tide was isolated by ion-exchange chromatography on DEAE-cellulose and by partition chromatography; treatment with trypsin gave Val·Glu(NH_2)·Lys and the heme-containing octapeptide. On treatment of the tripeptide with FDNP followed by acid hydrolysis, only DNP-valine was found in the ether extract. The aqueous phase was treated with FDNB, and DNP-glutamic acid and di-DNP-lysine were isolated by paper chromatography. Only the valine and lysine derivatives were radioactive. Of the amino acids in the octapeptide, only the valine was labeled.

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Separate permeases for the accumulation of methyl-β-D-galactoside and methyl-β-D-thiogalactoside in *Escherichia coli*

Studies on the accumulation of β-D-thiogalactosides in cells of *Escherichia coli* led MONOD and his collaborators[1,2] to postulate the existence of a specific transport system termed "galactoside-permease".

The following facts were considered by the same authors to imply that galactosides and thiogalactosides entered the cell by the same transport system[1,2,3]: (a) the capacity to accumulate labeled thiogalactoside is specifically inducible and formation of β-galactosidase is simultaneously induced; (b) β-galactosides are found to inhibit competitively the accumulation of thiogalactosides; (c) the rate of hydrolysis of *o*-nitrophenyl-β-D-galactoside by intact cells and their capacity to accumulate thiogalactoside were correlated under a variety of conditions.

On the other hand, data obtained in this laboratory raised the possibility that a single permease might not mediate the entrance of all galactosides[4]. Therefore, an attempt was made to test whether the same system is responsible for the transport of galactosides and thiogalactosides. For this purpose the accumulation of methyl-β-D-galactoside and methyl-β-D-thiogalactoside, both 1-^{14}C labeled, was studied. Since methyl-β-D-galactoside, unlike the thiogalactoside, can be hydrolyzed by β-galactosidase, a mutant strain (K12-W2244, kindly supplied by Dr. J. LEDERBERG) which lacks the enzyme was used.

TABLE I

ACCUMULATION OF METHYL-β-D-GALACTOSIDE AND METHYL-β-D-THIOGALACTOSIDE

Cells of W2244 (a Lac_4^- strain unable to synthesize β-galactosidase but with inducible thiogalactoside permease) were grown in M-56 medium[2], containing 0.4% glycerol, with and without inducer. The cells were harvested during exponential growth, washed, resuspended in the same medium containing 20 μg/ml chloramphenicol and $1.5 \cdot 10^{-4}$ *M* [1-^{14}C]methyl-β-D-galactoside or $1.0 \cdot 10^{-4}$ *M* [1-^{14}C]methyl-β-D-thiogalactoside, respectively. After 10-min shaking at 37° the cells were centrifuged, the supernatant carefully removed, the cells resuspended in water, boiled 5 minand re-centrifuged. The supernatant radioactivity was determined.

	Methyl-β-D-thiogalactoside		*Methyl-β-D-galactoside*	
	*counts/min**	*μmoles/g dry wt.*	*counts/min**	*μmoles/g dry wt.*
Non-induced cells	2	0.4	89	17.8
Induced cells	151	31.0	167	33.5

* Values corrected by 23 counts/min, the radioactivity in noninduced cells with 0.02 *M* NaN_3.

The results shown in Table I indicate clearly that cells grown in the absence of inducer concentrate methyl galactoside readily, but fail to concentrate the thiogalactoside. Cells grown in the presence of inducer (thiogalactoside or galactoside at $5 \cdot 10^{-4}$ *M*) concentrate both galactosides. The difference in the ability of the uninduced cells to concentrate these compounds cannot be ascribed to differing affinities of a single transport system because the Michaelis constants for the thiogalactoside[2] and the galactoside are $4.3 \cdot 10^{-4}$ *M* and $1.4 \cdot 10^{-4}$ *M* (Fig. 1), respectively. These are too close to explain the 40-fold difference in accumulation.

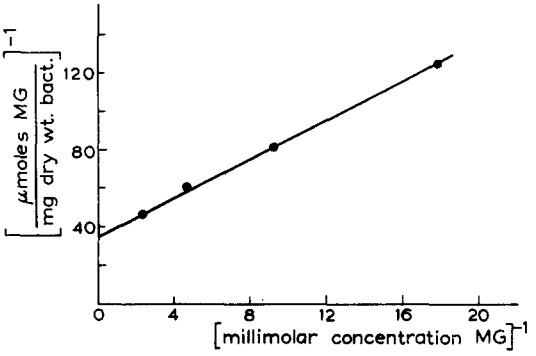

Fig. 1. Amount of methyl-β-D-galactoside (MG) accumulated in the cells as a function of its external concentration.

It appears, therefore, that *E. coli*, strain K12, contains in addition to the inducible system which accumulates methyl-β-D-thiogalactoside a constitutive one for methyl-β-D-galactoside. For the purpose of the discussion to follow, these two systems are designated, "thiogalactoside permease" and "galactoside permease" respectively.

No evidence is available as to whether the additional accumulation of methyl galactoside, which occurs in induced cells as compared with uninduced, can be attributed to the galactoside permease or the thiogalactoside permease.

The galactoside permease has the following properties:

(a) The labeled compound accumulated by the cells is unchanged methyl galactoside as shown by chromatography followed by radioautography.

(b) The amount of methyl galactoside accumulated per cell increased rapidly with time, reaching a constant value after about 5 min at 22°.

(c) The amount of methyl galactoside accumulated per cell is a function of the external concentration and follows the Langmuir adsorption isotherm (Fig. 1). The concentration of the galactoside in uninduced cells can be as high as 40 times that of the medium.

(d) The amount of labeled methyl galactoside found in the cells is proportional to the number of cells in the range $8 \cdot 10^8$ to $4 \cdot 10^9$ cells.

(e) Methyl galactoside accumulation is inhibited by azide and competitively inhibited by methyl thiogalactoside.

Detailed studies of the specificity of galactoside permease and of its relationship to β-galactosidase activity *in vivo* are currently under way.

It has been considered that, at low inducer concentration, the penetration of inducer and the attendant synthesis of the specific permease is the rate-limiting step in enzyme induction[3,5]. This hypothesis is now complicated by the finding that galactoside permease is essentially constitutive and therefore should not affect the kinetics of induction.

Finally, the inhibition of methyl galactoside accumulation by methyl thiogalactoside has further implications. The fact that the thiogalactoside can competitively inhibit without itself being accumulated in the cell would suggest that to penetrate the cell a molecule must meet at least two requirements: (a) it must be capable of specific interaction with the "entrance site", and (b) it must then be capable of actually penetrating into the cell. This finding points out the danger of assuming that a particular exogenous molecular species enters a cell by a given permease because it competively interacts with that permease.

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