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Mutations Affecting Transport of the Hexitols D-Mannitol, D-Glucitol, and Galactitol in *Escherichia coli* K-12: Isolation and Mapping

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Mutants of *Escherichia coli* K-12 unable to grow on any of the three naturally occurring hexitols D-mannitol, D-glucitol, and galactitol and, among these specifically, mutants with altered transport and phosphorylating activity have been isolated. Different isolation procedures have been utilized, including suicide by D-[^{3}H]mannitol, chemotaxis, and resistance to the toxic hexitol analogue 2-deoxy-arabino-hexitol. Mutations thus obtained have been mapped in four distinct operons. (i) Mutations affecting an enzyme II-complex^{mtl} activity of the phosphoenolpyruvate-dependent phosphotransferase system all map in gene *mtlA*. This gene has previously been shown (Solomon and Lin, 1972) to be part of an operon, *mtl*, located at 71 min on the *E. coli* linkage map containing, in addition to *mtlA*, the *cis*-dominant regulatory gene *mtlC* and *mtlD*, the structural gene for the enzyme D-mannitol-1-phosphate dehydrogenase. The gene order in this operon, induced by D-mannitol, is *mtlC A D*. (ii) Mutations in gene *gutA* affecting a second enzyme II-complex^{gut} of the phosphotransferase system map at 51 min, clustered in operon *gutC A D* together with the *cis*-dominant regulatory gene *gutC* and the structural gene *gutD* for the enzyme D-glucitol-6-phosphate dehydrogenase. The *gut* operon, previously called *sbl* or *srl*, is induced by D-glucitol. (iii) Mutations affecting the transport and catabolism of galactitol are clustered in a third operon, *gatC A D*, located at 40.5 min. This operon again contains a *cis*-dominant regulatory gene, *gatC*, the structural gene *gatD* for galactitol-1-phosphate dehydrogenase, and gene *gatA* coding for a third hexitol-specific enzyme II-complex^{gat}. Other genes coding for two additional enzymes involved in galactitol catabolism apparently are not linked to *gatC A D*. (iv) A fourth class of mutants pleiotropically negative for hexitol growth and transport maps in the *pts* operon. Triple-negative mutants (*mtlA gutA gatA*) do not have further transport or phosphorylating activity for any of the three hexitols.

Wild-type strains of *Escherichia coli* K-12 will grow on D-mannitol and D-glucitol (D-sorbitol) as the sole carbon source, whereas the majority of strains are unable to grow on galactitol (dulcitol), the third of the naturally occurring hexitols. Most strains, however, will take up hexitols, including galactitol, by an apparent great multitude of transport systems. Thus, D-mannitol (mtl) is taken up by two systems, D-glucitol (gut) is taken up by three, and galactitol (gat) is taken up by one.

Obviously, before any of these transport systems can be analyzed biochemically, the exact number of hexitol uptake systems should be determined as well as their nature and properties. Furthermore, mutants should be available in which only one transport system is active at a time. In this and the following paper (17), the hexitol transport systems were analyzed by genetic, biochemical, and kinetic methods.

Hexitol metabolism is known to be initiated in the *Enterobacteriaceae* either (i) by dehydrogenation of the free hexitol followed by an adenosine 5'-triphosphate-dependent phosphorylation of the resulting ketose, or (ii) by the immediate phosphorylation of the free hexitol (29). Thus, mutants of *Klebsiella aerogenes* have been shown to grow on D-mannitol by means of a D-arabinitol (D-arabitol) dehydrogenase converting free D-mannitol to D-fructose (5, 29). In such mutants, an active transport system can be measured that accumulates free D-mannitol (17).

As a general rule, however, members of the

Enterobacteriaceae seem to metabolize hexitols by the second type of pathway. Here the process of bringing a hexitol into the cytoplasm involves its phosphorylation by a phosphoenolpyruvate-dependent phosphotransferase system (7, 26, 29), as represented by reactions 1 and 2.

$$\text{HPr} + \text{PEP} \xrightarrow{\text{enzyme I}} \text{P-HPr} + \text{pyruvate} \quad (1)$$

$$\text{P-HPr} + \text{hexitol} \xrightarrow{\text{enzyme II-complex}} \text{hexitol-phosphate} + \text{HPr} \quad (2)$$

where PEP represents phosphoenolpyruvate, HPr and enzyme I represent two soluble proteins, P-HPr represents the phosphorylated derivative of HPr, and enzyme II-complex is a membrane-bound, substrate-specific protein-phosphatidylglycerol complex. To such complexes will be given, as a suffix, the name of the operon to which they belong, e.g., enzyme II-complex^{mtl} and enzyme II-complex^{gut} for complexes coded by genes of the *mtl* and *gut* operons, respectively. The hexitol-phosphates are dehydrogenated in the following reaction by a nicotinamide adenine dinucleotide-dependent hexitol-phosphate dehydrogenase to a ketose-phosphate. This pathway has been described previously (18, 28) for D-mannitol and D-glucitol in *E. coli* K-12. Since the name D-glucitol should be substituted for the name D-sorbitol, we propose to rename the operon previously called *sbl* by us (18) and *srl* by W. Epstein (cited in reference 30) as the "*gut*" operon.

A dehydrogenase inducible by galactitol and presumably converting galactitol-1-phosphate into tagatose-6-phosphate has been found in *E. coli* B (32). It seems reasonable to expect also a P-HPr-dependent enzyme II-complex^{gat} and eventually a similar pathway for galactitol transport and catabolism in *E. coli* K-12.

To enhance the probability of obtaining mutants with defects in different parts of the uptake systems, several methods have been utilized: penicillin selection (8), suicide with radioactive hexitols (21), chemotaxis (2), selection strains lacking for any of the hexitol-phosphate dehydrogenases (28), and resistance to the toxic analogue 2-deoxy-arabino-hexitol.

In this paper it will be shown that each method can be used to isolate mutants. The mutations have been mapped and found to be located in four distinct operons. The nature and the properties of the transport systems involved will be described in the accompanying paper (17).

MATERIALS AND METHODS

Chemicals. Casein acid hydrolysate (CAA) (salt free, vitamin free) was obtained from Nutritional Biochemicals Corp., Cleveland, Ohio; sodium phosphoenolpyruvate, D-mannitol-1-phosphate, D-glucitol-6-phosphate, and galactose-6-phosphate were from C. F. Boehringer u. Söhne, Mannheim, Germany. Galactitol-1-phosphate was prepared from galactose-6-phosphate by the method of Wolff and Kaplan (31). 2-Deoxy-D-glucose (D-glucose free) was from Calbiochem, Lucerne, Switzerland. This substance served as the starting material for the preparation of 2-deoxy-arabino-hexitol (11) by borohydride reduction. D-[^{14}C]mannitol, D-[^{3}H]mannitol, and D-[^{3}H]glucitol were obtained from New England Nuclear Corp., Boston, Mass.; D-[^{14}C]glucitol was from New England Nuclear Corp. and from ICN, City of Industry, Calif.; [^{14}C]galactitol was from The Radiochemical Centre, Amersham, England.

Thin-layer chromatography. The purity of commercial and recrystallized polyols and of the analogue 2-deoxy-arabinohexitol was checked by chromatography on Kieselgel plates in the system ethylacetate-pyridine-water (120:50:40, vol/vol/vol), spraying the dried plates very lightly with alcaline potassium permanganate. Polyol-phosphates were developed on cellulose plates in 1 M ammonium acetate (pH 5.0)-95% ethanol-disodium ethylenediaminetetraacetic acid (30:70:1, vol/vol/vol) and in absolute methanol-25% ammonium hydroxide-water-disodium ethylenediaminetetraacetic acid (60:10:30:1, by volume). The presence of phosphates was revealed by treatment with a molybdate-perchloric acid reagent (12) followed by ultraviolet irradiation.

Bacterial strains. The origin and genotypes of strains used in this study are summarized in Table 1 (see also Fig. 6). The origin, phenotype, and genotype of hexitol transport mutants, whose isolation from strain L141 is described in Table 2, are described in Table 3. Strain JC411 (6), the original parent of all transport mutants, was obtained through the courtesy of E. C. C. Lin. The *leu*$^{+}$ derivative L141 was isolated from strain JC411 by P1 transduction. From strain L146 (Table 2) a derivative, L147, resistant to 40 μg of nalidixic acid per ml, was isolated, followed by the selection of the *gut*$^{+}$ revertant L160. Strain L177 is a derivative of strain L146, resistant to 4 μg of nalidixic acid per ml but sensitive to 10 μg/ml. Since the diploid L177/F' 108 is *nal*$^{+}$, the mutation of strain L177 seems to be at the classical *nalB* locus (30).

Culture media and growth conditions. To the standard minimal medium described previously (29) the following additions were made where necessary (final concentrations): casein acid hydrolysate to 1%; amino acids to 20 μg/ml; thiamine to 1 μg/ml; carbon sources to 0.2% unless indicated otherwise. MacConkey indicator plates contained 40 g of MacConkey agar base (Difco Laboratories, Detroit, Mich.) per liter. To this was added 10 g of hexitols before autoclaving (15 min, 120 C), or other sterilized carbohydrates to 1% after autoclaving. Growth was determined turbidimetrically in either a Klett colorimeter (filter 42) or a Bausch & Lomb spectrophotometer at 420 nm. For an exponential culture, 20 Klett units equaled 10^{8} bacteria per ml, and 0.1 absorbance unit equaled 4.5×10^{7} bacteria per ml.

Mutagenesis and penicillin selection. Ethyl

TABLE 1. *Origin and genotype of E. coli K-12 strains*[a]

Strain	Parent	Reference	Source	Genotype
236	AB313/11	28	E. C. C. Lin	Hfr *thi leu strA mtl* $C^+A^-D^+$ *gutC*$^+$*A*$^+$*D*$^+$
238	AB313/11	28	E. C. C. Lin	Hfr *thi leu strA mtl*C^c*A*$^+$*D*$^+$ *gutC*$^+$*A*$^+$*D*$^+$
239	238	28	E. C. C. Lin	Hfr *thi leu strA mtl*C^c*A*$^+$*D*$^-$ *gutC*$^+$*A*$^+$*D*$^+$
KL16-*gat*$^+$	KL16	20	*	Hfr *str*$^+$ *nal*$^+$ *mtl*$^+$ *gut*$^+$ *gatC*^{c}*A*$^+$*D*$^+$
KL98-3*gat*$^+$	KL98-3	20	*	Hfr *str*$^+$ *nal*$^+$ *gatC*^{c}*A*$^+$*D*$^+$
KL96-*gat*$^+$	KL96	20	*	Hfr *str*$^+$ *nal*$^+$ *gatC*^{c}*A*$^+$*D*$^+$
AT2427	K10	25	A. Böck	Hfr *thi cysC rel*
BM113	K10	25	A. Böck	Hfr *alaS*^{ts} *pheA pyr rel thi*
NP3151	K10	27	A. Böck	Hfr *fda*^{ts} *gnd str*$^+$
KLF8		20	K. B. Low	F'108 *lys*$^+$ *recA/ thi thr leu cysC lysA rec*$^+$
KLF3-1		20	K. B. Low	F'131 *gat*$^+$ *his*$^+$ *supD/ argG metB leu his recA strA*
DFF1		20	K. B. Low	F'150 *his*$^+$ *zwf*$^+$*/ argG metB leu his recA strA*
KLF43		20	K. B. Low	F'143 *thy*$^+$ *rec*$^+$ *tyrA*$^+$*/ pyrD trp his tyrA recA thyA*
J441			*	F$^-$ *recA mtlC*$^+$*A*$^-$*D*$^+$ *gutC*^{c}*A*$^+$*D*$^+$
L141	JC411	6	L. Gorini	F$^-$ *lacY galT xyl thi leu*$^+$ *his argG metB strA*

[a] Genetic markers are according to Taylor and Trotter (30). The isolation of strains marked with an asterisk (*) are described in this paper.

TABLE 2. *Enzyme activities of parent and temperature-sensitive strains*[a]

Strain	Uptake activity[b]						Dehydrogenase activity[c]					
	D-Mannitol			D-Glucitol			D-Mannitol-1-phosphate			D-Glucitol-6-phosphate		
	25 C	42 C	37 C	25 C	42 C	37 C	25 C	42 C	37 C	25 C	42 C	37 C
238	8.65	8.60	6.20				5.50		2.80			
≤243	4.60	4.56	5.47				≤0.01		≤0.01			
≤240				9.10	8.00	8.45				0.90	0.40	0.83
≤244				8.45	6.00	20.00				1.00	0.05	0.01

[a] Cultures were pregrown at 26 or 37 C in minimal medium-glycerol and harvested for uptake assays at 3.5×10^8 bacteria per ml and for dehydrogenase tests. Cells grown at 26 C were either tested immediately at 25 C or after 30 min of incubation at 42 C for transport activity; cell extracts of cells grown at 26 C were tested immediately or after 5 min of incubation at 42 C for dehydrogenase activity.

[b] Nanomoles per minute per milligram of protein.

[c] Micromoles per minute per milligram of protein.

methane sulfonate mutagenesis in tris(hydroxymethyl)aminomethane-hydrochloride buffer (0.2 M, pH 7.5) was done by the method of Tanaka et al. (29).

To mutagenize cells with *N*-methyl-*N'*-nitro-*N*-nitrosoguanidine (MNNG), exponentially growing cells were concentrated by centrifugation to 10^{10} bacteria per ml in 0.2 M acetate, pH 5.0. To 0.5 ml of this suspension was added 0.1 ml of MNNG (3 mg/ml in the same buffer), and the cells were incubated at 37 C while being shaken for 2 h. Finally, the MNNG was destroyed by diluting the cells in 25 ml of Penassay broth and growing them out for 24 h followed by one recycling in minimal medium-D-glucose (A. Garen, personal communication). The penicillin selection was done by the method of Davis (8). To isolate mutants with an altered K_m of transport systems for hexitols, a fresh overnight culture grown in 10 mM D-mannitol was diluted to 2×10^8 bacteria per ml into 1 liter of prewarmed minimal medium containing 1 μM hexitol as the carbon source. The culture was grown under vigorous shaking until a high percentage of dividing cells could be observed in a microscope. At this time, 1 ml of 1 mM hexitol and 2×10^8 U of penicillin G were added. The inactivation was followed by plating and counting of the surviving bacteria. Inactivation usually amounted to 10^{-2} after 2 h and 2×10^{-3} to 5×10^{-3} after 4 h. At this time, the cells were quickly filtered under sterile conditions. They were then suspended in 10 ml of minimal medium containing 0.4% CAA and 10 mM hexitol. The procedure was repeated if necessary.

Suicide mutant selection. The suicide mutant selection method, first described for the selection of bacteria mutants by Lubin (21), was modified in the following way to select for mutants with an altered K_m or temperature-sensitive transport system. Cultures from single colonies were grown out after independent mutagenesis at 42 C in minimal medium with D-glucose or D-glucitol as the carbon source to counterselect for unspecific auxotrophic or temperature-sensitive mutants. The cultures were diluted 1:100 in minimal medium containing 10 mM D-mannitol, and

TABLE 3. *Origin, phenotype, and genotype of hexitol transport mutants*[a]

Strain	Parent	Isolation	Mtl	Gut	Gat	Frc	*mtl*			*gut*			*gat*			*pts*	
							C	*A*	*D*	*C*	*A*	*D*	*C*	*A*	*D*	*I*	*H*
L141	JC411	Leu$^+$ transductant	−	+	+	+	+	−	+	+	+	+	c	+	+	+	+
L142	L141	Mtl$^+$ revertant	+	+	+	+	+	−	+	c	+	+	c	+	+	+	+
L162	L141	Mtl$^+$ revertant	+	+	+	+	+	+	+	+	+	+	c	+	+	+	+
L143	L142	2-Deoxy-arabino-hexitol resistant	−	s	+	+	+	−	+	c	−	+	c	+	+	+	+
L144	L142	2-Deoxy-arabino-hexitol resistant	−	−	+	+	+	−	+	c	−	+	c	+	+	+	+
L146	L143	Glucitol resistant	−	−	−	+	+	−	+	c	−	+	c	−	+	+	+
L156	L146	Transduction	+	−	−	+	c	+	+	c	−	+	c	−	+	+	+
L148	L146	Revertant	+	+	−	+	+	−	+	c	+	+	c	−	+	+	+
L170	L148	Transduction	−	−	−	+	c	−	−	c	−	+	c	−	+	+	+
L153	L146	Conjugation	−	+	+	+	+	−	+	+	−	+	c	+	+	+	+
L173	L146	Transduction	+	+	+	+	c	+	+	c	+	+	c	+	+	+	+
L175	L173	Penicillin	−	−	−	−	c	+	+	c	+	+	c	+	+	−	(+)

[a] *C*, Gene determining inducibility or constitutivity of the hexitol pathways indicated; *A*, gene specifying the enzyme II-complex; *D*, gene specifying the hexitol-phosphate dehydrogenase. Mtl, Gut, Gat, and Frc, Phenotypes of D-mannitol, D-glucitol, galactitol, and D-fructose fermentation, respectively; *mtl*, *gut*, *gat*, and *frc*, corresponding genotypes. +, Positive or wild type; −, negative or mutated; c, constitutive; s, sensitive and negative. *ptsI*, Gene specifying enzyme I; *ptsH*, gene specifying protein HPr of the phosphotransferase system.

grown out at 25 C to 2 × 10^9 bacteria per ml. After centrifugation and one washing, 2 × 10^7 bacteria per ml were suspended in minimal medium and kept for 60 min at 42 C. Thereafter 2 × 10^6 to 5 × 10^6 bacteria were inoculated in a wide, screw-cap tube containing 0.9 ml of minimal medium and 10 μM D-[^{3}H]mannitol (3,130 mCi/mmol) at 42 C. The tubes were shaken for 3 days at 42 C, and the percentage of surviving bacteria was determined by plating and counting before, during, and after treatment. After 3 days the inactivation was ended by adding to each culture 5 ml of minimal medium containing 10 mM D-mannitol and growing out the cultures at 25 C. The survivors were screened for a D-mannitol-negative phenotype on indicator plates and for a temperature-sensitive phenotype on minimal plates at 25 and 42 C.

Isolation of transport mutants by chemotaxis. Mutagenized cells of the constitutive strain 238 were grown out in minimal medium-glycerol at 30 C. Bacteria (2 × 10^8/ml) of an exponential culture were inoculated in the middle of a soft-agar plate (2) containing 50 μM D-mannitol and incubated at 30 C until a clear chemotactic ring had moved 3 to 5 cm away from the inoculum. Cells of the center were then placed in the middle of a new plate, and the procedure was repeated 20 times. Every 5th time, a plate was used with D-mannitol being replaced by D-glucose to counterselect for nonmotile mutants. The next D-mannitol-containing plate was inoculated from the chemotactic ring of the glucose-containing plate. After the last run, cells were plates for single colonies, and these were tested for altered motility on D-mannitol plates. The same procedure with strain L148 was used to select for mutants with altered motility on D-glucitol-containing plates.

Interrupted mating, F-duction, and P1 transduction. Standard conditions were used in conjugation and F-duction experiments (20, 30) and in P1 transductions (3), except that glycerol was substituted for D-glucose in the Luria broth medium. All recombinants and transductants were purified twice on the selection plates before being tested for unselected markers. The markers *cysC*, *pheA*, *uraP*, *nalB*, *gut*, *gat*, and *fda*^{ts} were tested on minimal medium plates, whereas marker *alaS* had to be tested on MacConkey D-glucitol indicator plates at 25 and 42 C. The *gutC*$^+$*A*$^+$ *mtlA*$^-$*D*$^+$ colonies are white and the *gutC*^{c}*A*$^+$ *mtlA*$^-$*D*$^+$ colonies are red on D-mannitol indicator plates.

Uptake assays. Uptake assays were done, as described previously (18), by taking samples at time 10 and 20 s and at 0 s, with cells kept for 5 min prior to the test at 0 C. Standard final concentrations for hexitols were: D-[^{3}H]mannitol (20 Ci/mol), 5 μM; D-[^{3}H]glucitol (10 Ci/mol), 25 μM; [^{14}C]galactitol (5.5 Ci/mol), 25 μM. All uptake activities are expressed as nanomoles per minute per milligram of protein.

Preparation of cell-free extracts. The preparation of cell-free extracts was as described previously (18). Strain L146, triple negative for the three hexitol-specific enzyme II-complexes and grown in minimal medium plus casein acid hydrolysate plus 10 mM D-glucose, was used to prepare the enzyme I-HPr extract.

Assay of enzyme II-complex and enzyme I activities. The rate of formation of [^{14}C]hexitol-phosphate from [^{14}C]hexitol in vitro can be used to determine the enzyme II-complex activity, and was measured as described previously (18). Final concentrations were as follows: D-[^{14}C]mannitol (2.5 Ci/mol), 30 μM; D-[^{14}C]glucitol (5 Ci/mol), 0.21 mM; and [^{14}C]galactitol (5.5 Ci/mol), 0.5 mM. In the test, 0.1 ml of extract of strain L146 (15 to 25 mg of protein per ml) and 0.02 ml of extract of the strains to be tested

for enzyme II-complex activity (1 to 5 mg of protein per ml) were added. Samples were taken after 1, 5, and 10 min. Enzyme I activity was measured in a similar way with D-[^{14}C]mannitol as substrate, 0.02 ml of extract of strain L156 for enzyme II-complex^{mtl} activity, and 0.01 to 0.1 ml of extract to be tested for enzyme I activity. Enzyme I- and enzyme II-complex activities are expressed as nanomoles per minute per milligram of protein.

Assay of hexitol-phosphate dehydrogenase activities. Mannitol-1-phosphate dehydrogenase (EC 1.1.1.17) activity was measured, as described previously (18), by following the rate of reduction of nicotinamide adenine dinucleotide at 340 nm; D-glucitol-6-phosphate dehydrogenase (EC 1.1.1.140) activity was measured by following the rate of reduction of a tetrazolium dye (18). To test galactitol-1-phosphate dehydrogenase activity, the assay mixture contained in a total volume of 0.5 ml: water, 0.25 ml; 1 M Na_2CO_3, 0.05 ml; 20 mM nicotinamide adenine dinucleotide, 0.05 ml; 10 mM galactitol-1-phosphate, 0.10 ml; and cell extract. The rate of reduction of nicotinamide adenine dinucleotide at 340 nm was followed at 30 C. All hexitol-phosphate dehydrogenase activities are expressed as micromoles per minute per milligram of protein.

Protein determination. All protein determinations were done by the biuret (22) method, with bovine serum albumin (Sigma Chemical Co.) in 0.1 M tris(hydroxymethyl)aminomethane-hydrochloride buffer as the calibrating protein.

RESULTS

Isolation of mutants with altered hexitol transport activities. By means of the modified penicillin selection described above, mutants were isolated which are unable to grow at low (1 μM) D-mannitol concentrations but able to grow on high concentrations. Among 34 mutants isolated (series PHK) from the constitutive strain 238, 27 were mutants with low D-mannitol transport and enzyme II-complex^{mtl} activity (from 0.5 to 10%). They grow slowly (generation times from 90 to 360 min versus 72 min for strain 238) at all D-mannitol concentrations. Seven mutants, however, do not grow at all on low concentrations of D-mannitol, whereas they have generation times of 95 min on 10 mM substrate. All seven have been shown to be double mutants of the genotype *gutC^{c}A$^+$D$^+$ mtlC^{c}A$^-$D$^+$*. This system indeed behaves like a D-mannitol transport system, with a K_m for D-mannitol 10^3 times higher than that of the true D-mannitol transport system for this substrate (17).

As an alternative method of isolating transport mutants with an altered K_m, the suicide technique with D-[^{3}H]mannitol, was tried. To test the effectiveness of the method in a reconstruction experiment, 2×10^6 cells of the constitutive transport-positive strain 238 were mixed in one tube with 4×10^4 cells of the transport-negative mutant 236. The results indicate (Fig. 1) that the rate of inactivation of strain 238 is nearly 5.5 times faster than for strain 236. This inactivation is especially dramatic if a *recA* derivative (strain J441) is used. In Fig. 1 are also shown the inactivation curves of two previously mutagenized (MNNG) cultures of strain 238. The culture in tube C1N1 was pregrown at 25 C on 10 mM D-mannitol to counterselect for *mtl*-negative mutants. The ratio of *mtl$^-$/mtl$^+$* cells at the beginning of the suicide experiment was 3/2,275 (0.013%) and 6/94 at the end (6.0%). The culture in tube C1NG11 was only pregrown in D-glucitol, and the inactivation obviously slows down after 1 day, because 90% of the survivors are *mtl*-negative mutants (series SHK).

Among 87 such SHK mutants tested, none was found to have temperature-sensitive growth on D-mannitol. Therefore, nine independent D-mannitol transport-negative mutants of the PHK and SHK series were plated on minimal medium plates with D-mannitol as the carbon source, and spontaneous or MNNG- and ethyl

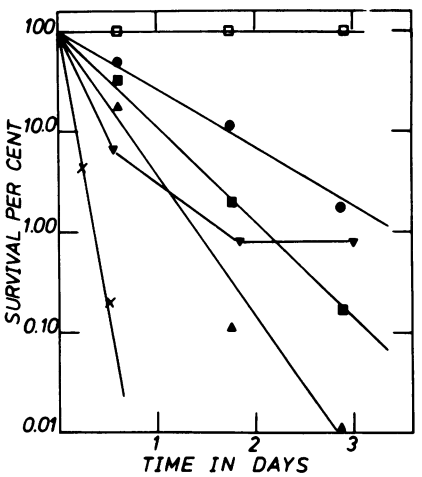

FIG. 1. *Suicide of strains 236 and 238 by D-[^{3}H]mannitol. To exponentially growing cells that had been pregrown on D-glucose or D-glucitol (C1NG11), washed, and suspended at 42 C in minimal medium was added D-[^{3}H]mannitol (10 μM; 3,130 Ci/mol), and the inactivation rate was determined by plating. Cells of strains 238 (▲) (2×10^6 bacteria per ml) and 236 (●) (4×10^4 bacteria per ml) were mixed in one tube. Other cultures were J441 (recA) (×) (2×10^6 bacteria per ml), C1N1 (■) (2×10^6 bacteria per ml), and C1NG11 ( ) (5×10^6 bacteria per ml), and two mutagenized cultures of strain 238. One culture (□) is a control of strain 238 without radioactive D-mannitol.*

methane sulfonate-induced positive revertants were isolated. These were either screened immediately for a D-mannitol temperature-sensitive phenotype or subjected to two penicillin selection cycles at 42 C followed by growth on D-mannitol at 25 C. From the 9 parents, 26 temperature-sensitive mutants were isolated. The growth behavior of two such mutants on D-glucose, D-glucitol, and D-mannitol at 25 and 42 C can be seen in Fig. 2. Although both were selected as revertants of mutants with a defect in D-mannitol uptake, and although the temperature sensitivity can only be seen when the cells are growing on D-mannitol or D-glucitol, none has a temperature-sensitive D-mannitol or D-glucitol uptake (Table 2). Instead, the D-mannitol-1-phosphate dehydrogenase and/or the D-glucitol-6-phosphate dehydrogenase is inactivated at 42 C. In cell extracts of strain L243, no dehydrogenase activity was detected, even when the cells were grown and tested at 25 C. The complete genotype of strain L243 was found to be $mtlC^cA^+D^{ts}$ $gutC^+A^+D^{ts}$ and of strain L244 to be $mtlC^cA^-D^+$ $gutC^cA^+D^{ts}$. During the screening of the mutants, commercial D-mannitol containing 2% D-glucitol was used. Therefore, strain L244 growing at 42 C would be inhibited by the accumulating D-glucitol-6-phosphate. Growth shown in Fig. 2 was on purified D-mannitol containing 0.005% D-glucitol, and thus no growth inhibition at 42 C was observed.

The selection of mutants with an altered or missing motility on soft-agar D-mannitol plates thus far has permitted the isolation of two types of transport mutants. The majority, as in the case of the two other selection procedures, have a reduced D-mannitol uptake activity always correlated with a similarly reduced enzyme

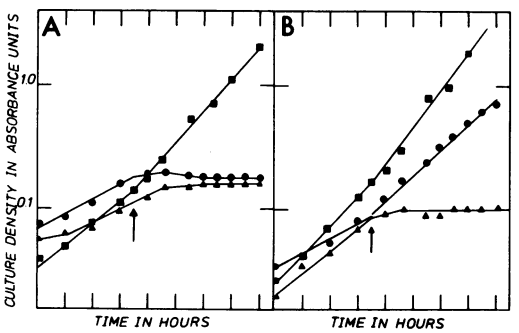

FIG. 2. *Growth of strains L243 and L244 at 25 and 42 C. Cultures of strains L243 (A) and L244 (B) growing exponentially on* D-*glucose* (■), D-*mannitol* (●), *or* D-*glucitol* (▲) *at 25 C were shifted rapidly at the time indicated by the arrow to 42 C, and further growth followed.*

II-complex^{mtl} activity (series MHK). Few mutants can be separated from the rest, since they will move on plates if the concentration of D-mannitol is raised to 5×10^{-4} M. These mutants again have been shown to be of the $gutC^c$ type.

The last two selection procedures utilized are based on the intracellular accumulation of toxic intermediates. Solomon and Lin (28) have shown that the addition of D-mannitol to a strain with an inactive D-mannitol-1-phosphate dehydrogenase growing on a second carbon source causes a rapid inhibition of growth. The culture, however, will resume growth after a while. We repeated the procedure with strain L243 growing on glycerol plus D-mannitol at 42 C, with strain L244 growing on glycerol plus D-glucitol at 42 C, and with a mutant lacking a galactitol-1-phosphate dehydrogenase growing on glycerol plus galactitol. Isolated in each case were a few true revertants (phenotype: hexitol$^+$ Frc$^+$), a few pleiotropic mutants with a defect in enzyme I of the phosphotransferase system (phenotype: hexitol$^-$ Frc$^-$), and in the majority of the cases mutants with a mutation in the corresponding transport system (phenotype: Mtl$^-$ Frc$^+$, Gut$^-$ Frc$^+$, or Gat$^-$ Frc$^+$). This elegant selection procedure, however, requires mutants with a defect in a hexitol-phosphate dehydrogenase. Thus, we chose 2-deoxy-arabino-hexitol as an analogue possibly toxic for the wild-type strains also. This substance might eventually be phosphorylated by a hexitol transport system, since it is 2-deoxy-D-mannitol or 2-deoxy-D-glucitol at the same time, but it never could be dehydrogenated to a ketose-phosphate.

When added to cells growing on glycerol, the analogue indeed rapidly inhibits growth of a strain with a constitutive D-glucitol transport system (L148) and allows only weak growth of a similar D-mannitol mutant (L156). It does not inhibit growth of a mutant with an inactive D-glucitol transport system either due to a mutation in gene *gutA* (strain L170) or in gene *ptsI* (strain L175), nor of a mutant with an active, but inducible, D-glucitol transport system (strain L162). Strain L153, with a constitutive galactitol system, is not affected either. The growth inhibition caused by the 10 mM analogue in strain L148 can be prevented by the addition of 1 mM D-glucitol. As expected, 2-deoxy-arabino-hexitol strongly inhibits the uptake of D-glucitol and weakly inhibits the uptake of D-mannitol (Fig. 3). Furthermore, although it is a good substrate for the system coded by gene *gutA*, the analogue is not an

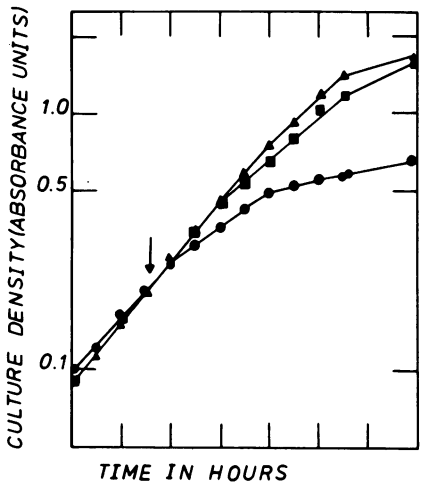

FIG. 3. *Growth of different hexitol transport mutants in the presence of 2-deoxy-arabino-hexitol. To cells growing exponentially in minimal medium-glycerol at 37 C was added 2-deoxy-arabino-hexitol (10 mM) at the time indicated by the arrow. The strains tested: L148 (gutC^{c}A$^+$D$^+$), ●; L156(mtlC^c A$^+$D$^+$), ■; and L170 (gutC^{c}A$^-$D$^+$), ▲. No growth inhibition (similar to L170) was observed in L162 (gutC$^+$A$^+$D$^+$), L175 (gutC^{c}A$^+$D$^+$ptsI), and L153 (gatC^{c}A$^+$D$^+$), and in strain L148 if 1 mM D-glucitol was added together with the analogue.*

inducer for the *gut* operon. This does explain the resistance of the inducible strain L162 towards the analogue.

Since *gutA* and *pts* mutants are resistant to the substance, we expected it to be transported and phosphorylated by the enzyme II-complex^{gut}, with P-HPr as the phosphate donor. From strain L148 grown in the presence of the analogue, we have in fact isolated a new phosphate found intracellularly in high concentrations. This phosphate (R_{Pi} = 1.06) can be separated on DC cellulose plates in the system ammonium-acetate-ethanol-water-ethylenediaminetetraacetic acid from fructose-6-phosphate (R_{Pi} = 0.92) and glucitol-6-phosphate (R_{Pi} = 0.58).

Sequential isolation of a triple mutant lacking all hexitol transport systems. From strain L141 (*mtlC$^+$A$^-$D$^+$ gutC$^+$A$^+$D$^+$*) the mutant L142 (*gutC^{c}A$^+$D$^+$*) was isolated as a mannitol-positive revertant (18). The *gutC^c* Mtl$^+$ revertants can be distinguished from true *mtl$^+$* revertants by a different coloration on MacConkey D-mannitol indicator plates, by their slower growth rate on D-mannitol (Table 4), and by their sensitivity towards 2-deoxy-arabino-hexitol. When derivatives of strain L142 resistant to this analogue were isolated, four classes were detected: (i) Gut$^-$ Frc$^+$ mutants of the genotype *gutC^{c}A$^-$*D$^+$ or *D$^-$*, (ii) Gut$^-$Frc$^-$ mutants of the genotype *gutC^{c}A$^+$D$^+$ ptsI*, (iii) Gut$^+$Frc$^+$ mutants of the genotype *gutC^{c}A$^-$D$^+$* (e.g., L144), and (iv) Gut$^-$Frc$^+$, sensitive to D-glucitol, of the genotype *gutC^{c}A$^-$D$^+$* (e.g., L143).

The different coloration of class iii mutants on MacConkey D-glucitol indicator plates and their slow growth in minimal D-glucitol medium (Table 4), together with the sensitivity of class iv mutants towards D-glucitol, suggested the existence of a second D-glucitol transport system in strain L142, which is perhaps missing or inactive in the class i and ii mutants.

Strains L141, L142, L143, and L144 happened to be all of the rare *E. coli* K-12 strains able to grow on galactitol (Table 4). As will be shown in a subsequent paper, this hexitol is dissimilated by a metabolic pathway different from the D-mannitol and D-glucitol pathways. Strain L146, however, a derivative of L143 resistant to D-glucitol, is no longer able to grow on galactitol. Since strain L146 has also lost the galactitol transport system (Table 4) and, since galactitol-positive revertants of strain L146 are D-glucitol sensitive again, we have to assume that this galactitol system does indeed transport D-glucitol too. If the enzyme D-glucitol-6-phosphate dehydrogenase is absent due to

TABLE 4. *Growth, uptake, and enzyme II-complex activities of some mutants*[a]

Strain	D-Mannitol			D-Glucitol			Galactitol		
	GT	TP	EII^{mtl}	GT	TP	EII^{gut}	GT	TP	EII^{gat}
L141	NG	0.1	0.01	72	8.50	2.10	105	12.00	0.60
L142	95	0.1	0.50	72	10.00	1.90	92	11.90	0.50
L143	NG	0.1	0.01	NG	1.80	0.01	93	8.90	0.50
L144	NG	0.1	0.01	NG	0.01	0.01	92	10.30	0.60
L146	NG	0.1	0.01	NG	0.01	0.01	NG	0.01	0.01
L173	60	8.0	2.00	84	9.10	2.80	93	15.00	1.00
L175	NG	0.1	0.70	NG	0.01	1.70	NG	0.10	0.90

[a] NG, No detectable growth; GT, generation time in minutes; TP, transport activity in nanomoles per minute per milligram of protein; and EII, enzyme II-complex activity in nanomoles per minute per milligram of protein.

polar effects, as in the case of mutant L143 and its derivative L146 (Table 5), cells with an active galactitol transport system (e.g., L143) are sensitive to D-glucitol and cells lacking it (e.g., L146) are resistant. The 14% dehydrogenase activity still present in the class iii mutant L144 obviously is sufficient to prevent the accumulation of toxic levels of D-glucitol-6-phosphate even in the presence of an active galactitol transport system.

The pedigrees of all derivatives of strain L141 mentioned so far are summarized in Table 3, and their properties are described in Table 4.

Mutant L146 was obtained through three mutations inactivating a D-mannitol, a D-glucitol, and a galactitol transport system. This strain does not grow on any of the three hexitols nor is it able to transport or phosphorylate a hexitol. Thus, all transport systems of the phosphotransferase type and also enzymes able to phosphorylate hexitols apparently have been eliminated. All hexitol-positive revertants isolated so far from strain L146 have regained at least one of the three systems described.

Mapping of mutations affecting the hexitol transport systems. The different mutations leading to constitutive synthesis or to an alteration of the enzyme II-complex and hexitol-phosphate dehydrogenase activities have been mapped by conjugation and P1 transduction.

(i) Genes clustered in the mtl operon. It has been suggested (28) that genes *mtlC*, *mtlA*, and *mtlD* form an *mtl* operon located at 71 min on the *E. coli* linkage map. Most point mutations in gene *mtlA*, coding the enzyme II-complex^{mtl}, have polar effects on the expression of gene *mtlD*, the structural gene for the D-mannitol-1-phosphate dehydrogenase. Mutations leading to a temperature-sensitive dehydrogenase activity indeed map in *mtlD*. Mutations in the control gene *mtlC*, termed *mtlC*^{c}, leading to the constitutive expression of *mtlA* and *D* are *cis* dominant over *mtlC*$^{+}$. These are further indications for the existence of an *mtlC A D* operon. This operon, as shown previously (18), is inducible only by D-mannitol.

All mutations thus far analyzed that inactivate the enzyme II-complex^{mtl} map in the gene *mtlA*.

(ii) Genes clustered in the gut operon (formerly sbl or srl). An enzyme II-complex^{gut} has been shown to be induced by D-glucitol together with a D-glucitol-6-phosphate dehydrogenase (18). All mutations thus far analyzed that inactivate this enzyme II-complex map in gene *gutA*. They are closely linked to control gene *gutC* and to *gutD*, the structural gene of the D-glucitol-6-phosphate dehydrogenase as determined by mapping of mutant L244. Most *gutA* mutants (e.g., L143 or L146 [Table 5]) show polar effects on the expression of the gene *gutD*, and the polarity is relieved in the revertant L148. Diploids of the genotype *gutC*^{c}*A*$^{-}$*D*$^{-}$/*F'*143 *gutC*$^{+}$*A*$^{+}$*D*$^{+}$ remain Mtl^{-}, and diploids *gutC*$^{cA+}$*D*$^{+}$/*F'*143 *gutC*$^{+}$*A*$^{+}$*D*$^{+}$ have an Mtl^{+} phenotype. Thus, the *gutC*^{c} mutation again is *cis* dominant over the wild-type *gutC*$^{+}$.

As summarized in Fig. 4 and 6, the markers *gutC A D* are covered by F'143 and F'108. According to interrupted matings with deriva-

TABLE 5. *Transport and dehydrogenase activities of point mutants isolated by different methods*

Strain	Hexitol	Transport activity		Dehydrogenase activity	
		nmol/min per mg of protein	%	μmol/min per mg of protein	%
238	D-Mannitol	5.40	100	1.15	100
239	D-Mannitol	11.20	210	0.00	0
PHK 15	D-Mannitol	0.11	2	0.35	30
SHK 20	D-Mannitol	0.45	8	0.13	11
SHK 22	D-Mannitol	0.20	4	0.75	65
SHK 23	D-Mannitol	0.16	3	0.08	7
SHK 330	D-Mannitol	0.33	7	0.10	9
MHK 50	D-Mannitol	0.16	3	0.02	2
MHK 53	D-Mannitol	0.32	6	1.06	93
MHK 54	D-Mannitol	0.15	3	0.68	58
L142	D-Glucitol	10.00	100	2.74	100
L143	D-Glucitol	0.01	1	0.00	0
L144	D-Glucitol	0.01	4	0.38	14
L146	D-Glucitol	0.01	1	0.00	0
L148	D-Glucitol	10.20	100	2.60	99
L151 gutC^{c}	D-Glucitol	8.30	83	0.01	1

tives of Hfr KL16, they map at 51 to 52 min on the *E. coli* linkage map, in good agreement with the preliminary mapping by W. Epstein (cited in reference 30). The *gut* gene cluster co-transduces in P1 transductions (Table 6) to 25% with the marker *alaS* and to 39% with the marker *nalB*, placed at 51 min on the linkage map. According to these data, *alaS* and *gut* are separated by 0.75 min, and *nalB* and *gut* are separated by 0.50 min.

Transductions involving the markers *nalB*, *uraP*, *pheA*, *cysC*, and *recA* are not very reproducible, and the data found in the literature

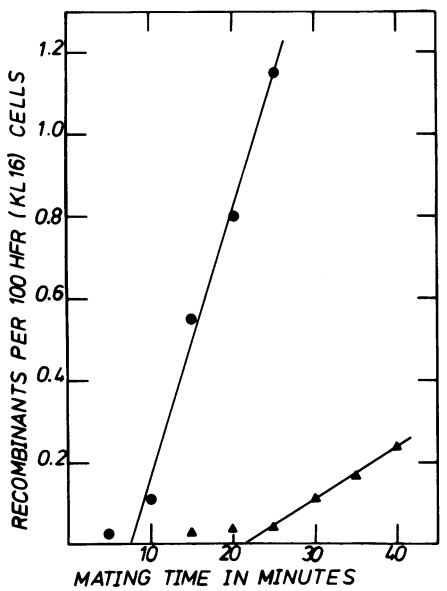

FIG. 4. *Interrupted mating to determine the time of entry of the markers gutCAD and his. Samples of a mating at 37 C between Hfr L204 (his⁺ gut⁺ gat⁺ str⁺) and L146 (F⁻ his⁻ gutC^cA⁻D⁺ gatC^cA⁻D⁺ strA) were plated on minimal medium-streptomycin plates selective for his⁺ (▲) and gut⁺ (●) recombinants.*

TABLE 6. *Linkage of the markers gutCAD, alaS, and nalB*[a]

Donor	Recipient	Selected marker	No. tested	Linkage of non-selected marker (%)
P1.AT2427	L177	*gut⁺*	164	*nalB* (39) *cysC* (2) *gutC* (93)
P1.BM113	L146	*gut⁺*	263	*alaS* (25) *pheA* (0) *gutC* (16)

[a] Selection of *gut⁺* recombinants in the transduction involving *alaS* was done at 30 C.

(cited in reference 30) differ by as much as 0.5 min. In this study also, *uraP* (data not shown), *pheA*, or *cysC* transductants might have been missed, all available strains being very leaky.

In an independent study, the phenotypic expression of the *nalB* marker was found to be allele, strain, and medium dependent. A linkage of 40% between *nalB* and *gut* and of 70% between *recA* and *gut* was established (R. Curtiss III, personal communication), indicating the gene order *alaS recA gutCAD nalB*.

The low linkage of markers *gutC* and *gutA* in one transduction (16 versus 93%) cannot be explained (counterselection, direction of the *gut* operon?).

All data together, however, strongly suggest the existence of a *gutC A D* operon closely linked to *nalB* and *alaS*.

(iii) Genes clustered in the gat operon. Interrupted matings with a series of Hfr strains made *gat⁺* by P1 transduction from strain L153 indicated that this gene cluster is located midway between *his* (linkage 71%) and *nalA* (linkage 64%) (Table 7, Fig. 5 and 6). This location is confirmed by the fact that the type I episome F'150 (20) of strain KL96 does not cover *gat*, whereas the type II episome F'131 (20), originating from the same strain, does. Our F'131 includes the markers *his gnd gat* and *mglP* but not *nalA* (Fig. 6).

No linkage between markers *his*, *gnd*, or *nalA* and our *gat* gene cluster has been found in P1 transductions (Table 8). Assuming 1.5-min maximal co-transduction with phage P1, the *gat* markers seem to be located near 40.5 to 40.8 min on the linkage map, close to markers *mgl* and *frc*.

cis-dominant *gatC^c* mutants, polar *gatA* mu-

TABLE 7. *Linkage of the markers gatCAD, his, and nalA as determined by conjugation*[a]

Donor	Recipient	Selected marker	No. tested	Recombinants (%)
L204	L160	*his*	146	*nalA gat⁺ his⁺* (40) *nal⁺ gat⁺ his⁺* (31) *nalA gat⁻ his⁺* (23) *nal⁺ gat⁻ his⁺* (5)
L204	L160	*gat*	138	*nal⁺ gat⁺ his⁺* (34) *nal⁺ gat⁺ his⁻* (30) *nalA gat⁺ his⁺* (18) *nalA gat⁺ his⁻* (17)

[a] *his⁺* and *gat⁺* recombinants from the mating shown in Fig. 5 were purified and tested as described in the text. Strains were: L204, *his⁺ nal⁺ gut⁺ gatC^cA⁺D⁺ str⁺*; and L160, *his⁻ nalA gutC^cA⁺D⁺ gatC^cA⁻D⁺*.

tants, and temperature-sensitive *gatD* mutants have been isolated (unpublished data) and shown to be closely linked (90%) in the *gatC A D* operon.

(iv) Pleiotropic hexitol transport-negative strains. Several pleiotropically negative strains like L175 (Table 4) unable to grow on hexitols,

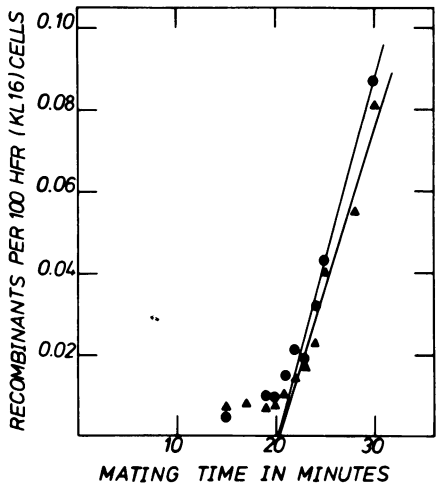

FIG. 5. *Interrupted mating to determine the time of entry of the markers gatCAD and his. Samples of a mating at 37 C between Hfr L204 (his⁺ gut⁺ gatC^cA⁺D⁺ nal⁺ str⁺) and L160 (F⁻ his⁻ gutC^cA⁺D⁺ gatC^cA⁻D⁺ strA nalA) were plated on minimal medium-streptomycin plates selective for his⁺ (●) and gat⁺ (▲) recombinants.*

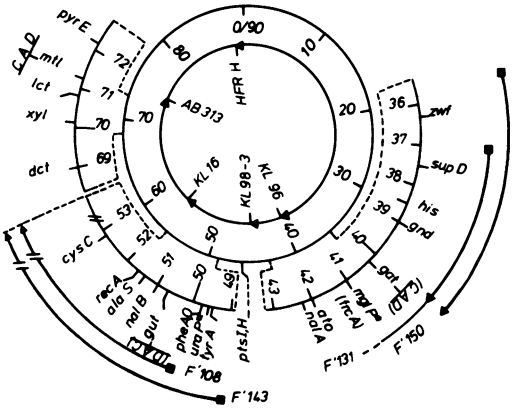

FIG. 6. *Simplified genetic map of E. coli K-12. The map was drawn according to the data given by Taylor and Trotter (30) and K. B. Low (20). Symbols are as described previously, except for srl which, for reasons discussed in this paper, has been changed to gut. Arrows and squares indicate the origins and ends, respectively, of Hfr and F' strains. Broken lines in the case of the type II episome F-131 are used to indicate that the exact length is not known.*

TABLE 8. *Linkage of the markers gatCAD, nalA, gnd, and his as determined by P1 transduction*

Donor	Recipient	Selected marker	No. tested	Linkage of unselected markers (%)
P1.L153	NP3151	*gat*	176	*gnd* (0)
P1.L153	L160	*gat*	200	*nalA* (0) *his* (0)
P1.L153	L147	*his*	100	*gat*⁺ (2)
		gat	100	*his* (1)

fructose, or glucose and having only low HPr and enzyme I activities (17) have been isolated by the methods described above. These mutants revert on hexitols, fructose, or glucose to the wild-type phenotype. The corresponding mutations are transferred in interrupted matings involving strain KL16 nearly 5 min after *gut*, indicating their location in the *pts* operon.

DISCUSSION

As shown in the present paper, D-glucitol is taken up and phosphorylated in *E. coli* K-12 by three transport systems, D-mannitol is taken up by two, and galactitol is taken up by one. Mutations in the three genes *mtlA*, *gutA*, and *gatA*, however, are sufficient to eliminate all transport and phosphorylating activities for hexitols. As will be shown in the accompanying paper (17), each gene does code for an enzyme II-complex of the phosphoenolpyruvate-dependent phosphotransferase system. These enzyme II-complexes phosphorylate more than one hexitol. Thus, D-glucitol is phosphorylated and transported by the *mtlA*-, *gutA*-, and *gatA*-coded systems, D-mannitol is phosphorylated and transported by the systems coded by *mtlA* and *gutA*, and galactitol is phosphorylated and transported by the *gatA*-coded system.

The aim of the present study was to find mutations for every transport system and enzyme involved in hexitol uptake and phosphorylation and, if possible, to find different types of mutations. A series of selection procedures has been used, and many mutants with defects in genes *mtlA*, *gutA*, and *gatA* or *pts* were detected. Unfortunately, they represent only a few classes of transport mutants. Thus, mutants with a temperature-sensitive enzyme II-complex activity have not been found, although mutants with temperature-sensitive hexitol-phosphate dehydrogenase activity were detected. Mutants with a low affinity of D-man-

nitol for the enzyme II-complex^{mtl} have been isolated recently. The analysis of such mutants or of mutants with altered translocation and feedback properties, together with the precise mapping of many transport mutants, might provide further information on the number of genes and molecules involved in group translocation.

If the data presented here are not sufficient to give the precise location of the genes and operons coding for hexitol transport systems, they clearly indicate the existance of three, and only three, such transport systems. The triple mutant *mtlA gutA gatA* has lost all transport and phosphorylating activities for hexitols, and hexitol-positive revertants invariably regain one of these activities.

In *K. aerogenes*, growth and transport of D-mannitol and D-glucitol normally also proceed via an enzyme II-complex^{mtl} and enzyme II-complex^{gut} (14, 29), followed by dehydrogenation by means of specific hexitol-phosphate dehydrogenases. When Mtl$^+$ revertants from a *mtlC*$^+$*A*$^-$*D*$^-$ *gut*$^+$ mutant of *Klebsiella* were isolated, the majority were shown to transport (17) and metabolize (5, 29) free D-mannitol. Revertants from a similar mutant of *E. coli* always had the genotype *mtlC*$^+$*C*$^-$*D*$^+$ *gutC*^{c}*A*$^+$*D*$^+$. Thus, *E. coli*, in contrast to *Klebsiella*, apparently is unable to revert in a single step from *mtlC*$^+$*A*$^-$*D*$^-$ to Mtl$^+$. *E. coli* K-12, in fact, is unable to grow under aerobic or anaerobic conditions on D-arabinitol, L-arabinitol, ribitol, or xylitol at high or low temperatures. Even after heavy mutagenesis by MNNG, no positive mutants have been found (unpublished data). Recently pentitol-positive mutants of *E. coli* K-12 have been reported (F. Stevens and T. T. Wu, Fed. Proc. **33**:156, 1974). The metabolism was shown to proceed via the propandiol transport system and a galactose dehydrogenase.

Some metabolic pathways in the *Enterobacteriaceae* have different enzymes for aerobic and anaerobic growth (9, 10, 24). Our strains with a defect in the corresponding *A* or *D* genes are negative under aerobic, as well as anaerobic, conditions. Selection of revertants under both conditions gives identical results. Mutants with temperature-sensitive dehydrogenases have a temperature-sensitive phenotype under both conditions. Thus, a second set of enzymes for anaerobic conditions seems to be ruled out.

The presence of an adenosine 5′-triphosphate-dependent D-mannitol kinase in *E. coli* Crookes has been reported (15). To obtain a high activity of this kinase, however, a heat-stable cofactor had to be added in addition to adenosine 5′-triphosphate. This cofactor obviously is generated during the assay from adenosine 5′-triphosphate. The enzyme has a pH optimum near 7.6, requires Mg^{2+}, and has a K_m for D-mannitol below 10^{-5} M. The heat-stable cofactor might well have been phosphoenolpyruvate (or P-HPr) since the K_m, Mg^{2+} requirement, and pH optimum are exactly as reported for the enzyme II-complex^{mtl} (17, 28, 29) in *E. coli* K-12. In this bacterium after the removal of enzyme II-complex^{mtl} and enzyme II-complex^{gut}, no further phosphorylation of D-mannitol is observed.

On the basis of chemotaxis tests, Adler and Epstein (2) have postulated the existence of three chemoreceptors for hexitols. We have been able to confirm and extend these data by means of our constitutive mutants having alternatively only one of the three transport systems described (unpublished data). As in the case of D-glucose (1), the enzyme II-complexes, or parts of them, are the chemoreceptors for hexitols since both have the same specificity and regulation. Furthermore, both activities are inactivated simultaneously by mutations in *mtlA*, *gutA*, or *gatA*. In strains where hexitols are transported only by low-affinity systems, the K_m for chemotaxis rises too. Chemotaxis towards hexitols is inhibited by D-glucose, and chemotaxis towards D-glucose is inhibited by D-mannitol. This was taken as an indication for the recognition of D-mannitol by the D-glucose chemoreceptor and vice versa. Uptake of D-mannitol by a D-glucose transport system or of D-glucose by hexitol transport systems has not been detectable. Instead, it could be shown that the inhibiton caused by D-glucose can only be seen if an active enzyme II-complex^{glc} is present. Most enzyme II-complexes, especially those for D-glucitol and galactitol, are strongly inhibited by hexose-phosphates (13, 16; unpublished data) in vivo and in vitro, usually in a noncompetitive way. Thus, the inhibition of chemotaxis or transport through systems involving enzyme II-complexes must not necessarily be competition for the same enzyme but might be indirect.

The same argument holds for a class of D-mannitol transport mutants of *Salmonella typhimurium* (4). These mutants show a concentration-dependent growth rate on D-mannitol from 0.5 to 10 mM substrate. Growth is severely inhibited by α-methylglucoside in contrast to the wild-type strains, where growth inhibition is barely detectable. Mtl$^+$ revertants of our series with the genotype *mtlC*$^+$*A*$^-$*D*$^+$ *gutC*^{c}*A*$^+$*D*$^+$ (e.g., L148) have a K_m for growth on

D-mannitol of 0.5 mM (versus 3 μM for an *mtl*$^+$ strain). In contrast to *mtl*$^+$ cells, this growth of *gutC*^{c}*A*$^+$ mutants on D-mannitol is severely inhibited by α-methylglucoside. Since uptake and phosphorylation of D-mannitol in such suppressed mutants has been shown to proceed via the enzyme II-complex^{gut} (18), an enzyme strongly inhibited by α-methylglucoside-phosphate (unpublished results), the hypothesis proposing uptake of D-mannitol via an enzyme II-complex^{glc} seems unnecessary.

Thus, it might be concluded that up to now no indications for additional hexitol transport or phosphorylating systems in our strains have been found.

Hexitol transport and metabolism in the *Enterobacteriaceae* proceed via identical pathways, with the genes for each pathway being clustered in one operon. Each operon consists of a regulatory gene specifically responding to one inducer and the structural gene for the dehydrogenase specific for the phosphate of the inducer. This gene is located distal from the *cis*-dominant regulatory gene, whereas a third gene coding for an enzyme II-complex is located proximal. The enzyme II-complex in every case has a very high affinity for its inducer and, at the same time, a low affinity for other hexitols. The structure and properties of hexitol operons and metabolic pathways thus did not change strongly during their evolution.

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