Biochimica et Biophysica Acta, 401 (1975) 285–298

BBA 77049

THE CHARACTERIZATION OF ENERGIZED AND PARTIALLY DE-ENERGIZED (RESPIRATION-INDEPENDENT) β-GALACTOSIDE TRANSPORT INTO *ESCHERICHIA COLI*

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(Received March 3rd, 1975)

SUMMARY

Unidirectional fluxes of [^{14}C]lactose by whole cells of *Escherichia coli* under highly energized and partially de-energized (in the presence of CN^-) conditions are analyzed kinetically.

When the cells are energized, the value for V influx is 0.45 ± 0.01 mM internal concentration increment/s and K_t is 0.26 ± 0.03 mM. At an external concentration of 0.61 mM the steady-state internal concentration is 0.25 M, reached after about 1h. The maximum steady-state concentration ratio is $2 \cdot 10^3$.

The efflux process under these conditions is non-saturable, being linearly dependent upon internal concentration over the range 25–250 mM with a first-order rate constant of $8.8 \pm 0.2 \cdot 10^{-4}$ s^{-1}.

The transport in the presence of CN^- is active, with a maximum concentration ratio (internal concentration/external concentration) of 104, and the uptake is mimicked by anoxia (<70 ppm O_2).

The effects of CN^- are to lower the V for influx and to change the efflux from a non-saturable to a saturable process with a value for K_t (60 mM) intermediate between that for energized efflux (>250 mM) and influx (0.3–0.6 mM), the latter value not changing appreciably. Partial de-energization thus affects both the influx and efflux processes.

INTRODUCTION

Since the original description of the lactose transport system 18 years ago [1], it has been known that the accumulation of intracellular substrate is energy dependent. The net flux of substrate at any time is composed of both influx and efflux. Whether the effects of energy are upon influx only, efflux only, or some combination of the two, the result of energization is an asymmetry in these fluxes. While it is generally

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accepted that the influx process obeys saturation kinetics with values for the concentration of external β-galactoside that yields half-maximal rate, K_t, in the millimolar range, measurements of efflux have yielded conflicting results. The efflux component under various conditions has been reported either to be non-saturable [1-4] or to exhibit a saturable component [5–8].

In the present study we used an assay procedure which would disturb the system as little as possible. The cells were not subjected to appreciable changes in osmotic strength, temperature, or growth conditions, and during the transport assay the bacterial suspension was vigorously aerated and provided with glycerol as an energy source. Under these conditions, both the initial rate of uptake and the steady-state internal concentration of lactose are 5-fold higher than have been previously reported [5] and the cells are capable of retaining solute for a period of hours.

In this paper we show that efflux does not exhibit saturation kinetics even under these conditions of high internal lactose concentration. These high internal concentrations of lactose are dependent on oxidative metabolism and we report here that the effect of both anoxia and cyanide is to markedly decrease, but not eliminate, the internal concentration of lactose that can be accumulated. The effect of this partial de-energization is on both the influx and efflux components of lactose transport.

EXPERIMENTAL PROCEDURE

Chloramphenicol was obtained from Sigma Chemical Corp., [1-^{14}C]lactose was obtained from Amersham/Searle Corp. and had a specific activity of 0.1–1 Ci/mol. All other chemicals were of reagent grade.

Escherichia coli strain ML 308-225 was used exclusively and was grown at 37 °C with shaking in a modification of the medium described by Phares [9] in which the buffer concentrations were changed to 9.9 g Na_2HPO_4 and 4.12 g KH_2PO_4 per l (0.1 M, pH 7) and supplemented with 0.4 % glycerol. The cells were harvested by centrifugation, and washed with the same medium in which the NH_4Cl was replaced with an equimolar concentration of KCl and to which 50 μg per ml chloramphenicol was added (wash medium). The cells were suspended in the wash medium supplemented with 0.4 % glycerol to give a cell density which permitted less than 10 % of the substrate to be transported (usually less than 100 μg dry weight/ml). The suspension was aerated and stirred for at least 30 min at 25 °C prior to the assay. For the experiments with cyanide and in the absence of oxygen, mannose was substituted for glycerol in the transport medium. Control experiments show that uptake in the presence of mannose is identical to that in the presence of glycerol under energized conditions.

The transport assay was carried out essentially according to the method of Stock and Roseman [10] which utilizes a rapid mixing device and fast filtration through glass fiber filters. This method allows samples to be taken as soon as 2 s after the initiation of the reaction. The observations of these authors [10] that the Reeve-Angel glass fiber filters (no. 984H) retain greater than 98 % of the bacteria and that negligible efflux of lactose occurred during the brief filtration period was confirmed. The filters were counted in 5 ml of PCS scintillation fluid (Amersham/Searle Corp.).

It was determined that when this scintillation fluid was used the same counting efficiency was obtained whether the [^{14}C]lactose was free in solution or intracellular. A blank obtained after heating the reaction mixture for 4 min at 100 °C and carrying

out the filtration and counting in an identical manner was subtracted from all of the experimental points.

The bacterial cultures were tested for their inability to utilize lactose by plating on lactose/eosin/methylene blue agar, by *o*-nitrophenylgalactoside hydrolysis, and by identifying the accumulated substrate as lactose according to the method of Winkler and Wilson [5].

The values for kinetic constants were determined from a weighted least squares evaluation of the data [11]. Each point was evaluated by the Q test [12] for spurious data. Unless specified otherwise, all errors reported are standard error of the estimate.

Kinetic Derivations

Net flux of solute across the bacterial membrane is composed of two counterparts, influx and efflux, and is usually measured by monitoring the concentration of internal substrate ($[S_{in}]$) as it proceeds from zero to some steady-state value. The above may be stated mathematically as

$$\frac{d[S_{in}]}{dt} = v_i - v_e \tag{1}$$

where v_i is the influx rate and v_e is the efflux rate.

If it is assumed that influx and efflux are mathematically independent processes, then it may be stated that influx is only dependent upon the external concentration of substrate, $[S_{ex}]$, and that efflux is only dependent upon $[S_{in}]$. This has been called the "independent" type of derivation [13]. Further, if conditions are maintained such that the absolute amount of substrate removed from the external compartment is so small compared to the total amount that the concentration change is negligible then v_i is a constant.

It is well established that the rate of transfer of many substances across a biological membrane depends upon the substrate concentration in a hyperbolic manner, expressed by:

$$v = \frac{V[S]}{K_t + [S]} \tag{2}$$

where V is the maximal rate and K_t is the concentration of solute which yields a rate of $V/2$. Although this is formally similar to the Michaelis-Menten expression, no attempt will be made here to associate V and K_t with any kinetic constants.

If the transfer phenomenon is not saturable the rate of transfer is directly proportional to the substrate concentration and is therefore a first-order process, expressed mathematically as:

$$v = k[S] \tag{3}$$

where k is the first-order rate constant.

With the above considerations, four types of measurements are considered below for the kinetic analysis of the unidirectional fluxes.

Initial time course of uptake. Eqn 1 predicts that, at early times (i.e. low $[S_{in}]$) the uptake curve ($[S_{in}]$ vs time) is linear and passes through the origin, with the slope equal to the rate of influx. Consequently, measurement of the initial, linear rates of

flux at different external concentrations of substrate will yield the dependency of the influx rate upon the external concentration.

Dilution of $[S^s_{in}]$. By this method a suspension of bacteria is first allowed to reach steady state. Upon dilution of the external substrate to a new, lower concentration the efflux rate decreases from a high value to a lower value as $[S_{in}]$ decreases, while the influx rate remains constant (since $[S_{ex}]$ is diluted very quickly and then does not change), and direct measurement of efflux can be made. For this situation, the integration of Eqn 1 with efflux defined by Eqn 3 yields

$$\ln \frac{[S^{s1}_{in}]-[S^{s2}_{in}]}{[S_{in}]-[S^{s2}_{in}]} = kt \tag{4}$$

where $[S^{s\,1}_{in}]$ is the steady-state $[S_{in}]$ before dilution and $[S^{s\,2}_{in}]$ is the steady-state $[S_{in}]$ after dilution.

Although this method utilizes a more direct approach to the determination of efflux kinetics, it can only be used when the amount of radioactivity left in the bacteria after dilution is significant. Thus, if the transport activity is low (e.g. under energy-depleted conditions), the measurement of efflux becomes very difficult, and it is necessary to use other methods.

Steady-state level of accumulation. At steady state, since neither the internal concentration nor the external concentration are changing, the rate of influx must equal the rate of efflux. If the external concentration is maintained constant throughout the time course of the experiment and influx is independent of efflux, then the efflux rate at steady state must equal the initial rate of uptake. Therefore, values of the efflux rate at various internal concentrations can be obtained by simply measuring the initial rate of uptake and the steady-state internal concentration for various external concentrations.

Approach to steady state. As originally pointed out by Kepes [2] and by Horecker et al. [14], if the efflux component is non-saturable and is described by Eqn 3, the integration of Eqn 1 over the time course of the uptake yields

$$\ln ([S^s_{in}]-[S_{in}]) = -kt+\ln[S^s_{in}] \tag{5}$$

where $[S^s_{in}]$ is the steady-state internal concentration. Thus, a plot of $\ln ([S^s_{in}]-[S_{in}])$ vs time will yield a straight line with a slope of $-k$.

Likewise, if efflux is saturable and takes the form of Eqn 2, the integration yields

$$[S^s_{in}]\ln([S^s_{in}]-[S_{in}])+[S_{in}] = -K^e_t \frac{v_i t}{[S^s_{in}]}+([S^s_{in}]-[S_{in}])+(K^e_t+[S^s_{in}])\ln[S^s_{in}] \tag{6}$$

From this equation, a plot of $[S^s_{in}]\ln([S^s_{in}]-[S_{in}])+[S_{in}]$ vs $v_i t/[S^s_{in}]+\ln([S^s_{in}]-[S_{in}])$ will yield a straight line with a slope of $-K^e_t$.

RESULTS AND CONCLUSIONS

As stated above, the bacterial densities which were used for the assay were low enough so that very little lactose was actually transported. Thus, $[S_{ex}]$ changed by very little and so the rate of influx can be assumed to be constant over the time course of uptake.

It has been reported [5, 15] that the rate of influx of galactoside at steady state is identical to the initial rate of uptake, demonstrating that influx is indeed independent of efflux. This assumption was verified here by allowing a suspension of bacteria to reach steady state and then diluting the suspension into medium containing non-radioactive lactose at the same external concentration. The initial, linear loss of radioactivity corresponding to the steady-state efflux rate was found to be 0.24 ± 0.02 mM/s. This compares very well with the value for the initial rate of uptake (determined as described below) of 0.23 ± 0.02 mM/s.

Energized uptake

Time course of uptake. Fig. 1 presents data for the uptake of lactose, illustrating the high steady-state internal concentrations attained and the long times required to reach them under our conditions. Fig. 2 illustrates the abrupt loss of accumulated substrate upon the addition of the uncoupler 2,4-dinitrophenol, even under conditions where the cells are vigorously aerated and are provided with a glycolytic substrate (glycerol). Also shown in this figure is the ability of the cells to retain solute for long periods.

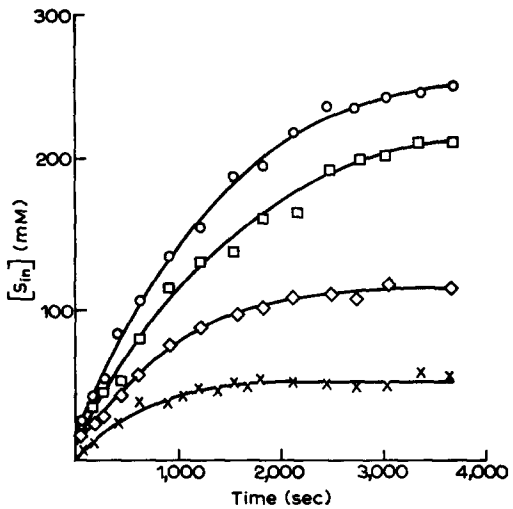

Fig. 1. Transport of [^{14}C]lactose into *E. coli*, assayed as described in the text. The external concentrations are 0.052 mM (×), 0.14 mM (◇), 0.3 mM (□) and 0.61 mM (○).

Influx. For the calculation of the initial rates, data gathered between 3 s and 4 min was approximated by a least squares straight line, and the initial rate was taken to be the slope of this line. In each instance, this initial uptake was linear and the intercept was small. Fig. 3 shows the dependence of initial influx rate upon external concentration of lactose and the weighted least squares line fitted to these data. This figure also shows the data plotted by the method of Hanes [16]. The value for V^i is 0.45 ± 0.02 mM/s and the value for K_t^i is 0.26 ± 0.03 mM.

Efflux. The most direct method for determining the kinetics of efflux is to dilute a suspension of bacteria at steady state and monitor approach to a new, lower value of

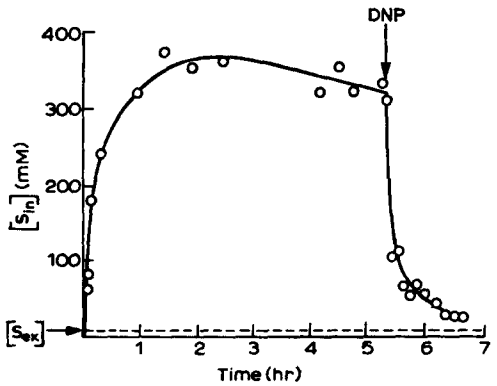

Fig. 2. The effect of 2,4-dinitrophenol on lactose uptake. The external lactose concentration is 4.1 mM. At the time indicated by the arrow sufficient dinitrophenol (DNP) was added to give a final concentration of 1 mM.

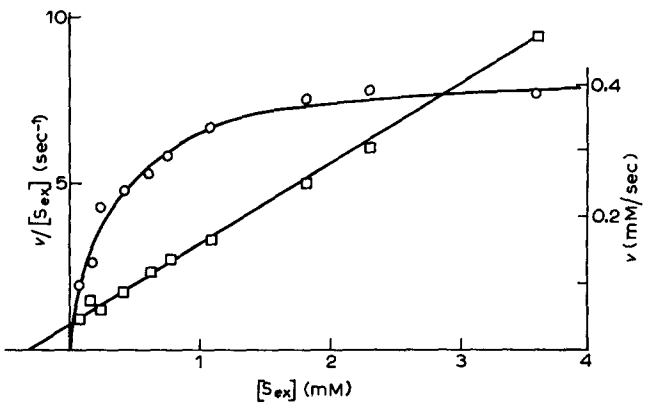

Fig. 3. Determination of V^i and K_t^i for lactose influx while bubbling with air. The upper curve (○) is the initial rate, v_i plotted as a function of the external lactose concentration, $[S_{ex}]$. The straight line (□) is the least squares fit to $v_i/[S_{ex}]$ plotted as a function of $[S_{ex}]$.

$[S_{in}^s]$, as described in the section Kinetic derivations. However, technical difficulties may be encountered when this is attempted due to the low amount of radioactivity which remains after dilution and efflux.

As a result of the high transport activity reported here, however, it was possible to perform this experiment without any special techniques such as cold centrifugation [6]. The results are presented in Fig. 4. Fig. 5 shows the data plotted as dictated by Eqn 4. The plot is clearly linear (the linear correlation coefficient is 0.996), indicating non-saturable efflux with a determined value for k of $9.1 \pm 0.3 \cdot 10^{-4}\ s^{-1}$.

Fig. 6 shows a plot of the initial rate vs steady-state internal concentration, which, as shown in Kinetic derivations, yields the dependence of efflux on $[S_{in}]$. The relation is linear, described by a first-order rate constant of $8.7 \pm 0.9 \cdot 10^{-4}\ s^{-1}$, over an internal concentration range of 50–250 mM. This value agrees well with that found by the more direct method, as described above.

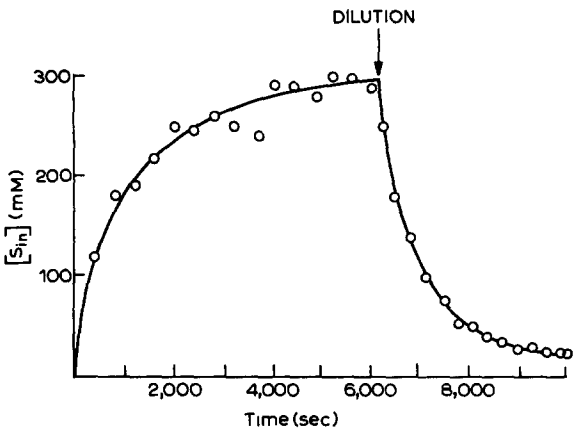

Fig. 4. Measurement of the efflux of lactose upon dilution. Initial external lactose concentration is 0.36 mM; at 6200 s, 0.1 ml of the suspension was added to 1.9 ml medium and samples taken as indicated.

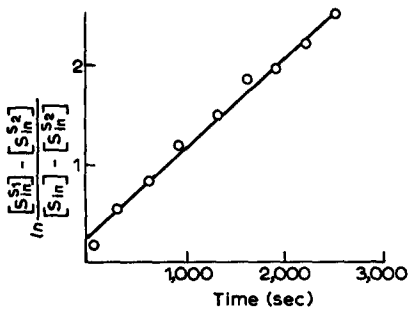

Fig. 5. Determination of k for efflux from dilution. The data are taken from Fig. 4. The value for k from this plot is $9.1 \pm 0.3 \cdot 10^{-4}\ s^{-1}$ and the linear correlation coefficient is 0.996.

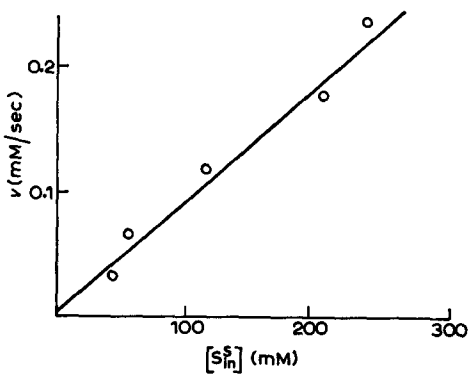

Fig. 6. Initial rate of uptake as a function of the steady-state internal concentration. The intercept of the ordinate is $5.3 \cdot 10^{-3}$ mM/s and the slope is $8.7 \pm 0.9 \cdot 10^{-4}\ s^{-1}$. The linear correlation coefficient is 0.986.

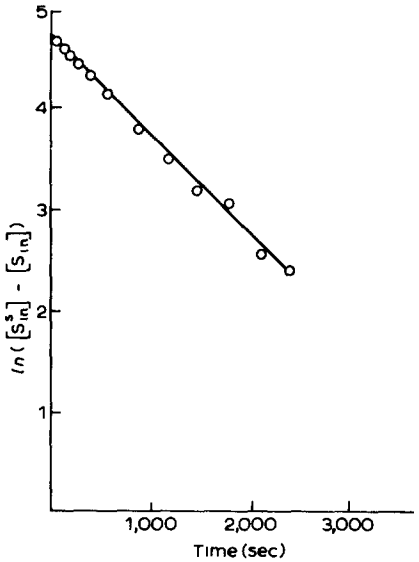

Fig. 7. Determination of k for efflux by the integrated rate equation for non-saturable efflux. The data are taken from Fig. 1, 0.14 mM external concenration of lactose. The value for k from this plot is $10.2 \pm 0.6 \cdot 10^{-4}\ s^{-1}$ and the linear correlation coefficient is 0.998.

Efflux was also analyzed by the measurement of the approach to steady state. Since this method involves evaluating the difference between $[S^s_{in}]$ and $[S_{in}]$, values for $[S_{in}]$ were chosen between $0.1 \times [S^s_{in}]$ and $0.9 \times [S^s_{in}]$. Fig. 7 shows such a determination for one uptake curve. From seven determinations, a value was obtained for k of $8.6 \pm 2.1 \cdot 10^{-4}\ s^{-1}$, also agreeing well with the more direct method.

When data for a transport process exhibiting non-saturable efflux are plotted according to Eqn 6 (that predicted by saturable efflux), a perpendicular line will be obtained, since the slope (the value for K_t^e) approaches infinity. Fig. 8 shows the data of Fig. 7 plotted by Eqn 6, clearly showing that the value for K_t^e is very high.

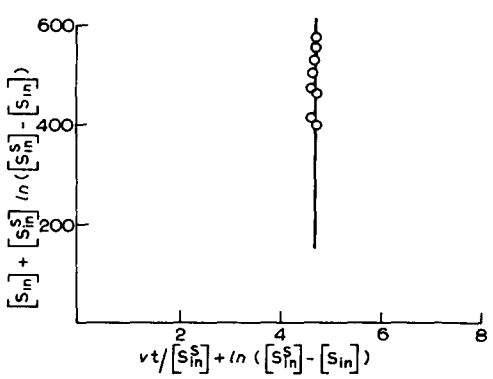

Fig. 8. The data of Fig. 7 plotted according to the integrated rate equation for saturable efflux.

The effects of respiratory inhibition

Time course of uptake. In order to investigate the effects of anoxia on uptake, an assay was performed under anaerobic (<70 ppm O_2) conditions by placing the apparatus in a glove box and flushing extensively with argon. The resulting 80 % inhibition of the initial uptake relative to that with air bubbling is shown in Fig. 9.

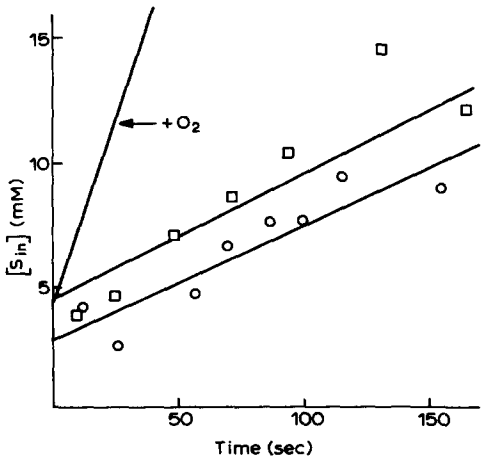

Fig. 9. Time course of oxygen-independent uptake. ○, uptake in the absence of oxygen; □, uptake in the presence of oxygen and 10 mM KCN. The lines are the least squares fit to the data. The line labeled "$+O_2$" is the uptake with air bubbling and without KCN.

Also shown here is the identical phenomenon in the presence of cyanide, which most probably acts by inhibiting cytochromes *o* and a_2 [17]. The characterization of this partially de-energized, oxygen-independent lactose uptake was investigated.

Influx. Data were collected and analyzed as described for energized uptake and the results are presented in Fig. 10. The value for V^i in the presence of 10 mM CN^- is 0.091 ± 0.05 mM/s and for K_t^i is 0.38 ± 0.09 mM compared to the above values for energized (air) uptake of 0.45 ± 0.01 mM/s and 0.26 ± 0.03 mM. Thus, the effect of CN^- on the system reported here is to decrease the value for V^i while not appreciably changing the value for K_t^i.

Efflux. To determine the efflux component of respiration-independent uptake, the integrated rate equations were applied to data collected over the time course of approach to steady state (Fig. 11). The data plotted according to Eqn 5 (non-saturable efflux; presented in Fig. 12) clearly yielded a non-linear relationship, the slope becoming more negative with time.

Eqn 6 can be rearranged to:

$$\ln([S_{in}^s]-[S_{in}]) = -\frac{V^e K_t^e t}{(K_t^e+[S_{in}^s])^2} + \frac{K_t^e+[S_{in}^s]\ln([S_{in}^s]-[S_{in}])}{K_t^e+[S_{in}^s]} \qquad (7)$$

So it is clear that if efflux is saturable, a plot of $\ln [S_{in}^s]-[S_{in}])$ vs t will bend downwards, since the second term is dependent on $\ln ([S_{in}^s]-[S_{in}])$, which will decrease.

When plotted according to Eqn 6 (Fig. 13), the efflux is found to exhibit saturation kinetics, with values for V^e of 0.14 ± 0.01 mM/s and K_t^e of 59 ± 5 mM.

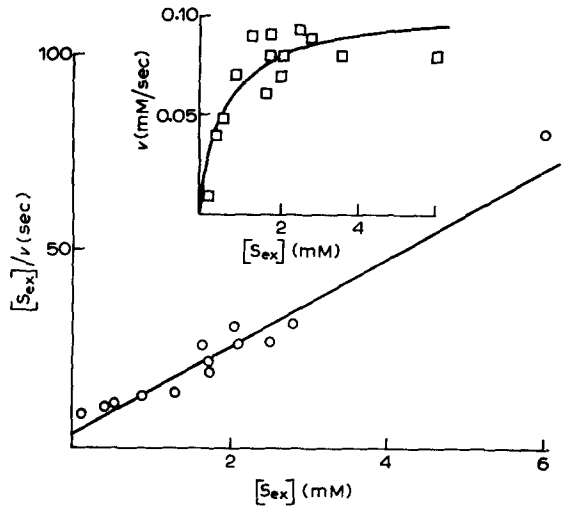

Fig. 10. Determination of V^i and K_t^i for influx in the presence of cyanide. The line (○) is the weighted least squares fit to the data. The linear correlation coefficient is 0.952. The inset (□) is a plot of the initial rate as a function of the external lactose concentration.

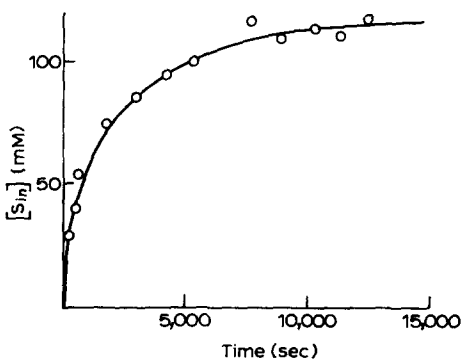

Fig. 11. Uptake of lactose in the presence of cyanide. The external lactose concentration is 2.49 mM and the concentration of KCN is 10 mM.

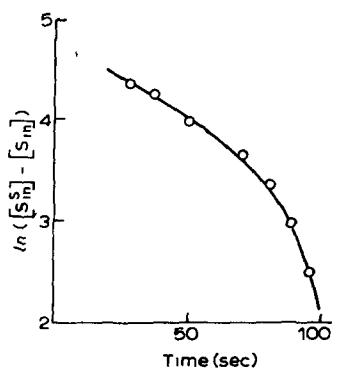

Fig. 12. The data of Fig. 11 plotted according to the integrated rate equation for non-saturable efflux.

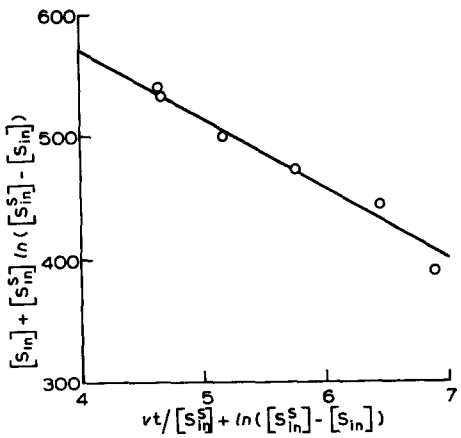

Fig. 13. The data of Fig. 11 plotted according to the integrated rate equation for saturable efflux. The value for V^e is 0.141 ± 0.004 mM/s and the value for K_t^e is 59 ± 5 mM. The linear correlation coefficient is 0.986.

DISCUSSION

Table I presents the determined values for the kinetic constants measured under the conditions reported here.

Bender [18] has advocated the use of the quantity V/K_t for comparing enzyme activities, since it will probably always be a meaningful constant. This number corresponds to the pseudo first-order rate constant k and can be directly related to the magnitude of the unidirectional flux. In addition, it can be shown that the maximum possible transmembrane concentration gradient of substrate (R_m) is equal to the quotient of the value for V/K_t for influx divided by that for efflux.

From Table I it is clear that in our hands partial de-energization by respiratory inhibition both decreases influx (lowering the value for V/K_t from 1.73 s^{-1} to 0.25 s^{-1}) and increases efflux (from $8.8 \cdot 10^{-4}$ s^{-1} to $2.4 \cdot 10^{-3}$ s^{-1}). Specifically, the ef-

TABLE I

THE EFFECTS OF PARTIAL DE-ENERGIZATION ON THE UNIDIRECTIONAL FLUXES

	Air	Cyanide
Influx		
V^i (mM · s^{-1})	0.45 ± 0.01	0.097 ± 0.005
K_t^i (mM)	0.25 ± 0.03	0.38 ± 0.09
V^i/K_t^i (s^{-1})	1.73	0.25
Efflux		
k ($\times 10^4$ s)	8.8 ± 0.2	–
V^e (mM · s^{-1})	–	0.14 ± 0.01
K_t^e (mM)	–	59 ± 5
V^e/K_t^e ($\times 10^4$ s)	8.8	24
R_m	1970	104

flux is changed to a saturable process with a relatively high value for K_t^e, and the value for V^i is decreased while K_t^i remains essentially unchanged. This result is in contradiction to any theory that claims energization acts on one or the other process, perhaps by inducing a 'conformational change' on only one side of the membrane. The results of Fig. 6 clearly show that no matter what value K_t^e may take upon energization, V^e must increase above 0.14 mM/s, thus demonstrating that energy coupling is not simply a matter of increasing the value of K_t^e.

The value of R_m under completely energized conditions is approximately $2 \cdot 10^3$. In terms of the chemiosmotic theory of Mitchell [19, 20], if the system functions as a 1 : 1 symport of a proton with lactose [21], a total proton-motive force of -200 mV would be required, which is similar to reported values [22–24].

The high degree of transport here achieved has allowed the determination of the kinetics of energized efflux over a very great internal concentration range, from 50 to 250 mM. The efflux rate, as determined by several independent methods, was always found to be non-saturable with a first-order rate constant of $8 \cdot 10^{-4}$–$9 \cdot 10^{-4}$ s^{-1} (corresponding to a $t_{1/2}$ of about 14 min and a permeability coefficient (P) of 2×10^{-8} cm $\cdot$ s^{-1}). If the data of Fig. 6 are treated as a saturable process (as in Fig. 3), values are calculated for V^e of 1.14 mM/s and for K_t^e of 1.33 M.

Concerning the nature of the efflux process, it is noteworthy that one of the effects of partial de-energization is to lower the value for K_t^e from that of the energized condition (>1.33 M) to 59 mM. This is still much higher than that for influx, 0.3–0.6 mM, which does not change appreciably. Thus, it is possible that the conflicting results reported by various workers on the saturable character of the efflux process [6] may be due to varying degrees of energization, and the efflux process under completely energized conditions is indeed non-saturable.

In this regard, Maloney and Wilson [8] have recently found that the efflux component of methylthiogalactoside accumulation could best be described by the sum of two fluxes, one saturable and one non-saturable. While it is true that comparisons of transport activity by different strains are hazardous, it is noteworthy that the steady-state level of lactose accumulation by this strain (X9003) was approx. 15 mM for an external concentration of 0.17 mM, reached after approx. 10 min. Under our conditions, with the ML strain lactose is accumulated to approx. 100 mM for an external concentration of 0.14 mM, obtained after 30 min (Fig. 1).

In a detailed paper, Winkler and Wilson [5] reported an analysis of the unidirectional fluxes of lactose by the same strain used here. While the values for K_t^i compare favorably (0.26 ± 0.003 mM compared to 0.6 ± 0.5 mM), the previously reported value for V^i is approx. one-sixth of that reported here (0.45 ± 0.01 mM/s compared to 0.07 ± 0.05 mM/s) and actually agrees well with the value found in the presence of cyanide (0.097 ± 0.005 mM/s). In addition, the efflux component was found to be saturable, with a value for K_t^e of 16 mM, resembling the efflux reported here in the presence of cyanide. While the reported value for V^e was considerably smaller than for that found here in the presence of cyanide (0.002 mM/s compared to 0.14 ± 0.01 mM/s), because of the difficulties inherent in direct efflux measurements the authors considered it to be low. Actually, a value for V^e can be deduced from the data of that paper by the following equation, which holds true at steady state:

$$V^e = \frac{V^i[S_{ex}]\,(K_t^e + [S_{in}])}{[S_{in}]\,(K_t^i + [S_{ex}])} \tag{8}$$

Substituting the reported values ($V^i = 0.07$ mM/s, $K_t^i = 0.6$ mM, $K_t^e = 16$ mM, $[S_{in}^s] = 75$ mM, $[S_{ex}] = 1$ mM) and solving, V^e is found to be 0.053 mM/s, in much better agreement with that reported here for respiration-inhibited conditions.

In this regard, we have found that if the bacterial density is increased to above 600 μg dry weight/ml the uptake resembles very much that for a lower density suspension bubbled with argon (data not shown), leveling off at a much lower steady-state internal concentration at a much earlier time. Thus, it seems to be easy to effect oxygen deprivation and consequent inhibition of transport.

That the energized uptake here is not limited by respiration is indicated by the fact that bubbling the suspension of bacteria with pure oxygen instead of air has essentially no effect on the values for the kinetic constants for either influx or efflux (data not shown).

Finally, it should be emphasized that the fluxes reported here are purely phenomenological, and as such can equally well be used to describe several different models. Thus, for example, one way to interpret the results here would be that the addition of CN^- decreases the number of carriers available for influx (thus, decreasing V^i) while not changing the nature of the influx process itself (thus, not changing K_t^i). Likewise, since there is evidence that at least part of efflux can be catalyzed by the carrier [5, 6], it is reasonable to think that the increased efflux induced by CN^- is due to an increase in the number of carriers catalyzing this flux. The change in the saturation characteristics of efflux upon de-energization could be explained by the existence of two efflux processes. One (non-saturable) would exist under energized conditions, while a saturable component (possibly being the same carriers removed from participation of influx) additionally comes into play as energy is removed.

This possibility cannot be investigated by examining the rate of uptake under partially de-energized conditions, as above. This is because, due to the saturation of the influx process, the internal concentration of lactose in the presence of CN^- never becomes high enough to detect the possible non-saturable component. To further examine this point, we incubated bacteria under energized conditions with lactose until steady state ($[S_{in}^s] = 300$ mM) was reached. CN^- was then added and the initial rate of the induced efflux was found to be 0.33 mM/s (data not shown). Assuming the CN^- acts instantaneously on both fluxes, if the efflux in the presence of cyanide is due only to the saturable process described above, this rate could be no higher than the determined value for V^e, 0.14 mM/s. If the efflux, however, is the sum of both the saturable and non-saturable component, the rate is calculated to be 0.4 mM/s. Thus, it appears that the non-saturable component does indeed exist in the presence of CN^-.

ACKNOWLEDGEMENTS

This investigation was supported in part by United States Public Health Service Research Grant NS 5964 and United States Public Health Service Research Career Development Award NS 23604 (to W.G.S.), and a National Science Foundation Traineeship (to J.R.L.).

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