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How Close to the Theoretical Diffusion Limit do Bacterial Uptake Systems Function?

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Abstract. Using a 10 cm flow-through cuvette in a high precision spectrophotometer linked to a mini-computer, the growth rate dependence of *Escherichia coli* on glucose concentration has been studied. The specific growth rate vs bacterial mass of single cultures consuming small amounts of glucose was followed. The data were analyzed with the computer programs described previously. For neither batch nor chemostat-cultured organisms did growth follow the Monod growth law. Rather, the growth rate vs residual glucose concentration has an almost abrupt change in slope, indicative of a passive diffusion barrier prior to an uptake system possessing hyperbolic dependency. Calculations showed that the diffusion through the outer membrane via the porin channels could quantitatively account for the deviations from hyperbolic dependency. Long term chemostat culture alters the bacteria so that the maximum specific growth rate is reduced, but the initial dependence on glucose concentration is increased approaching more closely the theoretical limit. Therefore there was both a change in the outer membrane channels and the uptake activity of the cytoplasmic membrane.

Key words: *Escherichia coli* – Growth rate – Low nutrient concentration – Precision measurement – Diffusion-limited growth – Theoretical maximum growth

Over a period of years, workers in this laboratory have developed a mini-computer linked spectrophotometer system which is capable of accurately, sensitively, and quickly measuring the specific growth rate of bacterial cultures turbidometrically (Wang and Koch 1978; Koch 1979b). These techniques are applied in this paper to cultures which during the course of the measurement depleted the carbon source from the medium. From such data, the dependence of growth rate on carbon source concentration can be inferred. To interpret such data, statistical procedures were developed to distinguish among a range of models for the entry process controlling growth (Koch 1982). The models vary from a series of saturable (enzyme-like) steps for transport into the cell to an array of saturable processes operating in parallel.

For glucose-limited growth in M 9 minimal medium both for a culture previously grown in a low but adequate glucose concentration and for a culture from a slow, long-term chemostat, the best fit was consistent with a two step process: a diffusion limited step followed by an enzyme-like (permease) step. The quantitative fit is here shown to be due to the resistance to flow through the outer membrane via diffusion through the porin channels. The concept that diffusion through the porin channels of small neutral hydrophilic nutrient molecules could restrict the growth of Gram negative bacteria when they are available in the environment in low concentration is examined theoretically.

Materials and Methods

Bacterial Strains and Culture Condition. All experiments were carried out with *Escherichia coli* strain ML 308 and a substrain selected therefrom. Organisms were cultured in the chemostat described in Koch (1971). Cultures were checked for contamination by tested for the constitutive nature of galactoside utilization. This is a useful test since almost no organisms in nature are constitutive for lactose utilization. Growth rates of cells transferred (usually directly from the chemostat) were measured in the long flow-through cell described by Wang and Koch (1978). The turbidity measurements of the Cary model 16 were sampled at a rate of 0.6/s by the Wang model 720C minicomputer, which corrected the values for deviations from Beer's Law. Then the minicomputer regressed the logarithms for data values obtained during a 200 s period and then reinitiates turbidity measurements. The specific growth rate for each 200 s interval together with the estimate of the initial cellular dry weight formed a data pair for FORTRAN IV computer programs that fits the data from a large number of pairs using nonlinear least squares to a variety of models (Koch 1982) on a CDC 6600 computer. The heart of the fitting procedure is a library package sub-routine of IMSL that minimizes the residual sum of the squares (RSS) using the Levenberg-Marquardt alogarithm. The range of models includes empirical and theoretical models for transport and growth. The derivation of the theoretical models has been given previously (Koch 1982).

Results

Growth of Batch Cultures of ML 308 on Glucose

When *Escherichia coli* grows on a carbon source, a part of the carbon source may be converted into other compounds that then may be slowly used after the original source is consumed. If the original carbon source is a low level of glucose (0.02%) strain ML 308 produces very little of these secondary compounds and essentially none at the forty-fold smaller concentration present in the growth chamber (Koch 1979b). The

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experimental evidence to validate this aspect for the present studies is that after the growth rate reaches zero in a run, if the computer measurements of optical density are followed for 10–15 h the turbidity changes are very small. In fact, while making such test, we found how necessary it is to have the air 100% saturated with water vapor. If saturation is not complete, the culture appears to grow slowly, but this artifact was eliminated when additional precautions were made to ensure saturation with water. Studies reported previously (Koch 1979b) also showed that the growth yield was constant at different ambient concentrations of glucose.

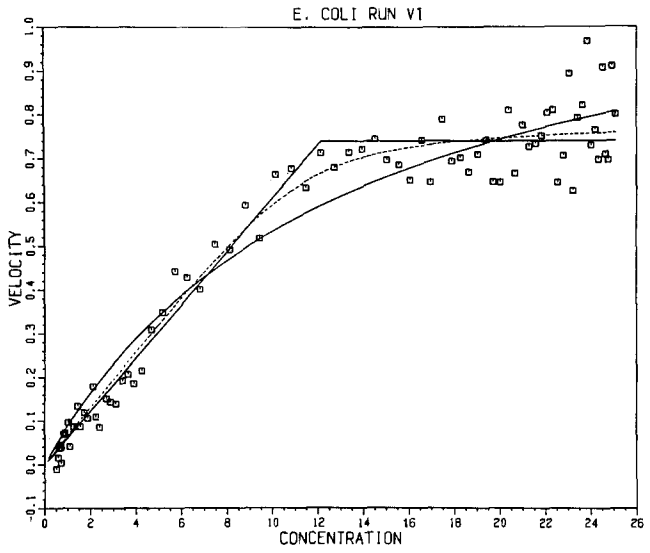

Fig. 1. Growth kinetics of batch grown ML308. Each point (running from right to left) indicates the velocity of growth (specific growth rate, h^{-1}) versus the residual glucose concentration (μM) for successive 200 s intervals. The data have been fitted by a minimization of the squared deviation to three models. The Diffusion: Enzyme model fits best (*dashed line*), see Table 1. This model represents an intermediate case between the hyperbolic (*smooth solid line*) Michaelis-Menten and the bilinear (Blackman) case (*two solid line segments*)

Figure 1 shows the specific growth rate vs glucose concentration data of a single run with 78 data pairs which was analyzed in great detail previously (Koch 1982). There it was fitted to 16 different models. Only three of those are shown in the Fig. 1: the Monod model (e.g., Michaelis-Menten hyperbolic dependence); the Blackman Case (e.g., two straight line splines, one passing through the origin, the second line horizontal); and the Diffusion: Enzyme model (e.g., the model that the outer membrane has a non-carrier mediated passive channels restricting flow, and the nutrient then passes through the cytoplasmic membrane with a carrier [or permease] which has irreversible enzyme [hyperbolic] kinetics).

The former two models represent extremes of the third model (Powell 1967; Koch and Coffman 1970). The parameters of each model best fitting the data are given in Table 1 as are the formulas. As the parameter J rises from zero to infinity, the shape of the curve varies from the Michaelis-Menten to the Blackman Case. [J is equal to V/AP, where V is the maximum velocity, A is area of the cell, and P is the permeability constant (see below).] The best fitting curve is intermediary between the two, but closer to the Blackman Case. Although the Diffusion: Enzyme model provides a better fit, the fit is only very slightly better than that for the Blackman Case.

Growth of Long Term Chemostat Cultured ML308

Figure 2 shows the growth response of bacteria transferred to the growth measuring apparatus from a steady state chemostat culture which had been growing for more than a month with a doubling time of half a day ($D = 0.06\ h^{-1}$). The maximum growth rate is smaller so the experimental errors are larger. We therefore repeated the growth studies six times in a 36 h period. Since there are pipetting errors, different dilution factors, and the growth yield and maintenance energy do vary slightly, we extrapolated to find the value of bacterial dry wt. that corresponded to complete utilization of the glucose for each run by least square linear regression.

Table 1. Least square fit to batch and chemostat cultured *Escherichia coli* ML 308

Model	Batch grown	$\bar{R}^{2a}$	Long-term chemostat culture	$\bar{R}^a$
Monod[d]		0.94251		0.92558
V_{max}: maximum velocity	1.23 h^{-1}		0.536 h^{-1}	
K_m: half-saturating conc.	13.0 μM		0.597 μM	
E_G: efficiency	104 s^{-1}		984 s^{-1}	
Blackman[e]		0.95407		0.91724
a_0': maximum velocity	0.740 h^{-1}		0.422 h^{-1}	
a_1: slope	0.061 $\mu M^{-1}\ h^{-1}$		0.446 $\mu M^{-1}\ h^{-1}$	
Diffusion: Enzyme[f]		0.95663		0.93248
V: maximum velocity	0.779 h^{-1}		0.467 h^{-1}	
K: half-saturating conc.	0.396 μM		0.156 μM	
J: $= V/AP$[c]	11.48 μM		0.630 μM	
E_{G^+}[b]: efficiency	72 s^{-1}		651 s^{-1}	

[a] $\bar{R}^2$ is a quantity that approaches 1 more closely as better fits are obtained. See Koch (1982)

[b] E_G was calculated as indicated in the text. For the Diffusion: Enzyme case K_m is replaced by $K+J$. E_{max} values for glucose at 37° calculated on the assumption that the equivalent radius is 0.8 μm for batch growth and 0.7 μm for chemostat culture are 2800 s^{-1} and 3700 s^{-1}

[c] A is the area of the cell and P is the permeability

[d] Monod equation $v = V_{max}\, S/(K_m + S)$

[e] Blackman equations $v = a_1 S$; $S \leq a_0'/a_1$, and $v = a_0$; $S > a_0'/a_1$

[f] Diffusion: Enzyme equation $v = V(S+K+J)\,(1-[1-4SJ/(S+K+J)^2]^{1/2})/2J$

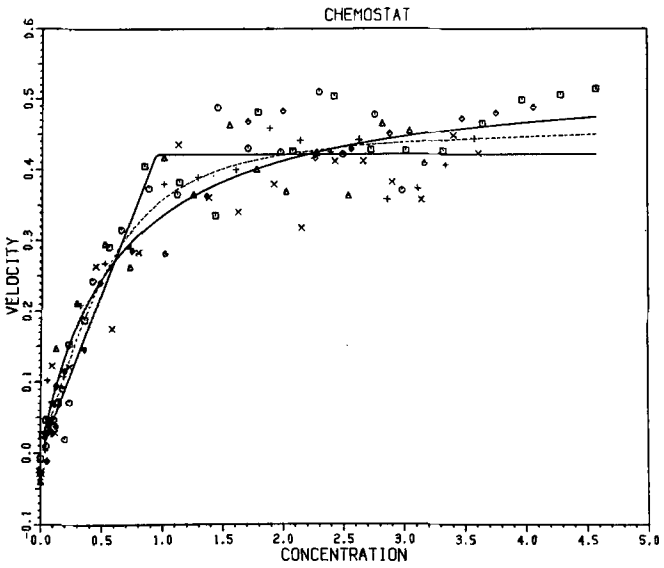

Fig. 2. Growth kinetics of cells cultured in a 11.7 h chemostat for 34 days. The graph is similar to that of Fig. 1, except different runs, are indicated by separate types of symbols. Each run was initiated by taking a sample directly from the chemostat. The Michaelis-Menten and the bilinear models are fitted with solid lines the Diffusion:Enzyme model with a dashed line

These values were additional input to a variation of the computer program previously described (Koch 1982). This revised program calculated the residual glucose concentration from the limits of each run, fitted the curves, and plotted the data. The 108 data points from the six runs are shown in Fig. 2. The best fits to the same three models as for Fig. 1 are also shown. The same general features are apparent for both type cultures, i. e., that a hyperbolic dependency fits less well than the Blackman Case, while the Diffusion:Enzyme model fits best. The fitted parameters for these models are given in Table 1. Also given are the values of $\bar{R}^2$ (see below). This coefficient is closest to 1 for the Diffusion:Enzyme Model; consequently it is the model of choice.

The chemostat culture had been maintained for 34 days at a doubling time of 11.5 h ($D = 0.060\ h^{-1}$). The change in the pattern of growth rate dependence relative to the results of Fig. 1 were probably due to genetic changes. At least they were not reversed by a hundred-fold growth in 0.02% glucose, a low but excess level of glucose. This control experiment was necessary because it had previously been reported (Koch 1979b) that after short term chemostat culture (several days), *E. coli* retains a slow growth character on glucose-minimal (although not on nutrient broth) for several doublings.

Discussion

Ten years ago I published (Koch, 1971) some general considerations about the growth of microorganisms at low nutrient concentrations. A comparison of available experimental data was made against the extreme theoretical model that assumed that any substrate molecule which diffuses up to the bacterial surface would be consumed. The available data at that time only included experiments made with laboratory strains of *Escherichia coli*. The best experimental performance was some fifty-fold poorer that the theoretical diffusion limit. Consequently, I offered a series of conjectures, but felt that this discrepancy was most likely attributable – not to a mechanistic explanation – but to an evolutionary one; namely, *E. coli* in nature has been adapted over hundreds of millions of years to an environment that when limiting for carbon and energy resource was also an environment of high viscosity. Such conditions should apply during growth in the intestines of animals. Calculations were presented at that time that showed that under such conditions the actual uptake systems of *E. coli* for those substances which it can utilize in large amounts, would be evenly matched with the diffusion-limited movement of the molecules through the viscous medium up to the cells. There was no corroborating evidence, it was just that the alternatives seemed less likely.

Critical data in this regard have become available through the experiments of Matin and Veldkamp (1978). Van Es in these workers' laboratory had isolated a *Spirillum* sp. and a *Pseudomonas* sp. from pond water by enrichment in L-lactate-limited chemostat culture at different dilution rates; the spirillum being the better competitor at slower dilutions rates. Matin and Veldkamp estimated the apparent K_m for lactate uptake in two independent ways: from the concentration of free lactate in chemostat cultures, and from the rate of [U^{14}C]-lactate uptake by resting cell suspensions. Although the former measurements depend very critically on the mixing in the chemostat and the speed with which the cells are separated from the culture medium, and the latter measurements depend on the physiological state of the bacteria and the very early kinetics of tracer uptake, the data by the two approaches were consistent. Since the workers also presented size measurements, it was a straightforward matter to calculate the efficiency of uptake for either type of data for comparison with the theoretical maximum as detailed previously (Koch 1971). It is merely a question of computing the first order rate constant from the quotient of V_{max} and K_m and then converting units to s^{-1}. For uptake, Matin and Veldkamp reported $V_{max} = 14.7$ nmol/(mg protein · 30 s) and $K_m = 5.8\ \mu M$. Therefore assuming 85% protein and 27% solids:

$$E_T = [14.7 \times 10^{-9}\ \text{mol/(mg protein} \times 30\ \text{s)}] \times [850\ \text{mg protein/g dry wt.}] \times [0.27\ \text{g dry wt./g wet wt.}] [10^3\ \text{g wet cells/l}]/[5.8 \times 10^6\ \text{mol/l}] = 19.4\ s^{-1}.$$

Similarly E_G, the efficiency from growth measurements, can be estimated from growth data. Matin and Veldkamp reported K_m to be 23 µM determined from lactate level in the chemostat. The maximum growth rate, μ_{max}, was 0.35/h. Therefore:

$$E_G = \mu_{max}/K_m = [0.35/3600\ \text{s}] \times [270\ \text{g dry wt./l}] \times [\text{g lactate/0.38 dry wt.}] \times [\text{mol/90 g lactate}]/[23 \times 10^{-6}\ \text{mol/l}] = 33.4\ s^{-1}.$$

The theoretical maximum efficiency of growth or uptake for an organism depends on the size of the cell and the diffusion constant of the substrate. The equivalent radius of 0.433 µm that can be computed from the measured volume of 0.34 µm^3. A diffusion constant of lactate in dilute aqueous medium at 28° C of 8.90×10^{-6} cm^2/s can be computed from data in physical chemistry handbooks. Therefore:

$$E_{max} = 3D/R^2 = [3 \times 8.90 \times 10^{-6}\ \text{cm}^2\text{/s}]/[0.433 \times 10^{-4}\ \text{cm}]^2 = 14200\ s^{-1}.$$

Thus, it can be seen that the theoretical limit is 733 or 426 times larger than the experimental values for E_T and E_G

respectively. Either discrepancy is, of course, an underestimate because the actual organism is not spherical and has a considerably higher ratio of surface to volume; so that the true E_{max} is larger. The corresponding numbers for the *Pseudomonas* sp. are: $E_T = 3.83/s$; $E_G = 15.4/s$; and $E_{max} = 9250/s$. For reference, $E_G = 54\,s^{-1}$ and $E_{max} = 2800\,s^{-1}$ were the values calculated previously (Koch 1971) for *E. coli* at 37° C while growing on glucose.

E_{max} is largest for the spirillum because it is smallest. It is smallest for *E. coli* because it is the largest of the three, but in addition, its growth substrate, glucose, is larger than lactate and diffuses more slowly. It is thus evident from these comparisons that *E. coli* is actually closer to its diffusion limit in taking up glucose than either oligotroph is at taking up lactate. These differences remain when correction for the difference in growth temperature is made.

Therefore, the important conclusion can be made that both these aquatic organisms isolated by the Groningen group are very far from approaching the physical diffusion limit. It can be believed that these organisms are well adapted to oligotrophy, by virtue of their source and method of enrichment. Consequently the conjecture which I made ten years ago that evolution had developed transport systems for oligotrophs to the level to match diffusion in the environment is negated and hereby retracted. But it will be resurected in a considerably different guise below.

This is the reason that we have carried out the experiments presented here – to look anew for the cause of the discrepancy between the theoretical and actual efficiency. Comparison of the properties of cells taken from the two types of cultures show that the experimental value of E_G calculated from either the Michaelis-Menten fit or from the initial slope of the Diffusion:Enzyme fit increased nine-fold as *E. coli* was cultured in the chemostat under glucose limitation at a slow dilution rate. However, even after 34 days at a doubling time of 11.5 h of selective pressure, E_G was still 3.7 to 5.6-fold less than E_{max}. (Evidence that the new organism from the chemostat was not a contaminant was that it still possessed the lac constitutive character of the original organism. Moreover, contaminating organisms even if they were oligotrophs like those studied by Matin and Veldkamp would be clearly at a great selective disadvantage relative to the original strain.)

In an experiment not shown, there was essentially no change in the initial slope of the specific growth rate vs glucose concentration plot with cells taken from a chemostat after 3 days of culture. If there were a single mutant cell possessing a nine-fold greater growth rate at the concentration of free glucose in the chemostat, it would increase in proportion to the parental organisms by 4×10^{10}-fold in 50 h. Since the chemostat was operated with a steady state population of 4×10^{10} cells, not even one mutant was initially present nor did a single mutant arise during the first 22 h of chemostat culture. During that time, there were $4 \times 10^{10} \times 0.693 \times 22\,h/11.5\,h = 5 \times 10^{10}$ cell divisions; consequently, the mutation rate to the type isolated from the 34 day chemostat must be less than 2×10^{-9} per generation. This calculation clearly shows that improved ability to grow at low glucose concentration is not an induced facility nor does it arise at mutation rates even as low as that for the development of say, streptomycin resistance.

But an avid facility for glucose uptake did arise during the 34 days of chemostat culture either as a very rare mutant, but perhaps due to a succession of more probable mutations. Let us assume that the mutant organism first developing in the chemostat simply arose as a duplication of the phosphotransferase system (PTS) or that part of it which is limiting for glucose uptake.

Such a cell type should grow at twice the growth rate permitted to the majority of the organism in a chemostat. A mutant which doubles in proportion to the total number once every chemostat doubling time would rapidly take over the population. For the long duration chemostat experiment, where the dilution rate and the total number of cells in the culture vessel were as above, one mutant individual would grow to half of the total population in 16.3 days (or even more rapidly if triplet and higher multiplet gene families arise). Consequently, the duplication rate must be large enough so that this had happened in the previous 18 days of chemostat operation. During that time there were $4 \times 10^{10} \times 0.693 \times 18 \times 24\,h/11.5\,h = 1 \times 10^{12}$ divisions. Therefore it follows that duplications or other mutations that would double or more than double the growth rate under the low glucose concentrations that prevail in the chemostat had a collective mutation rate of 10^{-12} per generation. If a mutation arose directly with the nine-fold advantage which the organisms from the long term chemostat possess, the same type of calculation yield a mutation rate of 2×10^{-13} per generation. Consequently, the mutation rate is bracketed in the range $10^{-9} - 2 \times 10^{-13}$ per generation.

Once a tandem duplicate forms, then further improvement can arise by unequal crossing over yielding triplet genes and these in turn could yield higher order tandem repeats (see Koch 1979a). Certain regions of the chromosome are normally duplicated (see Anderson and Roth 1977; Riley and Anilionis 1978) in one out of 1000 to 10,000 cells. Obviously, the relevant region for the present experiments must be naturally duplicated much less frequently than such regions. In any case, one interpretation is that a duplication was further amplified to give nine or more copies.

Still this is not enough to reach the theoretical level. Consequently, if we assume that mutations giving still higher levels of function of the growth limiting process at low external substrate concentration could occur, then either: 1) such mutations are extremely rare, 2) they do not improve the growth because there is not enough room in the membrane, or 3) they carry with them such deleterious effects that they do not grow faster under chemostat conditions. There are other alternatives because uptake at the cytoplasmic membrane is not the only part of the total transport mechanism where a bottle-neck can occur. For Gram negative organisms, the limitation could result from passage through the outer membrane. Its structure is becoming increasingly well understood (see DiRienzo et al. 1978; Nikaido 1979). The outer membrane consists of two leaflets. The outside half contain lipopolysaccharide of sufficient hydrophilic character to impede hydrophobic substances flowing through the membrane. The inner half is largely phospholipid with little permeability to hydrophilic substances. However, there are channels through the membrane that allow the passage of small molecules (molecular weight less than 600). These porin channels are made of three molecules of matrix protein (porin) according to work of Inouye (1974) and Nakae (1976).

The radius of the pore (0.6 nm) and the number of porin triplets per cell is known from permeability limits and by measurement of the protein content. Even though porin is very abundant (1.1×10^5 porin monomers per cell, Steven et al. 1977), the total pore area on the surface of a bacterium, A_p, is only $4\pi(0.6\,nm)^2 \times 1.1 \times 10^5 = 0.166 \times 10^6\,nm^2$. This is about

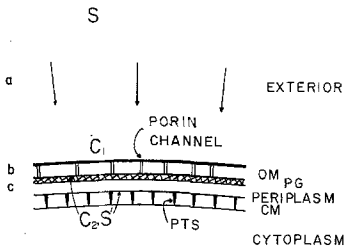

Fig. 3. Diffusion up to the cytoplasmic membrane. This diagram depicts the barriers to diffusion through the medium up to the outer membrane, through the porin channels, through the peptidoglycan, and finally through the periplasm up to the cytoplasm. The diagram is roughly to scale considering *Escherichia coli* as an equivalent sphere. Diffusion through these separate regions follow different physical laws. However all can be written in the form of Fick's law. As shown in the text by eliminating the concentrations at the intermediary regions, C_1 and C_2 the overall transport can be expressed in terms of the bulk concentration S and the concentration at the cytoplasmic membrane S'

1/48th of the total surface area of bacteria (8×10^6 nm^2). Consequently even if the glucose diffused through the pores as it does in bulk solution, transport is slowed 48-fold. But there are two additional considerations that further impede diffusion. These factors were brought together by Renkin (1954). The first factor arises because of the chance that the permeant molecule hits the edges of the pore on its initial approach and may not enter the pore. The second factor arises because diffusion inside a narrow pore is slower than in bulk solution. The resultant drag for this second factor can be computed based on an equation derived by Stokes on the assumptions that the pore is a right circular cylinder and that the molecule behaves as a rigid sphere. Renkin derived an expression that takes both of these factors into account. From his expression, calculated that the correction factor, f, of 7.6×10^{-3} for that glucose with an assumed hydrated radius of 0.42 nm passing through a pore of 0.6 nm radius. This means that a molecule of glucose that diffused into the entrance of a pore would effectively diffuse through it 7.6×10^{-3} times as fast as would a molecule of the same molecular weight but with zero radius. Since D, the diffusion constant of glucose in H_2O at 37°C, is 9.0×10^{-6} cm^2 s^{-1}, glucose would have an effective diffusion constant in the outer membrane of $9.0 \times 10^{-6} \times 7.6 \times 10^{-3}/48 = 1.42 \times 10^{-9}$ cm^2 s^{-1}. Thus, it can be seen that the outer membrane provides a substantial barrier even for small molecules. Of course the membrane is thin ($x = 7.5$ nm) and the thinner it is, the less impediment to transport it would be.

To quantitate the effect, let us rewrite Fick's diffusion law for outer membrane as:

$$dq/dt = (A_p f D/x)(C_1 - C_2) = b(C_1 - C_2), \quad (1)$$

where dq/dt is the flux through the outer membrane driven by the concentration difference $C_1 - C_2$ (see Fig. 3). A_p is the collective area of pores in the entire membrane, f is the Renkin factor, and x is the thickness of the outer membrane. (The quantity in the first set of parentheses in the middle expression is designated b in the last form given.) In terms of the quantities specified above b is 1.514×10^{11} nm^3/s. Taking the total area of the outer membrane to be 8×10^{-6} nm^2, this corresponds to a permeability coefficient for the membrane of 0.189×10^{-2} cm/s. Nikaido (1979) has also made a similar calculation and arrives at a value of 0.37×10^{-2} cm/s. The difference between the two calculations is negligible but due to three causes. I have corrected the diffusion constant of glucose to 37° C. Secondly, he chose x to be 5 nm, and thirdly, he chose the surface area of the bacteria to be 3×10^6 nm^2.

The formulation as given on the right-hand side of equation (1) combining the area and permeability factors is useful when we wish to include in the calculation the effects of the other two transport stages external to the cytoplasm diagrammed in Fig. 3. We can write for diffusion through the bulk solution up to cell:

$$dq/dt = 4\pi RD(S - C_1) = a(S - C_1). \quad (2)$$

This is the Von Smoluchowski equation for diffusion up to an absorbing sphere (see Koch 1960, 1971). S is the concentration in the bulk phase and C_1 is the concentration at the external surface of the outer membrane.

On the other hand, diffusion through the periplasmic space up to the cytoplasmic membrane is governed by Fick's law:

$$dq/dt = (4\pi R^2 D/y)(C_2 - S') = c(C_2 - S'), \quad (3)$$

where y is the thickness of the periplasmic space (7.5 nm). C_2 is the concentration on the internal surface of the outer membrane and S' is the concentration on the external surface of the cytoplasmic membrane. It is assumed that diffusion through this space which contains peptidoglycan and periplasmic proteins would take place at the same rate that it would in free solution. Combining these three equations in such a way as to eliminate C_2 and C_1, we obtain:

$$dq/dt = (1/[1/a + 1/b + 1/c])(S - S'). \quad (4)$$

In this form, it is clear that a series of steps each of which formally obeys an equation of the form of Fick's law is equivalent to a single diffusion step with a coefficient that results by combining the coefficients for the individual steps according to the formula for combining electrical resistances in parallel. To express them as apparent permeabilities, we divide a, b, and c by the total area of the cell. For the case of our idealized *E. coli*, numerical evaluation with the values presented above yields:

$a/A = 1.24 \times 10^{-1}$ s^{-1},

$b/A = 1.89 \times 10^{-3}$ cm s^{-1}, and

$c/A = 1.3 \times 10^{1}$ cm s^{-1}.

In this format, it is clear that diffusion through the periplasmic space (third term) has the greatest permeability and gives the least resistance to flow. Even if the suggestion of Stock et al. (1977) is correct and the periplasmic space is 10% of the cellular volume, it still would be negligible compared to the other terms. Diffusion through the environment external to the outer membrane (first term) has a hundred times larger effect, while the diffusion through the outer membrane through the porin channels (second term) has a further hundred times greater restrictive effect in controlling the overall resistance. In fact, the effect of the external diffusion and the diffusion through the periplasmic space together only cause a 1.30% slowing beyond that due to the outer membrane. Of course, the external environment would be more important if its viscosity were high. Alternatively, the periplasmic space would be more important if the peptidoglycan were closely packed so that diffusion were strongly impeded or under conditions where the periplasmic space is much larger as in cases where plasmolysis has taken place. In what follows we shall assume that the diffusion component of

Table 2. Apparent K_m for growth of wild-type *E. coli* on glucose-minimal medium

Reference	Half-saturating concentration (μM)
Monod (1942)	22
Moser (1958)	40
Jannasch (1968)	44
Koch (1971)	19
Shehata and Marr (1971)	<1
Von Meyenburg (1971)	<1
Bavoil et al. (1977)	3
Dykhuizen (1978)	40
Nikaido and Rosenberg (1981)	6
Koch and Wang (this paper)	13

transport is 1% resistive than that due of the contribution of the outer membrane alone.

In the previous paper (Koch 1982), methods for the statistical analysis of growth data were provided, and the same body of data shown in Fig. 1, i.e., Run VI concerning growth of ML 308 at 37° C in 5 μg/ml glucose, was used as an example, but interpretation of the fitted parameters was deferred to this paper. These fitted parameters will be considered first; then the results of growth experiments from cells from the chemostat will be discussed. The parameters to the standard hyperbolic model of Monod and the preferred Diffusion:Enzyme model are given on the left hand side of Table 1.

First, comment is needed about the K_m value fitted to the Monod model. Previous values from the literature for glucose-limited growth of *E. coli* strains from the literature are given in the Table 2. Large variations have been reported from a high of 44 μM from the work of Jannasch (1968) to a low of below 1 μM reported by von Meyenburg (1971) and by Shehata and Marr (1971). Such a large variability might be ascribed to strain differences. We are inclined to attribute them to the prior growth conditions, to the experimental methods, and degree of adherence to balance growth conditions.

It is to be noted that it was from the intermediary values from Monod's and my laboratory that the efficiency of glucose uptake was originally calculated (Koch 1971). The failure to approach high efficiency would also be adduced by the Monod fit to Run VI data. For the Diffusion:Enzyme model it is the sum of the K and J values of 0.396 μM $+11.48$ μM $= 11.88$ μM and V of 0.779 h^{-1} which are to be utilized in this type of calculation given above. For E_G, 72 s^{-1} is calculated. This is not much different than the Monod fit of 104 s^{-1}, since both approaches approximate the initial slope. However, if the outer membrane were hypothetically stripped off the cell, then only the values for V and K from the Diffusion:Enzyme model would enter the calculation and a higher value for E_G of 623 s^{-1} results. This is much larger (factor of 9) than the previous values, but still 4 to 5 times slower than the theoretical maximum.

Now, this implies that if the outer membrane of the Escherichia cell were striped off without damaging the cell, it would be capable of much faster growth at low substrate concentrations. Thus it can be concluded that wild type batch grown ML 308 does have an excess of transport capacity via the phosphotransferase system in its cytoplasmic membrane than is allowed to function in the presence of the Gram negative envelope at low glucose concentrations. If this same type of calculation is repeated for the long-term chemostat cultured cells from the data of Table 1 a value for E_G of 3280 s^{-1}. This is essentially the diffusion limit, E_{max} $= 3700$ s^{-1}, and it is concluded that the cytoplasmic membrane of this evolved strain have an uptake system of a capability to match diffusion at their cytoplasmic membrane.

The permeability of the outer membrane can be calculated from $J = V/AP$, assuming $A = 131$ cm^2 of bacterial surface per mg of dry weight. The calculation yields P equals 2.0×10^{-3} cm s^{-1} for the batch grown cells of Run VI. Bavoil et al. (1977) have been carried out a similar calculation from their own data in a different, less precise way and obtain 1.8×10^{-3} cm s^{-1}. As noted above a correction of about one percent must be added to correct for the other two diffusion-like steps. Consequently, the corrected value for the permeability of the outer membrane is 2.02×10^{-3} cm s^{-1}, this may be compared with the value for b/A of 1.89×10^{-3} cm s^{-1} calculated from the biophysical considerations of the outer membrane. This would appear to be excellent agreement considering the uncertainty of the precise number of porin molecules and the effective internal dimension of the pores. Consequently, it can be concluded: 1) that after long-term adaptation in chemostat culture, the cytoplasmic membrane of *E. coli* has achieved an efficiency approaching the theoretical; 2) that the porin system provides the resistance to flow that prevents the batch grown *E. coli* from approaching the theoretical maximum; and 3) that long-term cultivation on glucose limitation leads to increased numbers, kinds, or pore diameter of the outer membrane channels in *E. coli*.

Finally, we should note that the fresh water oligotrophs studied by Matin and Veldkamp (1978), even when isolated by chemostat enrichment do not approach the efficiency of *E. coli* grown batch culture, much less that of the chemostat-selected *E. coli*. This apparent failure to adapt to their environment may have important implications to microbial ecology. Possibly the porins in the *Pseudomonas* sp. and *Spirillum* sp. are of smaller radius or are at lower density in the outer membrane. Possibly the inner membrane cannot contain enough of one kind of permease or some other transport function necessary for growth becomes limiting. Possibly negatively charged molecules necessarily move slower through the porins of these organisms or through the cytoplasmic membrane. In any case the present study shows that is possible for the efficiency of uptake to approach the limit set by diffusion in the surrounding medium.

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