BBA 76005

TRANSPORT OVERSHOOT DURING BIOTIN UPTAKE BY *SACCHAROMYCES CEREVISIAE**

JEFFREY M. BECKER AND HERMAN C. LICHSTEIN

Department of Microbiology, University of Cincinnati College of Medicine, Cincinnati, Ohio 45219 (U.S.A.)

(Received March 6th, 1972)

SUMMARY

Biotin permeation into non-proliferating cells of *Saccharomyces cerevisiae* (JB 112), which requires biotin, adenine, and tryptophan for growth, was determined by liquid scintillation spectrophotometry of the radioactive vitamin retained by the cells. After a large concentration difference of intracellular to extracellular biotin was generated, the accumulated biotin flowed out of the cells. This phenomenon was designated as overshoot and was investigated further. The outflow of biotin was not due to the breakdown of cellular permeability barriers, nor was the rate temperature or pH dependent. Efflux did, however, follow first-order kinetics.

Some factors which might alter biotin permeability were tested; these experiments indicated that (a) another protein component of transport was not synthesized during the course of uptake and (b) general cellular metabolism did not affect the onset of overshoot. Cells incubated without biotin retained uptake capacity while the presence of biotin in the incubation medium caused a 50% decrease in the initial rate of uptake after 40 min. Thus the active accumulation of biotin was inhibited by biotin of the intracellular pool in the yeast. This transinhibition of uptake and the continued efflux of intracellular biotin results in an overshoot effect.

INTRODUCTION

In most active transport systems the *trans* concentration (*ie.* the concentration on the side of the membrane toward which transport is occurring) of the transported substance approaches asymptotically a maximum intracellular value; the steady-state intracellular level of substrate is defined by the balance between efflux and influx. Reported deviations from these data include the overshoot phenomenon, where the intracellular concentration of the transported substance obtains a maximum value followed by an outflow of the substance from the cell[1].

Previous publications noting overshoot in microbial systems include potassium uptake in yeast[2], proline in *Escherichia coli*[3], glutamate in *Staphylococcus aureus*[4], lipoic acid in *Streptococcus faecalis*[5], and sulfate in *Salmonella typhimurium*[6]. Only

* Presented in part at the 70th Annual Meeting of the American Society for Microbiology, Boston, Mass., 26 April–1 May 1970. Taken from a dissertation submitted by the senior author in partial fulfillment of the requirements for the Ph.D. degree in microbiology.

Dreyfuss and Pardee[6] studied overshoot *per se* and proposed that entry of sulfate into *S. typhimurium* was inhibited by a high-energy compound formed during transport. This feedback inhibition of entry and the maintenance of a constant exit rate decreased the influx relative to efflux after a short time (1 min) thus creating an overshoot.

In the course of investigation of biotin permeation into yeast, overshoot was observed in this laboratory[7]. The experiments herein reported were undertaken to determine (a) whether this observation was in fact transport overshoot, (b) how the phenomenon could be explained since no high energy biotin compound is formed in the course of biotin transport in yeast, and (c) whether this phenomenon plays a role in the regulation of intracellular biotin levels.

MATERIALS AND METHODS

Organisms and growth conditions

S. cerevisiae (JB 112), a biotin, tryptophan, and adenine auxotroph obtained from Dr Thomas R. Manney, Case-Western Reserve University, was employed in all uptake studies. The yeast was maintained on agar slants (ACG) containing 1 % yeast extract, 1 % casitone, 0.5 % K_2HPO_4, 0.5 % glucose, and 1.5 % agar. *Lactobacillus plantarum* (ATCC 8014) was used for the microbiological assay of (+)-biotin, and was maintained on APT agar (Baltimore Biological Laboratory) as a stab culture. The yeast was grown in the medium of Hertz[8] containing 0.25 ng of (+)-biotin per ml and modified by the substitution of glucose for sucrose and by the addition of 0.2 mM tryptophan and 0.2 mM adenine. The amounts of biotin, tryptophan, and adenine corresponded to the minimal concentrations yielding maximum growth.

Measurement of biotin uptake

The yeast cells were harvested from growth media at the mid-exponential phase by centrifugation or by filtration on HA membrane filters (Millipore Corp., Bedford, Mass.), washed 3 times with distilled water and resuspended in distilled water to yield a suspension of known concentration (usually 1 mg dry cells per ml).

The uptake reaction mixture (double strength) contained 0.10 M potassium phosphate (pH 4.0), 2 % glucose, and (+)-[^{14}C]biotin (4 μM, 57.5 mCi/mmole). Changes in concentration and/or substitutions are specified where relevant. The non-proliferating cells and uptake reaction mixture were temperature equilibrated, added together in equal volumes, and incubated at 30 °C in a shaking water bath (100 strokes per min). Samples, usually 1.0 ml, of the complete mixture were removed at intervals by means of a pipette and deposited on moistened HA membrane filters. The cells were washed with distilled water and if total biotin uptake was measured, the washed cells and filter were added directly to a counting vial (Packard Instrument Company, Downers Grove, Ill.). If free and bound biotin uptake were assayed separately, the washed cells and filter were added to a tube containing 5 ml of distilled water and placed in a boiling water bath for 10 min. The soluble boiled cell extract contained free biotin. Bound biotin was released from the boiled cell debris by autoclaving at 121 °C in 3 M H_2SO_4 for 1 h.

15 ml of Bray's[9] liquid scintillation solution were added to each sample and radioactivity measured in a Packard Tri-Carb liquid scintillation spectrophotometer.

Corrections for quenching were made by using an external standard, and counts per min were converted to biotin concentration.

L. plantarum was employed for the assay of (+)-biotin since it responds to this of all the forms of the vitamin found in nature. The general procedure for this assay was that of Wright and Skeggs[10] for which a complete description of the medium and assay procedure has been outlined elsewhere[11].

Chromatography of cellular extracts

Free biotin was extracted by incubating the cells in boiling water for 10 min. A small amount (0.01 ml) of this extract was spotted on a filter-paper strip and chromatographed by the descending technique[11]. Chromatograms were assayed for biotin both by the bioautographic technique of Wright and co-workers[12] and by a radioautography method. For radioautography 1-cm sections of the chromatograms were placed in scintillation vials to which 15 ml of Bray's solution were added. The radioactivity was measured in the Tri-Carb scintillation spectrophotometer.

Chemicals

Crystalline (+)-biotin was purchased from the Sigma Chemical Co., St. Louis, Mo. [*carbonyl*-^{14}C]biotin (57.5 mCi/mmole) was obtained from Amersham-Searle Co., Des Plaines, Ill. The purity of the labeled biotin was checked by bio-and radiochromatography. (±)-Desthiobiotin was purchased from Nutritional Biochemicals Corp., Cleveland, Ohio. The biotin analogs (+)-homobiotin and (+)-norbiotin were gifts from Hoffmann–LaRoche, Inc., Nutley, N. J. 2-Deoxy-D-glucose uniformly labeled with ^{14}C was a gift from A. H. Romano, University of Cincinnati. Iodoacetic acid and NaN_3 were obtained from Matheson Coleman and Bell, Cincinnati, Ohio. Cycloheximide was purchased from Calbiochem, Los Angeles, Calif.

RESULTS

State of intracellularly accumulated biotin

An important criterion in transport studies is to determine whether the accumulated substance is metabolized by the cells. Although Chang and Peterson[13] reported that several strains of yeast did not convert (+)-biotin to other vitamers or degrade the vitamin to metabolites, Leonian and Lilly[14] were not able to recover all the biotin offered to cells of *S. cerevisiae* "old process". It was necessary, therefore, to establish whether *S. cerevisiae* (JB 112) metabolized biotin under the conditions used in the present studies.

Non-proliferating cells were prepared and 4.8 ng/ml of radioactive biotin (57.5 mCi/mmole) added to the uptake reaction mixture. Menstruum, free, and bound biotin were measured by both radioactive and microbiological assay methods. Any vitamin activity which disappeared from the reaction mixture appeared within the cells as free or bound biotin (Fig. 1). Since both assay methods gave essentially identical values (Table I) the added biotin appeared not to be degraded or converted to biotin vitamers. Hence, the determination of biotin uptake by either microbiological or radioactive measurement of the intracellular appearance of the vitamin during incubation of cells in uptake reaction mixture appears to be a valid method for the assay of the transport of biotin by these cells.

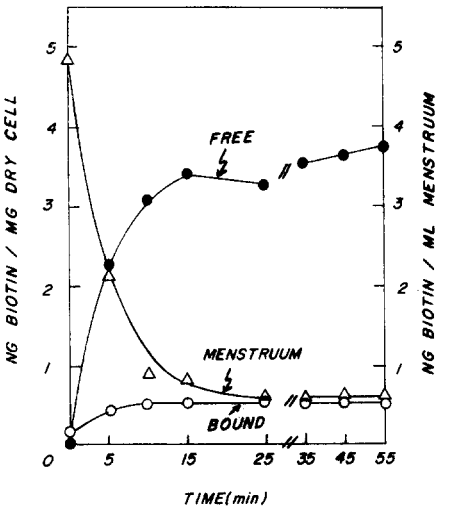

Fig. 1. Accumulation of biotin into cells of *S. cerevisiae* (JB 112). Biotin (4.8 ng/ml) was added to non-proliferating cells; its disappearance from the menstruum and its intracellular accumulation was measured by microbiological assay with *L. plantarum* and by radioactive assay. △, biotin in the reaction mixture or menstruum biotin; ●, intracellular free biotin; ○, intracellular bound biotin. The left ordinate is for free and bound biotin; the right ordinate is for menstruum biotin.

TABLE I

RECOVERY OF BIOTIN DURING INTRACELLULAR ACCUMULATION INTO CELLS OF *S. cerevisiae* (JB 112)

The data from Fig. 1 are represented in this table. At each time interval the menstruum, free, and bound biotin concentrations were totalled and are recorded in the second column. Data in parentheses are from microbiological assay, whereas the first-mentioned values in each column are from radioactive assay.

Time after start of experiment (min)	*Total biotin (ng/ml)*	*Recovery (%)*
0	4.92 (4.90)	100 (100)
5	4.60 (4.70)	93 (96)
10	4.27 (4.55)	87 (92)
15	4.55 (4.60)	92 (93)
25	4.26 (4.40)	87 (89)
35	4.40 (4.60)	89 (93)
45	4.60 (4.60)	93 (93)
55	4.70 (4.60)	96 (93)

Parameters of biotin uptake

Rogers and Lichstein[7] reported the characteristics of the biotin uptake system in *S. cerevisiae* (139). Since the present study concerns biotin transport in another yeast strain, a complete characterization of uptake was carried out, a summary of which is presented in Table II. All parameters for both yeast strains were essentially identical, except for temperature optimum (35 °C for JB 112 and 30 °C for 139). The requirement for ions was not established for strain 139. Moreover, the stimulation by Na^+ or K^+ for JB 112 was not replaced by Mg^{2+}, Mn^{2+}, Ca^{2+} or Li^+, nor was uptake inhibited by 10^{-4} M ouabain.

TABLE II

PARAMETERS OF BIOTIN UPTAKE IN *Saccharomyces cerevisiae* (JB 112)

Parameter examined	*Experimental observation*
pH dependence of initial velocity	Maximal at pH 4.0 in phosphate buffer
Temperature dependence of initial velocity	Maximal at 35 °C; Q_{10} (between 20 °C and 30 °C = 2.29)
Ionic requirement for maximal accumulation	Uptake in the absence of either Na^+ or K^+ is 40% of maximal
Energy requirement for maximal accumulation	Glucose (1%) increased 3-fold Iodoacetate (10 mM) inhibited 90% NaN_3 (10 mM) inhibited 97%
Structural specificity	Structural analogs desthiobiotin, norbiotin, and homobiotin inhibited more than 90%
K_m	$8.0 \cdot 10^{-7}$ M
V	$2.0 \cdot 10^{-7}$ moles/min per mg cell

The results of these experiments were indicative of an energy-dependent transport process with enzymatic properties of a carrier-mediated uptake. If an energy-dependent transport results in the accumulation of a solute against a large concentration difference, transport is considered to be active[15].

Biotin uptake versus time

Non-proliferating cells (1 mg/ml) were divided into four portions, and each added to an equal volume of uptake reaction mixture containing various amounts

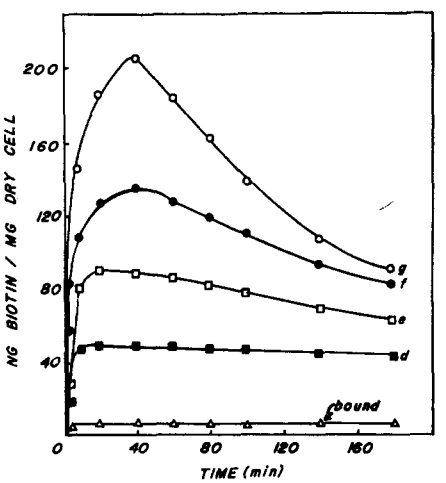

Fig. 2. Biotin uptake *versus* time: A family of curves. Non-proliferating cells (1 mg/ml) were incubated with various amounts of labeled biotin, and radioactivity retained by the cells determined. △, concentration of bound biotin; ■, cells incubated in 46 ng/ml of biotin; □, cells incubated in 71 ng/ml of biotin; ●, cells incubated in 94 ng/ml of biotin; ○, cells incubated in 173 ng/ml of biotin.

of radioactive biotin. Radioactivity retained by the cells was measured at several time intervals, and samples were taken for the assay of bound biotin. The data in Fig. 2 indicate that the bound biotin pool was saturated at low intracellular levels no matter how much biotin was incubated with the cells. The upper curves are essentially a measurement of the influx and efflux of biotin from the free pool. The higher the amount of biotin offered the cells initially, the greater was the rate of biotin uptake, the higher was the level of biotin reached intracellularly, and the faster was the rate of biotin efflux.

In order to calculate the concentration difference created by uptake, the intracellular biotin concentration at the peak of uptake (Fig. 2) was calculated on the basis of the free water content (0.0021 ml/mg dry cells) of yeast experimentally determined by Okada and Halvorson[16]. The extracellular biotin was determined by radioactive assay. The data in Table III indicate that: (1) a large concentration gradient of biotin was generated; (2) the onset of outflow was not dependent on a specific concentration difference; and (3) the rate of outflow was not related to the intracellular/extracellular ratio. Since the results were based on radioactive measurements and more biotin was accumulated than seen in Fig. 1, it was important to know whether biotin was metabolized.

TABLE III

INTRACELLULAR AND EXTRACELLULAR CONCENTRATIONS, CONCENTRATION RATIO, AND RATE OF LEAKAGE AT THE PEAK OF BIOTIN UPTAKE

The intracellular concentration at the peak of uptake in Fig. 2 and the extracellular biotin remaining are represented in this table. The rate of outflow was also calculated from the data in Fig. 2.

Concentrations at peak of uptake (μM)			*Rate of outflow (ng of biotin/min per mg dry cell)*
Intracellular	*Extracellular*	*Intracellular/ Extracellular*	
87.8	0.12	731	0.04
165.8	0.16	1036	0.10
226.8	0.39	582	0.40
314.0	0.85	369	1.00

Outflow and recovery of biotin

Non-proliferating cells (1 mg/ml) were incubated in the uptake reaction mixture containing radioactive biotin (50 ng/ml; 57.5 mCi/mmole). The cells were harvested at 20 min, resuspended in water, and aliquots taken for the assay of intracellular and extracellular biotin. The loss of intracellular biotin was followed quantitatively by its recovery in the menstruum (Fig. 3). At the end of leakage the cells still maintained biotin against a concentration gradient (note the different scales on the right and left abscissa). The sample indicated by the arrow was taken for biotin assay by biochromatography and radiochromatography. The sample was recovered at the R_F for (+)-biotin (0.83) with a 95 % yield of the material chromatographed by both radioactive and biological assay. Thus, after a large concentration difference was generated in the process of transport a release of unaltered biotin by the cells occurred during leakage.

Completeness of the cellular permeability barriers

A possible explanation for biotin leakage could be damage of permeability barriers under the experimental conditions. During biotin outflow the cell viability, measured by plating dilutions of the reaction mixture on Petri dishes containing

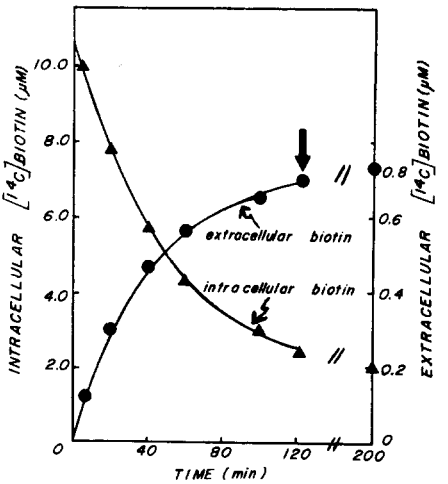

Fig. 3. Outflow and recovery of biotin. Non-proliferating cells were loaded with biotin under optimal conditions of uptake, harvested, and resuspended in water. Loss of accumulated biotin from the cells and its recovery in the menstruum were measured by radioactivity assay. Note the different scales on the right and left ordinate. ●, extracellular or menstruum biotin; ▲, intracellular biotin. The arrow denotes the sample taken to determine if the biotin that flowed out of the cell was altered.

ACG media, did not fall. Additionally there was no leakage of ninhydrin-reactive, Lowry-reactive, or 280 nm-absorbing materials. In contrast, when non-proliferating cells were offered 2-deoxy-D-glucose under the same conditions, the intracellular 2-deoxy-D-glucose concentration approached asymptotically a maximum which was not followed by leakage. Hence, the outflow of biotin does not appear to be caused by a general breakdown in the permeability barriers of the cell. Rather, a specific outflow of unaltered biotin is suggested.

The net outflow of biotin presumably requires a change in some component of the transport system. Outflow could be due to a modification of the inflow or outflow mechanism or both. Since biotin uptake had been characterized, it was important to determine the salient fratures of the efflux of biotin from non-proliferating cells of *S. cerevisiae* (JB 112).

Characteristics of biotin efflux

Non-proliferating cells (1 mg/ml) were divided into portions and each incubated with radioactive biotin at various concentrations, thus loading the cells to various intracellular concentrations of biotin. After 10 to 20 min of biotin accumulation, the cells were separated from the reaction mixture by filtration, resuspended in water, and the amount of radioactive biotin retained by the cells determined at intervals. A plot of the logarithm of the biotin concentration in the cell against time was found to fall on a straight line suggesting that efflux of biotin is a first-order process which is

indicative of diffusion or exit through a system which is not saturable within the range investigated[17].

To determine the effect of temperature on efflux, non-proliferating cells (0.5 mg/ml) were incubated with radioactive biotin (50 ng/ml, 57.5 mCi/mmole) in the uptake reaction mixture for 10 to 20 min, after which time portions were removed by filtration. The cells on the filter were resuspended rapidly in water which had been equilibrated to the temperature of efflux. The apparent Q_{10} between 25 °C and 35 °C was 1.31, which is in the range of diffusion reactions. Additional experiments demonstrated that in contrast to the sharp pH optimum and glucose dependency for biotin uptake, the pH of the menstruum and presence of glucose had no effect on the efflux of biotin from loaded cells. This finding indicates that reabsorption of biotin does not interfere with efflux measurements, because changes of menstruum pH values which affect influx do not affect efflux.

A comparison of efflux and influx is given in Table IV. The parameters listed in this table suggest that efflux occurs *via* a system other than the one mediating biotin influx. However, these data do not eliminate the possibility that the reverse reaction of active transport occurs. Depending on the site of coupling, either influx or efflux may show a stronger dependence on metabolism, temperature, or other factors.

TABLE IV

COMPARISON OF INFLUX WITH EFFLUX

Parameter	*Effect on influx*	*Effect on efflux*
Kinetics	Saturation (Michaelis–Menten)	First order
Glucose	Stimulates 3-fold	No effect
Temperature (Q_{10})	2.29	1.31
pH	Dependent	Independent

Synthesis of another component of biotin transport

The possibility was tested that a component which inhibited influx was synthesized during biotin uptake. The synthesis of an inducible exit system has been shown for galactosides in *E. coli*[18]. Uptake was measured in the presence of 10 μg/ml of cycloheximide which blocks protein synthesis in yeast[19] and is a potent growth inhibitor of *S. cerevisiae* (JB 112). The inhibitor had no effect on the initial uptake or subsequent outflow of biotin. Also, the absence of tryptophan and adenine in the uptake reaction mixture prevented further growth of the cells and indicated that protein synthesis would be minimal under the conditions of the uptake experiments.

Alteration of biotin uptake by cellular metabolism

If cellular energy reserves were depleted during biotin uptake, then the concentration difference generated would not be maintained; the biotin accumulated would flow out of the cell. To test this idea cells were incubated with uptake reaction mixture containing radioactive biotin (500 ng/ml, 57.5 mCi/mmole) after harvesting

or were incubated in uptake reaction mixture without biotin before addition of radioactive vitamin. Three different temperatures were utilized because general cellular metabolism should be quite different at each of the temperatures. The top portion of Fig. 4 represents biotin uptake and outflow at different temperatures with biotin being added to the cells immediately after harvest from the growth medium. The bottom portion of Fig. 4 represents the results of biotin uptake after the cells were incubated at 30 °C for 10 min in a reaction mixture without biotin and then equilibrated to the temperature of uptake. The uptake pattern at each temperature was identical suggesting that the properties of the cells with respect to biotin transport and retention capacity were not altered by cellular metabolism under the conditions employed.

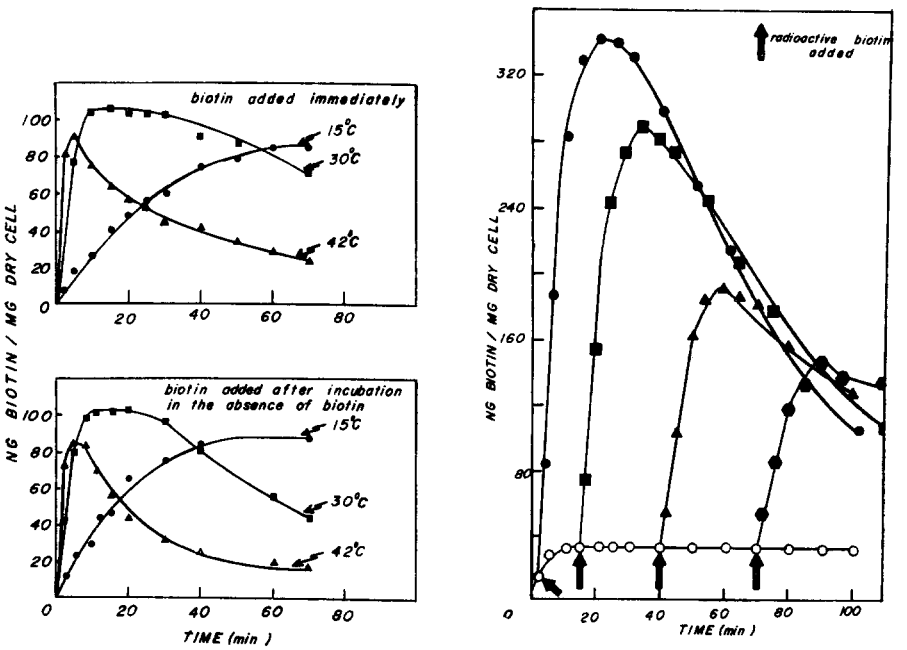

Fig. 4. Biotin uptake before and after incubation of cells in reaction mixture without biotin. Non-proliferating cells were divided into 6 portions, three of which were temperature equilibrated and incubated with biotin (500 ng/ml) in the uptake reaction mixture (represented in the top half of the figure). The other portions were incubated at their respective temperature for 10 min before incubation with the same uptake reaction mixture (represented in the bottom half of the figure).

Fig. 5. Effect of cellular biotin on uptake of extracellular biotin. Non-proliferating cells were incubated in uptake reaction mixture with 5 ng/ml of biotin. At 2, 15, 40, and 70 min portions of this mixture were removed, washed, and resuspended in a second uptake reaction mixture with 500 ng/ml of biotin. ○, uptake of cells incubated with 5 ng of biotin/ml; ●, additional biotin at 2 min; ■, additional biotin at 15 min; ▲, additional biotin at 40 min; ⬢, additional biotin at 70 min.

Transinhibition of biotin uptake by intracellular biotin

An observation indicating that a feedback inhibition or a negative exchange diffusion (a phenomenon where the presence of a substrate of a transport system on the *trans* side of the membrane inhibits the flux of a transported substance) was involved in the control of biotin uptake was made by the following experiment.

Non-proliferating cells were incubated in an uptake reaction mixture containing a small amount of radioactive biotin (5 ng/ml, 57.5 mCi/mmole). At 2, 15, 40, and 70 min, portions of this mixture were separated from the main vessel by filtration, washed, and resuspended in a second uptake reaction mixture containing radioactive biotin (500 ng/ml, 57.5 mCi/mmole). The data in Fig. 5 indicate that the ability of the cells to accumulate this additional biotin decayed remarkably. This decay of uptake capacity was brought about solely by the presence of biotin intracellularly since cells that were incubated under identical conditions, but without biotin, did not show the marked decay of uptake.

An attempt was made to restore biotin uptake capacity by chasing from the cell the biotin that had been accumulated. However, a restoration of uptake ability to the levels taken up at the 2-min biotin addition was not accomplished by exchange of intracellular biotin with extracellular homobiotin. This lack of restoration could be due to the replacement of biotin by homobiotin as the transinhibitor or to the biotin remaining in the cells after the chase acting as the species of intracellular biotin responsible for the transinhibition.

DISCUSSION

A unidirectional influx of substrate resulting from an active transport system would lead to unlimited intracellular accumulation of substrate unless some mechanism was operative allowing efflux or feedback inhibition. Thus, the steady-state intracellular level of substrate is defined by the balance between influx and efflux. The original, and possibly the simplest, efflux mechanism described was that of "leaks" allowing substrate to escape from the cell[20]. The leak was given the necessary role of protection against osmotic damage due to the rise of internal osmolarity from substrate inflow followed by water inflow.

In one of the first models of bacterial transport, Cohen and Monod[21] proposed that the accumulation of galactosides in *E. coli* was mediated by a stereospecific entry mechanism which was catalyzed by a "permease". This accumulated galactoside leaked from the cell by non-specific simple diffusion; the final intracellular concentration being determined by the ratio of the rate of entry to the rate of exit of the galactoside.

Refinements to the scheme of non-specific outflow were suggested when Horecker *et al.*[18] and Kepes[22] formulated models where an inducible exit reaction of facilitated diffusion was activated by intracellular sugars. Other amendments included an *E. coli* mutant lacking an exit mechanism for galactosides[23] and two reports in which the authors proposed that exit of glucose from bacteria required energy[24,25].

The coupling of energy to transport in bacteria has been studied by Wilson and co-workers[26,27], who stated that the apparent K_m for efflux was decreased when energy was coupled to uptake to provide the establishment and maintenance of a concentration gradient. This feedback type mechanism was proposed earlier by Koch[28], who hypothesized that metabolic energy decreased the affinity of the carrier for intracellular galactoside.

Comparison of the parameters of biotin influx and efflux in *S. cerevisiae* (JB 112) helps focus attention to the contrasts between the unidirectional fluxes (Table V).

TABLE V

PARAMETERS OF BIOTIN UPTAKE IN THREE ORGANISMS

Parameters	*L. plantarum*	*S. cerevisiae* (*139*)	*S. cerevisiae* (*JB 112*)
pH optimum	7.0	3.8	4.0
Glucose stimulation	7×	13×	3×
K_m (M)	$3.15 \cdot 10^{-8}$	$3.32 \cdot 10^{-7}$	$8 \cdot 10^{-7}$
Q_{10}	2.58	2	2.29
Temperature optimum (°C)	37	30	35

The contrasts between the two fluxes seem quite marked; biotin uptake in yeast appears to fulfill the criterion of a "pump and leak" mechanism of accumulation with the important addition that a control on influx is imposed by the transinhibition from intracellular biotin. An important question with respect to biotin transport is whether transinhibition of influx can account for the overshoot observation.

Since biotin metabolism in these yeast cannot be detected, one can assume that the intracellular concentration of biotin is governed by the relative rates of influx and efflux. We have shown that influx is mediated by a system characteristic of active transport and inhibited by intracellular biotin. Efflux appears to be limited by diffusion only. We feel, therefore, that a rapid influx of biotin is followed by inhibition of this influx accompanied by efflux that depends on the intracellular concentration accumulated. A more difficult observation to explain is that the transinhibition appears to be time-dependent—the longer biotin is in the intracellular pool the greater is the transinhibition. Whether a small amount of biotin is metabolized to a species that is responsible for transinhibition or whether biotin interacts directly with the biotin transport system cannot be detected.

Reports of direct feedback inhibition by the transported substance itself are found in the literature. Cummins and Mitchison[29] demonstrated a non-competitive inhibition of adenine uptake into yeast cells by intracellular adenine. Choline-*O*-sulfate and cysteine acted as feedback inhibitors of choline-*O*-sulfate uptake in cells of *Penicillium notatum*[30]. Grenson *et al.*[31] reported that large pools of amino acids inhibited further accumulation of these amino acids into yeast cells. In a series of studies, Ring, Gross and Heinz[32,33] showed that uptake of amino acids in *Streptomyces hydrogenans* was inhibited in cells preloaded with a neutral amino acid. These examples of transinhibition were not accompanied by an observation of overshoot. Perhaps these systems include an efflux mechanism different from that observed in this report.

An interesting comparison can be made of the parameters of biotin uptake in the three organisms studied by this laboratory. The data (Table V) demonstrate the remarkable similarities among the transport systems. That a transport system was conserved throughout evolutionary processes suggests the importance of biotin in the life of these cells. In addition, an argument exists for the importance of a mechanism that rids the cell of excess accumulated biotin.

The growth of several organisms including *Rhizobium*[34], *Clostridium*[35] and *Neurospora*[36] is inhibited under certain conditions by concentrations of biotin in the growth menstruum greater than that required for maximum growth. Over the past

few years work in this laboratory has demonstrated numerous controls on the synthesis[37], accumulation[38,39], excretion[11], and degradation[40] of biotin by various microorganisms. All of these mechanisms presumably work to ensure different cells that the intracellular biotin concentration does not exceed certain levels. If intracellular biotin were toxic in some way and if a transport system with high affinity for biotin were present in the cells, then a mechanism to rid the cell of this accumulated biotin would be necessary. According to this view the overshoot of biotin uptake in the yeast, *S. cerevisiae* (JB 112), is the manifestation of the mechanism which rids the cell of excess biotin.

ACKNOWLEDGEMENT

This investigation was supported in part by a grant from the National Science Foundation (GB 8087).

REFERENCES

1 A. C. Burton, *J. Cell. Comp. Physiol.*, 14 (1939) 327.
2 A. Rothstein and M. Bruce, *J. Cell. Comp. Physiol.*, 51 (1958) 145.
3 H. Tristam and S. Neale, *J. Gen. Microbiol.*, 50 (1968) 121.
4 J. A. Hamilton, *J. Gen. Microbiol.*, 50 (1968) 441.
5 D. Sanders and F. R. Leach, *Biochim. Biophys. Acta*, 82 (1964) 41.
6 J. Dreyfuss and A. B. Pardee, *J. Bacteriol.*, 91 (1966) 2275.
7 T. O. Rogers and H. C. Lichstein, *J. Bacteriol.*, 100 (1969) 557.
8 R. Hertz, *Proc. Soc. Exp. Biol. Med.*, 52 (1943) 15.
9 G. A. Bray, *Anal. Biochem.*, 1 (1960) 279.
10 L. D. Wright and H. R. Skeggs, *Proc. Soc. Exp. Biol. Med.*, 56 (1944) 95.
11 J. Birnbaum, C. H. Pai and H. C. Lichstein, *J. Bacteriol.*, 94 (1967) 1846.
12 L. D. Wright, E. L. Cresson and C. A. Driscoll, *Proc. Soc. Exp. Biol. Med.*, 86 (1954) 480.
13 W. S. Chang and W. H. Peterson, *J. Bacteriol.*, 58 (1949) 33.
14 L. H. Leonian and V. G. Lilly, *J. Bacteriol.*, 49 (1945) 291.
15 V. P. Cirillo, *Ann. Rev. Microbiol.*, 15 (1961) 197.
16 H. Okada and H. O. Halvorson, *Biochim. Biophys. Acta*, 82 (1964) 547.
17 B. D. Davis, R. Dulbecco, H. N. Eisen, H. S. Ginsberg and W. B. Wood, *Microbiology*, Harper and Row, New York, 1967, p. 1445.
18 B. L. Horecker, J. Thomas and J. Monod, *J. Biol. Chem.*, 235 (1960) 1586.
19 M. R. Siegel and H. G. Sisler, *Biochim. Biophys. Acta*, 87 (1964) 83.
20 W. D. Stein, *The Movement of Molecules Across Cell Membranes*, Academic Press, New York, 1967, p. 351.
21 G. N. Cohen and J. Monod, *Bacteriol. Rev.*, 21 (1957) 169.
22 A. Kepes, *Biochim. Biophys. Acta*, 40 (1960) 70.
23 Y. Halpern and M. Lupo, *Biochim. Biophys. Acta*, 126 (1966) 163.
24 P. E. Hoffee, E. Englesburg and F. Lamy, *Biochim. Biophys. Acta*, 79 (1964) 337.
25 H. Wiesmeyer and M. Cohn, *Biochim. Biophys. Acta*, 39 (1960) 440.
26 H. H. Winkler and T. H. Wilson, *J. Biol. Chem.*, 241 (1966) 2200.
27 P. T. S. Wong, E. R. Kashket and T. H. Wilson, *Proc. Natl. Acad. Sci. U.S.*, 65 (1970) 63.
28 A. Koch, *Biochim. Biophys. Acta*, 79 (1964) 177.
29 J. E. Cummins and J. M. Mitchison, *Biochim. Biophys. Acta*, 139 (1967) 108.
30 N. Bellenger, P. Nissen, T. C. Wood and I. H. Segel, *J. Bacteriol.*, 96 (1968) 1574.
31 M. Grenson, M. Crabeel, I. M. Wiame and J. Bechet, *Biochim. Biophys. Acta*, 151 (1968) 204.
32 K. Ring, W. Gross and E. Heinz, *Arch. Biochem. Biophys.*, 137 (1970) 243.
33 K. Ring and E. Heinz, *Biochem. Z.*, 344 (1966) 446.
34 S. G. Murphy and G. H. Elkan, *J. Bacteriol.*, 86 (1963) 884.
35 F. M. Clark and W. R. Mitchell, *Arch. Biochem.*, 3 (1944) 158.
36 A. Nason, N. O. Kaplan and H. A. Olderwurtel, *J. Biol. Chem.*, 201 (1953) 435.
37 C. H. Pai and H. C. Lichstein, *Arch. Biochem. Biophys.*, 114 (1966) 138.
38 T. O. Rogers and H. C. Lichstein, *J. Bacteriol.*, 100 (1969) 565.
39 J. R. Waller and H. C. Lichstein, *J. Bacteriol.*, 90 (1965) 843.
40 R. J. Baumann, Ph.D. Thesis, University of Cincinnati, 1970.