to circumvent drastic oscillations in the rate of

TABLE 1. *Strains used*

Designation	Genotype	Phenotype	Reference
M25	a *his6 ura1 lys1* α *ade6 leu1*	Wild type	12
M62	a *his6 ura1 dur1-E145* α *ade6 leu1 dur1-E145*	Urea carboxylase minus	13
M104	a *his6 ura1 dal2-N18* α *ade6 leu1 dal2-N18*	Allantoicase minus	7
M85	a *his6 ura1 dal1-N16* α *ade6 leu1 dal1-N16*	Allantoinase minus	7
M927	a *his6 ura1 dal1-N16 dal2-N18* α *ade6 leu1 dal1-N16 dal2-N18*	Allantoinase and allantoicase minus	

uptake as cells made the transition from growing to resting state (8).

Transfer of cells from one medium to another. In a number of the experiments we had to transfer cell samples from one medium to another. This was done by filtering the culture through membrane filters and suspending the harvested cells in fresh medium. All filtrations were performed with nitrocellulose filters (0.45-μm pore diameter; Millipore Corp.) and were completed in less than 15 to 20 s. The extent of cell loss during this procedure was determined by using radioactively labeled cells and was found to be negligible (1).

Assay of allantoate uptake. At zero time, a 2- to 10-ml portion of the culture to be assayed was transferred to a prewarmed flask containing 0.1 to 0.3 mM [^{14}C]allantoate (specific activity, 0.2 μCi/μmol). This concentration of allantoate was two- to sixfold greater than the apparent Michaelis constant of the transport system. The cells were then incubated at 30°C in a shaking-water bath under conditions identical to those used for growth. At the times indicated, 1.0-ml samples of the culture were removed and transferred to Amicon microporous filters (0.45-μm pore diameter). The filters were then washed five times with 4 ml of cold, buffered medium containing 0.5 M NaCl. NaCl was included in the wash medium to raise its ionic strength. This eliminated nonspecific binding of allantoate to cationic groups on the cell surface. The temperature of the wash solution did not appear to be significant, since the same values of accumulation were observed when cells were washed with medium at either 27 or 4°C. Washed filters were placed in 5 ml of aqueous scintillation fluid (Aquasol, New England Nuclear Corp.), and their radioactivity content was determined 16 to 24 h later. The incubation in Aquasol was needed to allow the filters to become transparent. Failure to do this resulted in unevenly quenched samples and loss of assay precision. All data are expressed as amounts of radioactive allantoate accumulated per milliliter of culture unless otherwise indicated.

Synthesis of radioactive allantoate. Radioactive allantoate was synthesized by treating [^{14}C]allantoin with 1 N potassium hydroxide for 30 min at 75°C. [2,7-^{14}C]allantoin was synthesized by the procedures of Sumrada and Cooper (8). The product was separated from the reactants on a Dowex-1-acetate resin as described below. The allantoate-containing fractions were pooled and lyophylized. The final product was obtained at a yield of 85 to 90%. The specific activity of allantoate was determined by carefully weighing a sample, dissolving it in a precisely known volume of water, and measuring the radioactivity content of the solution. The scintillation spectrometer used for these measurements was operating at an efficiency of 89%.

Paper chromatography. Compounds to be analyzed were spotted on Whatman no. 541 filter paper. The chromatogram was developed for approximately 64 h in a descending orientation with butanol–ammonium hydroxide–95% ethanol (80:30:10) as the solvent system. After development, the paper was air dried in a fume hood and then cut into strips (1.0 by 1.5 cm), and the radioactivity content of each strip was determined.

Ion-exchange chromatography. Ion-exchange chromatography was performed by the procedures described earlier by Sumrada and Cooper (8).

RESULTS

Purity and authenticity of radioactive allantoate. Characterization of the allantoate uptake system has been based on the observed intracellular accumulation of [^{14}C]allantoate. Since this compound is not available commercially, we synthesized it and outline experiments below which demonstrate its purity and authenticity. A sample of our radioactive preparation was dissolved in 5 mM Tris buffer (pH 7.0) along with several milligrams of authentic, nonradioactive allantoate and passed over a Dowex-1-X8–acetate ion-exchange resin. The chromatogram was then developed with a 400-ml gradient of 0 to 0.5 M NaCl. All of the radioactive material was eluted as a single sharp peak which was congruent with elution of authentic allantoate detected colorimetrically (3) (see Fig. 1C for a typical elution profile). No other radioactive material was observed in the column eluate. This observation indicates that the purified allantoate was completely free of allantoin, the compound used as starting material for its synthesis. In addition, none of the radioactive preparation was retained by a Dowex 50-X8–H^+ resin, indicating the absence of any urea, a breakdown product of allantoate. The synthesized material comigrated with authentic allantoate as a single

species on a descending paper chromatogram (data not shown). The solvent system used to develop the chromatogram was capable of resolving all of the allantoin pathway intermediates; none was observed as contaminants of our purified allantoate.

An additional test of authenticity and purity was performed with wild-type and mutant strains of *S. cerevisiae* in a biological assay. Here samples of the radioactive preparation were incubated for varying lengths of time with extracts derived from various strains (see legend to Fig. 1 for methods of preparation). As shown in the inset of Fig. 1B, the radioactive material was rapidly degraded when wild-type or allantoinase-minus (*dal1*) strains were used to prepare the extract. However, no detectable conversion of substrate to $^{14}CO_2$ was observed when either allantoicase (*dal2*) or urea carboxylase-minus (*dur1*) strains were used. These are the expected results if the radioactive preparation contained only allantoate.

Chemical lability of allantoate. Allantoate is stable in basic solution, but readily hydrolyzes under acidic conditions. Since *S. cerevisiae* normally grows at pH values ranging from 3.3 to 6.0, we were concerned about the possibility of allantoate being chemically degraded in the growth medium to urea which could then be accumulated by the low-K_m (14 μM) urea active transport system (3, 10). Several experiments were performed to measure the stability of allantoate under various potential assay conditions and to evaluate the effects of allantoate hydrolysis on our uptake assay. As shown in the inset of Figure 1A, 8% of the initially added allantoate was degraded to urea during incubation (180 min) at pH 3.0. This amount of conversion would seriously compromise our assays of allantoate uptake. At pH 6.0 to 6.5, no chemical degradation was detected. Therefore, the latter pH range was chosen for all subsequent assays.

Two experiments were performed to ascertain whether or not our uptake assay was being compromised by urea production and uptake. First, we measured uptake of radioactive material (added initially as [^{14}C]allantoate) in the presence and absence of excess nonradioactive urea (0.1% final concentration). If radioactive urea was being produced and accumulated, the inclusion of nonradioactive urea in the assay mixture would greatly dilute it. We observed the same rate of allantoate uptake and final levels of accumulation regardless of whether or not carrier urea was present. A second experiment was performed in which allantoate uptake was compared in *dal2* and *dal2 dur3* mutant strains (10). Active transport of urea does not occur in the latter strain. Allantoate uptake was exactly the same in both strains. In toto, these observations argue that our allantoate uptake assay was not being compromised by simultaneous accumulation of urea produced chemically in the assay mixture.

Reversibility of the allantoinase reaction. The above experiments provided confidence that our uptake assay was sound. Therefore, we began the characterization of allantoate uptake with an allantoicase-minus (*dal2*) strain to separate uptake of allantoate from its metabolism. One of the characterization experiments involved demonstrating that accumulated allantoate was not altered once it entered the cell. A *dal2* culture was permitted to accumulate allantoate for 8 to 10 h. At that time the cells were harvested and washed, and an extract was prepared. This extract was passed over an anion-exchange resin, and the elution profile shown in Fig. 1A was obtained on chromatography. Elution of material in peak II coincided with that expected for authentic allantoate. Material in peak I was unexpected, but was eluted from the resin in a position normally occupied by allantoin. To determine whether or not this material was allantoin, we treated it with 1.0 N KOH at 75°C for 30 min. After this treatment, which is known to convert allantoin to allantoate, we again passed the unknown material over an anion-exchange resin. As shown in Fig. 1B, the base-treated material now behaved in a manner identical to allantoate. This tentatively identified the material in peak I as allantoin and suggested that, contrary to many previous reports, the allantoinase reaction was freely reversible (the ratio of material, peak I:peak II = 0.65). This conclusion was further supported by repeating the above experiment with a strain that possessed defective forms of both allantoinase (*dal1*) and allantoicase (*dal2*). As shown in Fig. 1C, only a single radioactive species was observed during this experiment. Nonradioactive carrier allantoate was added to a sample of the cell extract prepared as described above (Fig. 1C) and then recovered from solution by repeated crystallization. The specific activity of the crystallized material remained reasonably constant through three crystallizations (Table 2), suggesting that all of the accumulated radioactive material remained as allantoate once it entered the cell. In view of these observations, all subsequent experiments were performed with a *dal1 dal2* double mutant.

Accumulation of allantoate against a concentration gradient. An important attribute of active transport is its ability to concentrate solute against a concentration gradient. As

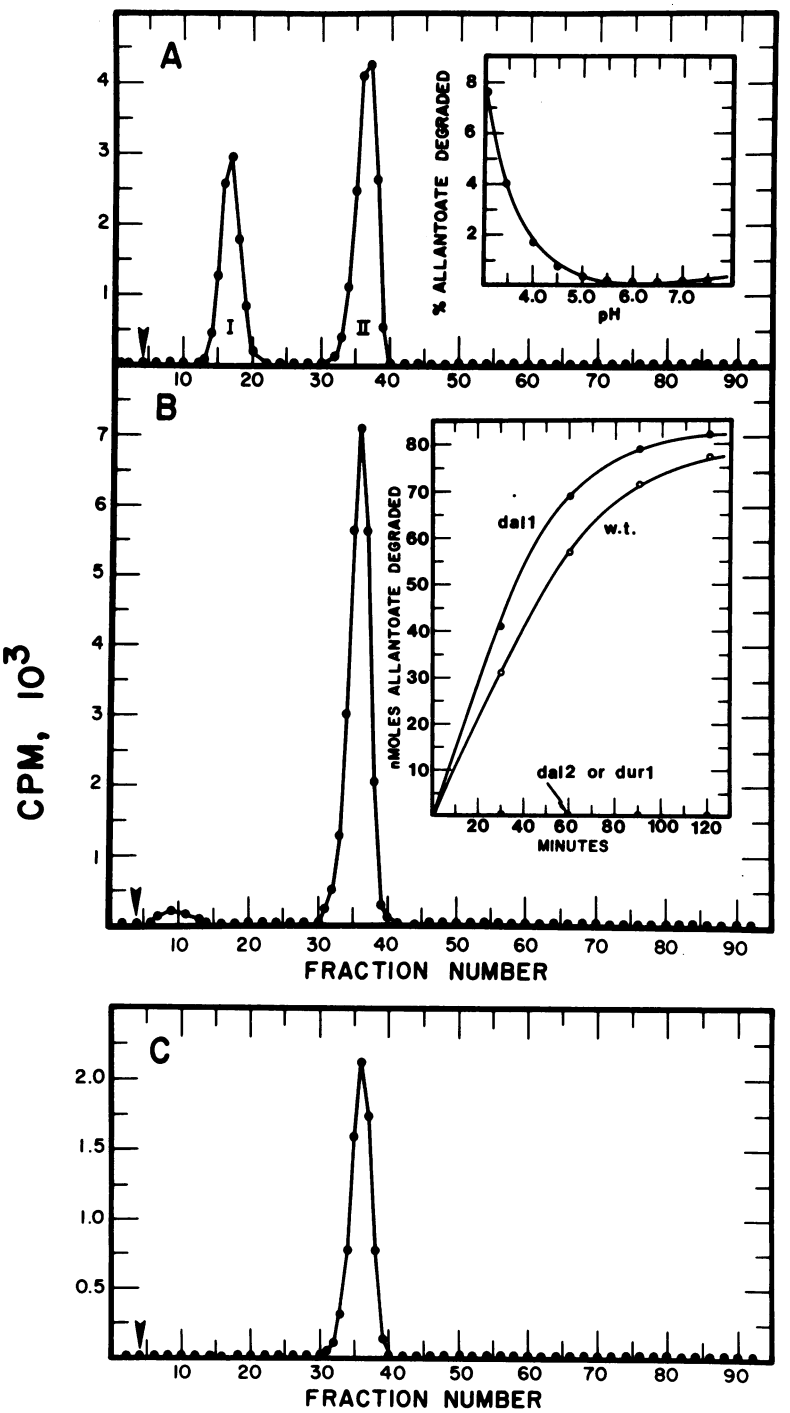

FIG. 1. *(A) Separation of cellular constituents derived from an allantoicase-minus strain that was permitted to accumulate [^{14}C]allantoate. A resting cell culture of strain M104 was incubated for 10 h with 0.3 mM [^{14}C]allantoate. After incubation, the cells were harvested by filtration, washed with cold glass-distilled water, and resuspended in ethanol. Precipitated material was removed by centrifugation and then resuspended in cold 0.1 N KOH (cold KOH at this concentration was directly shown to have no effect whatever on allantoin stability). The supernatant solutions from both the ethanol and KOH washes of the precipitated material*

TABLE 2. *Crystallization of radioactive material accumulated by S. cerevisiae*[a]

Crystallization	mg/ml	cpm/ml	cpm/mg
I	357	52,240	147
II	122	20,220	166
III	68	10,380	152
IV	42	6,920	167

[a] Crystallization was accomplished with 95% ethanol at 4°C. Crystals were harvested by filtration, dried, and carefully weighed. The dried material was then dissolved in a precisely known volume of water, and the amount of radioactivity contained in the solution was determined.

shown in Fig. 2A, allantoate accumulated in nondividing cells for over 30 h before a plateau was reached. In dividing cells, a plateau was never attained. To ascertain whether or not the intracellular allantoate concentration exceeded that in the surrounding medium, we incubated a culture of cells (density, 247 Klett units or 7.4×10^7 cells per ml) in the presence of 0.03 mM [^{14}C]allantoate. At various times 1.0-ml samples of the culture were removed for assay. The cells and medium were separated by filtration, and the amount of radioactive allantoate in each fraction was determined. As shown in Fig. 2B, nearly all of the allantoate was removed from the medium. At 330 min 27.1 and 1.5 nmol of allantoate were observed in the cells and medium, respectively. If one assumes the volume of a yeast cell is four times its dry weight (2, 3, 8), the above values are consistent with intra- and extracellular concentrations of 4.5 and 0.0015 mM, respectively, or a 2,900- to 3,000-fold concentration of allantoate. If, alternatively, one uses the average volume of yeast cells (43 μm^3) determined with a Coulter Counter channelyzer (10-μm latex beads were used as a standard), a 5,500- to 5,600-fold concentration was found.

Efflux and exchange of preloaded allantoate. The enormous intracellular concentration of allantoate prompted us to determine whether or not preloaded allantoate could be easily removed from cells. This was done by permitting cells to accumulate allantoate (0.15 mM) for 40 min and then resuspending them in fresh, prewarmed, preaerated medium devoid of allantoate. Thereafter, we sampled the culture and measured the amount of radioactivity that the samples contained. As shown in Fig. 3A, no detectable efflux of preloaded allantoate was observed during the 80 min of incubation.

The allantoate uptake system also appeared to be incapable of an exchange reaction. This was shown by preloading cells with [^{14}C]allantoate and then adding a large excess (0.1% final concentration) of nonradioactive allantoate to the medium. As shown in Fig. 3B, addition of nonradioactive allantoate to the test culture halted further accumulation of radioactive allantoate. However, intracellular [^{14}C]allantoate was not lost.

The efflux and exchange characteristics just described are similar to those reported for the allantoin (8, 11) and several amino acid uptake systems (4–6). In the case of allantoin uptake, we have found that efflux and exchange occur under certain conditions, but both processes are

were combined, diluted to a proper ionic strength (<1.0 mmho conductivity), and passed over a Dowex-1-acetate resin. Elution of bound compounds was accomplished by the procedures described in the text. The arrow indicates the beginning of the salt gradient. (B) A sample of the pooled supernatants analyzed in A was incubated with 1.0 N KOH for 30 min at 75°C. After incubation (during which time any allantoin present would have been converted quantitatively to allantoate), the sample was analyzed by ion-exchange chromatography as described in A. (C) Results obtained when the experiment described in A was repeated with a dal1 dal2 double mutant (M927, allantoinase and allantoicase minus). (Inset to A) Effect of pH on the stability of [^{14}C]allantoate. A sample of [^{14}C]allantoate was incubated with 0.2 M citrate buffers (at the pH values indicated in the figure) for 1 h at room temperature. At that time a cell extract prepared from strain M104 (allantoicase minus) was added to the sample along with all of the cofactors needed to convert urea to CO_2 by the reaction catalyzed by urea amidolyase (13). After an additional incubation period, 0.2 ml of perchloric acid was added to the reaction mixture to liberate any $^{14}CO_2$ that had been formed. This radioactive CO_2 was then trapped in hyamine hydroxide, and its radioactivity content was determined. (Inset to B) Degradation of chemically synthesized [^{14}C]allantoate by extracts derived from wild-type and mutant strains of S. cerevisiae. All four strains of yeast used in the experiment (M25, M85, M104, M62) were grown to cell densities of 75 Klett units in glucose-ammonia medium containing 0.25 mM oxalurate. Cells were harvested by centrifugation, resuspended in breakage buffer (0.5 M Tris-acetate [pH 7.5], 5% glycerol, 0.2 mM EDTA, and 20 μl of beta-mercaptoethanol per 100 ml of buffer) and broken in a Braun homogenizer (2 min). The homogenate was clarified by centrifugation for 20 min at 21,000 × g (15,000 rpm in an SS34 rotor). Portions of each extract were placed in closed vessels along with a reaction mixture (1.0 ml) that consisted of 200 mM Tris acetate (pH 7.9), 100 mM KCl, 2.5 mM $MgSO_4$, 10 mM $KHCO_3$, 5 mM ATP, and 0.35 mM [^{14}C]allantoate. At the times indicated, 0.2 ml of perchloric acid was added to each vessel. Radioactive CO_2 that was evolved during the degradative process was absorbed with hyamine hydroxide. The data are expressed as nanomoles of allantoate degraded, which is one half the number of nanomoles of CO_2 evolved.

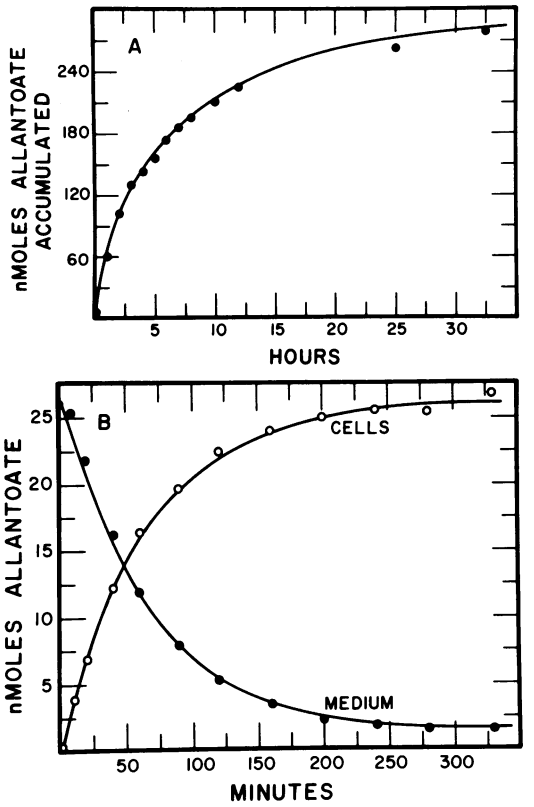

FIG. 2. *(A) Time-dependent accumulation of [^{14}C]allantoate by a resting culture of strain M927 (final cell density, 290 Klett units). A sample of the culture was incubated in the presence of radioactive allantoate (6.0 mM final concentration; specific activity, 0.003 μCi/μmol). At the times indicated, 1.0-ml samples were removed from the incubating culture and processed as described in the text. The data are expressed as nanomoles of allantoate accumulated per milliliter of culture. (B) [^{14}C]allantoate accumulation against a concentration gradient in a resting cell culture of strain M927. Data are presented as nanomoles of radioactive allantoate per milliliter of medium or per number of cells that were originally present in 1 ml of culture.*

very slow. To test whether or not a similar slow rate of allantoate efflux and exchange occurs, we repeated the two experiments described above on a very protracted time scale. As shown in Fig. 4, there was no detectable efflux of allantoate over the 9-h course of the experiment. Exchange, on the other hand, occurred with a half-life of approximately 4 h.

Apparent Michaelis constant of the allantoate transport system for allantoate. Figure 5 depicts a Lineweaver-Burk plot of the initial rate of allantoate uptake observed at increasing external allantoate concentrations. A linear response was found, indicating that the uptake process is saturable at high allantoate concentrations. Such linearity would not have been expected if allantoate was taken into the cell by two uptake systems with markedly different apparent Michaelis constants (see the analogous curve for the urea uptake systems in reference 3). Extrapolation of this plot yields an apparent Michaelis constant of approximately 50 μM.

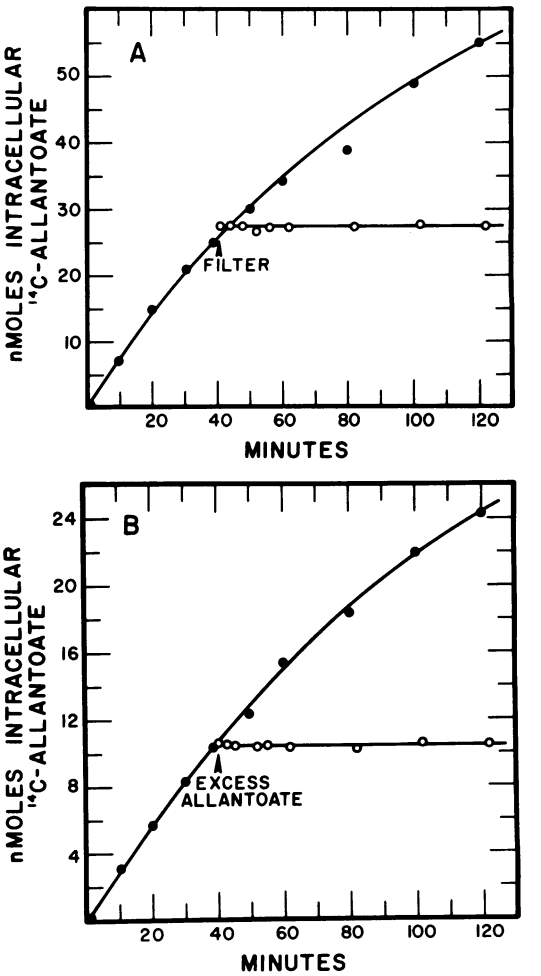

FIG. 3. *(A) Suspension of preloaded, resting cells (strain M927 at 258 Klett units) in allantoate-free medium. (B) Measurement of exchange of previously accumulated [^{14}C]allantoate with nonradioactive allantoate added to the medium. The experiment, whose results are depicted in this figure, was conducted in a manner identical to that described for A. In this case, however, the [^{14}C]allantoate concentration was 0.3 mM and the test portion of the culture was transferred to medium containing a final concentration of 0.1% allantoate rather than allantoate-free medium.*

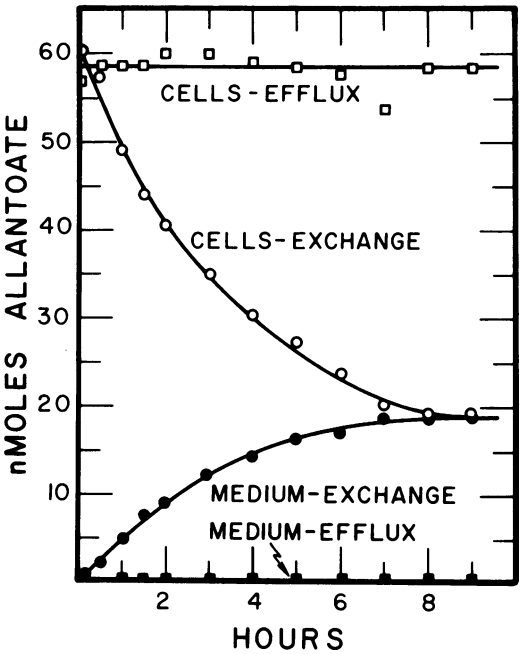

FIG. 4. *Efflux and exchange of [^{14}C]allantoate from a resting cell culture of strain M927 (final cell density, 290 Klett units). Cells were allowed to accumulate [^{14}C]allantoate (final concentration in the culture medium, 0.1 mM) for 2 h. At that time (zero time in the figure), the culture was divided into two portions. One portion was harvested by filtration and resuspended in prewarmed, preaerated buffered medium devoid of [^{14}C]allantoate (□, ■). The second portion was treated in the same manner, except the cells were resuspended in medium containing excess allantoate (0.1% final concentration) (○, ●). At the times indicated in the figure, 1.0-ml samples were removed from each test culture, and the radioactivity content of both cells and medium was determined.*

pH optimum of allantoate uptake. Allantoate uptake occurred over a very narrow range of pH with an optimum at pH 5.75 (Fig. 6). Uptake occurred at 6 and 54% of the maximum rates when the pH was 3.5 and 6.5, respectively. This is a higher pH value than observed for optimum urea (pH 3.0 to 3.5), oxalurate (pH 3.0), or allantoin (pH 5.0 to 5.5) uptake (2, 3, 8).

Requirement of a driving force for uptake of allantoate. To test the energy dependence of allantoate uptake, we monitored the initial rates of uptake in the presence of several compounds known to inhibit various steps in energy metabolism. As shown in Table 3, allantoate uptake was severely depressed by the proton ionophores dinitrophenol and carboxyl cyanide-*m*-chlorophenyl hydrazone, but was apparently insensitive to the action of cyanide, arsenate, and fluoride. Control experiments with the

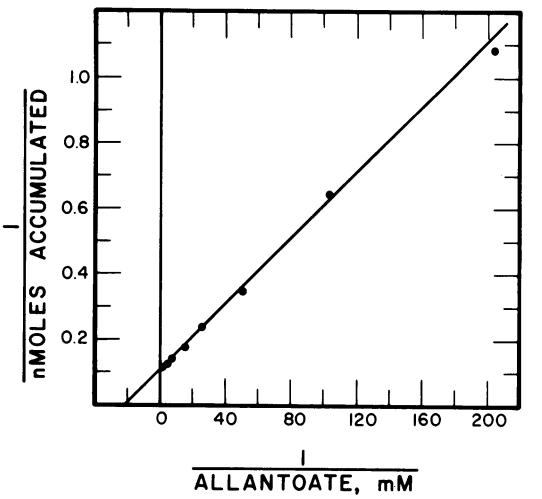

FIG. 5. *Lineweaver-Burk plot of the rate of allantoate accumulation (nanomoles accumulated per 20 min) observed at increasing extracellular concentrations of [^{14}C]allantoate. A resting cell culture of strain M927 was prepared and assayed as described in the text (final cell density, 247 Klett units).*

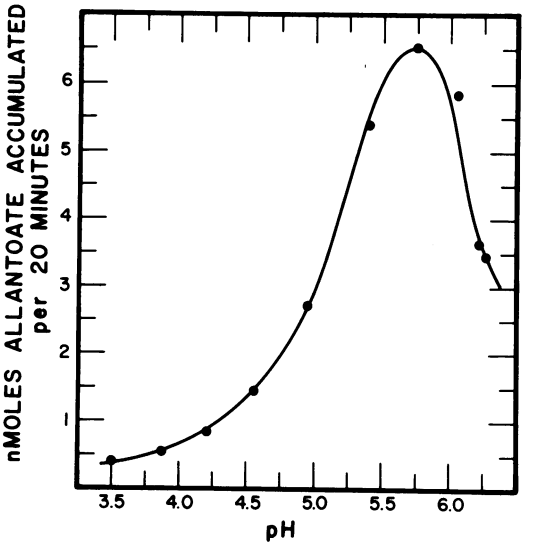

FIG. 6. *Effect of pH on the rate of allantoate accumulation in resting cell culture of strain M927 (cell density, 266 Klett units). Portions of the culture were placed in small vessels in a shaking 30°C water bath. At zero time, citrate buffers of known pH were added to each portion (0.1 M final concentration). After 1 min, 1.0 ml of each portion was transferred to a vial containing 0.15 mM [^{14}C]allantoate. The cells were then allowed to accumulate allantoate for 20 min before being processed as described in the text. A precise measurement of the pH was made for each of the buffered suspensions. This was necessary due to minor changes in pH that occurred when the cell suspension was added to the buffered solution.*

TABLE 3. *Energy requirement for allantoate accumulation in S. cerevisiae*[a]

Inhibitor	Allantoate accumulation (nmol/20 min)
None	9.8
1 mM cyanide	10.0
5 mM arsenate	10.2
5 mM fluoride	10.0
0.1 mM CCCP	0.9
1 mM DNP	0.5

[a] A resting cell culture of strain M927 was prepared as described in the text (cell density, 290 Klett units). Portions of the culture were transferred to small vessels containing one of the inhibitors noted in the table. After 5 min of incubation, 0.15 mM [^{14}C]allantoate was added to each vessel. After an additional 20 min of incubation, samples were removed and processed as described in the text. Sampling was continued in this manner for 2 h. The data reported in the table were derived from the slopes of the incorporation curves. CCCP, Carboxyl cyanide-*m*-chlorophenyl hydrazone; DNP, dinitrophenyl.

urea active transport system demonstrated that the latter three inhibitors were inhibiting cellular energy metabolism as expected.

Although energy was required to accumulate allantoate, it did not seem to be required to maintain preaccumulated allantoate within the cell. Addition of dinitrophenol to cultures that had been accumulating allantoate for 40 min prohibited further accumulation, but did not result in any loss of radioactive material accumulated before addition of the inhibitor (Fig. 7A). Treatment with nystatin brought about release of previously accumulated allantoate, albeit at a much slower rate than observed for urea (3), allantoin (8), or oxalurate (2) (Fig. 7B).

DISCUSSION

Attempts to characterize allantoate transport were at first complicated by a pH-dependent lability of the substrate and a previously unnoticed reversibility of the reaction catalyzed by allantoinase. However, once these problems were successfully managed, characterization of the system proceeded normally. Our results suggest that allantoate is accumulated in *S. cerevisiae* by way of a low-K_m (50 μM), energy-dependent active transport system. Organisms grown in minimal glucose-ammonia medium were able to concentrate allantoate approximately 3,000- to 6,000-fold. This assumes that intracellular allantoate is uniformly distributed throughout the cell volume, a highly unlikely possibility. This degree of concentration required special care be taken to determine whether or not allantoate was chemically altered after it entered the cell. Normal metabolism of allantoate by either allantoinase or allantoicase was not possible because all of the experiments reported here were performed with mutant strains lacking these enzyme activities. In addition, accumulated radioactive allantoate was re-

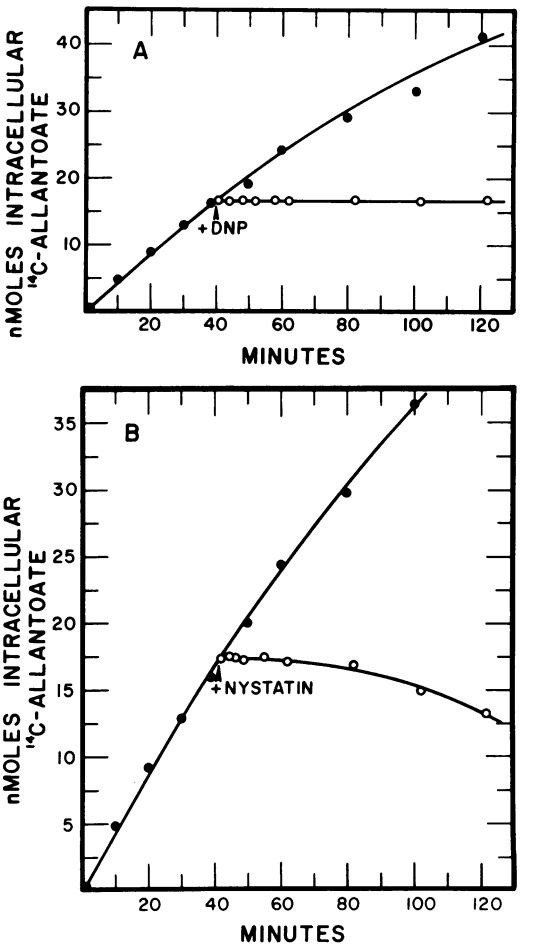

FIG. 7. *(A) Maintenance of intracellular allantoate in the presence of dinitrophenol (DNP). At zero time, a portion of a resting cell culture of strain M927 was added to a flask containing 0.1 mM [^{14}C]allantoate. Samples (1.0 ml) were removed, thereafter, for about 40 min. At that time the culture was divided in half. One portion received dinitrophenol (1.0 mM final concentration), whereas the remaining portion remained untreated. Sampling was then continued as before, and all of the samples were processed as described in the text. (B) Nystatin-mediated release of [^{14}C]allantoate that had been previously accumulated by resting cells. This experiment was performed in the same way as that described in A. In this case, however, nystatin (18 μg/ml, final concentration) was added in place of the dinitrophenol.*

covered from cells and shown, by several criteria, to be chemically unmodified.

The remarkable degree of intracellular concentration, the slow rate of exchange between intra- and extracellular allantoate and the absence of detectable allantoate efflux from the cell are all characteristics shared with the allantoin uptake system. These similarities might suggest that allantoin and allantoate share a common uptake system. We disagree with such a conclusion because allantoin uptake is totally prevented by addition of arsenate or fluoride to the culture medium, whereas allantoate uptake proceeds normally under these conditions. An alternative and more tenable conclusion is that allantoate is sequestered within the cell vacuole in a manner similar to allantoin. This view is supported by preliminary data that we reported earlier concerning the intracellular localization of allantoin, allantoate, and urea (reference 3, Table 5; radioactive material in strain M104 was at least 50% allantoate due to the presence of functional allantoinase). The possibility that two transport systems may be operating in tandem (one at the plasma membrane and another at the vacuole membrane) requires that our current results be interpreted conservatively. A clear understanding of the details of allantoate transport will necessitate being able to clearly distinguish which transport process is operating in a unidirectional manner: transport of allantoate into the cell or into the vacuole. This is beyond our technical capability at present due to the lack of appropriate mutant strains.

We have made several serious but unsuccessful attempts in the past to isolate mutant strains lacking allantoate uptake. The results presented in this report demonstrate why our efforts failed. We used Wickerham's medium which has a pH of 3.3 during cell growth. At this pH allantoate would spontaneously decompose to urea, and hence two nitrogen sources would be present under our selection conditions precluding isolation of the desired mutants. By using medium that is well buffered at pH 6.0, we should be able to obtained allantoate uptake-defective mutants and can then use them along with strains defective in the *dal4* gene product (allantoin uptake defective) to determine the extent of relationship between the allantoin and allantoate uptake systems.

ACKNOWLEDGMENTS

We thank Roberta Sumrada for synthesizing the [^{14}C]allantoin which was the starting material to produce the [^{14}C]-allantoate used in this work.

This work was supported by Public Health Service grants GM-19386, GM-20693, and GM-24383 from the National Institute of General Medical Sciences. T.G.C. was the recipient of a Public Health Service Research Career Development Award (KO4-GM-00091), also from the National Institute of General Medical Sciences.

LITERATURE CITED

1. **Bossinger, J., and T. G. Cooper.** 1976. Sequence of molecular events involved in induction of allophanate hydrolase. J. Bacteriol. **126**:198–204.
2. **Cooper, T. G., J. McKelvey, and R. Sumrada.** 1979. Oxalurate transport in *Saccharomyces cerevisiae*. J. Bacteriol. **139**:917–923.
3. **Cooper, T. G., and R. Sumrada.** 1975. Urea transport in *Saccharomyces cerevisiae*. J. Bacteriol. **121**:571–576.
4. **Crabeel, M., and M. Grenson.** 1970. Regulation of histidine uptake by specific feedback inhibition of two histidine permeases in *Saccharomyces cerevisiae*. Eur. J. Biochem. **14**:197–204.
5. **Greth, M. L., M. R. Chevallier, and F. Lacroute.** 1977. Ureidosuccinic acid permeation in *Saccharomyces cerevisiae*. Biochim. Biophys. Acta **465**:138–151.
6. **Kotyk, A., and L. Rihova.** 1972. Transport of α-aminoisobutyric acid in *Saccharomyces cerevisiae*. Biochim. Biophys. Acta **288**:380–389.
7. **Lawther, R. P., E. Riemer, B. Chojnacki, and T. G. Cooper.** 1974. Clustering of the genes for allantoin degradation in *Saccharomyces cerevisiae*. J. Bacteriol. **119**:461–468.
8. **Sumrada, R., and T. G. Cooper.** 1977. Allantoin transport in *Saccharomyces cerevisiae*. J. Bacteriol. **131**: 839–847.
9. **Sumrada, R., and T. G. Cooper.** 1978. Control of vacuole permeability and protein degradation by the cell cycle arrest signal in *Saccharomyces cerevisiae*. J. Bacteriol. **136**:234–246.
10. **Sumrada, R., M. Gorski, and T. G. Cooper.** 1976. Urea transport-defective strains of *Saccharomyces cerevisiae*. J. Bacteriol. **125**:1048–1056.
11. **Sumrada, R., C. A. Zacharski, V. Turoscy, and T. G. Cooper.** 1978. Induction and inhibition of the allantoin permease in *Saccharomyces cerevisiae*. J. Bacteriol. **135**:498–510.
12. **Whitney, P. A., and T. G. Cooper.** 1970. Requirement for HCO_3^- by ATP:urea amido-lyase in yeast. Biochem. Biophys. Res. Commun. **40**:814–819.
13. **Whitney, P. A., and T. G. Cooper.** 1972. Urea carboxylase and allophanate hydrolase: two components of ATP:urea amido-lyase in *Saccharomyces cerevisiae*. J. Biol. Chem. **249**:1349–1353.
14. **Wickerham, L. J.** 1946. A critical evaluation of the nitrogen assimilation tests commonly used in the classification of yeasts. J. Bacteriol. **52**:293–301.
15. **Zacharski, C. A., and T. G. Cooper.** 1978. Metabolite compartmentation in *Saccharomyces cerevisiae*. J. Bacteriol. **135**:490–497.