Selection and Neutrality in Lactose Operons of *Escherichia coli*

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ABSTRACT

The kinetics of the permeases and β-galactosidases of six lactose operons which had been transduced into a common genetic background from natural isolates of *Escherichia coli* were investigated. The fitnesses conferred by the operons were determined using chemostat competition experiments in which lactose was the sole growth-limiting factor. The cell wall is demonstrated to impose a resistance to the diffusion of galactosides at low substrate concentrations. A steady state model of the flux of lactose through the metabolic pathway (diffusion, uptake and hydrolysis) is shown to be proportional to fitness. This metabolic model is used to explain why an approximately twofold range in activity among the permease alleles confers a 13% range in fitness, whereas a similar range in activity among alleles of the β-galactosidase confers a 0.5% range in fitness. This metabolic model implies that selection need not be maximized when a resource is scarce.

EVER since the discovery of extensive allozyme polymorphisms in natural populations (HARRIS 1966; LEWONTIN and HUBBY 1966) the causes of molecular polymorphism and evolution have been hotly debated. The neutralists regard polymorphism as molecular noise—a balance between the random sampling of unselected alleles reducing the genetic variation and mutation pumping it back into the population. Evolution is a consequence of the chance spread of new mutants through the population. In contrast, the selectionists claim that much, possibly most, molecular evolution has been driven by natural selection, and that polymorphisms are, by and large, maintained by it too.

An important approach has been the experimental determination of the fitness effects of allozymes which are known to have differences in activity or expression. Some experimental studies provide little evidence for the action of natural selection (*e.g.*, see HARTL and DYKHUIZEN 1981, 1984), but others support its presence (*e.g.*, see ZERA, KOEHN and HALL 1985). In either event, there is usually nothing to connect the biochemical differences to fitnesses, except a set of arbitrary speculations, assumptions and hopes. Yet to bridge this gulf is to make considerable progress toward predicting the future course of molecular evolution, neutral or selected.

The challenge, then, is to provide a biochemical model of fitness. To achieve this goal, the ideal experimental system must be well characterized at the levels of molecules, organisms, populations and environment. Moreover, the system must be amenable to manipulation by the experimenter so that rigorous controls can be conducted to confirm or reject any suspected relations.

The lactose operon of *Escherichia coli* inhabiting the simple environment of a chemostat (a continuous culture device) comes close to this experimental ideal. The operon encodes a permease which transports lactose across the cell membrane and a β-galactosidase which hydrolyzes lactose to glucose and galactose. These eventually enter central metabolism as glucose-6-phosphate. Lactose is the sole source of carbon and energy and limits growth-rate in the chemostat (DEAN, DYKHUIZEN and HARTL 1988a, b). Control experiments with glucose are used to determine that only differences among lactose operons affect fitness.

Previous work with this experimental system (DEAN, DYKHUIZEN and HARTL, 1986, 1988a, b; DYKHUIZEN, DEAN and HARTL 1987) used laboratory mutants and modulation of expression of the K12 operon using an artificial inducer which itself could not be metabolized. This work demonstrated that the relations between the activities of both the permease and the β-galactosidase and fitness were concave. When the operon is fully induced, the β-galactosidase resides on top of a fitness plateau whereas the permease resides on the shoulder. Hence, small changes in β-galactosidase activity have little effect on fitness, whereas equivalent changes in permease activity have more pronounced fitness effects. However, the fitnesses conferred by naturally occurring variants of the operon were not investigated. The data were interpreted using a simple metabolic model of KACSER and BURNS (1973, 1981) by assuming that fitness was proportional to the flux of lactose. The analysis suggested

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that steps in the pathway other than the β-galactosidase and permease exert a considerable influence on the flux and hence fitness. The steps responsible for exerting the influence were not identified.

To address these problems, the fitness effects of six naturally occurring lactose operons in *Escherichia coli* are investigated. An exact biochemical model for the lactose flux is constructed and used to demonstrate that fitness is directly proportional to flux. This model can be used to predict which steps in the pathway will be the targets for natural selection as *E. coli* adapts to the chemostat. Together with information on additional phenomena that might impose alternate selection pressures in natural environments, this provides some insight into the evolutionary determinants that might have moulded the activities at each metabolic step in natural populations. The model can be used to predict fitness in chemostats solely from a knowledge of the kinetics at each step. The model enables a partitioning of the contributions of naturally occurring kinetic variation at each enzyme step to fitness. The analysis suggests that the naturally occurring kinetic variation of the lactase activity of the β-galactosidase is unlikely to be subject to intense selection because the allele activities remain on the fitness plateau. In contrast, the naturally occurring kinetic variation of the permease does cause pronounced differences in fitness. Surprisingly, the metabolic model predicts that the higher the concentration of lactose, the more exposed the permease becomes to selection.

MATERIALS AND METHODS

Media, growth conditions and buffers: Minimal medium (DAVIS salts) is 40 mM K_2HPO_4, 15 mM KH_2PO_4, 7.6 mM $(NH_4)_2SO_4$, 1.7 mM trisodium citrate and 1 mM $MgCl_2$ (pH 7.3). Rich medium (LB) is, per liter, 10 g NaCl, 10 g Bacto tryptone and 5 g Bacto yeast extract. 15 g/liter of Bacto agar were added for plates. These are supplemented as indicated. Z buffer is 60 mM Na_2HPO_4, 40 mM NaH_2PO_4, 10 mM KCl, 1 mM $MgCl_2$ and 50 mM β-mercaptoethanol (pH 7.3) (MILLER 1972). Potassium phosphate buffer is 60 mM K_2HPO_4, 40 mM KH_2PO_4, 1 mM $MgCl_2$ (pH 7.3). All growth experiments, enzyme assays and galactoside uptake experiments were conducted at 37° unless otherwise specified.

Strains: The strains used in this study are presented in Table 1. Six operons represent three pairs of β-galactosidase electromorphs from six natural worldwide isolates of the *E. coli* Reference Collection (ECOR) (OCHMANN and SELANDER 1984). The various operons derived from the K12 strain are: DD320 which carries a small deletion of the entire *lac* operon; G11 which carries a small deletion of the *lacY* encoded permease; CSH64 which has a "wild-type" K12 operon; JL3300 which has constitutive K12 operon (it carries a mutation inactivating the *lacI* encoded repressor) that is otherwise "wild type."

All studies were conducted on operons which had been moved into the DD320 background using the generalized transducing phage P1 (*cml clr100*) according to the methods of MILLER (1972). The selection was for growth on minimal medium plates containing 0.2 g/liter of lactose. Constitutive operons of strains TD9 through TD63 were isolated as described in MILLER (1972) using minimal medium containing 0.2% (w/v) phenylgalactopyranoside as a carbon source and in the absence of an inducer. These operons were again transduced into DD320 for competition experiments.

Competition experiments: Competition experiments were conducted according to the methods described by DYKHUIZEN and DAVIES (1980), DYKHUIZEN and HARTL (1980, 1983), and DEAN, DYKHUIZEN and HARTL (1986, 1988a). The medium was Davis salts containing 0.01% (w/v) of a carbon source (lactose or glucose) which was essential to growth and became the sole resource limiting the rate of growth at steady state (DEAN, DYKHUIZEN and HARTL 1988a).

The fitness effects encoded by the *lac* operons were determined with lactose as the sole growth-rate limiting resource in the presence of 0.1 mM isopropyl 1-thio-β-D-galactopyranoside (IPTG—a nonmetabolizable inducer). Under these conditions, inducible *lac* operons are expressed to the same degree as constitutive ones (DYKHUIZEN and DAVIES 1980; DEAN, DYKHUIZEN and HARTL 1988a). This addition of IPTG is necessary to prevent spurious selection caused by newly arising constitutive mutants from rapidly taking over an otherwise inducible chemostat population. The medium was not supplemented with IPTG if both strains were constitutive. Control experiments were conducted with glucose as a source of carbon and in the absence of IPTG.

Relative fitness was determined as follows. A spontaneous mutation (*fhuA*) conferring resistance to the bacteriophage T5 was isolated from each strain. Competition experiments were conducted between a strain carrying one *lac* operon and the *fhuA* marker and another coisogenic strain carrying a different *lac* operon but which was sensitive to T5. The proportion (R) of the population which was resistant to T5 was monitored by counting colonies formed after plating cells in the presence and absence of excess T5 phage (approximately 10^9 phage per plate) on rich medium. An estimate of the selection coefficient per hour was determined as the slope (s) of the regression line of $\ln(R/(1-R))$ against time. Relative fitness was determined as $w = 1 - sH$ where H was the holding time of the chemostat (approximately 3 hr). The effect upon fitness of the *fhuA* mutation was determined by comparing the absolute values of the selection coefficients obtained from two competition experiments: in the first a strain was resistant to T5 and its competitor was sensitive, and in the second it was sensitive and the competitor was resistant. Resistance to T5 conferred no detectable effect on fitness and so the selection coefficients from replicate experiments were pooled as described by SNEDECOR and COCHRAN (1967). A selection coefficient applies to a strain placed in competition with the common competitor (TD1 or TD2) unless specified otherwise.

Cell extracts: After overnight growth in DAVIS salts with 0.2% (w/v) of sodium succinate and 0.1 mM IPTG, the cells were centrifuged and the supernatant discarded. For studies of *o*-nitrophenyl-β-D-galactopyranoside (ONPG) hydrolysis the pellet was resuspended in the same volume of Z buffer, sonicated when ice cold, centrifuged to remove the cellular debris and the supernatant diluted tenfold with more Z buffer. Potassium phosphate buffer was substituted for studies of lactose hydrolysis.

Hydrolysis of ONPG: An aliquot of 0.1 ml of extract was placed in a cuvette with 0.9 ml of a solution of ONPG in Z buffer and the initial production of ONP was monitored at 420 mm. Typically, duplicate assays were conducted at each of the six concentrations of ONPG used (ranging from 2.2 mM to 0.07 mM). One absorbance unit is equivalent to

TABLE 1

Strains

Strain	Genotype	*lac* operons transduced into DD320	β-Galactosidase mobility	Source
Laboratory strains				
DD320	Δ*lac*			DYKHUIZEN and DAVIES (1980)
G11	*lacI*$^+$*Z*$^+$Δ*Y*			WINKLER and WILSON (1966)
TD1	*lacI*$^+$	CSH 64	?	MILLER (1972)
TD2	*lacI*$^-$	JL 3300	?	LANGRIDGE (1974)
Natural isolates				OCHMAN and SELANDER (1984)
TD9	*lacI*$^+$	ECOR 4	7	Human
TD10	*lacI*$^+$	ECOR 16	2	Leopard
TD11	*lacI*$^+$	ECOR 67	7	Domestic goat
TD13	*lacI*$^+$	ECOR 70	2	Lowland gorilla
TD14	*lacI*$^+$	ECOR 68	5	Giraffe
TD63	*lacI*$^+$	ECOR 45	5	Domestic pig

439 μM ONP. The hydrolysis of ONPG was also investigated in the absence of β-mercaptoethanol.

Hydrolysis of lactose: Lactase activities were determined by continually monitoring the reduction of NAD to NADH at 340 nm by the action of galactose dehydrogenase on the galactose produced. The six concentrations of lactose ranged from 18 mM to 1.125 mM in the presence of 10 mM NAD and 0.01 mg/ml of galactose dehydrogenase. The addition of galactose demonstrated that the coupling enzyme was in at least 200-fold excess. DD320 was used as a control. Estimates of rates of hydrolysis were obtained by assuming that the production of galactose and NADH were equimolar.

ONPG translocation: Cells were grown in chemostats at a dilution rate of approximately 0.28 hr^{-1}. The chemostat medium was Davis salts containing 0.01% (w/v) of sodium succinate which became the sole resource limiting the rate of growth at steady state (DEAN, DYKHUIZEN and HARTL 1988a). The addition of IPTG to a final concentration of 0.01 mM ensured that inducible operons were expressed at constitutive levels (DYKHUIZEN and DAVIES 1980; DEAN, DYKHUIZEN and HARTL 1986, 1988a).

Uptake of ONPG by intact cells was determined by the method of KOCH (1964). 0.9 ml of chemostat grown culture was added to 0.1 ml of an ONPG solution in Davis salts and the production of ONP by intracellular β-galactosidase was monitored. Under these conditions the rate increase in absorbance is proportional to the rate of production of ONP (KOCH 1964). The 15 concentrations of ONPG ranged from 5 mM to 0.039 mM. The increase in absorbance at 420 nm caused by the settling of the cells was estimated in the absence of ONPG. In each experiment strain TD1 was used as a standard.

Facilitated diffusion was investigated by treating cells of strain TD1 using the uncoupler carbonyl cyanide *m*-chlorophenylhydrazone (CCCP) at a concentration of 0.05 mM in the presence of 10 mM EDTA prior to uptake studies. Also, the transport of ONPG was investigated after the cell wall of strain TD10 had been permeabilized by the method of LEIVE (1965). For this study, the entire volume of the chemostat growth chamber was removed and half the culture centrifuged and washed in 2 ml of 100 mM Tris-HCl, pH 7.8. After 2 min in the presence of 0.5 mM EDTA, the cells were centrifuged and resuspended in the original chemostat medium. The remaining half of the culture was used as a control after being centrifuged and resuspended in 2 ml of Davis salts for 2 min, and again centrifuged and resuspended in the remaining medium from the chemostat. Uptake of ONPG was determined as described above.

Lactose translocation: The active uptake of lactose was investigated by standard methods (WINKLER and WILSON 1966; GHAZI and SHECHTER 1981; WRIGHT, REID and OVERATH 1981). 0.5 ml of an overnight culture grown in Davis salts with 0.2% sodium succinate and 0.1 mM IPTG were used to inoculate 50 ml of the same medium and grown with vigorous shaking into mid log-phase. At a density of approximately 10^8 cells/ml, the culture was centrifuged and resuspended in 5 ml of fresh Davis salts with 0.1% (w/v) sodium succinate and 0.005 mg/ml of chloramphenicol, and incubated with gentle vortexing at regular intervals for 45 min. Cells of strain TD2 grown in chemostats with lactose or succinate as the limiting nutrient were also prepared for lactose uptake studies. The entire volume of each 30 ml chemostat was used and the cells concentrated in 1.2 ml after centrifugation.

Aliquots of 0.1 ml of prepared cells were added to 0.01 ml of various concentrations of lactose (from 16 mM to 0.03125 mM) containing [D-glucose-1-^{14}C]lactose at a specific activity of 0.625 Ci/mol. After 10 sec, 0.5 ml of a stop solution was added (100 mM potassium phosphate buffer containing 5 mM cold lactose and 2 mM mercuric chloride). The cells were immediately filtered onto 0.45 μm HA millipore filters and washed with 20 ml of the same stop solution. The filters were air dried for half an hour before being placed in 4.5 ml of aqueous counting solution and the counts determined. Uptake was determined to be linear for at least 15 sec. Strain DD320 was used as a control.

Protein assay and units of activity: For all the above kinetic studies, the amount of cell protein was estimated by the method of BRADFORD (1976) from standard curves prepared using immunoglobulin G. V_{max} units are μM min^{-1} mg^{-1} cell protein and D units are min^{-1} mg^{-1} cell protein.

RESULTS

Competition experiments: All competition experiments were conducted between operons placed in the DD320 genetic background, and each strain carrying an operon from a natural isolate was placed in competition TD1 which carries the K12 operon. Some typical results are illustrated in Figure 1. The absence of detectable levels of selection during competition for glucose demonstrates that the strong selection,

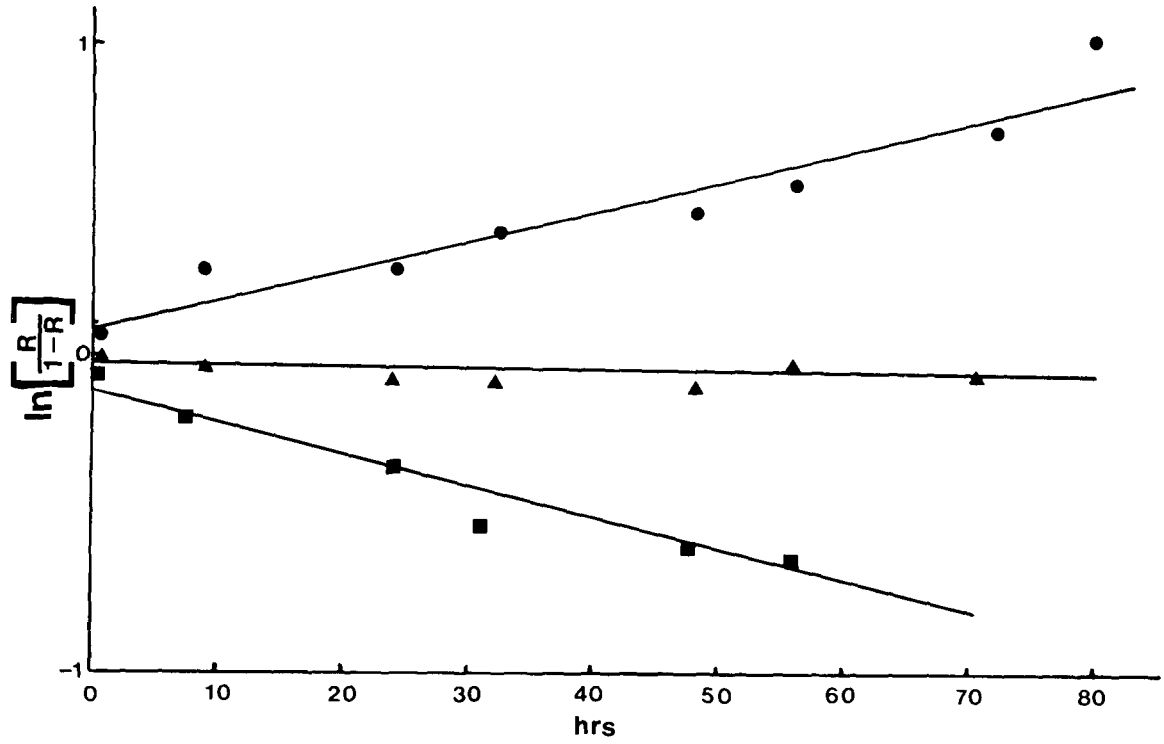

FIGURE 1.—A typical example of selection conferred by an operon from a natural isolate. Lactose limitation–dots TD9.T5R *vs.* TD1 squares TD1.T5R *vs.* TD9: glucose limitation–triangles, TD1.T5R *vs.* TD9.

observed against TD9 when in competition for lactose with TD1, is caused by differences encoded by the lactose operons. The symmetrical selective response, caused by a strain carrying the genetic marker in the first and its competitor carrying it in the second of a pair of competition experiments for lactose, demonstrates that no detectable selective effect is conferred by the *fhuA* marker.

During competition for lactose, four of the six strains (TD9, TD11, TD13 and TD14) were markedly inferior to the control strain TD1, one was selectively neutral within the resolution of the technique (TD63), and one (TD10) was strongly favoured. No evidence of selection was detected during competition for glucose confirming that the observed selection on lactose is due to differences among the lactose operons. Duplicates of strains TD1, TD9, TD10 and TD11 were constructed in parallel with the original ones, but using separate phage stocks, and were placed in lactose-limited competition. The estimated selection coefficient of each replicate was statistically indistinguishable from its respective partner.

A constitutive mutant of each ECOR operon was isolated and placed in competition for limiting lactose with the constitutive K12 operon of strain TD2 in the absence of IPTG. The selection coefficient of each constitutive operon was statistically indistinguishable from its inducible counterpart. This demonstrates that, in the presence of IPTG, the repressors of the ECOR operons do not interact with their operators sufficiently to affect fitness by modifying expression of the operons. The pooled results are presented in Table 2.

The strains selectively inferior to TD1 and TD2 fell into two fitness classes, TD9 with TD14 and TD11 with TD13. Competition experiments demonstrated that any selection between the strains within a fitness class was below the resolution of the technique. The existence of these two fitness classes was further confirmed by a competition experiment between TD13 and TD14. The selection coefficient of 2.4%/generation was not significantly different from expected value of 1.99%/generation (obtained as the difference between the selection coefficients between the fitness classes). Note that β-galactosidases of the same electrophoretic mobility are not associated with the same selection coefficients.

Galactoside hydrolysis: The action of the β-galactosidase of strain K12 toward lactose is complicated by several side reactions of which the synthesis of allolactose, the natural inducer of the *lac* operon, is by far the most significant (HUBER, KURZ and WALLENFELS 1976). During initial rate studies approximately 50% of the lactose utilized is converted into allolactose whilst the rest is hydrolyzed to the monosaccharides glucose and galactose. However allolactose is also an excellent substrate for β-galactosidase (HUBER, KURZ and WALLENFELS 1975). Consequently a marked acceleration is initially detected when monitoring the production of glucose or galactose as the

TABLE 2

Pooled selection coefficients

Strain	Limiting nutrient: Lactose n[a]	Lactose S/gen[b]	Lactose ±SE	Glucose n[a]	Glucose S/gen[b]	Glucose ±SE
Laboratory mutants						
TD1		ND[c]			ND	
TD2 *vs.* TD1	1	−0.0034	±0.0028		ND	
Natural isolates						
TD9 *vs.* TD1	4	−0.0334	±0.0023	2	+0.0045	±0.0078
TD10 *vs.* TD1	4	+0.0782	±0.0038	2	−0.0012	±0.0030
TD11 *vs.* TD1	5	−0.0532	±0.0023	4	−0.0008	±0.0024
TD13 *vs.* TD1	3	−0.0537	±0.0038	2	−0.0035	±0.0043
TD14 *vs.* TD1	4	−0.0338	±0.0029	1	−0.0005	±0.0031
TD63 *vs.* TD1	3	−0.0003	±0.0039	2	+0.0007	±0.0020
TD11 *vs.* TD13	1	+0.0036	±0.0073		ND	
TD13 *vs.* TD14	2	−0.0243	±0.0031		ND	
TD9 *vs.* TD14	2	−0.0024	±0.0039		ND	

[a] n, number of competition experiments.
[b] The selection coefficients apply to the strain on the far left of the table. gen = generation.
[c] ND, not determined.

pool of allolactose approaches a quasi-steady-state. At this point the rate remains constant for at least 10 min.

In growing cells, the rate of production of allolactose should be approximately equal to the rate of its consumption (DEAN, DYKHUIZEN and HARTL 1986). Therefore the estimates of the kinetic constants were based on measurements taken at the quasi-steady-state because these will best reflect hydrolysis of lactose *in vivo*. Despite the fact that both lactose and allolactose were simultaneously hydrolyzed, the data conformed to a Michaelis-Menten expression when analyzed using Eadie-Hofstee plots (these are particularly sensitive to deviations from linearity). An analysis of the kinetic mechanism of HUBER, KURZ and WALLENFELS (1976) has shown that only changes in glucose concentrations can, in principle, cause deviations from linearity (DEAN 1987). Apparently, the affinity of all the β-galactosidases for glucose was sufficiently low that the Michaelis-Menten model provided a satisfactory approximation.

Kinetic estimates for hydrolysis of lactose and ONPG by the β-galactosidases are presented in Table 3. The estimates of V_{max} (μM min^{-1} mg^{-1} cell protein) vary substantially among the strains for both substrates. In contrast, only the β-galactosidases from the natural isolates in TD13 (toward both substrates) and TD63 (toward ONPG alone) have Michaelis constants which are significantly different from the others.

Galactoside translocation: The uptake of ONPG by intact cells was monitored by the production of ONP produced by the action of intracellular β-galactosidase (in the absence of β-mercaptoethanol). No extracellular β-galactosidase activity was detected in the chemostat medium. Strain G11, which carries a deletion of the entire permease and an active β-galactosidase induced by 0.1 mM IPTG, hydrolyzed ONPG very slowly (less than 0.3% of TD1 at 5 mM ONPG). This demonstrated that the cell membrane was relatively impermeable to this galactoside.

Table 4 presents the results of ONPG hydrolysis in the absence of β-mercaptoethanol together with those for the flux to ONP. The activity of each β-galactosidase was sufficiently high that the observed variation in hydrolysis kinetics would have a negligible effect on the flux to ONP (see the section on *Control Theory*). These results must therefore reflect only the translocation kinetics.

The uncoupler, CCCP, strongly inhibits uptake by increasing K_t, the apparent Michaelis constant for translocation (Figure 2). This confirms reports in the literature that the translocation of galactosides by the *lacY* encoded permease is primarily energy dependent rather than by facilitated diffusion (WINKLER and WILSON 1966; PAGE and WEST 1981).

In contrast, lactose uptake by strain TD1 grown under identical conditions is noticeably biphasic (Figure 3). KACZOROWSKI, ROBERTSON and KABACK (1979), ROBERTSON, *et al.* (1980) and GHAZI and SHECHTER (1981) have demonstrated that high K_t component is associated with facilitated diffusion. The difference between the behavior of the permease towards ONPG and lactose is not readily accounted for, but the uptake of ONPG was studied over a much longer time (approximately 3 min) whereas the studies of lactose uptake were possible only for a brief 10 sec. The longer time period might allow the proton motive force to regenerate, thereby accounting for the dif-

TABLE 3

Kinetic parameters of the β-galactosidases

	ONPG[a]				α-Lactose				
		V_{max}		K_m			V_{max}		K_m	
Strain	n^b	(units)[c]	±SE	(mM)	±SE	n^b	(units)[c]	±SE	(mM)	±SE
Laboratory mutants										
TD1	11	11.879	±0.134	0.1737	±0.0042	8	1.0569	±0.0104	2.4216	±0.0102
TD2	3	11.059	±0.276	0.1677	±0.0016	4	0.9901	±0.0324	2.2843	±0.0512
Natural isolates										
TD9	11	10.165	±0.104	0.1659	±0.0029	3	0.9185	±0.0072	2.4173	±0.1303
TD10	8	11.809	±0.246	0.1745	±0.0034	5	1.0569	±0.0227	2.2525	±0.0748
TD11	8	9.485	±0.178	0.1699	±0.0037	5	0.7781	±0.0085	2.4046	±0.0733
TD13	9	7.785	±0.099	0.1388	±0.0019	8	0.8549	±0.0118	3.5274	±0.1026
TD14	6	7.843	±0.169	0.1657	±0.0069	4	0.6821	±0.0261	2.4959	±0.0706
TD63	8	10.845	±0.366	0.1499	±0.0041	4	0.8872	±0.0061	2.3519	±0.1311

[a] β-Mercaptoethanol present.
[b] n, number of independent determinations.
[c] Units, μM of product released/min/mg cell protein.

TABLE 4

Kinetic parameters of galactoside uptake

	Substrate					
	ONPG				Lactose	
	β-Galactosidase[a]		Permease		Permease	
Strain	V_{max} (units)[b]	K_t (mM)	V_{max} (units)[b]	K_t (mM)	V_{max} (units)[b]	K_t (mM)
Laboratory mutants						
TD1	10.026	0.0657	0.8341	0.4621[c]	0.1723	0.0559
TD2	ND	ND	0.8341	0.4621[c]	ND	ND
Natural isolates						
TD9	8.556	0.0672	0.7174	0.6119[d]	0.1379	0.0487
TD10	10.113	0.0511	1.0255	0.2218	0.2436	0.0364
TD11	6.828	0.061	0.7256	0.6472[e]	ND	ND
TD13	6.082	0.364	0.7256	0.6472[e]	ND	ND
TD14	8.609	0.0588	0.7174	0.6119[d]	ND	ND
TD63	9.419	0.0578	0.8341	0.4621[c]	ND	ND

Diffusion constant (ONPG): $D = 17.5784 \times 10^{-4}$ (min^{-1} mg^{-1})[f]
Diffusion constant (lactose): $D = 6.6605 \times 10^{-4}$ (min^{-1} mg^{-1})[g]

[a] β-Mercaptoethanol absent.
[b] Units, μM min^{-1} mg^{-1} cell protein.
[c] Pooled estimate of TD1, TD2, and TD63.
[d] Pooled estimate of TD9, TD14.
[e] Pooled estimate of TD11, TD13.
[f] Pooled estimate of all above strains.
[g] Pooled estimate of TD1, TD9, and TD10.

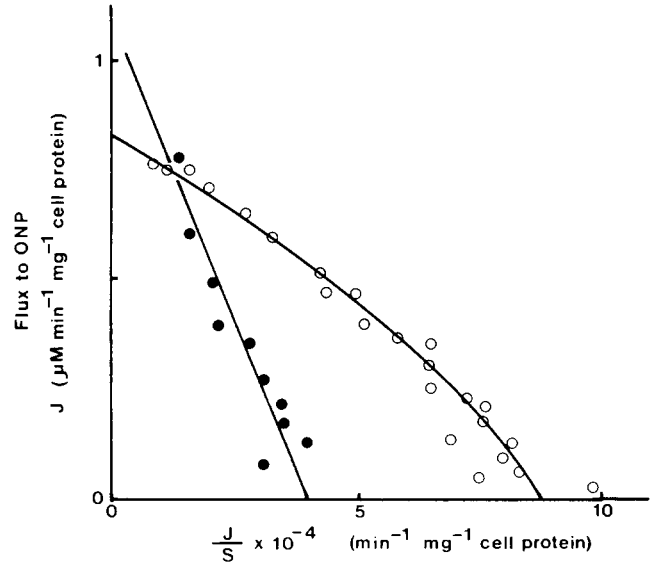

FIGURE 2.—An Eadie-Hofstee plot (velocity, J; substrate concentration, S) showing the effect of a metabolic poison on translocation of ONPG in cells of strain TD1. Circles, untreated cells; dots, cells treated with the uncoupler CCCP. Each point represents the mean of at least three determinations.

ference. Obviously, reliable estimates of the kinetic parameters for active lactose translocation when lactose concentrations are very low can not be obtained from this data set. Estimates of active uptake of lactose by the permease were obtained from cells grown in batch cultures with excess succinate.

Although detailed kinetic models for galactoside translocation by the *lacY* encoded permease are complicated (PAGE and WEST 1981), numerous studies confirm that the initial kinetics follow a Michaelis-Menten expression in cell vesicles and in intact cells with permeabilized outer walls (WINKLER and WILSON 1966; KACZOROWSKI, ROBERTSON and KABACK 1979; ROBERTSON *et al.* 1980; GHAZI and SHECHTER 1981). An Eadie-Hofstee plot should yield a straight line of slope-K_t and a y-axis intercept of V_{max}. Clearly, the plots in Figures 4 and 5 are nonlinear. When the cell wall of strain TD10 is permeabilized by the method of LEIVE (1965) the plot becomes straight demonstrating that the cell wall imposes a considerable resistance to uptake at low concentrations of galactosides.

The model originally proposed by BEST (1955), and which has been invoked and rederived from time to time (*e.g.*, see DABES, FINN and WILKE 1973; KOCH and WANG 1982; WEST and PAGE 1984), can be used to obtain estimates of the kinetic parameters at the

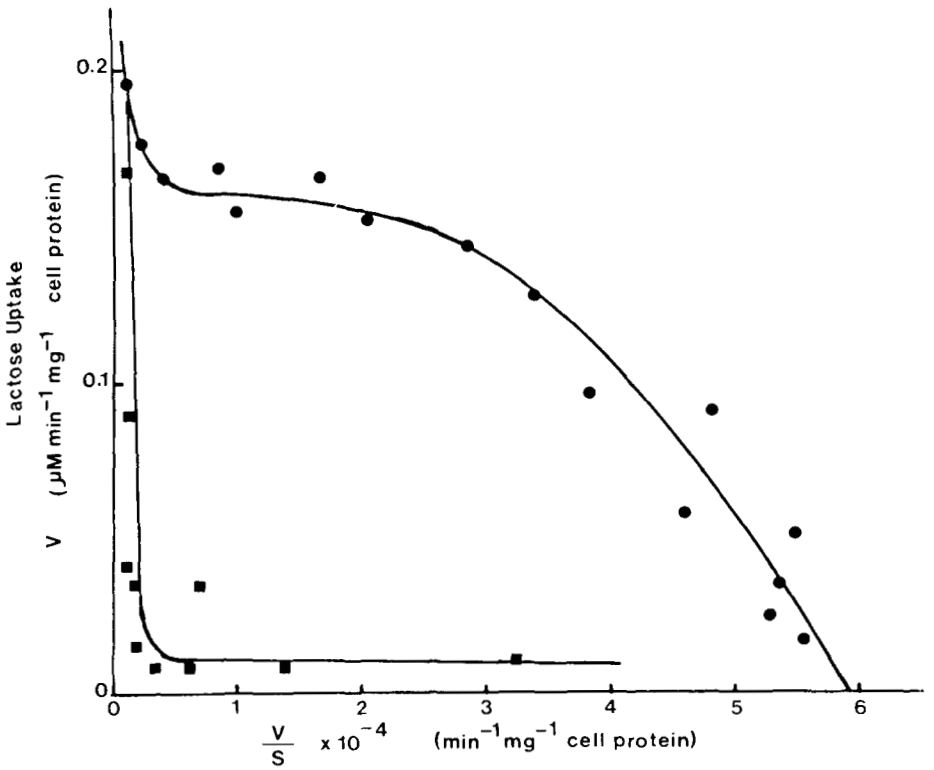

FIGURE 3.—An Eadie-Hofstee plot (velocity, J; substrate concentration, S) of the translocation of lactose by cells of strain TD1 grown in batch culture (circles), and in succinate-limited chemostat cultures (squares). Each point represents the mean of at least two determinations.

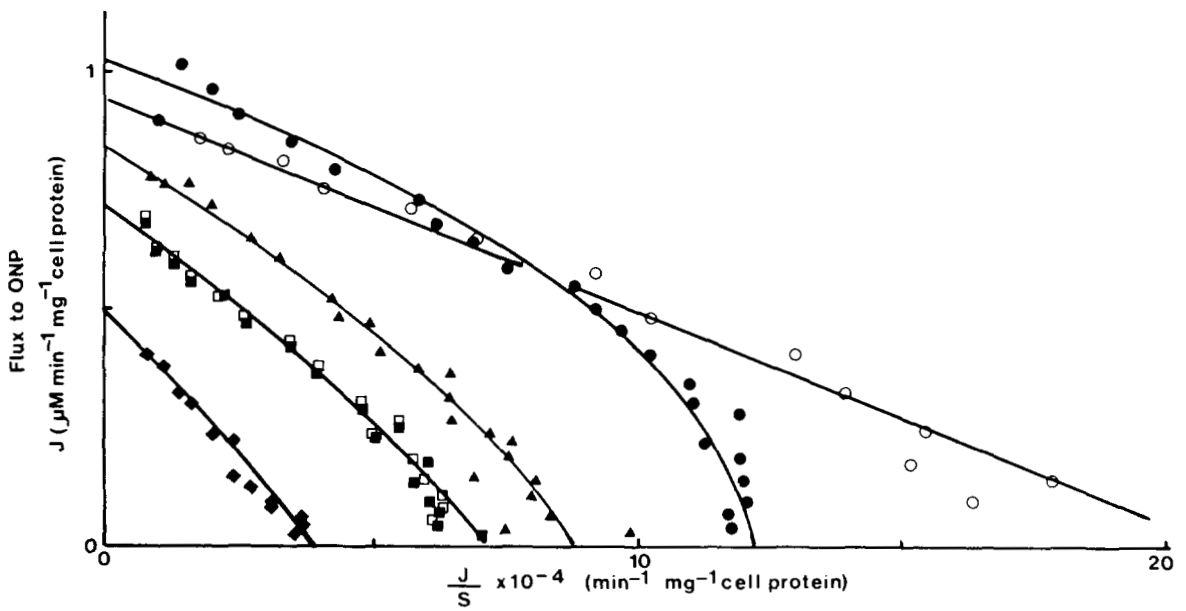

FIGURE 4.—An Eadie-Hofstee plot (velocity, J; substrate concentration, S) of the active uptake of ONPG by chemostat grown cultures of various strains. Solid squares, TD11 and TD13; open squares, TD9 and TD14; solid triangles, TD1; dots, TD10; circles, TD10 treated with EDTA. Each point represents the mean of at least four determinations.

diffusion and translocation steps. The diffusion step is modeled after Fick's law as

$$J = D(G_m - G_p), \tag{1}$$

where J (expressed in μM min^{-1} mg^{-1} cell protein) is the steady state rate of uptake, D (expressed in min^{-1} mg^{-1} cell protein) is a constant related to the diffusion of the substrate, G_m is the concentration of galactoside in the medium, and G_p is the concentration in the periplasmic space. Active uptake by the *lac* permease is modeled as

$$J = V_{\text{maxperm}}G_p/(K_t + G_p), \tag{2}$$

where V_{maxperm} and K_t are the maximum rate of uptake and the apparent Michaelis constant. Elimination of G_p by substitution yields the quadratic

$$J = \tfrac{1}{2}[V_{\text{maxperm}} + DK_t + DG_m] - \tfrac{1}{2}\sqrt{\{[V_{\text{maxperm}} + DK_t + DG_m]^2 - 4V_{\text{maxperm}}DG_m\}}. \tag{3}$$

This model obviously ignores effects caused by the

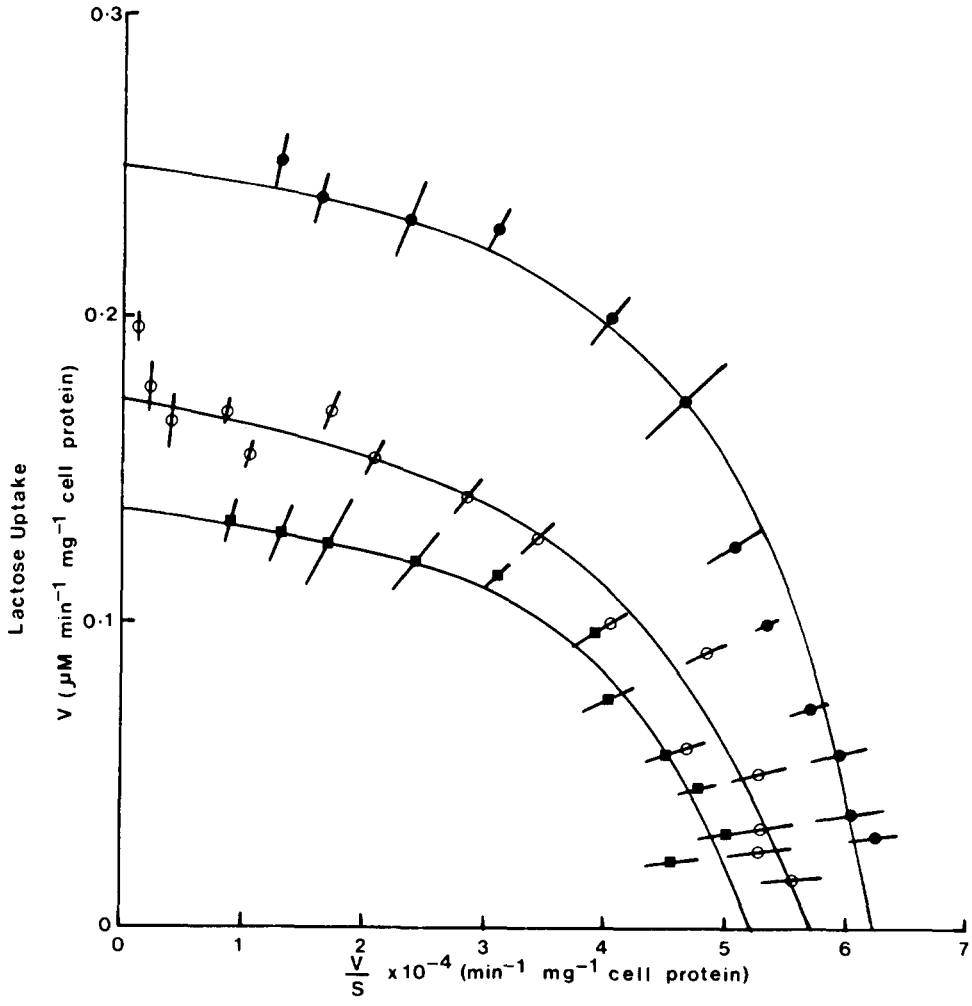

FIGURE 5.—An Eadie-Hofstee plot (velocity, J; substrate concentration, S) of the active uptake of lactose by cells grown in batch culture. Circles, TD1; dots, TD10; squares, TD9. Each point represents the mean of at least three determinations.

leakage of accumulated galactosides and hydrolytic products from cells and the exit of them from cells via the permease and variation in the activities of the β-galactosidases. The low rate of ONPG hydrolysis by intact cells of strain G11 (which contains a deletion of the entire permease but an active β-galactosidase), and the negligible rate of lactose uptake by the deletion strain DD320, suggest that leakage across the cell membrane is insignificant. Moreover, WINKLER and WILSON (1966) have shown that the apparent Michaelis constant of exit of accumulated substrates through the permease is extremely high, that the uptake of galactosides in preloaded cells is similar to unloaded cells, and that there is no evidence of forced exchange.

Table 4 presents estimates of the kinetic parameters for both galactosides and the various strains used. Two nonlinear least squares algorithms, a modified Gauss-Newton and the Marquardt, were used to estimate the parameters and to cross check the fits. The initial analysis suggested that better error bounds could be obtained by fitting on a log scale because the residuals were better behaved. D, V_{max} and K_t/V_{max} were chosen as the three basic parameters, plus indicators for changes from strain to strain and from experiment to experiment. Estimates were somewhat dependent upon the particular form of the parameters chosen for the fit. Although D and K_t are independent variables, the estimates of D and K_t are strongly correlated, and acceptable fits to the data can be obtained for a range of values of D each compensated by changes in the estimate of K_t. Consequently, a single estimate for D was obtained using the whole data set because it was expected to be identical in all strains.

The estimate of D can be confirmed by comparing it to the theoretical expectation obtained using

$$D_{\text{theory}} = \frac{egduf}{ti} \tag{4}$$

where u is the number of cells per milligram of cell protein (1×10^9), d is the diffusion constant of the galactoside (assumed to be equal to that of sucrose at 5.23×10^{-10} m^2/sec, WEAST 1983), g is the number of porin pores per cell (1.1×10^5, STEVEN *et al.* 1971), e is the surface area of a single pore of radius 0.58 nm (NIKAIDO and ROSENBERG 1981), i is the length of the pores (7.5 nm, INOUYE 1974), and t is a conversion factor of 1.666×10^{-5} for cubic meters to liters and

seconds to minutes. f is the RENKIN (1954) factor

$$f = (1 - r)^2 (1 - 2.104r + 2.09r^3 - 0.95r^5) \quad (5)$$

where r is the ratio of the Stokes radius of the galactoside to the radius of the porin pore. Assume that the Stokes radius of lactose is the same as that of sucrose at 0.53 nm (DAVSON 1970) because both have similar structures and molecular weights yields. Then $D_{\text{theory}} = 4.831 \times 10^{-4}$ (min^{-1} mg^{-1} cell protein) which, considering the uncertainty of many of the above estimates, is remarkably close to the estimated value of $D = 6.6605 \times 10^{-4}$ (min^{-1} mg^{-1} cell protein). The higher value of D estimated for ONPG (Table 3) is readily explained because the ONP moiety is both smaller and less hydrophilic than that of lactose and consequently the Renkin factor will be considerably smaller. Assuming that ONPG has a Stokes radius 5% smaller than that of lactose gives a value of $D_{\text{theory}} = 12.2581 \times 10^{-4}$ (min^{-1} mg^{-1} cell protein) which is similar to the estimated value of $D = 17.5784 \times 10^{-4}$ (min^{-1} mg^{-1} cell protein).

Causes of kinetic variation among natural polymorphisms: The Michaelis constants of allozymes were determined under the same assay conditions. Consequently, differences among the Michaelis constants of allozymes must reflect differences among their amino-acid sequences. On ONPG and on lactose the Michaelis constants of the β-galactosidase of TD13 are significantly different from those of its electromorph TD10 (Table 3). Thus, the kinetic data not only indicates that some of the kinetic variation in β-galactosidase activity is encoded by the structural gene but that β-galactosidases of the same electrophoretic mobility need be neither identical by state nor by descent. Although the kinetic behavior of the β-galactosidase of TD10 is similar to those of strains TD1 and TD2, its permease is kinetically distinct from the others on both substrates (Table 4). This suggests that some of the variation in permease activity is encoded in the *lacY* structural gene.

Changes in the rates of transcription and translation are expected to cause proportional changes in the $V_{\max}$ values of both enzymes towards both lactose and ONPG. Table 5 presents the kinetic data normalized to that of the control strain TD1 (The kinetic behavior of the permease towards ONPG of strains TD1, TD2 and TD63 are so similar, as were those of strains TD9 with TD14 and TD11 with TD13, that the pooled estimates are presented). The normalized $V_{\max}$ values of the β-galactosidase and permease on ONPG and on lactose are similar in strain TD9. This provides tentative evidence supporting the notion that regulatory differences might cause as much variation in enzyme activities as differences among the structural genes.

Changes in amino-acid sequence may cause kinetic changes which only manifest themselves as changes in $V_{\max}$. Although the enzymes of strains TD14 and TD63 have Michaelis constants similar to those of TD1 on both substrates, the changes in the normalized $V_{\max}$ values are not proportional. Presumably, these changes are, at least in part, encoded by the amino acid sequences.

TABLE 5

Relative activities and relative fitnesses

	Substrate						
	ONPG		Lactose		Estimates used		
Strain	β-gal[a]	Permease	β-gal	Permease	β-gal	Permease	Fitness
Laboratory mutants							
TD1	1	1	1	1	1	1	1
TD2	0.964	1	0.993	ND[b]	0.979	(1)[c]	0.997
Natural isolates							
TD9	0.896	0.860[d]	0.871	0.876[d]	0.876[e]	0.876[e]	0.967
TD10	0.989	2.500	1.075	2.160	1.075	2.160	1.078
TD11	0.816	0.870[d]	0.741	ND	0.809	0.809[e]	0.947
TD13	0.609	0.870[d]	0.555	ND	0.555	(0.809)[e]	0.946
TD14	0.692	0.860[d]	0.626	ND	0.626	(0.860)[e]	0.966
TD63	1.058	1	0.864	ND	0.864	(1)[c]	1

[a] β-Mercaptoethanol present. β-gal = β-galactosidase.
[b] ND, not determined.
[c] Inferred values based only on ONPG data.
[d] Based on ratio of $V_{\max}$ values only—see text.
[e] Averaged assuming reduced expression only—see text.

CONTROL THEORY

The Flux: This section discusses the relations between enzyme activity and metabolic flux and the relation between metabolic flux and fitness. The relations between enzyme activity and the flux of lactose can be derived by following the approach taken by KACSER and BURNS (1981). Noting the complexities of the mechanism of β-galactosidase reduce to a simple Michaelis-Menten mechanism, that the hydrolysis of galactosides by β-galactosidase is irreversible, and that the Michaelis constant for the exit of galactosides from the cell is very large, the flux, J, is found to be

$$J = \frac{G_m}{\frac{1}{D} + \frac{K_t + G_p}{V_{\text{maxperm}}\, K_{m,p}} + \frac{K_m + G_c}{V_{\max\beta\text{gal}}\, K_{m,c}}} \quad (6)$$

where the subscripts perm and βgal refer to the permease and β-galactosidase steps and K_t and K_m are the Michaelis constants for uptake and hydrolysis. G_m, G_p and G_c are the concentrations of the galactoside in the medium, the periplasmic space and the cytoplasm respectively. $K_{m,p}$ and $K_{m,c}$ are the equilibrium constants from G_m to G_p and G_c, respectively.

If $K_{p,c}$ is the apparent equilibrium constant of galactoside uptake then $K_{m,c} = K_{m,p} * K_{p,c}$, and because a passive diffusion process must necessarily have an equilibrium constant of unity, so $K_{m,c} = K_{p,c}$. The equi-

librium constants of enzymes distal to β-galactosidase are also products of the intervening equilibrium constants across each step. These are huge because the hydrolysis of galactosides is essentially irreversible. Consequently changes in expression of enzymes downstream from the hydrolysis step can only affect this flux indirectly—for example by altering the proton motive force and hence the activity of the permease.

Enzymes are unsaturated: Faster growth-rates caused by increases in the concentration of lactose would not be possible if either enzyme were already saturated. Yet the chemostat population grows at approximately one half of its maximum rate in an identical medium in batch culture with lactose in gross excess at 584 μM. The concentration of lactose entering the chemostat is 0.01% (292 μM). However, the concentration must be far lower in the chemostat than in the feed medium because the chemostat population consumes this lactose for growth and energy. No other nutrient limits growth—a doubling of the concentration of lactose in the feed also doubles the cell density (DEAN, DYKHUIZEN and HARTL 1988a). Consequently, most of the lactose entering the chemostat must be consumed.

It is possible to calculate the concentrations of lactose in the chemostat, the cytosol, the periplasmic space and the chemostat medium by working from the β-galactosidase through the diffusion step as follows.

At steady state in the chemostat $J = H^{-1}Y^{-1} = 0.28Y^{-1}/60/342.3$ μM min^{-1} cell^{-1}. $Y = 1.19 \times 10^6$ cells μg^{-1} lactose consumed (DYKHUIZEN and DAVIES 1980) and noting there are 10^9 cells mg^{-1} cell protein gives $J = 0.0115$ μM min^{-1} mg^{-1} cell protein. The β-galactosidase follows the Michaelis-Menten equation $J = V_{\max\beta\text{gal}}\, G_c/(K_m + G_c)$. Using the estimates for TD1 in Table 3 gives $G_c = 26.64$ μM which is far below the $K_m = 2421.6$ μM.

The net flux through the permease at steady state is $J = V_{\max\text{perm}}\,(G_p - G_c K_{eq})/(K_t + G_p)$. With $K_{pc} = 442$ (see below) and using the estimates for the kinetic parameters of TD1 in Table 4, gives $G_p = 4.06$ μM which is far below the $K_t = 55.9$ μM.

From Equation 1, and the estimate for D in Table 4, the concentration of lactose in the chemostat medium is estimated to be $G_m = 21.33$ μM. Thus, the concentration of lactose in the chemostat is approximately 7% of the concentration entering in the feed medium. This justifies the assertion made above that the concentration of lactose in the chemostat must be far lower than that entering it.

From flux to fitness: As described, the first step in the pathway is not a saturable process. Hence, all the steps in this pathway are unsaturated; equation 6 reduces to

$$J = \frac{G_m}{\frac{1}{D} + \frac{K_t}{V_{\max\text{perm}}} + \frac{K_m}{V_{\max\beta\text{gal}}\, K_{p,c}}}. \qquad (7)$$

A useful parameter for discussions of metabolic flux is the control coefficient of a step which is defined as

$$C^J_{X_i} = d\ln J/d\ln X_i \qquad (8)$$

where X_i is the "activity" at step i. For unsaturated enzymes unsaturated $X_i = V_{\max i}/K_{mi}$. A control coefficient is the tangent to the curve of a log-log plot of J against X_i and in integrated form corresponds to the ratio of a proportional increase in flux to a proportional increase in enzyme activity. The summation theorem (KACSER and BURNS 1973) states

$$\sum_{i=1}^{n-1} C^J_{X_i} = 1 \qquad (9)$$

and demonstrates that control is shared among all steps of a pathway.

Normalizing the flux to that of strain TD1 and using primes to distinguish values in the second strain yields

$$J'/J = j = \frac{\frac{1}{D} + \frac{K_t}{V_{\max\text{perm}}} + \frac{K_m}{V_{\max\beta\text{gal}}\, K_{p,c}}}{\frac{1}{D} + \frac{K'_t}{V'_{\max\text{perm}}} + \frac{K'_m}{V'_{\max\beta\text{gal}}\, K_{p,c}}}. \qquad (10)$$

All the parameters in the above equation have been estimated save for $K_{p,c}$ the apparent equilibrium constant of the permease step. Data from WINKLER and WILSON (1966) show that the apparent equilibrium constant $K_{p,c}$ of ONPG lies between 30 and 150. This is sufficiently large that a 50% reduction in the activity of the β-galactosidase towards ONPG is estimated to reduce the *in vivo* flux between 0.002% and 0.09%. These are sufficiently small that the estimated parameters for ONPG uptake by intact cells are not detectably affected by the kinetic polymorphism displayed by the β-galactosidase.

WINKLER and WILSON (1966) also show that the apparent equilibrium constant $K_{p,c}$ at very low lactose concentrations is approximately 175. WRIGHT, REIDE and OVERATH (1981) obtain a value of 709 under similar conditions. Assume that the average of 442 is the best estimate of this apparent equilibrium constant when lactose is scarce as in chemostat competition experiments.

The fitnesses and normalized enzyme activity data for mutants and laboratory strains presented by DYKHUIZEN, DEAN and HARTL (1987) can be used to supplement those for the operons from the natural isolates studied here. This is only possible because the absolute activity of the K12 permease and the diffu-

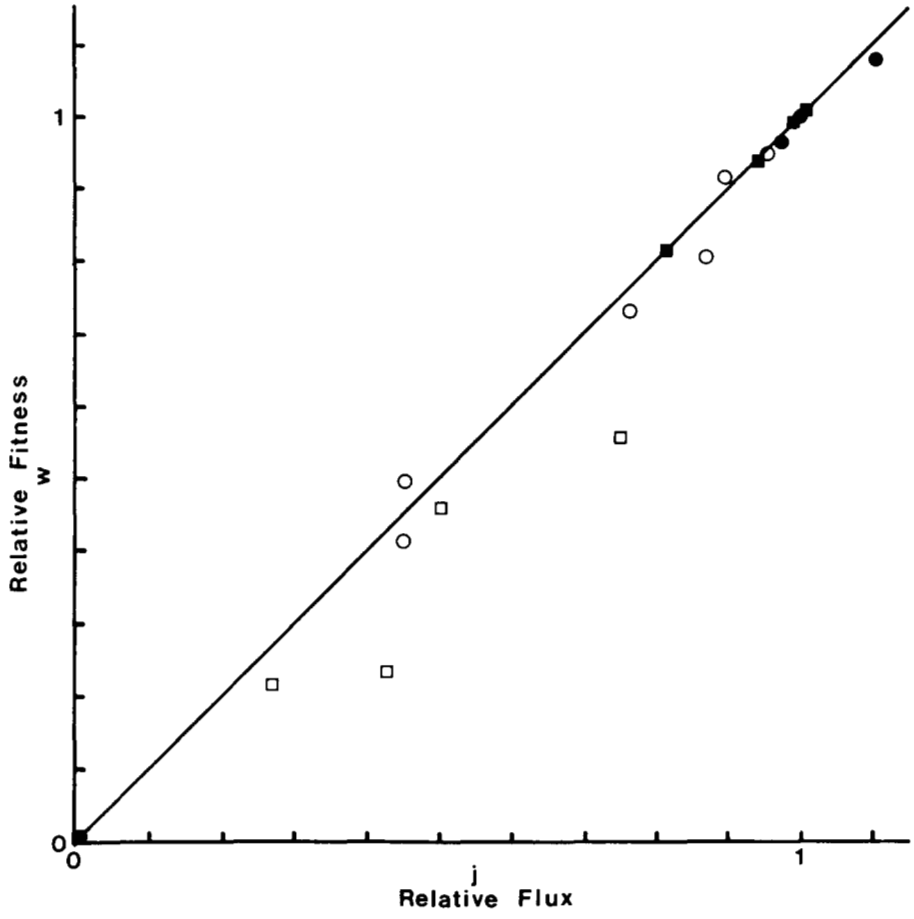

FIGURE 6.—The relation between observed relative fitness and relative flux. Dots–observed lactose uptake by strains DD320, TD1, TD11 and TD10: data presented by DYKHUIZEN, DEAN and HARTL (1987)–open squares estimated lactose flux–open squares, *ebg* mutants (from HALL 1984); solid squares, β-galactosidase mutants (from DEAN, DYKHUIZEN and HARTL 1986); circles, coordinate regulation of an inducible operon by IPTG (from DYKHUIZEN and DAVIES 1980).

sion constant of the cell wall of the DD320 genetic background have been determined. Together, these data can be used to plot relative fitness against relative flux. Figure 6 shows that the relation is a straight line of slope 1. Thus, fitness is directly proportional to flux, *i.e.*, $w = j$. Note that a nonlinear relation between flux and fitness would imply that other metabolic steps have a significant influence on the flux. Consequently, Equation 10 is a biochemical model of fitness. Equation 10 can be rewritten as

$$w = j = \frac{1}{C_d^j/d + C_p^j/p + C_\beta^j/\beta} \qquad (11)$$

where d, p and β are the rates of diffusion, permease activity and β-galactosidase activity normalized to those of TD1 and C_d^j, C_p^j, and C_β^j are the respective control coefficients. The cell wall structures are identical (because of the common genetic background) so $d = 1$ for all strains.

The reciprocal of the equation 11 is a three dimensional form of the well known Lineweaver-Burke plot and so the slopes of the plane are direct estimates of the control coefficients. Estimates of the control coefficients can be obtained using the data collected for various laboratory mutants and presented by DYKHUIZEN, DEAN and HARTL (1987), two laboratory mutants, TD1129.2 and TD2108.1 (A. M. DEAN, unpublished results) and the data from the natural isolates presented in this paper. Using multiple linear regression, as proscribed by SNEDECOR and COCHRAN (1967), the sum of the estimated control coefficients equals 1.04 ± 0.09 which is not significantly different from unity. Normalizing the estimates yields

$$C_d^w = C_d^j = 0.8450 \pm 0.0844$$

$$C_p^w = C_p^j = 0.1512 \pm 0.0290$$

$$C_\beta^w = C_\beta^j = 0.0037 \pm 0.0003.$$

These agree well with the estimates for the laboratory strains alone and presented by DYKHUIZEN, DEAN and HARTL (1987).

Figure 7 is a three dimensional representation of the fitness surface showing the approximate positions of the data points. The dots are for the natural isolates and the open circles for the data presented in DYKHUIZEN, DEAN and HARTL (1987). The rising slope on the left-hand side of the figure shows that small increases in permease activity have marked effects on fitness whereas the plateau shows that substantial increases in lactase activity have negligible effects. Theoretically if lactase activity became infinite the maximum gain in fitness would be a paltry 0.375% com-

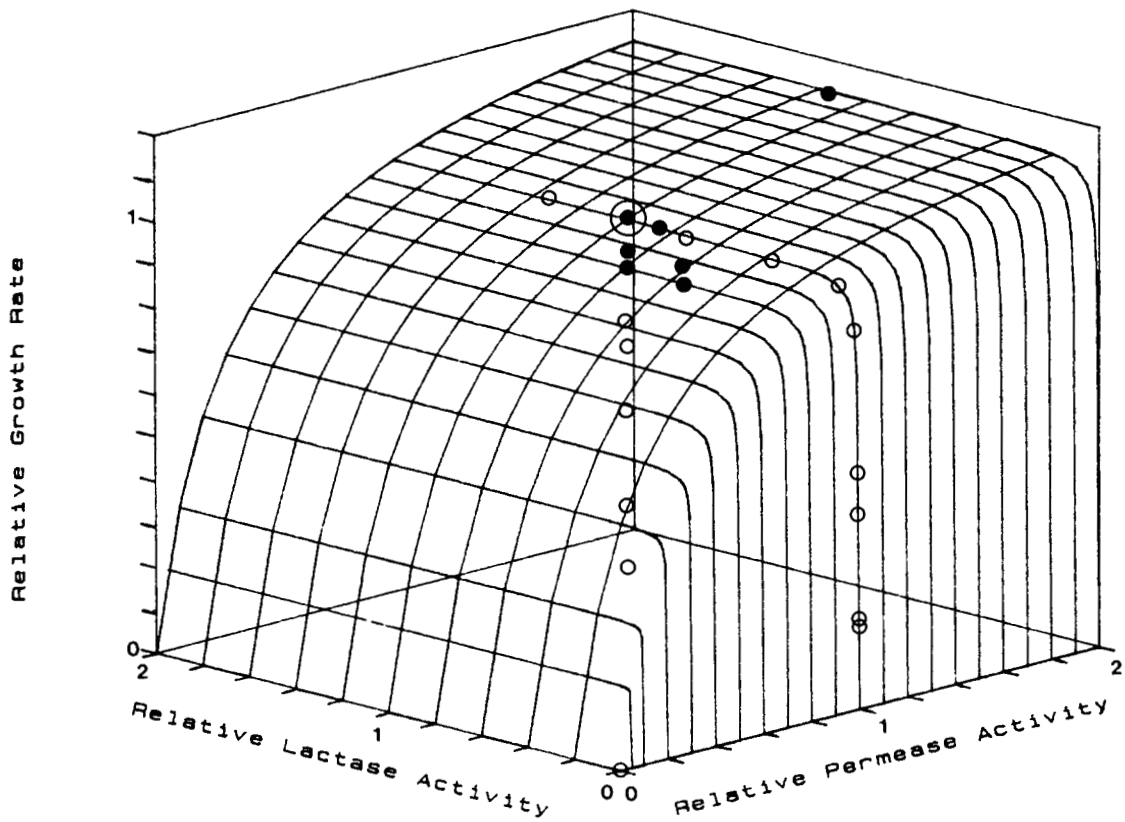

FIGURE 7.—The adaptive surface of the *lac* permease and β-galactosidase in lactose-limited chemostat cultures. Dots, strains with operons from natural isolates; circles, mutant strains discussed in DYKHUIZEN, DEAN and HARTL (1987)–*ebg* mutants (from HALL 1984); β-galactosidase mutants (from DEAN, DYKHUIZEN and HARTL 1986); coordinate regulation of an inducible operon by IPTG (from DYKHUIZEN and DAVIES 1980).

pared to the 17.818% gain if permease activity were infinite.

DISCUSSION

When strains of *E. coli* are placed in competition for growth-rate limiting concentrations of lactose, fitness is proportional to flux (Figure 6). Consequently, natural selection will favor those strains with increased kinetic activity at metabolic steps with large control coefficients. As permease mutants with greater activity are selected onto the fitness plateau the summation theorem (Equation 9) dictates that virtually all of the control will come to reside in the cell wall. Presumably, continued selection might increase the number of diameter of the porin pores, or perhaps reduce the thickness of the outer wall. Taken to its logical limit, control theory alone would suggest that the ultimate chemostat adapted *E. coli* should have no cell wall at all, whence the control shifts back onto the permease and the β-galactosidase. At least one of the two remaining steps now has a rather large control coefficient. Assuming that further increases in activity are possible, further rounds of selection will occur until the permease and β-galactosidase are so efficient that the diffusion of substrates from the active site of one enzyme to another limits fitness.

The activities of these enzymes do not approach, even remotely, the diffusion rates of their substrates. This serves to demonstrate that other agents of selection must prevent the above scenario from ever happening. These forces may provide the key to understanding enzyme activity and the distribution of control among the metabolic steps. The considerable resistance of the cell wall to the diffusion of lactose at low concentrations may be a necessary consequence of an ability to survive osmotic shock. Constitutive cells die if a sudden excess of lactose floods into the cell and the membrane potential collapses (Figure 2; DYKHUIZEN and HARTL 1978; WILSON, PUTZRATH and WILSON 1981; GHAZI, THERISOD and SCHECTER 1983). This phenomenon might serve to prevent increases in permease activity in natural environments if populations are alternately subjected to lactose starvation and inundation (DYKHUIZEN, DEAN and HARTL 1987). The small control coefficient of the constitutively expressed β-galactosidase may be due to the fact that no other selective forces are of sufficient magnitude to prevent activity from increasing up to the plateau (DEAN, DYKHUIZEN and HARTL 1986).

Although the natural polymorphisms of the permease and β-galactosidase have similar ranges in activity, that of the permease accounts for a 13% range in

fitness whereas that of the β-galactosidase accounts for a 0.5% range. This is simply a consequence of the permease activities being on the shoulder of the concave fitness function, whereas those of the β-galactosidase remain upon the plateau (Figure 7). This observation might be considered surprizing because the lactase activity of the most efficient β-galactosidase is 0.47×10^{-3} min^{-1} mg^{-1}, whereas the activity of lactose uptake by the most inefficient permease is much higher at 2.8×10^{-3} min^{-1} mg^{-1}. The reason can be found in metabolic control theory. Equation 6 shows that the activity of an enzyme must be weighted by the equilibrium constant from the pathway's first substrate to the enzyme's substrate—this is simply the product of the intervening equilibrium constants of 1 × 442. For β-galactosidase, this constant is so large that even low lactase activities are effectively pushed onto the flux/fitness plateau. Thus, consideration of the evolution of enzymes is incomplete without a knowledge of the equilibrium constants which are as important in determining the distribution of flux control as the activities of the enzymes themselves.

Inducible operons are strongly selected against in the chemostat environment because the levels of lactose are insufficient to keep the operon fully induced (DYKHUIZEN and DAVIES 1980; DYKHUIZEN, DEAN and HARTL 1987). Thus, the control coefficients of the permeases and β-galactosidases in inducible strains will be considerably larger than those of the constitutive strains in lactose-limited chemostats. In natural habitats the high levels of lactose necessary to induce the operons will also start to saturate the permease. The diffusion step can not become saturated, and the V_{max} of the β-galactosidase step is so much larger than that of the permease that the intracellular lactose concentration will barely approach the K_m, even at maximum flux. Thus, in natural habitats one expects the flux control coefficient of the permease to approach unity and the range in flux to be increased from 13% to 61%. Therefore, the chemostat environment tends to maximize the flux control coefficients of the β-galactosidase and porin pores, but minimizes that of the permease.

We usually associate the scarcity of a resource with increased competition and often equate this with increased selection. If fitness remains proportional to flux as lactose concentrations are increased, this intuitive reasoning leads us to believe that the selection upon the β-galactosidase will be reduced. This is true only because the control coefficient is reduced. Equally, however, this is only possible because more of the control has shifted to the permease which may now be exposed to an even greater selective pressure. In other words—the more abundant the resource, the more intense the selection.

The large control coefficients possible at the permease step, the possibility of trade offs between activity and lactose killing, the probably heterogeneous environments to which a permease is necessarily physically exposed, and the extensive kinetic variation that confers a wide range in fitness, suggests that this polymorphism might be subject to natural selection. Indeed, the polymorphism might be protected if selection favours different permease alleles in different spacially variable environments. If true, this would also suggest that migration rates are sufficiently low to keep the demes from coalescing into a single panmictic population. In alternate environments the permease variation might constitute a stochastic additive scale while the very nature of metabolism provides the concave fitness function necessary for the SAS-CFF model of GILLESPIE (1978).

But if there is ample opportunity for selection as a factor in maintaining the permease polymorphism, there is singularly little evidence for it protecting the variation in lactase activity of the β-galactosidases. So small are the control coefficients that even the most intense selection imposed by the chemostat can barely estimate it. Clearly, one should not conclude that selection acts on the functional differences between alleles even if the trait to which they contribute is under intense selection. This suggests, together with the small range in fitness conferred by the naturally occurring alleles of β-galactosidase, that natural selection itself may have driven these alleles sufficiently far along the fitness plateau for their fate to be largely determined by random genetic drift; a notion that is explored in more detail by HARTL, DYKHUIZEN and DEAN (1985). The permease and β-galactosidase polymorphisms suggest that the alleles of different genes, contributing to the same trait, may be subject to entirely different modes of evolution—selection and neutrality.

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