Cation Transport in *Escherichia coli*

VIII. *Potassium Transport Mutants*

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ABSTRACT Analysis of K transport mutants indicates the existence of four separate K uptake systems in *Escherichia coli* K-12. A high affinity system called Kdp has a K_m of 2 μM, and V_{max} at 37°C of 150 μmol/g min. This system is repressed by growth in high concentrations of K. Two constitutive systems, TrkA and TrkD, have K_m's of 1.5 and 0.5 mM and V_{max}'s of 550 and 40 at 37 and 30°C, respectively. Mutants lacking all three of these saturable systems take up K slowly by a process, called TrkF, whose rate of transport is linearly dependent on K concentration up to 105 mM. On the whole, each of these systems appears to function as an independent path for K uptake since the kinetics of uptake when two are present is the sum of each operating alone. This is not true for strains having both the TrkD and Kdp systems, where presence of the latter results in K uptake which saturates at a K concentration well below 0.1 mM. This result indicates some interaction between these systems so that uptake now has the affinity characteristic of the Kdp system. All transport systems are able to extrude Na during K uptake. The measurements of cell Na suggest that growing cells of *E. coli* have very low concentrations of Na, considerably lower than indicated by earlier studies.

Cells of the gram-negative bacterium *Escherichia coli* share with most other cells the property of maintaining much higher intracellular concentrations of K than those in the extracellular medium. Intracellular K in *E. coli* is determined largely by the osmolarity of the external medium (Epstein and Schultz, 1965; Epstein and Schultz, 1968) and is virtually independent of the concentration of K in the external medium (Schultz and Solomon, 1961). Intracellular K ranges from 150 mM in cells grown in 80 mosM media to nearly 600 mM in cells grown in 1,200 mosM media (Epstein and Schultz, 1968). Osmotic regulation of intracellular K also occurs in *Salmonella* (Christian, 1955) and may be characteristic of many genera of bacteria.

Cells of *E. coli* K-12 are capable of high rates of K uptake. Such uptake has a K_m for external K of approximately 5 mM when measured in cells depleted of K by growth into late stationary phase (Schultz et al., 1963). Rapid rates of K uptake can be produced in exponential phase cells by a sudden increase in osmolarity (Epstein and Schultz, 1965). Under these conditions the K_m for uptake is approximately 1 mM. In both cases maximum rates are high, ranging from 130 to 240 μmol/g min at 30 and 37°C, respectively. Under both conditions

the major ionic movement maintaining electroneutrality during K uptake is proton extrusion. In stationary phase cells which have elevated pools of Na, part of the K taken up is exchanged for Na (Schultz et al., 1963).

In order to gain a more detailed knowledge of the process of K transport, mutants affecting this process were isolated. All the mutants studied to date were isolated by methods that enrich for mutants requiring higher K concentrations for growth than needed by the wild type. The mutants were isolated in two steps. Beginning with a wild-type strain and using mild ultraviolet mutagenesis to minimize the incidence of multiple mutations, only a single class of mutants was obtained (Epstein and Davies, 1970). These strains have mutations in any one of four closely linked *kdp* genes. The *kdp* mutants require 0.07 mM K to achieve a half-maximal rate of growth, differing from the wild type which has a constant rate of growth down to concentrations of 5 μM or less (Weiden et al., 1967). Mutants requiring more K for growth than the *kdp* mutants were not obtained by mutagenizing wild-type strains, but were readily obtained by mutagenizing a *kdp* mutant strain. In this way five types of double mutants were identified (Epstein and Kim, 1971), each carrying the original *kdp* mutation and a further mutation in five other genes labeled *trkA* through *trkE*. Each class was distinguished by growth tests on plates, and was shown to be due to a mutation in a distinct gene. The five *trk* genes are widely scattered on the *E. coli* chromosome, none lying extremely close to any other or to the *kdp* genes, although two (*trkA* and *trkB*) are close enough to be cotransduced by P1*kc*.

We here describe the kinetics of K transport in the mutants. All of the mutants differ from the wild type in K transport. The data below indicate that *E. coli* has three saturable K uptake systems, called the Kdp, TrkA, and TrkD systems by analogy with the genetic symbols for the genes affecting these processes. A fourth system which is not saturable, TrkF, appears to be present in all mutants. Two genes, *trkB* and *trkC*, are associated with defects in K retention. A preliminary report of some of this work has been presented (Epstein, 1970).

METHODS

Bacteria

The principal bacterial strains, all *Escherichia coli* K-12, are listed in Table I. The origin of parental strain FRAG-1 and the isolation of most of the mutants derived from FRAG-1 have been described (Epstein and Davies, 1970; Epstein and Kim, 1971). For convenience in referring to the different types of K transport mutants the abbreviations listed in the first column of Table I are used. A capital letter followed by a superscript minus sign refers to a defect in a single class of genes; K^- refers to a mutation in the *kdp* genes, and letters A^- through E^- refer to mutations in the *trkA* through *trkE* genes, respectively. Thus a strain listed as K^-A^- has mutations in the *kdp* and *trkA* genes, and other genes affecting K transport are wild type. The symbols Kdp, TrkA, and TrkD refer to the transport systems associated with the corresponding genes, while TrkF refers to a nonsaturable transport system apparently present in all of the mutants and seen clearly in mutants lacking the three saturable systems. No mutations affecting the TrkF system have been identified.

The derivative strains listed in Table I were obtained by P1*kc*-mediated transduction, taking advantage of the different K requirements of the mutants. K^-D^- strain TK1001

TABLE I

BACTERIAL STRAINS

Mutant class*	Typical strain(s)‡	Genotype	
		K transport related	Other§
Wild type	FRAG-1		
K^- (Trk^+)	FRAG-5	*kdpABC5*	
K^-A^- (TrkA)	TK133	*kdpABC5 trkA133*	
K^-B^- (TrkB)	TK110	*kdpABC5 trkB110*	
K^-C^- (TrkC)	TK118	*kdpABC5 trkC118*	
	TK121	*kdpABC5 trkC121*	
K^-D^-	TK1001	*kdpABC5 trkD1*	
K^-E^- (TrkE)	TK142	*kdpABC5 trkE142*	
$K^-A^-D^-$ (TrkA/D)	TK401	*kdpABC5 trkA401 trkD1*	
	TK1002	*kdpABC5 trkA133 trkD1*	
A^-	TK1005	*trkA133*	
A^-D^-	TK1004	*trkA401 trk D1*	*malA*
	TK1030	*trkA401 trkD1*	*nadA*

* Symbols in parentheses are those used in Epstein and Kim, 1971.

‡ The prefix 2K was used in place of TK in referring to these strains in Bhattacharyya et al., 1971.

§ All strains also have *gal rha lacZ* and *thi* mutations (Epstein and Kim, 1971).

was obtained as a transductant of TK401 capable of growth in medium containing 0.1 mM K but not in medium containing no added K (K0 medium, see below). The A^- and A^-D^- strains were obtained by transduction to growth on K0 plates of the corresponding parent carrying a *kdp* mutation. Derivatives carrying the F100 and F141 episomes (previously designated F_1gal and F-41, respectively) were prepared essentially as described earlier (Epstein and Davies, 1970; Epstein and Kim, 1971). Transfer of F100 was selected for by the *nadA* marker, which was kindly provided by Gerald Tritz in strain UTH4679 and introduced into our strains by cotransduction with the *gal* marker. The *nadA* locus is cotransduced with *kdp* at a frequency of approximately 20%.

Media and Growth of Bacteria

In most experiments the phosphate-buffered media described earlier (Epstein and Davies, 1970; Epstein and Kim, 1971) were used. These are referred to by K concentration in millimolar; K0 contains no added K, while K115 contains 115 mM K. The small amount of K, usually between 5 and 20 μM, contaminating K0 medium had to be removed for studies with the Kdp system. Accordingly what is referred to as *K-free* medium was prepared by incubating K-starved wild-type *E. coli* in K0 medium containing glucose for approximately 30 min at 30°C, a time sufficient to allow the cells to remove all but traces of K (see Fig. 4 below and Epstein and Davies, 1970). The bacteria were then removed by filtration through 0.45-μm pore size membrane filters (Millipore, type HA). A few experiments were done with maleate-buffered media containing: 8 mM $(NH_4)_2SO_4$, 0.4 mM $MgSO_4$, 6 μM $FeSO_4$, 1 mM Na_2HPO_4, 5 mM Na citrate, 3 μM thiamine HCl, and either 60 mM of K, Na, or Tris maleate, or 90 mM Mg maleate. The desired K concentration was attained by mixing sufficient K maleate medium with one of the other maleate media.

Except as noted below, cells were grown with agitation at the same temperature as was to be used for transport measurements, and glucose at 2 g/liter was present during both growth and subsequent steps. Cells were harvested in the exponential phase of growth

except for cells subjected to K limitation to derepress the Kdp system. The latter treatment was accomplished either by growing cells in low K medium, usually K0.05, for approximately 2 h after the time the cells had exhausted the medium of K, or by transferring cells from the growth medium to K0 containing glucose and agitating for a further 2 h.

Transport Studies

A good steady state for transport work is achieved by the simple expedient of suspending cells in a solution similar to growth medium and containing glucose but lacking a nitrogen source. Such solutions, referred to by the suffix N0 after the K designation of the medium (K0N0 contains neither K nor NH_4), were prepared by substituting osmotically equivalent amounts of NaCl for the $(NH_4)_2SO_4$ in growth medium. Chloramphenicol, 50 mg/liter, was added to inhibit protein synthesis. Wild-type cells maintain constant cell K for several hours when incubated with aeration in such solutions. To stimulate net K uptake the cells must either be depleted of K, or subjected to osmotic upshock (Epstein and Schultz, 1965). In most experiments K was depleted by a 30-min treatment with 10 mM 2,4-dinitrophenol (DNP) in K0N0 lacking glucose. This concentration of DNP removes over 90% of cell K, most of the loss occurring in the first minutes. The cells were then washed and incubated in K0N0 for approximately 30 min before transport was measured. Extensive spontaneous loss of K occurs in certain of the mutants when suspended in K0N0 (Fig. 1). This method of K depletion was used in some experiments. After incubation until the cells achieved a new, lower steady state, the cells were washed and suspended in fresh K0N0 for transport measurements. Uptake was initiated by adding K to the desired concentration. K concentrations up to 10 mM were achieved by adding KCl; in most cases compensatory amounts of NaCl were added so that the same osmolarity was maintained at all K concentrations. To achieve higher K concentrations, the cells were concentrated approximately 10-fold and added to tubes containing suitable mixtures of K115N0 and K0N0.

For studying transport by the osmotic upshock method, cells were grown in dilute medium consisting of K10 or K115 diluted with an equal volume of water. The cells were washed and transferred to similarly dilute K0N0, and net K uptake was initiated by adding 4 vol of cell suspension to 1 vol 2.5 M glucose in dilute N0 solution containing five times the desired final K concentration.

Cell samples were collected on gridded 0.45-μm pore size membrane filters (Millipore), washed briefly with 0.4 M glucose (0.8 M glucose for the upshock experiments) containing 1 mM Tris Cl (pH 7), dried, and analyzed for K with an internal standard flame photometer (Instrumentation Laboratory, Inc., Lexington, Mass., model 143). K uptake under the conditions described above is linear until the cells have taken up approximately one-half of the K they will ultimately take up, after which time net uptake progressively slows down. All rates measured are initial rates.

The inclusion of Tris Cl in the wash solution and more extensive washing made it possible to obtain reliable measurements of cell Na by the filter method. It was necessary to wash most batches of filters with 10 mM NH_4Cl and then water to avoid very high and rather variable filter blanks for Na. Due to the large amount of Na in the media and the relatively small amounts in the cells, errors due to trace contamination of cell samples with medium are much larger than is the case for K samples.

Transport rates are expressed as micromoles taken up per minute and gram dry weight. The latter was estimated from measurements of the turbidity of the cell suspensions in a Bausch and Lomb Spectronic 20 colorimeter (Bausch & Lomb, Inc., Rochester, N.Y.) and a calibration curve for that instrument. This method is less precise than the

direct measurements of pellet volume used earlier (Epstein and Schultz, 1965) but is much more convenient. Variance in this way of measuring transport affects our estimates of V_{max} but does not affect the K_m determinations, each of which is based on measurements of different samples of the same cell suspension. Previously published data relating cell water and cell surface area to dry weight (Schultz and Solomon, 1961) were used to convert filter data for cell K and Na to concentrations and to express the earlier flux rates mentioned in the introduction from units of picomoles per square centimeter second to the units used in this paper. 1 pmol/cm^2 s corresponds to 12.9 μmol/g min.

Growth rates were measured as described earlier (Epstein and Davies, 1970). For such studies cells were grown to midexponential phase in medium of the same type as to be used but containing the highest K concentration attainable in that medium. After washing, the cells were inoculated to a density of approximately 10^8 cells/ml in a series of 18 × 150-mm tubes containing media of different K concentrations and shaken at 37°C. Turbidity was measured at intervals in a Bausch and Lomb Spectronic 20 colorimeter, and the growth rate was measured over the interval where the logarithm of the turbidity increased linearly with time.

All chemicals used were reagent grade. ^{42}K was obtained from New England Nuclear Corp., Boston, Mass.

RESULTS

An initial screening of the mutants was performed by examining the rate at which K is lost from cells suspended in K0N0 containing glucose and chloramphenicol. The results are shown in Fig. 1. The K$^-$ mutant loses very little K in 2 h and in this regard is similar to the wild-type and K$^-$D$^-$ mutants which lose little, if any, K under these conditions (data not shown). The opposite extreme is seen in the K$^-$B$^-$ and K$^-$C$^-$ mutants, which lose K very rapidly. Between these extremes are the three other mutant classes, all of which lose K rather slowly. The K$^-$A$^-$D$^-$ mutants eventually lose almost all of their K, retaining less than 10% after 2 h at 37°C. The difference between the two rapid loss mutants and the others is even greater than suggested by the data of Fig. 1 because the rapid loss mutants were examined at 25°C to permit a more accurate determination of the rate, while the others (except for the K$^-$ strain) were examined at 37°C. A way of analyzing the results is to assume that mutants defective in uptake will lose K only as fast as it normally leaves the cells. The normal exit rate can be estimated from the rate at which K exchanges in the steady state, which occurs with a half time of approximately 20 min at 30°C in wild-type strains (Epstein and Schultz, 1966). By this criterion the K$^-$B$^-$ and K$^-$C$^-$ mutants have a defect in K retention since they lose K with a half time of 7.5 and 3.5 min, respectively. All of the other mutants lose K with a half time exceeding 40 min and were therefore suspected of having defects in K uptake.

Measurements of K uptake confirmed the classification inferred from the data in Fig. 1. We have identified four separate K transport systems in *E. coli* whose properties are summarized in Table II. The kinetic properties are from data presented below; the genetic properties are from published data (Epstein and Davies, 1970; Epstein and Kim, 1971).

The lowest rates of transport are seen in the triple K$^-$A$^-$D$^-$ mutants which lack all three saturable uptake systems. Transport rates in such strains are

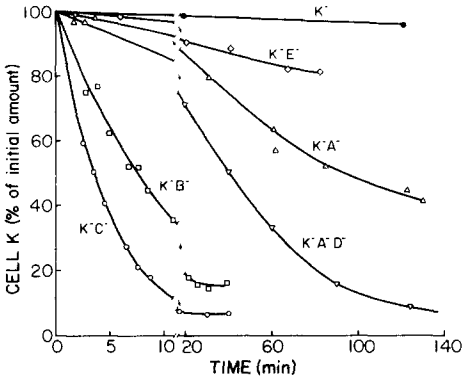

FIGURE 1

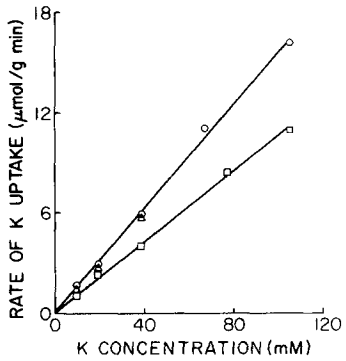

FIGURE 2

FIGURE 1. Spontaneous loss of K after suspension in K0 buffer. Cells in the exponential phase of growth were collected and washed by filtration, and suspended at a density of approximately 250 μg/ml in K0N0 containing 2 mg glucose and 50 μg chloramphenicol per milliliter. At the indicated times samples were taken for measurement of intracellular K. ●, K^- strain FRAG-5, temperature 25°C; ◇, K^-E^- strain TK142, 37°C; △, K^-A^- strain TK133, 37°C; ▽, $K^-A^-D^-$ strain TK1002, 37°C; □, K^-B^- strain TK110, 25°C; ○, K^-C^- strain TK118, 25°C. Note change in scale of abscissa after 10 min.

FIGURE 2. Dependence of K uptake rate on K concentration in $K^-A^-D^-$ mutants at 37°C. Data for three separate experiments are shown. Exponential phase cells of strain TK1002 (○, □) or strain TK401 (△) were depleted of K by incubation in K0N0 buffer containing glucose and chloramphenicol for 130 min (□, △) or 180 min (○). Then the cells were concentrated by filtration and added to a series of tubes containing mixtures of K0N0 and K115N0 to achieve the indicated final K concentrations. The initial rate of uptake at each concentration is plotted.

linearly dependent on external K concentration up to 105 mM, the highest concentration tested (Fig. 2). Data for three experiments are shown. In each case linearity is seen, although the absolute values differed somewhat in one of the experiments. If this uptake process is saturable the K_m is very high indeed, since no evidence of curvature is seen over the range examined. This process is referred to as the TrkF system. No mutants affecting it have been identified and it appears to be present in all strains studied.

To determine the role of each of the three mutations in the $K^-A^-D^-$ strains, derivatives of such a strain carrying only two of the mutations were constructed. The most dramatic restoration of transport occurs when the wild-type allele at the *trkA* locus is introduced, resulting in a K^-D^- strain. As shown in Fig. 3, this strain takes up K by a process very similar to that previously noted in wild-type strains not subjected to K limitation (Schultz et al., 1963). This is called the TrkA system. For the experiment of Fig. 3 done at 25°C the K_m was 1.8 mM, and V_{max} was 310 μmol/g min. In an experiment at 30°C a K_m of 1.4 mM and a V_{max} of 470 μmol/g min were obtained.

A very high affinity system for K transport called the Kdp system can be demonstrated in A^-D^- mutants as well as other strains which are wild type for

TABLE II

PROPERTIES OF K UPTAKE SYSTEMS OF *E. coli**

System	K_m	V_{max}‡	Genetics	Other characteristics
	mM	*μmol/g min*		
Kdp	0.002	150	Four linked *kdp* genes	Repressible by K
TrkA	1.5	550	Single *trkA* gene	Constitutive
TrkD	0.5	40	Single (?) *trkD* gene	Constitutive
TrkF	>500	–	No mutations known	Constitutive (low rate, linearly dependent on K concentration)

* Kinetic data are from this paper; genetic data are from Epstein and Davies, 1970, and from Epstein and Kim, 1971.

‡ Rates at 37°C for Kdp and TrkA, at 30°C for TrkD.

the *kdp* genes. In A^-D^- strain TK1004 grown in K5 medium, K uptake rates were 7.5, 9.1 and 10.8 μmol/g min at external K concentrations of 0.01, 0.1, and 3 mM, respectively. The affinity of this system for K is so high that we were unable to obtain reliable estimates of the K_m from chemical measurements of K uptake. We therefore used ^{42}K in an experiment in which the cells were allowed to deplete the medium of K (Fig. 4). Initial cell K in such experiments is relatively high, approximately 40 mM in the experiment of Fig. 4, because the Kdp system allows the cells to scavenge traces of K in the wash and resuspension solutions. If efflux is negligible the external specific activity will remain constant, and radioactivity in the external medium is directly proportional to K concentration. A double reciprocal plot of the rate of K uptake versus K concentration shown in the inset of Fig. 4 indicated a K_m of 2.5 μM. Another experiment of this type yielded a value of 2 μM. The assumption of negligible efflux is not critical for these estimates of the K_m. Efflux will increase external K concentration and at the same time reduce the specific activity of external K, two effects which tend to cancel out each other.

The rate of uptake by the Kdp system varies over a wide range depending on the way the cells are grown. The system is not evident at all in cells grown in K115 medium regardless of genotype, while all strains which are not mutant for the *kdp* genes express the system at a high rate, ranging from 140 to 160 μmol/g min at 37°C after K limitation of the cells for approximately 2 h. K limitation in the presence of chloramphenicol does not lead to appearance of the system, suggesting that this type of control is due to repression of synthesis of one or more protein components of the Kdp system.

The Kdp system appears to be regulated indirectly by the K concentration of the growth medium to permit the cells to meet their K needs for growth. Growth of wild-type strains in K5 medium does not lead to a detectable derepression of the system as measured by uptake at or below 0.02 mM K where the contribution of the TrkA and TrkD systems (see below) is very small and can be estimated. However, as already noted, growth of A^-D^- strains in K5 medium leads to derepression ranging from 8 to 15 μmol/g min at 37°C in different experiments. These levels are less than 15% of the maximum derepressed levels of the system but not much above the net uptake rate of 6 μmol/g min needed to maintain

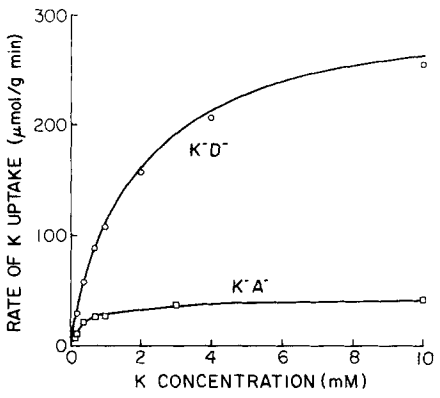

FIGURE 3

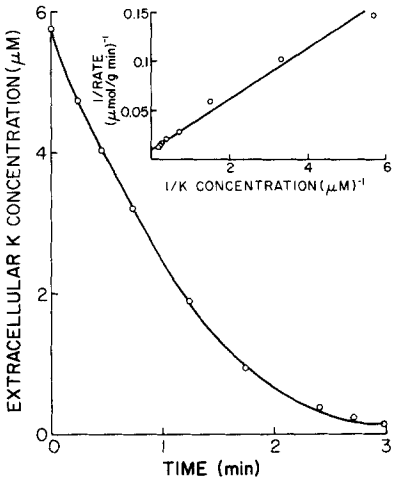

FIGURE 4

FIGURE 3. Kinetics of K uptake by the TrkA and TrkD systems. Initial rates of uptake were measured after depletion of cell K with DNP. ○, K^-D^- strain TK1001 at 25°C; the curve drawn is for a saturable process with a K_m of 1.8 mM and a V_{max} of 310 μmol/g min. □, K^-A^- strain TK133 at 30°C; the curve represents process with a K_m of 0.46 mM and a V_{max} of 42 μmol/g min.

FIGURE 4. Measurement of the K_m of uptake by the Kdp system. Wild-type strain FRAG-1 grown at 37°C was subjected to K limitation at 37°C for 90 min, then depleted of K by DNP treatment, and suspended to a density of 51 μg/ml in K-free medium containing 2 mg glucose and 50 μg choramphenicol per milliliter. This suspension was incubated at 21°C for several minutes, then at zero time ^{42}K (5,000 cpm/nmol) was added to 5.7 μM. Radioactivity remaining in the medium was measured in samples obtained by rapid filtration at the times indicated. This measured radioactivity was converted to chemical concentration of K assuming that specific activity remained constant (see text). External specific activity was calculated by dividing initial radioactivity by the initial external K concentration, the latter obtained indirectly as the change in cell K from control samples before addition of K to the final samples when over 99.5% of the added K had been taken up. The inset is a double reciprocal plot of the rate of uptake over each time interval versus the average external K concentration during that time interval. The straight line represents a process with a K_m of 2.5 μM and a V_{max} of 96 μmol/g min.

physiological K concentrations during exponential growth at 37°C. The Kdp system appears to be the only one subjected to physiological regulation of expression by external K since K limitation did not produce any detectable changes in the properties of the TrkA or TrkD systems.

The *trkD* locus affects a minor K transport system not previously detected in wild-type cells since it is overshadowed by the TrkA system. In K^-A^- mutants this system of modest affinity and rate is seen in relative isolation (Fig. 3). The experiment shown reveals a K_m of 0.46 mM and a V_{max} of 42 μmol/g min at 30°C. In two other experiments, both done at 25°C, the corresponding figures were 0.59 and 0.71 for the K_m, and 19 and 24 μmol/g min for the V_{max}.

The one K^-E^- mutant isolated to date has much lower rates of K uptake than wild-type strains. However, results of uptake studies were so extremely variable that no clear picture of the kinetics has emerged. In six experiments at 25°C uptake was saturable; the K_m ranged from 0.2 to 2 mM, while estimates of the V_{max} ranged from 20 to 50 μmol/g min. This result suggested that such strains may lack uptake by the TrkA system whose function is somehow affected by the *trkE* gene product. However, a mutant also lacking the TrkA system, a $K^-A^-E^-$ mutant, was constructed and found to have uptake characteristics very similar to those of the $K^-A^-D^-$ mutants (Fig. 2). We therefore conclude that the *trkE* gene product is needed to obtain high rates of transport via both the TrkA and the TrkD systems.

K uptake in K^-B^- and K^-C^- strains is rapid and similar to that found in K^- strains where uptake is primarily a reflection of the TrkA system. In two experiments with K^-C^- strain TK121 at 25°C, the K_m was 1.4 and 1.6 mM, and the V_{max} was 245 and 230, respectively. The results with K^-B^- strain TK110 were similar, with a K_m of 1.2 mM and a V_{max} of 500 μmol/g min at 30°C. These results, taken with the data of Fig. 1, indicate that the *trkB* and *trkC* genes affect K retention, but have little or no effect on K uptake.

The four K uptake systems have been examined in relative isolation; the TrkF system was studied in a mutant lacking the other three, while each of the saturable systems was studied in a strain lacking the other two and over a concentration range where uptake by the TrkF system is negligible. Is there an interaction between two systems when both are present, or do they act as independent parallel paths for K transport? If interaction is occurring, the kinetics of uptake when both are present will not be simply the sum of each system as measured alone. This kinetic test for the Kdp and TrkA systems is shown in Fig. 5. Here K uptake was measured in a wild-type strain grown either in K10 medium to repress the Kdp system, or subjected to K starvation to derepress the Kdp system. When grown under repressing conditions only a single component of uptake with a K_m of 1.2 mM is seen (lower curve), while in the derepressed cells uptake is nicely described by the upper curve, which is the sum of the saturable process seen in the repressed cells plus an additional component of uptake constant over the range of K concentrations tested. This latter component is typical of the Kdp system, and the result indicates independent functioning of the Kdp and TrkA systems. In this experiment net K uptake was produced by osmotic upshock, a procedure which tended to give lower and somewhat more variable maximum rates of uptake than when K depletion is used to stimulate uptake. Similar results were obtained in two other experiments of this type, including one in which the Kdp system was derepressed to only 20% of the extent seen in Fig. 5. We have ignored the role of the TrkD system in these experiments since that system makes a small contribution compared to the TrkA system.

A similar test for the TrkD and Kdp systems suggests that these systems do interact (Fig. 6). An A^- strain was tested with the Kdp system, either repressed or partially derepressed. The typical kinetics of uptake by the TrkD system (lower curve) are converted after Kdp derepression (upper curve) to uptake that

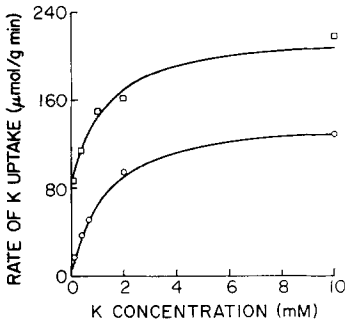

FIGURE 5

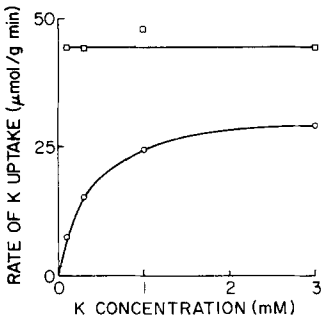

FIGURE 6

FIGURE 5. Effect of Kdp derepression on K uptake kinetics in a wild-type strain. Strain FRAG-1 was grown at 30°C either in K10 medium (○), or in K0.05 medium (□) for approximately 2 h after the time at which K in the medium was exhausted. Then the cells were collected and K uptake was measured by the osmotic upshock procedure. The lower curve is for a saturable process with a K_m of 1.2 mM and a V_{max} of 145 μmol/g min. The upper curve is the same curve shifted upwards by a constant 80 μmol/g min.

FIGURE 6. Effect of Kdp derepression in an A^- strain. Strain TK1005 was grown in Kdp repressing K115 medium (○) or in K0.3 medium (□) to partially derepress the Kdp system. K uptake was measured at 30°C after K depletion by treatment with DNP. The curve is for a saturable process with a K_m of 0.3 mM and a V_{max} of 33 μmol/g min.

appears to be independent of K concentration, as is typical for the Kdp system. Similar results were obtained in two other experiments of this type. Derepression of the Kdp system somehow suppresses all traces of a component of uptake with a moderate affinity for K, typical of the TrkD system.

A test for additivity of the TrkF and Kdp systems was performed by examining K uptake in the range above 10 mM in A^-D^- strain TK1004 after growth in K5 medium to partially derepress the Kdp system. The Kdp system has such a low K_m that uptake above 10 mM should be constant. Uptake at 87.5 mM K was 5.9 μmol/g min greater than at 12.5 mM K, the measurements being performed at 30°C. This difference is not far from expectations for the TrkF system, whose rate at 37°C increases from 8 to 11.5 μmol/g min over this range of K concentrations (Fig. 2). Tests for additivity of the TrkD and TrkF systems were performed in the same way using A^-K^- strain TK133. Calculations for the TrkD system indicate that the rate at 10 mM K is 95% of the maximum, so that at most an increase of some 2 μmol/g min is expected when going from 10 to an external K concentration of 100 mM. In two experiments at 37°C the increase from 10 to 100 mM was 6.0 and 6.4 μmol/g min, while in two at 30°C the increase was somewhat larger, 10.2 and 11.7 μmol/g min. These values are in the range expected for the TrkF system, and are more than can be attributed to the TrkD system.

Kinetic tests for independent function of the TrkD and TrkA systems performed at pH 7 are not sufficiently sensitive because the K_m's of the two systems are not very different, and the high rate of the TrkA system dominates uptake.

An attempt to resolve two components was made by measuring uptake at pH 5.5, a condition which stimulates the TrkD system approximately twofold while inhibiting the TrkA system by approximately 50% (data not shown). Rates of K uptake over a range of K concentrations from 0.05 to 10 mM in K^- strain FRAG-5 were approximately equal to the sum of uptake rates in K^-A^- strain TK133 and K^-D^- strain TK1001, a result consistent with independent functioning of the TrkA and TrkD systems. Double reciprocal plots of the data are curved for all three strains, preventing a simple kinetic resolution of two components of uptake in FRAG-5 at low pH.

The effect of increasing the gene dosage for the *kdp* and *trkA* genes was tested by comparing uptake of the corresponding systems in strains partially diploid for the genes with the isogenic haploid strain (Table III). The amount of product made from a gene is usually increased in such diploids, the increase ranging from two- to threefold. For the Kdp system the diploid strain transports at slightly over twice the rate in the haploid, suggesting that more of the *kdp* gene products are made in the diploid, and that the amount of one or more of these products determines the maximum rate for this system. For the TrkA system the maximum rate of uptake in the diploids is not consistently altered, but there seems to be a small reduction in K_m. If this reduction in K_m is due to the presence of the episome, then the alteration suggests that more *trkA* gene product is being made but that the amount of this product does not set the maximum rate for the system.

Electroneutrality during K uptake can be maintained by extrusion either of Na or of protons (Schultz et al., 1963). Cell Na was measured to test whether any of the mutants differed in the amount of Na extruded during K uptake. Since the cell Na values show much more scatter than the K data (see Methods) only those experiments with reasonably small scatter could be used. Most of the variability is probably due to contamination with medium Na not completely

TABLE III

GENE DOSAGE EFFECTS FOR THE TrkA AND Kdp SYSTEMS

System	Strain	K uptake at 25°C*	
		K_m	V_{max}
		mM	*μmol/g min*
TrkA	FRAG-5‡ (haploid)	1.5	160
	FRAG-5/F141 (diploid)	1.2	210
	TK1001 (haploid)	1.85	310
	TK1001/F141 (diploid)	1.5	270
Kdp	TK1030 (haploid)	§	71, 77‖
	TK1030/F100 (diploid)	§	147, 199‖

* K uptake was measured after depletion with DNP.

‡ The strain used in the first two experiments is a derivative of FRAG-5 which also has spontaneous *malA* and *strA* mutations.

§ Not measured.

‖ Results for two separate experiments are presented.

removed by the washing procedure since there were many values much higher than the majority but never any that were very much lower. An example of one of the best experiments of this type is shown in Fig. 7, where the fall in cell Na during K uptake by the TrkA system is shown. There is a linear relationship of cell Na to cell K, extrusion of 0.8 equivalents of Na accompanying uptake of 1 equivalent of K. Similar experiments with strains in which K uptake occurred via the Kdp system, the TrkD system, the TrkF system, or both the TrkA and TrkD systems yielded ratios ranging from 0.5 to 1. We doubt that any of these ratios are significantly different from each other since a range of 0.5 to 1 was obtained in five experiments of this type with K^-E^- strain TK142. Since it appears that all of the mutants studied here have very low cell Na when their cell K levels are at or near physiological levels, the apparent stoichiometry of K and Na movements may be determined more by the amount of Na that enters the cell during K depletion than by the properties of a particular K transport system. Our data do show that all four K transport systems are able to take up K in exchange for Na.

The filter measurements of cell Na suggest that cells not subjected to K depletion have very low concentrations of Na. Extrapolating the line of Fig. 7 to a cell K concentration of 200 mM, which is the physiological K concentration for cells in medium of this osmolarity (Epstein and Schultz, 1965), predicts that cell Na will be close to 0. In two experiments Na was measured in cells not subjected to K depletion and suspended in dilute medium of 120 mosM and an Na concentration of 58 mM. Cell Na values were 1 ± 3, and 10 ± 4 mM (SE, n = 21 for each), values considerably lower than those reported earlier based on analysis of cell pellets (Schultz and Solomon, 1961). The difference between filter and pellet Na analyses suggests that a considerable amount of Na in pellets is loosely bound to the cell envelope and is readily removed by brief washing.

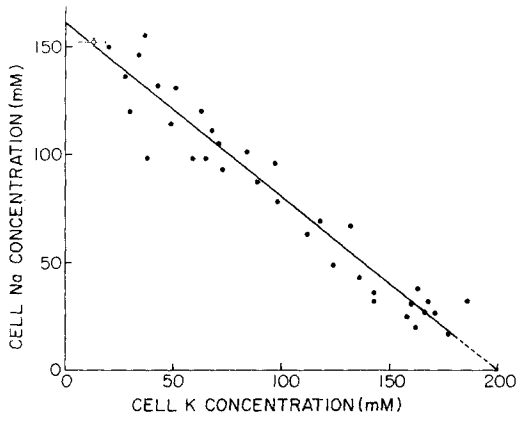

FIGURE 7. Relationship of cell Na to cell K during net K uptake in K^-D^- strain TK1001. Data are from the same experiment as shown in the top curve of Fig. 3. The point with bars at the upper left represents the mean and standard deviations for six samples of the control suspension before the addition of K. The line, drawn by the method of least squares, has a slope of −0.81.

The effect of the K concentration of the medium on the growth rate of the mutants is shown in Fig. 8. The results are plotted as the percent of the maximal growth rate since growth rate of all of the different mutants in K115 medium is the same within experimental error. The data of Fig. 8 is presented in terms of initial K concentration in the medium. Growth reduced the values somewhat, but the change was negligible for all but the lowest K concentrations, and even below 0.05 mM initial K, the change was never greater than 15% during the period used to determine growth rate. The logarithmic scale on the abscissa used to allow presentation of data for all strains in a single figure somewhat obscures the rather large differences between strains and the steep slopes of several of the curves. Using as an index the concentration of K required to achieve a half-maximal growth rate, the mutants range from the K^- mutants which require only 0.07 mM to the $K^-A^-D^-$ mutants which require 10 mM. Hill plots of the four mutant classes which rise steeply yielded slopes between 2 and 3, indicating that the growth dependence of these strains on external K can be described as cooperative.

Growth rates were measured in the media listed in Table IV to assess the effect of pH, osmolarity, and other cations on the K requirements of the mutants. For all of the mutants except the K^-E^- strain (see below) the curves relating growth rate to K concentration in the media of Table IV have the same shape as those of Fig. 8, and at high K concentrations all have the same growth rate as that of a wild-type strain in that medium. Thus changes are adequately described by the K concentrations giving half-maximal growth rate listed in the table. Increasing the osmolarity or reducing pH tends to raise the K requirements of the mutants, but the opposite effect is seen in K^-A^- and K^-B^- strains. The effect of pH on K^-A^- strains, as compared to K^- and K^-D^- strains, is accounted for by the different response of the TrkA and TrkD systems at low pH as noted above.

Growth in maleate-buffered media tested the role of the supposedly indifferent major medium cation on K requirements. In most cases substitution of either Tris or Mg for Na produced no major effect, although for several of the mutants

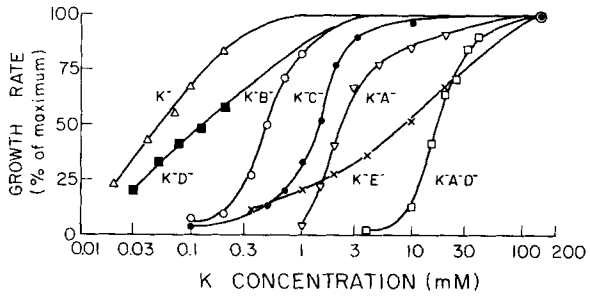

FIGURE 8. K dependence of growth rate for different classes of K mutants. Growth rates were determined turbidimetrically as described under methods in phosphate-buffered pH 7 media with glucose, 2 g/liter, except for strain TK142 where glycerol, 2 g/liter, was used in medium with a pH of 7.3. △, K^- strain FRAG-5; ■, K^-D^- strain TK1001; ○, K^-B^- strain TK110; ●, K^-C^- strain TK118; ▽, K^-A^- strain TK133; ×, K^-E^- strain TK142; □, $K^-A^-D^-$ strain TK401.

TABLE IV

EFFECT OF pH, CATIONS, AND OSMOLARITY ON K REQUIREMENT FOR GROWTH*

Mutant class	Strain	K concentration needed to achieve half-maximal growth rate (mM)						
		Phosphate-buffered media				Maleate-buffered media		
		pH 5.8	pH 7.0‡	Dilute	Hypertonic§	Na	Tris	Mg
		220 mosM	*240 mosM*	*120 mosM*	*820 mosM*	*150–190 mosM*		
K^-A^-	TK133	1.4	2.3	0.6	1.8	1.9	1.9	9.6
K^-B^-	TK110	0.5	0.5	0.3	0.9	0.6	0.6	0.7
K^-C^-	TK121	3.2	1.4	0.8	2.5	1.9	2.6	1.3
K^-D^-	TK1001	0.33	0.12	0.12	0.36	0.26	0.34	0.34
$K^-A^-D^-$	TK401	60	16	14	52	19	21	14
K^-E^-	TK142	1.7‖	9	1.9‖	9‖	7	18	13

* Growth rates at 37°C were measured as described under Methods. The carbon source was glucose except for the maleate-buffered media where glycerol was used. Curves like those of Fig. 8 were obtained, and interpolated to obtain the K concentration at which half-maximal growth rate occurred.

‡ These results are from Fig. 8.

§ Standard medium with approximately 0.6 M glucose.

‖ Growth rate of strain TK142 even at the highest K concentration was less than that of wild-type strains in these media.

Tris led to a somewhat higher K requirement. Mg has a rather large effect in the K^-A^- strain, shifting the curve toward one that is fairly close to that for the $K^-A^-D^-$ strain. The obvious conclusion, that Mg inhibits the TrkD system, was confirmed by kinetic studies which showed that in Mg medium the TrkD system has a V_{max} only 40% of that in the standard media. This effect of Mg is not a general one since the TrkA system is not inhibited by Mg.

Data for the K^-E^- strain is included in Table IV for the sake of completeness. The growth curve for that strain has a rather low slope (Fig. 8), so that small changes in the curve produce rather large shifts in the midpoint. Another complication, noted in Table IV, is that this strain does not achieve wild-type growth rates in several of the media even at the highest K concentration that can be attained in that medium.

DISCUSSION

The studies reported here demonstrate that K uptake in *E. coli* K-12 is a complex process comprising the four kinetically distinct systems whose properties are summarized in Table II. The Kdp system is rather typical of high affinity bacterial transport systems (Boos, 1974). It is repressible and is not made except when needed to scavenge K (in a wild-type strain) or to compensate for defects in other K transport systems. Transport by this system was detected some years ago when it was noted that K limitation leads to an increased rate of K exchange and that this increase requires concomitant protein synthesis (Goldman et al., 1966). The *kdpD* gene may have a regulatory role for this system since some revertants of the *kdpD2* mutation express the Kdp system at a partially constitutive level. Another gene, probably *kdpB*, seems to code for a periplasmic protein based on

the osmotic shock sensitivity of the Kdp system and an electrophoretic analysis of shock fluid proteins from wild-type and *kdp* mutants (D. B. Rhoads and A. Woo, unpublished observations). The high maximum rate and high affinity of the Kdp system enable it to compensate for other K transport defects be they uptake defects due to *trkA* or *trkD* mutations or retention defects such as those produced by *trkB* and *trkC* mutations. This compensation by the Kdp system explains why *kdp*$^+$ derivatives of all the mutants studied here (Table I) are wild type for growth since they require less than 50 μM K to achieve maximal growth rates. Only when the Kdp system is first eliminated is it possible to identify and select for other defects by growth tests. Except for its transport role, the Kdp system is nonessential since deletion and amber nonsense mutations of the *kdp* genes have been obtained (Epstein and Davies, 1970).

The TrkA system is responsible for the bulk of K uptake under most conditions. It has only modest affinity for K but has a very high maximum rate. So far only one component of this system has been identified, the product of the single *trkA* gene. The ease with which we have isolated amber nonsense mutations in *trkA* suggests that this gene product is also dispensable except for its role in K transport. Relatively little is known of the components making up the TrkD system. Only a few independent *trkD* mutations have been isolated so far, and we do not know how many different genes make up the *trkD* locus.

The single *trkE* mutation isolated so far remains the most mysterious. The mutation clearly interferes with function of the TrkA and TrkD systems. While *kdp*$^+$ derivatives of the K$^-$E$^-$ mutant are wild type for growth, we have not examined their transport properties to see if the Kdp system is altered.

The TrkF system is unique. It transports K at relatively low rates and shows no evidence of saturation; the kinetics are those of a diffusion-limited process. K accumulation by the TrkF system results in a concentration gradient approaching 10-fold, uptake which could be driven by the membrane potential alone if the latter is of the order of 60 mV.

Mutations affecting the TrkF system have not been identified to date. Such mutations are either not selected by any of the methods we have used, or they are disadvantaged in our media and possibly lethal. It is possible that the TrkF system transports K via a specific channel, one which participates in K uptake at much higher rates when the products of other K transport genes are present. An interesting result of our studies is that DNP treatment leads to rapid loss of K in all strains, and the rate of this loss is not significantly slower in K$^-$A$^-$D$^-$ triple mutants than in wild-type strains. Thus none of the mutations affect the path for rapid K efflux in DNP poisoned cells. K efflux during DNP treatment is dependent on the type of cation in the medium; efflux into Tris media is much slower than into Na media. Thus there appears to be a channel which permits rapid downhill (energetic) movement of K in exchange for another cation for which there is some specificity.

We have reported here only studies on net K uptake effected by the different transport systems. All four systems also mediate K exchange. Net K uptake rates differ from the rates of K exchange in two important ways: the V_{max} of net K uptake is higher, usually approximately fivefold higher than K exchange rates,

and the K_m for exchange was found to be much lower than the K_m for net uptake (Epstein and Schultz, 1966). Preliminary experiments suggest that this discrepancy is due primarily to unusual properties of the TrkA and TrkD systems. Both of these systems catalyze exchange with a lower V_{max} and a lower K_m than when they effect net K uptake. The Kdp and TrkF systems mediate exchange with kinetics similar to net uptake kinetics with the important exception that the Kdp-mediated exchange is inhibited at high extracellular K concentrations.

E. coli mutants defective in K transport have been described by others. In 1960 Lubin reported the isolation of a mutant of *E. coli* strain B which requires elevated K for growth and whose defect is in a region of the *E. coli* chromosome consistent with the location of *trkC* (Lubin and Kessel, 1960). This mutant is defective in K retention (Lubochinsky et al., 1964; Günther and Dorn, 1966). Mutants of *E. coli* B defective in K uptake at low external K concentrations (Damadian, 1968) are probably analogous to our K^- strains, since the defect has been mapped to a location consistent with *kdp* (Burmeister, 1969).

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