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QUANTITATIVE ASPECTS OF ACTIVE TRANSPORT BY THE LACTOSE TRANSPORT SYSTEM OF *ESCHERICHIA COLI*

PETER C. MALONEY and T. HASTINGS WILSON

Department of Physiology, Harvard Medical School, Boston, Mass. 02115 (U.S.A.)

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SUMMARY

Accumulation by the lactose transport system of *Escherichia coli* has been measured in cells induced so as to contain increasing numbers of membrane carriers. Carrier activity was assayed both by the rate of *o*-nitrophenyl-β-D-galactopyranoside entry and the initial rate of accumulation of methyl-1-thio-β-D-galactopyranoside. At the steady-state cells with a low number of carriers accumulated considerable amounts of substrate when compared to the fully induced control. This is consistent with the hypothesis that there are two distinct routes of both entry and of exit: a carrier mediated pathway and a diffusion component. When these two factors are evaluated quantitatively they account for the observed relationship between the number of carriers and the steady-state accumulation achieved.

INTRODUCTION

The lactose transport system of *Escherichia coli*, specified by the *Y* gene of the *lac* operon, is capable of promoting the movement of lactose and other galactosides across the membrane permeability barrier. Studies of this system have yielded important information regarding the genetic and biochemical control of active transport in living cells. However, descriptions of the quantitative aspects of the kinetics of entry, accumulation and efflux of galactosides remain incomplete.

In 1956 Rickenberg *et al.*[1] postulated that entry of galactosides into *E. coli* was mediated by a saturable active transport system, and that exit occurred by a diffusion-like process. Herzenberg[2] then demonstrated diffusion kinetics for the entry of *o*-nitrophenyl-β-D-galactopyranoside (ONPG) into transport-negative (Y^-) cells, and concluded that in wild type (Y^+) cells there was normally both a diffusion and a carrier mediated component for entry. The possibility that the exit of galactosides was carrier mediated was first suggested by Kepes[3]. Subsequently, Koch[4] provided strong evidence for the participation of the specific lactose carrier during efflux, although saturation kinetics for exit could not be shown. Winkler and Wilson[5] later demonstrated saturation kinetics for the exit of lactose in normal and metabolically poisoned

Abbreviations: ONPG, *o*-nitrophenyl-β-D-galactopyranoside; TMG, methyl-1-thio-β-D-galactopyranoside; TDG, D-galactopyranosyl-thio-β-D-galactopyranoside.

cells, and showed that in unpoisoned cells the affinity of the carrier for lactose was much lower for the efflux step than for the entry process. Various models have supposed that exit is either entirely diffusion in nature[1,3,6,7] or exclusively carrier mediated[8]. This communication is an attempt to evaluate quantitatively the relative contribution of these two components in galactoside transport. The formulation presented is consistent with several observations which are not readily accommodated by other views on the mechanism of active transport by this system.

MATERIALS AND METHODS

Bacterial strains

Wild type K12 *Escherichia coli* strain CA8000 was used in most of the experiments reported here. The various *lac* promoter mutants used, CA8005, UV5, UV89, N25 and S11 are all derivatives of strain CA8000 and have been described by Silverstone *et al.*[9]. The K12 strain X9003 which was isolated by Epstein[10] carries a deletion in the gene for β-galactosidase, but is otherwise normal for the *lac* operon.

Media and growth conditions

Mineral medium 63[11] was used, supplemented with 0.4% glycerol or 1% Difco-Bacto Tryptone as carbon source, with B_1 present at a final concentration of 4 μg/ml. Cultures were shaken at 37 °C in sidearm flasks, and growth was monitored turbidimetrically using a Klett–Summerson colorimeter (No. 42 filter). A Klett reading of 100 units corresponds to about 220 μg dry weight of cells/ml. For induction of the *lac* operon, isopropyl-1-thio-β-D-galactopyranoside (0.5 mM final concentration) was added to exponentially growing cultures for varying times. To obtain fully induced cultures, cells were grown in the presence of inducer for at least three generations.

Chemicals

Isopropyl-1-thio-β-D-galactopyranoside, methyl-1-thio-β-D-galactopyranoside (TMG), D-galactopyranosyl-thio-β-D-galactopyranoside (TDG) and *o*-nitrophenyl-β-D-galactopyranoside (ONPG) were purchased from Schwarz/Mann Co. Chloramphenicol was obtained from Sigma Chemical Co. [^{14}C]Methyl-1-thio-β-D-galactopyranoside was purchased from New England Nuclear Corp. and [^{14}C]lactose, labelled at carbon 1 of the glucose moiety, was obtained from Nuclear Chicago Corp.; both were purified by paper chromatography using 2-propanol–water (3:1) prior to use.

Assays

At the end of the growth period, chloramphenicol (50 μg/ml final concentration) was added, and the culture placed on ice. Cells were harvested by centrifugation at 4 °C, washed once with cold medium 63 containing chloramphenicol, and resuspended in this same medium to a final cell density of about 500 Klett units.

The rate of entry of ONPG was determined by incubating intact cells with this sugar and measuring the rate of appearance of the yellow product of hydrolysis, *o*-nitrophenol. Washed cells were incubated in the following medium (pH 7.0): 60 mM Na_2HPO_4, 40 mM NaH_2PO_4, 1 mM $MgSO_4$, 10 mM KCl, 50 μg/ml chloramphenicol, 1 mM β-mercaptoethanol and 1 mM ONPG. After incubation at 37°C for a suitable

period of time, three volumes of 0.6 M Na_2CO_3 were added and the concentration of *o*-nitrophenol determined with a Klett–Summerson colorimeter (No. 42 filter). To correct for the non-carrier mediated entry of ONPG parallel assays were carried out in which TDG was present at a concentration of 5 mM to inhibit entry on the carrier. This galactoside has a high affinity for the lactose carrier[1,3] but a low affinity for β-galactosidase[1]. The TDG-sensitive component of ONPG entry was taken as a measure of carrier activity.

β-Galactosidase was assayed using cells pretreated with toluene and deoxycholate[12]. The assay solution was that given above, as recommended by Miller[13]; incubation at 37 °C was terminated by addition of three volumes of 0.6 M Na_2CO_3 and the concentration of *o*-nitrophenol determined.

The transport and accumulation of TMG or lactose were measured in cells pre-equilibrated to 25 °C. At zero time cells were mixed with [^{14}C]TMG or [^{14}C]lactose of known specific activity and at timed intervals, 0.2-ml aliquots, containing approx. 100 μg dry weight of cells, were withdrawn. Cells were separated from the medium by placing the sample on pre-soaked membrane filters (0.6 μm pore size, Matheson-Higgins Co.); this was followed by a wash with 5 ml of medium 63 (25 °C). The total time from sampling until the completion of the wash was about 6 s. Under these washing conditions less than 5% of the intracellular galactoside was lost from the cells. Radioactivity was determined by liquid scintillation counting using Bray's solution[14], and the intracellular concentration of galactoside was calculated assuming that 1 mg dry weight of cells was equivalent to 2.7 μl cell water[5]. Maximal, steady state intracellular concentrations of TMG were attained after 15 min of incubation. A control experiment using both partially and fully induced glycerol grown cells indicated that the capacity to accumulate TMG was not stimulated by the presence of glycerol (0.4%) during the assay.

RESULTS

Assay for membrane carriers

The first objective of this study was the choice of a satisfactory assay for the relative number of membrane carriers. Earlier studies[1,2] had indicated that measurement of the rate of hydrolysis of ONPG by intact cells was suitable in this regard. Transport has been assumed to be the rate limiting step in this assay since in cells made permeable by treatment with toluene, the rate of hydrolysis of ONPG increases by a factor of 10 to 20. To further substantiate the reliability of this method, cells were induced for the *lac* operon so as to contain varying numbers of carriers, and the levels of ONPG entry compared with the initial rates of entry of the non-metabolizable galactoside TMG. As shown in Fig. 1, there was a linear correlation between these two types of carrier assays, at all levels of *lac* operon expression.

Relationship between number of carriers and accumulation of substrate

Having established that the ONPG entry assay measured the relative number of membrane carriers, it was possible to study the relationship between the capacity of the transport system to accumulate substrate and the number of carriers present. In the first experiment, a series of promoter mutants was examined. Fully induced levels of *lac* operon expression in these strains varies from 5% to 60% of the wild type

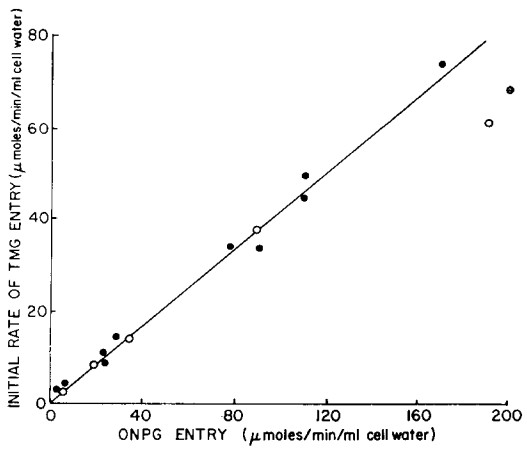

Fig. 1. Correlation between carrier mediated entry of ONPG and initial rate of entry of TMG. Cells grown with glycerol as carbon source were induced for varying times, harvested and assayed. In these experiments the level of carrier mediated ONPG entry in fully induced cells varied between 160 and 200 μmoles/min per ml cell water. The initial rate of entry of TMG was calculated from the intracellular concentration of TMG after 15 (●) or 30 s (○) incubation with substrate, after correction for the nonspecific uptake found in uninduced cells. In the assays of TMG entry, two different external substrate concentrations were used (0.1 mM and 0.28 mM). The initial rate of TMG entry is expressed as the maximal velocity calculated from the known $K_{t(entry)}$ value (0.8 mM) and the external concentrations used.

level because of mutations in the promoter region of the operon[9]. The data given in Fig. 2A demonstrate a linear correlation between the activity of the lactose carrier, as measured by ONPG entry, and the extent of *lac* operon expression, as determined by levels of β-galactosidase. Cells possessing only a fraction of the maximal number

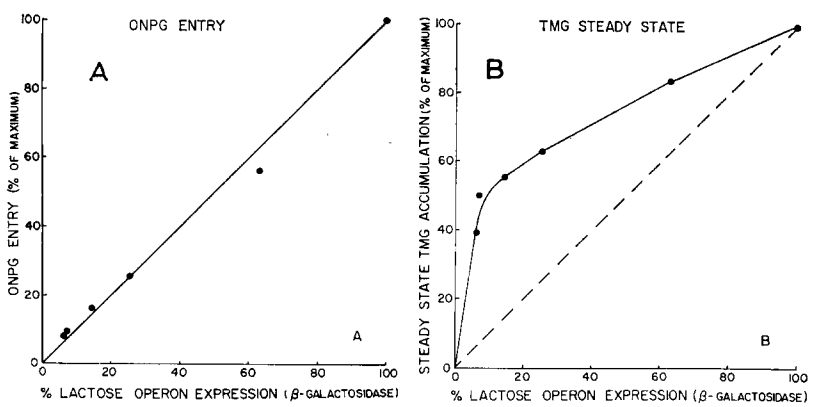

Fig. 2. Carrier mediated ONPG entry and steady state TMG accumulation in fully induced *lac* promoter mutants. Cells grown with tryptone as carbon source were induced for four generations before harvesting. For each strain and each assay, results are given as a percentage of the value found using the wild type parental strain CA8000. For this strain the level of carrier mediated ONPG entry was 212 μmoles/min per ml cell water; for the external concentration of TMG used (0.24 mM), the steady state level of TMG accumulation was 21.5 mM. The promoter mutants used (in order of increasing β-galactosidase content) were CA8005, S11, UV89, N25 and UV5. A: carrier mediated ONPG entry. B: TMG accumulation.

of carriers were capable of accumulating TMG to nearly maximal levels at the steady state. This is clearly shown in the experiment using the promoter mutants (Fig. 2B). For example, cells which had only 8% of the normal fully induced number of carriers accumulated TMG to about 40% of the level found in wild type cells.

This observation was explored in more detail in an experiment in which the kinetics of accumulation of TMG were measured in partially induced cells of the wild type strain (Fig. 3). Assays of ONPG entry and TMG accumulation indicated that when only small numbers of carriers were induced, cells were capable of accumulating considerable amounts of sugar. For example, cells with 3% carriers concentrated TMG to about 15% of the level found in fully induced cells; similar behavior was found for cells containing intermediate numbers of carriers. This experiment demonstrates that under conditions where this difference is seen, the initial rates of entry of TMG (30-s points) correlate well with the number of carriers present (see Fig. 1, open circles). It will be shown in the following section that these results are predicted by a simple model for the lactose transport system.

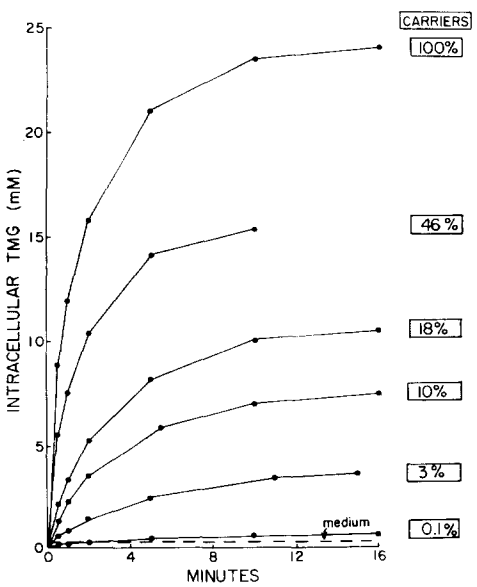

Fig. 3. TMG accumulation in cells with different numbers of membrane carriers. Cells of strain CA8000 grown with glycerol as carbon source were induced for varying times before harvesting for assay. The number of membrane carriers (see insets) was obtained from the level of ONPG entry, relative to the fully induced control (163 μmoles/min per ml cell water). The data for uninduced cells are included (0.1% carriers) although the measurement of such low carrier levels with the ONPG entry assay is subject to considerable error. Medium concentration of TMG was 0.28 mM.

When lactose itself was used as a substrate, a difference was also observed between the number of membrane carriers present and the capacity of the transport system to accumulate substrate. In this experiment (Fig. 4) strain X9003, which lacks β-galactosidase, was employed. The partially induced sample contained only 3% of the maximal number of membrane carriers (estimated from the comparison between

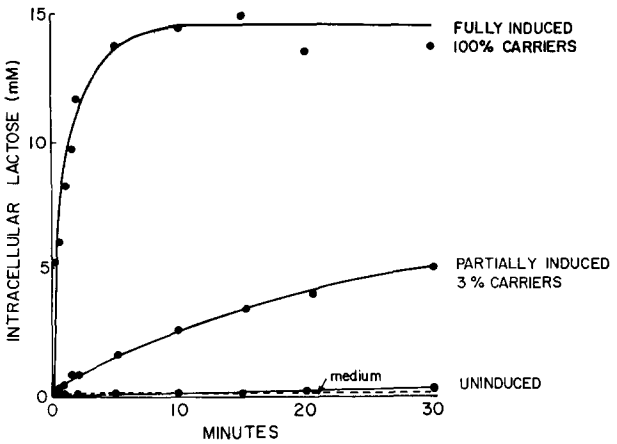

Fig. 4. Lactose accumulation in partially and fully induced cells. Strain X9003 (lacking β-galactosidase) grown in glycerol as carbon source was used. Medium concentration of lactose was 0.17 mM. The partially induced sample was exposed to inducer for 6 min before harvesting.

the initial rates of entry in partially and fully induced cells) while the intracellular concentration of lactose had reached 35% of the level attained in the fully induced control after 30 min of incubation.

THEORETICAL CONSIDERATIONS

Model for accumulation by the lactose transport system

Both entry and exit of lactose and related galactosides are viewed as occurring by way of two independent pathways. One pathway, represented by the specific lactose carrier, displays saturation kinetics, whereas the second pathway, which is independent of the lactose carrier, can be characterized by diffusion-like kinetics. This latter pathway is present in transport-negative cells and in uninduced cells in which the basal level of lactose carrier activity is blocked. This component will be designated "diffusion" although it is realized that it may result from a carrier mediated process with a poor affinity for galactosides.

Representing these two pathways by their simplest formulations, the rate of entry of substrate, $v_{(\text{entry})}$, is given by the sum of two terms, as in Eqn 1. The first term indicates the carrier mediated event, the second gives the contribution by "diffusion".

$$v_{(\text{entry})} = V_{(\text{entry})} \frac{S_0}{S_0 + K_{t(\text{entry})}} + D_{(\text{entry})} S_0 \tag{1}$$

In Eqn 1 $V_{(\text{entry})}$ is the maximal rate of entry of substrate on the carrier, S_0 is the external substrate concentration, $K_{t(\text{entry})}$ is the concentration for half saturation of the carrier mediated entry reaction, and $D_{(\text{entry})}$ is the coefficient which describes the "diffusion" pathway in the inward direction. Similarly, the rate of exit of substrate, $v_{(\text{exit})}$, is given by

$$v_{(\text{exit})} = V_{(\text{exit})} \frac{S_i}{S_i + K_{t(\text{exit})}} + D_{(\text{exit})} S_i \tag{2}$$

where $V_{(exit)}$ is the maximal rate of exit on the carrier, S_i is the internal substrate concentration, $K_{t(exit)}$ is the concentration for half saturation of the exit reaction, and $D_{(exit)}$ is the coefficient which characterizes the "diffusion" pathway for movement in the outward direction. Two assumptions are made. The first is that the coefficients describing the "diffusion" pathways are the same for both the inward and outward movement of substrate (given by D). The second assumption is that the maximal rates of the carrier mediated entry and exit reactions are the same (given by V)*. At the steady state of accumulation, the rate of entry of substrate must be equal to its rate of exit and one may write:

$$V\frac{S_0}{S_0+K_{t(entry)}}+DS_0=V\frac{S_i}{S_i+K_{t(exit)}}+DS_i \tag{3}$$

One method of determining whether this steady state equation adequately represents the actual system would be to compare measured values of S_i (the intracellular concentration of substrate) with those calculated from this equation, using cells with different numbers of carriers (different values of V) and using known values for the remaining variables, S_0, $K_{t(entry)}$, $K_{t(exit)}$ and D. In this present study this kind of comparison has been attempted, using TMG as the model substrate for the transport system. The external substrate concentration, S_0, is fixed for any experiment. The concentration for half saturation of the entry reaction, $K_{t(entry)}$, (0.8 mM TMG) was determined experimentally from the dependence of the initial rate of entry on the external substrate concentration using strain CA8000. Knowing both S_0 and $K_{t(entry)}$, the value of V for any sample could be obtained by calculation from the observed initial rate of entry of TMG. The value of D was determined experimentally for strain CA8000 from the kinetics of entry and equilibration of TMG into uninduced cells in which the low basal level of lactose carrier activity was blocked by the presence of a competitive inhibitor, D-galactopyranosyl-thio-β-D-galactopyranoside. Over the concentration range of TMG which was tested (0.03 mM to 3 mM) these kinetics could be described by a value of D equal to 0.14 μmoles/min per ml cell water for a gradient of 1 mM TMG. The concentration for half saturation of the exit reaction was not directly measured in the work presented here, since measurements of the initial rates of exit from preloaded cells are technically difficult[5]. Instead, a value for $K_{t(exit)}$ of 84 mM was calculated from Eqn 3 using the known values of $K_{t(entry)}$, D, and the experimentally determined values of S_0 (0.24 mM), S_i (21.5 mM) and V (106 μmoles/min per ml cell water) which were measured for the fully induced wild type cells described in Fig. 2. Assuming this value for $K_{t(exit)}$, it was possible to compare the observed steady-state levels of TMG with those calculated from Eqn 3, for cells with different numbers of carriers (Fig. 5). Although the experimental points do not fall precisely on the line, this comparison clearly shows that the model formulated in Eqn 3 predicts the observation that partially induced cells may accumulate substrate to high intracellular levels.

* Exchange diffusion occurs in this system[15], but including terms to accomodate this phenomenon does not alter the form of the steady-state equation.

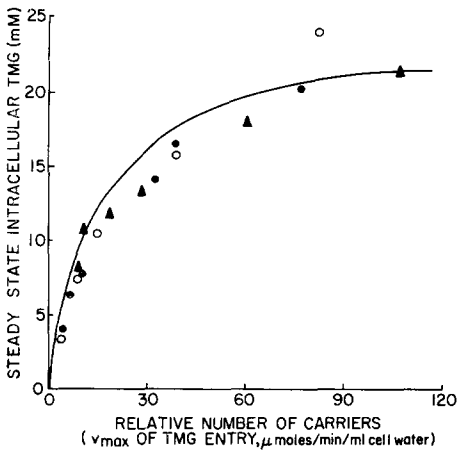

Fig. 5. Relationship between number of membrane carriers and the steady state accumulation of TMG. For each sample the relative number of membrane carriers is indicated by the value of V given, which was calculated from the level of carrier mediated ONPG entry as described in Fig. 1. The points indicate measurements of S_i for each sample: (○), strain CA8000 data from Fig. 3; (●), strain CA8000, experiments similar to the one in Fig. 3; (▲), various fully induced promoter mutants, data from Fig. 2. The line gives the value of S_i calculated from Eqn 3 using the experimentally determined values for S_0, V, D and $K_{t(entry)}$; the value of $K_{t(exit)}$ was assumed to be equal to 84 mM (see text).

DISCUSSION

A common assay for membrane transport activity is the measurement of the degree to which a substrate can be accumulated within a cell. Such "steady state" assays have been frequently utilized to study the changes in membrane carrier activity which occur under different experimental circumstances. A direct relationship between the number of functional carriers and the steady state level of accumulation would be expected if exit from the cell occurred only by diffusion. However, the experiments presented here indicate that this is not a valid assumption for the lactose transport system of *E. coli* since two components for efflux can be distinguished —a carrier mediated pathway, and a "diffusion" pathway.

As first noted by Koch[4], the presence of a relatively few carriers can give rise to a high steady state level of galactosides such as TMG. A model which can account for the difference between the number of carriers and the capacity for accumulation is described by Eqn 3. According to this model the steady state of accumulation depends upon the relative contribution of a carrier mediated and a "diffusion" component. This, in turn, is dependent upon the number of carriers present. This model represents a compromise between the view that exit occurs by "diffusion" alone, and that which assumes exit is entirely carrier mediated. If exit were by way of "diffusion" alone (and assuming that $S_i \gg S_0$), simplification of Eqn 3 shows that the capacity to accumulate substrate (S_i/S_0) would be linearly dependent on the number of carriers. If, however, there were no "diffusion" pathway, the capacity to accumulate would be determined solely by the ratio $K_{t(exit)}/K_{t(entry)}$ and would be independent of the number of carriers. As shown in Fig. 5, the behavior of the real system is intermediate between these two limits, indicating that for TMG accumula-

tion both pathways make significant contributions in determining the final steady state level of substrate within the cell.

According to the model which is presented here the contribution of "diffusion" to efflux varies with the number of membrane carriers present (Fig. 6). Thus, with about 5% of the fully induced level of carriers, the "diffusion" component of exit accounts for about 75% of the total efflux at the steady state, whereas in fully induced cells (100% carriers) the "diffusion" component accounts for less than 20% of efflux. The contribution of the "diffusion" pathway to entry is minimal with the concentrations usually employed.

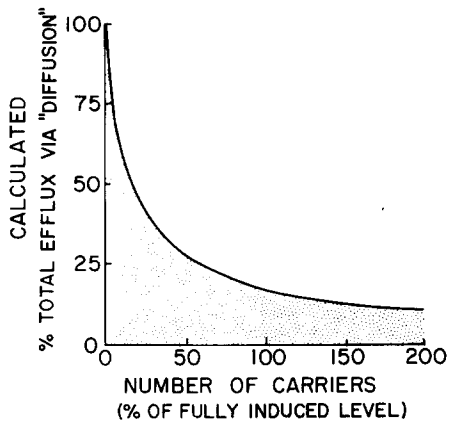

Fig. 6. Calculated efflux by "diffusion" at the steady state in cells with different numbers of membrane carriers. Eqn 3 was used to calculate the internal substrate concentration at steady state (S_i) for assumed V levels of 1 to 200 μmoles/min per ml cell water. Fully induced cells were assumed to have a V of 100 μmoles/min per ml cell water, similar to the levels actually found (see Fig. 1). The external substrate concentration (S_0) was taken as 1 mM. The values of D, $K_{t(entry)}$ and $K_{t(exit)}$ are given in the text. From the calculated values of S_i the fraction of the total efflux resulting from "diffusion" was determined using Eqn 2 (shaded area).

One would predict from this model that the difference observed between the number of membrane carriers present and the capacity to accumulate would become more striking for a substrate having a "diffusion" coefficient less than that of TMG. To test this prediction, the accumulation of lactose was measured in partially induced cells, since the reported "diffusion" coefficient for this sugar[5] is at least 10-fold lower than that measured here for its analogue TMG. A comparison of the data in Fig. 3 and Fig. 4 indicates that this prediction is fulfilled, since for cells containing 3% carriers, TMG is accumulated at the steady state to about 15% of the maximum found in fully induced cells, whereas lactose is accumulated to at least 35% of the maximum (note that lactose accumulation in partially induced cells had not yet reached its steady state by the time the experiment was terminated).

A further prediction of the formulation given here is that as the number of carriers increases beyond the normal fully induced level the contribution of "diffusion" to efflux (and entry) diminishes (Fig. 6). Thus, in cells with more than normal numbers of carriers the behavior of the transport system would approach the limit in which the steady state level of substrate is independent of the number of carriers. This is illustrated in Fig. 7, which shows the steady state level of TMG in cells with different

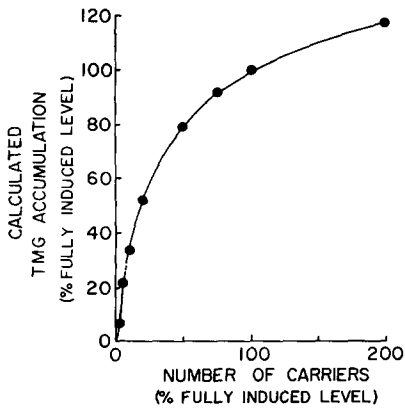

Fig. 7. Predicted steady state levels of TMG in cells with different numbers of membrane carriers. See legend to Fig. 6 for details of calculations. Levels of TMG in cells are given as a percentage of the level calculated for fully induced cells.

numbers of carriers, relative to the level in fully induced cells. The model predicts that cells with two copies of the *lac* operon (200% carriers) would show an accumulation of substrate only slightly increased over that found in normal fully induced cells (100% carriers). This is consistent with earlier observations[16,17] that cells with abnormally high numbers of membrane carriers do not show a parallel increase in their capacity to accumulate TMG.

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