JOURNAL OF BACTERIOLOGY, Aug. 1987, p. 3743–3749
0021-9193/87/083743-07$02.00/0

Vol. 169, No. 8

Evidence for Multiple K^+ Export Systems in *Escherichia coli*

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Received 18 February 1987/Accepted 6 May 1987

The role of the K^+ transport systems encoded by the *kefB* (formerly *trkB*) and *kefC* (formerly *trkC*) genes of *Escherichia coli* in K^+ efflux has been investigated. The rate of efflux produced by *N*-ethylmaleimide (NEM), increased turgor pressure, alkalinization of the cytoplasm, or 2,4-dinitrophenol in a mutant with null mutations in both *kef* genes was compared with the rate of efflux in a wild-type strain for *kef*. The results show that these two genes encode the major paths for NEM-stimulated efflux. However, neither efflux system appears to be a significant path of K^+ efflux produced by high turgor pressure, by alkalinization of the cytoplasm, or by addition of high concentrations of 2,4-dinitrophenol. Therefore, this species must have at least one other system, besides those encoded by *kefB* and *kefC*, capable of mediating a high rate of K^+ efflux. The high, spontaneous rate of K^+ efflux characteristic of the *kefC121* mutation increases further when the strain is treated with NEM. Therefore, the mutational defect that leads to spontaneous efflux in this strain does not abolish the site(s) responsible for the action of NEM.

Potassium (K^+) is the major cytoplasmic cation of growing bacterial cells (11, 12, 27), in which it plays a role in the activation of cytoplasmic enzymes (28), in the maintenance of turgor pressure (10, 23), and possibly in the regulation of cytoplasmic pH (4, 7, 22). The size of the K^+ pool is regulated by the osmotic pressure of the growth medium (9). In *Escherichia coli*, this regulation is believed to act at the level of control of the synthesis (14) and activity (11, 16, 24) of the uptake systems and the activity of efflux (19). Studies of efflux have been hampered by the limited knowledge of the genes which affect this process. Two genetic loci, *trkB* and *trkC*, have been identified by mutations that cause a requirement for high levels of K^+ in the growth medium because of enhanced rates of K^+ leakage (25). Recently, it was shown that these mutations affect two separate K^+ efflux systems, which thereby become overactive (5). These lesions can be suppressed by intragenic null mutations or by the insertion of transposons into the mutated gene. The only other mutant of *E. coli* with altered K^+ efflux is strain KHA, which was reported to have lost K^+/H^+ antiport activity (22). However, a detailed genetic analysis of this strain has not been presented.

K^+ efflux can also be elicited by a variety of cell treatments including addition of *N*-ethylmaleimide (NEM), alkalinization of the cytoplasm, or increased turgor pressure (18–20). In each case, it is believed that efflux occurs via K^+/H^+ antiporters (3, 20). The NEM effect on the K^+ pool is reversible even in the absence of protein synthesis (18), and this has led to the proposal that NEM exerts its action by titration of the glutathione pool rather than by covalent modification of a protein target. Thus, it has been proposed that control of K^+ retention is mediated by glutathione (17).

The experiments described here were initiated to ascertain the relative contributions of the systems encoded by *trkB* and by *trkC* to the K^+ efflux phenomena described above. To avoid confusion of these genes, which appear to affect only efflux, with other *trk* genes that affect the Trk system for K^+ uptake, we propose to rename these loci *kefB* and *kefC*; the mnemonic refers to K^+ efflux. We refer to the systems they encode as KefB and KefC. Our results confirm that both genes encode systems for the efflux of K^+, but they are important only in efflux produced by NEM. The KefB and KefC systems do not appear to mediate K^+ efflux under other conditions. The data imply the presence of at least one other K^+ efflux system, besides KefB and KefC, in *E. coli*.

MATERIALS AND METHODS

Strains. The bacterial strains used, all *E. coli* K-12, are listed in Table 1. Strains TK118-1 and TK121-1, spontaneous revertants of strains TK118 and TK121, respectively, were isolated as described previously (5). Strain MJF253 was constructed by mating TK110 with NK6051, at a 1:10 ratio of donor to recipient, and selecting tetracycline-resistant colonies. Recombinants carrying the *glt-lac* deletion of the donor, and the *kdpABC5* and *trkB110* mutations of the recipient, were identified by replica plating. One such recombinant was transduced, first to *purE*$^+$ and then to *kefB*::Tn*10* to yield strain MJF253. Strain MJF256 is a derivative of MJF253 with an insertion of λp*lac*Mu9 in *kefC*, selected as for Tn*10* insertions (5) by the method of Bremer et al. (6). Derivatives of these and other strains carrying insertion mutations were obtained by transduction. Strain FRAG-55 was constructed from FRAG-5 by transduction to *gal*$^+$, to *trpB*::Tn*10*, and then to *trp*$^+$ with a P1 lysate of a *galU* mutant. FRAG-59 is a derivative of FRAG-55 transduced to *rpsL crp*, then to *crp*$^+$ (and *rpsL*$^+$) with a lysate of a spontaneous revertant of TK110 carrying the *kefB1102* allele, and finally to *kefC*::Tn*10*. The *kefB1102* mutation is probably a small deletion because it increases cotransduction of *rpsL* with *crp* from a value of 0.45 (107/236) with a wild-type lysate to 0.61 (145/238). Then the *kefC*::Tn*10* mutation was introduced by transduction. Strain MJF291 is a spontaneous Pro$^+$ derivative of MJF256 and is believed to represent suppression of auxotrophy caused by an *argD* mutation (13). The origins of other strains are listed in Table

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TABLE 1. Bacterial strains

Strain	Genotype[a]		Origin or reference
	kef	Other	
C19	*kefC*::Tn*10*		5
FRAG-5		*gal*	8
FRAG-55		*galU*	—[b]
FRAG-59	*kefB1102 kefC*::Tn*10*	*galU*	—[b]
MJF253	*kefB*::Tn*10*	Δ(*glt-lac*)*5*	—[b]
MJF256	*kefB*::Tn*10* Φ(*kefC-lacZ*)*53*(λ*plac*Mu9-15)	Δ(*glt-lac*)*5*	—[b]
MJF291	*kefB*::Tn*10* Φ(*kefC-lacZ*)*53*(λ*plac*Mu9-15)	Δ(*glt-lac*)*5 argD*	—[b]
NK6051		Hfr Δ(*glt-lac*)*5 purE79*::Tn*10*	CGSC[c]
TK110	*kefB110*	*gal*	25
TK110-2	*kefB1102*	*gal*	—[b]
TK118	*kefC118*	*gal*	25
TK118-1	*kefC1181*	*gal*	Spontaneous revertant of TK118
TK121	*kefC121*	*gal*	25
TK121-1	*kefC1211*	*gal*	Spontaneous revertant of TK121
TK221	*kefB*::Tn*10 kefC121*	*gal*	Transductant of TK121
TK2309		*trkA405 trkD1 nagA kdpA*::Tn*10*	—[b]

[a] All strains are also *thi rha lacZ kdpABC5*, except TK2309, which is *kdpA*::Tn*10*, and NK6051, which is wild type for markers not listed.
[b] Strains constructed for this work, as described in Materials and Methods.
[c] Obtained from Coli Genetics Stock Center at Yale University.

1. Mating and transduction were done as described previously (8).

Growth conditions. In most experiments, cells were grown in phosphate-buffered minimal medium, described previously (8), containing the desired concentration of K^+. For transport experiments, cells were grown at the temperature used for transport. The carbon source was 10 mM glucose, and the following supplements were added where appropriate: proline, 0.1 to 1 mM; thiamine, 1 μg/ml; tetracycline, 12.5 μg/ml; kanamycin, 25 μg/ml.

Efflux produced by high turgor pressure. Cells were grown in phosphate-based medium containing 1 mM K^+ and 0.3 M NaCl to mid-log phase, collected by filtration, suspended at approximately 5×10^8 cells per ml in the same medium lacking a nitrogen source at a K^+ concentration as stated for each experiment, and incubated with aeration for 25 to 30 min to reach a steady state with respect to K^+ content. After control samples were taken, K^+ efflux was provoked by the addition of betaine to 1.5 mM. In some experiments, part of the cell suspension was preincubated with tetraethylammonium bromide, 10 mM, for 20 min before the addition of betaine.

Tris-EDTA treatment. Treatment with Tris-EDTA was done as described previously (21) to increase the permeability of the outer membrane to lipophilic cations (26) and to deplete the cells of K^+ (2).

NEM treatment. Cells were grown in medium containing 115 mM K^+ to a turbidity at 578 nm of 0.7 to 1.0 and then harvested by centrifugation at 5,000 × *g*. Cells were washed twice with a buffer similar to growth medium in which most or all K^+ was replaced by Na^+ to attain K^+ concentrations of 0, 2, or 20 mM and from which $(NH_4)_2SO_4$ and $FeSO_4$ were omitted. Cells were suspended at a turbidity at 578 nm of 30 to 60 in the same buffer containing glucose at 10 mM and shaken at 25°C for 15 to 25 min. K^+ efflux was initiated by diluting the suspension 50-fold into the same medium lacking K^+ and containing 0.5 mM NEM.

Diethanolamine treatment. Tris-EDTA-treated cells were washed twice by centrifugation at 10,000 × *g* with 200 mM K^+ 3-[tris(hydroxymethyl)amino]propanesulfonic acid (TAPS) buffer (pH 8.4) and immediately diluted into 200 mM 1,3-bis[tris(hydroxymethyl)methylamino]propane-TAPS (pH 8.4) containing glucose and KCl, each at 10 mM, to a final cell concentration of about 1.5 mg (dry weight) per ml. The suspension was shaken at 20°C for 30 to 60 min, at which time 100 mM diethanolamine was added from a 3 M stock solution titrated to pH 8.4 with *N*-2-hydroxyethylpiperazine-*N'*-2-ethanesulfonic acid (HEPES). The K^+ content of the cells was measured as a function of time.

Measurement of cell K^+ content. In the method referred to as the centrifuge assay, 1-ml samples of incubation mixtures were layered on silicone oil and the cells were centrifuged at 10,000 × *g* through the oil with a lag of less than 10 s between sampling and separation of cells from medium. The K^+ content of the cell pellet was determined by flame photometry (2). In some experiments, cell K^+ was measured by what is referred to as the filtration method. Cells were collected on 0.45-μm-pore-size membrane filters (Millipore Corp., Bedford, Mass.; type HA), washed with ice-cold glucose (of a concentration chosen to avoid osmotic shock and stated for each experiment)–10 mM Tris chloride (pH 7.5)–1 mM $MgCl_2$, and dried, and K^+ was determined by flame photometry as previously described (25).

The transmembrane K^+ concentration ratio was measured by using Tris-EDTA-treated cells incubated with the appropriate concentration of K^+ in 200 mM Na HEPES (pH 7.5) containing 10 mM glucose. Cell samples were collected by centrifugation at 10,000 × *g* through silicone oil and analyzed as described above after 30, 40, or 50 min of incubation. The intracellular concentration of K^+ was calculated from the measured turbidity of the cell suspension, after conversion of the turbidity measurement to dry weight and using a value of 1.45 ml of cytoplasmic volume per g (dry weight) (2).

Measurement of the membrane potential. The membrane potential was measured as described previously (2) by using Tris-EDTA-treated cells and the lipophilic cation tetraphenylphosphonium (TPP) at a final concentration of 10 μM. No corrections were made for the binding of TPP to the cells when calculating the potential by the Nernst equation.

Chemicals. Betaine, 1,3-bis[tris(hydroxymethyl)methylamino]propane, 2,4-dinitrophenol (DNP), diethanolamine,

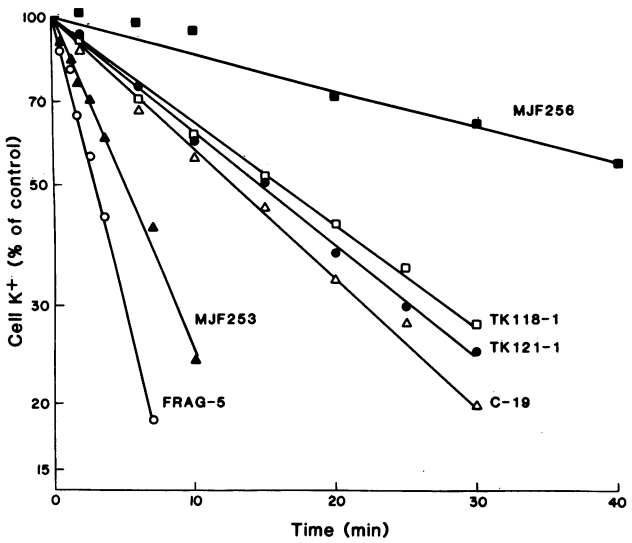

FIG. 1. K^+ efflux after NEM treatment of a wild-type strain and of strains with *kef* mutations. Cells suspended at 30°C in ammonia-free medium containing 2 mM KCl were diluted into ammonia-free, K^+-free medium containing NEM, 0.5 mM, at zero time. Measurements of cell K^+ by the centrifugation assay were taken at the times indicated. Symbols: ○, FRAG-5 (*kef*$^+$); ▲, MJF253 (*kefB*::Tn*10*); △, C-19 (*kefC*::Tn*10*); ●, TK121-1 (*kefC1211*); □, TK118-1 (*kefC1181*); ■, MJF256 [*kefB*::Tn*10* Φ(*kefC-lacZ*)*53*].

HEPES, NEM, and TAPS were from Sigma Chemical Co., St. Louis, Mo.; L-proline (analytical grade) was from Merck & Co., Inc., Rahway, N.J.; Rotatherm H silicone oil (*D* 1.05) was from Roth, Karlsruhe, Federal Republic of Germany; TPP-Cl was from ICN Chemicals Inc., Plainview, N.Y.; and [^{14}C]TPP (31 Ci/mol) was from Amersham-Buchler, Brunswick, Federal Republic of Germany.

RESULTS

Role of the KefB and KefC systems in NEM-induced K^+ efflux. It has been proposed that NEM-induced K^+ efflux represents activation of one or more K^+/H^+ antiporters (3). The availability of strains with null mutations (either point mutations or transposon insertions) in the *kefB* and *kefC* genes (5) enabled us to investigate the role of the systems encoded by these genes in NEM-induced K^+ efflux. Studies of a number of strains demonstrated a consistent correlation between the presence of functional KefB and KefC systems and the rate of NEM-induced K^+ efflux (Fig. 1). The rate of K^+ efflux was reduced 15-fold in mutant MJF256 lacking both the KefB and KefC systems. The effect of the loss of each of the two systems was additive, and the system encoded by the *kefC* gene was kinetically dominant. Thus, contrary to conclusions of a previous report and as suggested in a note added in proof to that report (5), the *kefB* and *kefC* genes encode the major NEM-activated K^+ efflux systems.

NEM-activated K^+ efflux in *kefC* pseudorevertant strains TK118-1 and TK121-1 was similar to that observed in strain C-19, which has a Tn*10* insertion in the *kefC* gene (Fig. 1). Similar results were obtained in comparisons of pseudorevertants of *kefB* strain TK110 with *kefB*::Tn*10* insertion mutant MJF253. These data support our earlier conclusion that the pseudoreversion event leads to an inactive gene product.

At moderate K^+ concentrations, the net rate of efflux of K^+ induced by NEM is a composite of exit via the KefB or KefC systems (or both) and recapture by the Trk uptake system. Mutations that eliminate Trk activity increase the apparent rate constant for efflux some sixfold when measured in medium containing 10 mM K^+ (Fig. 2). A similar change in the efflux rate in strain FRAG-5 was achieved by lowering the external K^+ concentration from 10 mM to 50 μM, a concentration well below the 1.5 mM K_m of the uptake system (Fig. 2). In the Trk$^-$ strain TK2309, there was only a threefold increase in the rate of K^+ efflux, from 0.16 to 0.46 min^{-1}, over the same concentration range. Therefore, the reduction in the efflux rate by increasing external K^+ concentrations is caused in large part by a stimulation of compensatory uptake of K^+ by the Trk system. Uptake by strain TK2309 over this concentration range (like that of strain TK401, [25]) is so low that most of the difference in the efflux rate can be attributed to inhibition of the Kef systems either by external K^+ or by the reduction of the transmembrane K^+ gradient.

Evidence for a third K^+ efflux system. The presence of a K^+/H^+ antiporter that is activated by alkaline cytoplasmic pH has been inferred from experiments in which weak permeable bases elicit K^+ efflux from cells incubated at alkaline pH (20). We sought to determine whether the products of the *trkB* and *trkC* genes play a major role in K^+ efflux elicited by alkaline pH. The rate of diethanolamine-induced K^+ efflux was unaffected, within experimental error, by transposon insertions that eliminated the function of both the KefB and KefC systems (Fig. 3). This result shows that efflux produced by diethanolamine is not dependent on either of these efflux systems. K^+ efflux after addition of diethanolamine was also unaffected by mutations that abolish the activity of the Trk uptake system (data not shown), suggesting that efflux does not occur by reversal of uptake.

K^+ efflux can also be elicited by the addition of the osmoprotectant betaine to cells that have accumulated K^+ in response to high osmolarity (Fig. 4). Efflux in this situation is believed to be produced by the increased turgor that results as betaine is accumulated. For these measurements, we used *galU* mutants to prevent the accumulation of trehalose (15, 29). In *galU*$^+$ strains grown in media of elevated osmolarity, cell K^+ concentration falls upon removal of a nitrogen source, presumably because cessation of growth leads to a

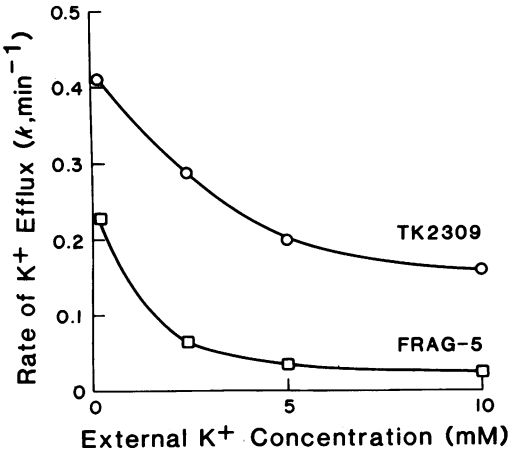

FIG. 2. Effect of external K^+ concentration on the rate of NEM-induced K^+ efflux. Efflux was measured as described in the legend to Fig. 1. Symbols: □, FRAG-5 (Trk$^+$); ○, TK2309 (Trk$^-$).

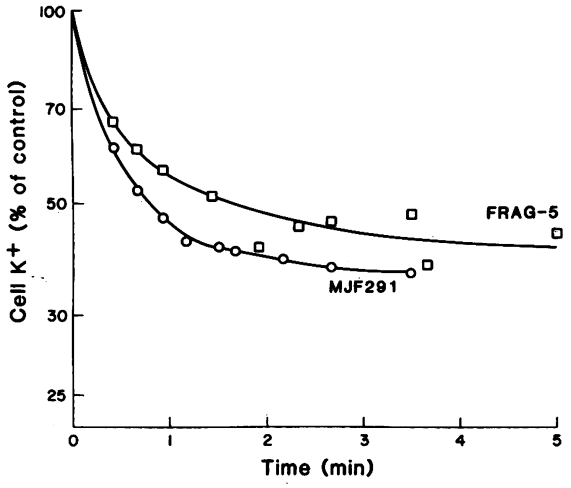

FIG. 3. K^+ efflux produced by diethanolamine. Cells were grown and washed as described in Materials and Methods. At zero time, diethanolamine-HEPES (pH 8.4) was added to a final concentration of 0.1 M. Cell samples for K^+ measurement were obtained by the centrifugation method. Symbols: □, FRAG-5 (*kef*$^+$); ○, MJF291 (*kefB* and *kefC* null).

higher pool of trehalose than in its absence, and there is a lag of about 20 s between the addition of betaine and the onset of rapid K^+ efflux. The initial rate constant of betaine-provoked K^+ efflux at 30°C at an external K^+ concentration of 0.3 mM in the double *kef* mutant FRAG-59 was 0.121 ± 0.018 (standard error) min^{-1} (n = 4), not significantly different from the rate of 0.113 ± 0.014 min^{-1} (n = 4) in the isogenic *kef*$^+$ strain FRAG-55. Efflux in strain FRAG-59 at an external K^+ concentration of 10 mM was 0.115 min^{-1} in a single experiment, not different from the rate at lower external K^+. We did not see any reduction of efflux in the presence of 10 mM tetraethylammonium (Fig. 4), a compound reported to inhibit efflux produced by reducing the osmolarity of the external medium (19).

Rapid K^+ efflux results when cells are treated with high concentrations of some uncouplers such as DNP (25). Efflux at 30°C produced by 10 mM DNP in double *kef* mutant FRAG-59 had a rate constant of 0.31 min^{-1}, not significantly different from the rate in *kef* wild-type strain FRAG-55 of 0.33 min^{-1} given the reproducibility of this measurement of about 0.02 min^{-1}. The rate was 0.36 min^{-1} in *kefC* null mutant CR19 and in double *kef* mutant MJF256. The conclusion that neither KefB nor KefC contributes to K^+ efflux after DNP treatment is also supported by earlier data (5).

All of the above data point to the existence of a third K^+ efflux system. After treatment with ethanolamine, the membrane potential was relatively constant at −190 to −210 mV, and there is no reason to suppose the membrane potential is markedly altered by adding betaine to cells in medium of high osmolarity. Electrogenic K^+ efflux could not have occurred under these two conditions in which cell K^+ was lower than the concentration in equilibrium with the membrane potential, so we infer that it is an electrically neutral process such as a neutral K^+/H^+ antiporter. Efflux after DNP treatment, when the membrane potential can be expected to be low, need not be electrically neutral.

Properties of the *kefB* and *kefC* mutations. *kefB* and *kefC* mutants are unable to grow at low K^+ concentrations, because of enhanced rates of K^+ efflux (25). The defect in the mutants is reflected in their inability to sustain high K^+ gradients at low external K^+ concentrations (Fig. 5). Strains carrying different *kefC* alleles (TK118 and TK121) sustain different K^+ concentration gradients, suggesting that different amino acid residues in the KefC protein are affected in these two mutants. The gradient of K^+ sustained is correlated with the concentration of K^+ needed to achieve rapid growth (25). Spontaneous revertants TK118-1 and TK121-1, in which growth on low K^+ was restored due to intragenic suppressors to the null state (5), as well as *kef* insertion mutants, sustain a K^+ gradient indistinguishable from that of the wild-type strain (Fig. 5).

The accumulation of TPP^+, a measure of the membrane potential, and the accumulation of K^+ were measured in the *kefC* mutant TK121 to determine whether the potential for K^+ fell to the level of the membrane potential. In *kef*$^+$ strain FRAG-5, the accumulation of K^+ exceeded that of TPP^+ when external K^+ was 0.1 mM (Table 2) as reported previously (1). In contrast, K^+ accumulation in mutant TK121

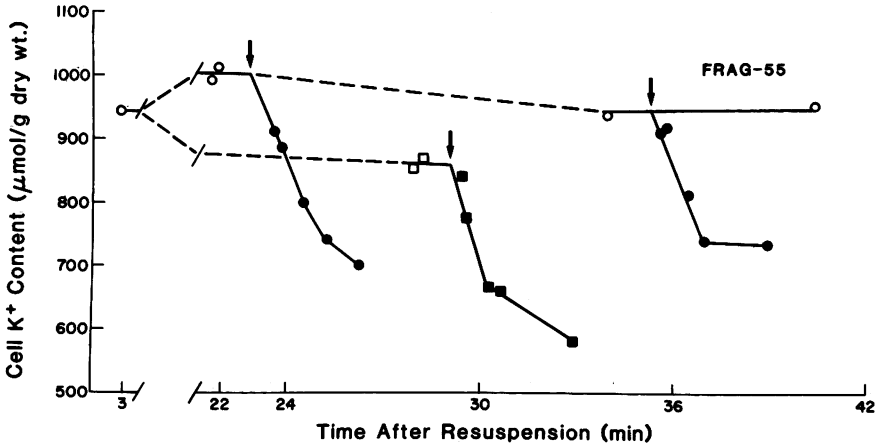

FIG. 4. K^+ efflux at 28°C after the addition of betaine to cells of *kef*$^+$ strain FRAG-55 grown in medium of high osmolarity. Cells were grown in minimal medium containing 1 mM K^+ and 0.3 M NaCl at 28°C, filtered, transferred at zero time to the same medium containing 0.3 mM K^+ but lacking ammonium, and incubated with aeration. At the times indicated by the arrows, cells received betaine to 1.5 mM (filled symbols). Cell K^+ was measured by the filtration method by using wash solution containing 0.9 M glucose. Symbols: ○, ●, control cells; □, ■, cells receiving tetraethylammonium chloride, 10 mM, at 10 min.

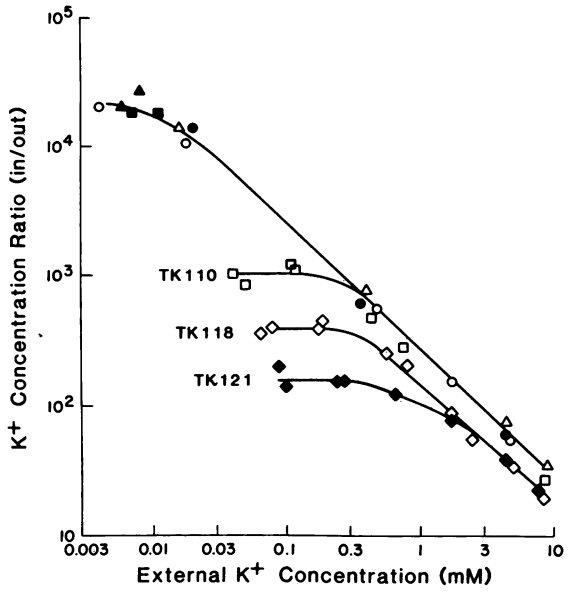

FIG. 5. The ratio of K^+ accumulation as a function of the external K^+ concentration in wild-type and *kef* mutant strains. Cells were grown at 37°C in minimal medium containing 115 mM K^+, harvested by centrifugation at 5,000 × *g*, treated with Tris-EDTA, washed again, and suspended in medium of the indicated K^+ concentration. Cell K^+ was measured by the centrifugation method 40 min later, by which time cell K^+ had reached a steady state. Symbols: ○, FRAG-5; △, C-19; ▲, MJF253; ■, MJF256; ●, TK121-1; □, TK110; ◇, TK118; ◆, TK121.

was less than that of TPP^+. In addition, the presence of high K^+ did not cause depolarization of the membrane in the mutant strain (Table 2). Together, these data indicate that K^+ efflux via the mutated *kefC* gene product is probably electroneutral.

The K^+ retention defect associated with the *kefB* and *kefC* alleles could arise from loss of control by glutathione (17). If so, addition of NEM should produce no change in the rate of K^+ efflux through the mutated Kef system. To abolish effects on the other (wild-type) Kef system in the strain, the *kefC121* mutant was studied in a strain with a *kefB*::Tn*10* insertion. In this strain, sensitivity to NEM is retained (Fig. 6). The spontaneous rate of K^+ efflux increased fourfold upon addition of NEM. Thus, the phenotype of the mutations that lead to inappropriate K^+ efflux is not necessarily caused by loss of control of the Kef systems by glutathione.

DISCUSSION

The data presented above show that *E. coli* cells have at least three K^+ efflux systems. Two of these systems, KefB and KefC, account for efflux at a rate of 100 to 200 μmol of K^+ min^{-1} g^{-1} after treatment with NEM. Strains in which both Kef systems are inactivated have control rates of K^+ efflux after treatment of cells with permeant amines (Fig. 3), with DNP, or in response to an increase in turgor pressure (Fig. 4). Efflux probably does not involve reversal in function of a K^+ uptake system, because efflux is unaffected in strains defective in uptake. Therefore, there must be at least one other system, besides KefB and KefC, capable of high rates of K^+ efflux. This other system (or systems) is probably a K^+/H^+ antiporter or other electroneutral system, because it mediates K^+ efflux under conditions (Fig. 3 and 4) in which electrogenic movement of K^+ does not result in efflux. No genetic loci have been identified that affect this other system(s), but it is possible that strain KHA which lacks K^+/H^+ antiporter activity (22) is mutated in the other system(s).

Despite our inability to demonstrate a role of the KefB and KefC systems in K^+ efflux produced by high turgor or diethanolamine, our data do not rule out such a role. If the maximum rate of efflux by other systems is very high, loss of efflux through KefB and KefC would not be apparent when K^+ efflux was not the rate-limiting step. This is a distinct possibility in the case of K^+ efflux produced by betaine, in which uptake of betaine may be much slower than the ability of the cell to expel K^+. A possible role of KefB and KefC in K^+ efflux produced by diethanolamine (Fig. 3) is less likely because the rate of K^+ efflux in this case is much more rapid and therefore more likely to be rate limiting.

The mode of action of NEM in eliciting K^+ efflux has been suggested to reflect the titration of the pool of a soluble

TABLE 2. K^+ accumulation and membrane potential in wild-type and *kefC* mutant strains[a]

Strain	K^+ concn (mM)	K^+_{in}/K^+_{out}	TPP^+_{in}/TPP^+_{out}
FRAG-5	0.1	8,000	780
	10	30	300
TK121	0.1	200	500
	10	35	350

[a] Measurements were done on cells treated with Tris-EDTA as described in Materials and Methods.

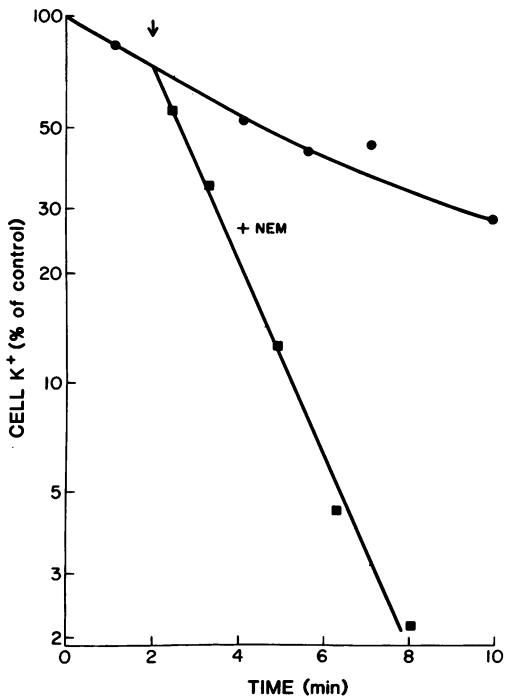

FIG. 6. Effect of NEM in mutant TK221 (*kefC121 kefB*::Tn*10*). Cells were grown at 25°C in medium containing 10 mM K^+. At zero time, cells were transferred by filtration to medium containing no K^+ and samples for cell K^+ were taken by the filtration method by using a wash solution containing 0.4 M glucose. At the time indicated by the arrow, NEM was added to 0.5 mM. Symbols: ●, control; ■, NEM. The initial half time of efflux was 4.7 min for the control and 1.1 min in the presence of NEM.

cytoplasmic sulfhydryl group, most probably glutathione (17). Indirect support for this proposal comes from the observation that other compounds which are known either to chemically modify glutathione or oxidize it to the disulfide form also elicit K^+ efflux (unpublished data). However, because NEM is a potent sulfhydryl reagent, it is probable that it affects many cellular functions, including the Trk uptake system. However, the principal effect of NEM is the activation of the KefB and KefC efflux pathways, since mutations which eliminate these pathways prevent K^+ loss. By contrast, mutations that reduce uptake do not reduce NEM-induced K^+ efflux (Fig. 2).

The *kefB* and *kefC* loci were first identified by mutations that caused an abnormal leakage of K^+ from the cell (8, 25). Subsequently, it was shown that K^+ loss was caused by altered function of two systems that did not cause leakage in the wild-type state nor when abolished by null mutations (5). It has been suggested that K^+ efflux is regulated by glutathione (17), and that reduced glutathione pools (produced by NEM or other conditions) cause rapid K^+ efflux. A loss of control by glutathione in *kef* mutants would explain their properties. This attractive idea does not apply to the *kefC121* mutation, which remains responsive to NEM (Fig. 6). Preliminary results indicate that NEM has no effect on several other *kef* alleles. Thus the K^+ efflux defect of some but not all *kef* alleles appears to be explained by loss of control by glutathione.

Several suggestions can be made to explain K^+ efflux in mutants like *kefC121*: overproduction of the *kefC* gene product, loss of energy coupling, or loss of control mediated by some state or compound (other than glutathione). Overproduction as a cause of efflux can be ruled out because expression of the cloned *kefC* gene at high levels does not produce an increase in K^+ efflux (I. R. Booth, unpublished data). The possibility that the *kef* mutations represent energy coupling mutations similar to those reported for the *lacY* gene (30) is unlikely at best. The K^+ efflux measurements were taken at pH 7.5, at which ΔpH is very small, and thus the pertinent driving forces are the K^+ gradient and the membrane potential. Uncoupling K^+ movements from those for protons would not produce efflux except when external K^+ is very low.

We are led to the conclusion that some other conditions, or compound besides glutathione, also control the KefB and KefC systems. At present, neither the nature of such control nor the physiological function of these systems is understood. As for the function, it is a subtle one, because loss of both systems results in no apparent defect except the loss of sensitivity to NEM. Just as our data do not exclude a role of KefB and KefC in K^+ efflux produced by turgor and permeant amines, we cannot exclude the possibility that these systems are controlled by turgor. Only when other systems for K^+ efflux are suitably inhibited or abolished will it be easy to determine the physiological function and control of the KefB and KefC systems.

ACKNOWLEDGMENTS

We thank A. Strøm for communicating to us information about trehalose synthesis before publication, B. Bachmann for providing strain NK6051, and John Westley for assistance in statistical analysis.

Studies in Osnabrück (E. P. Bakker and U. Dinnbier) were supported by grant SFB171 from the Deutsche Forschungsgemeinschaft, those in Aberdeen (I. R. Booth and A. Gajewska) by a grant from the Nuffield Foundation, and those in Chicago (W. Epstein) by grant PCM 7904641 from the National Science Foundation.

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