

Cation Transport in *Escherichia coli*

IX. Regulation of K Transport

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ABSTRACT Kinetics of K exchange in the steady state and of net K uptake after osmotic upshock are reported for the four K transport systems of *Escherichia coli*: Kdp, TrkA, TrkD, and TrkF. Energy requirements for K exchange are reported for the Kdp and TrkA systems. For each system, kinetics of these two modes of K transport differ from those for net K uptake by K-depleted cells (Rhoads, D. B., F. B. Walters, and W. Epstein. 1976. *J. Gen. Physiol.* **67**:325-341). The TrkA and TrkD systems are inhibited by high intracellular K, the TrkF system is stimulated by intracellular K, whereas the Kdp system is inhibited by external K when intracellular K is high. All four systems mediate net K uptake in response to osmotic upshock. Exchange by the Kdp and TrkA systems requires ATP but is not dependent on the protonmotive force. Energy requirements for the Kdp system are thus identical whether measured as net K uptake or K exchange, whereas the TrkA system differs in that it is dependent on the protonmotive force only for net K uptake. We suggest that in both the Kdp and TrkA systems formation of a phosphorylated intermediate is necessary for all K transport, although exchange transport may not consume energy. The protonmotive-force dependence of the TrkA system is interpreted as a regulatory influence, limiting this system to exchange except when the protonmotive force is high.

Potassium transport in *Escherichia coli* serves to maintain high intracellular K concentrations which are determined chiefly by the osmolarity of the external medium (Epstein and Schultz, 1965, 1968). Maintenance of a given K content determined by medium osmolarity but independent of medium K concentration implies the existence of mechanisms that regulate K influx quite precisely.

Some evidence for regulation was seen in the fact that K-depleted cells had high rates of K uptake by a process that had a K_m in the millimolar range (Schultz et al., 1963), whereas K/K exchange in the steady state occurred at a considerably lower rate and had a very high affinity for K (Epstein and Schultz, 1966). An even more striking regulatory response is produced by an osmotic upshock. Cells react to an upshock by taking up K until they achieve a higher K content corresponding to the new medium osmolarity (Epstein and Schultz, 1965). These studies were done with wild-type cells, before it was known that *E.*

coli has four distinct K transport systems. These four systems, whose properties are summarized in Table I, have been characterized genetically (Epstein and Davies, 1970; Epstein and Kim, 1971) and on the basis of the kinetics for net K uptake by K-depleted cells (Rhoads et al., 1976). One regulatory influence has been documented, namely, that the high affinity Kdp system is repressible by growth in high K media, and is only derepressed when other K transport systems are not adequate to satisfy the cells' needs for K (Rhoads et al., 1976). Because the other systems are constitutive, regulatory influences must operate at the level of the function rather than the level of formation of the systems.

In this paper we examine two different modes of K transport for each of the four systems: K/K exchange in the steady state and net K uptake produced in K-replete cells by osmotic upshock. In each case significant differences in the properties of these two modes of K transport are seen as compared with net K uptake in K-depleted cells. We suggest that these differences reflect regulatory

TABLE I
K TRANSPORT SYSTEMS IN *E. coli**

System	Characteristics of net K uptake				
	Kinetic parameters		Energy requirements	Genetics	Other characteristics
	K_m mM	V_{max}^\ddagger $\mu\text{mol/g/min}$			
Kdp	0.002	150	ATP	Four linked <i>kdp</i> genes	Repressible by K
TrkA	1.5	550	ATP, PMF§	Single <i>trkA</i> gene	Constitutive
TrkD	0.5	40	ATP, PMF§	Single (?) <i>trkD</i> gene	Constitutive
TrkF	>500	—	PMF	No mutations known	Constitutive, low rate, linearly dependent on K concentration

* Genetic data from Epstein and Davies (1970), and Epstein and Kim (1971); kinetic data from Rhoads et al. (1976); energy requirements from Rhoads and Epstein (1977) and unpublished data.

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

§ Protonmotive force.

influences on the systems. In attempting to determine what forces could produce such regulation, we examined energy requirements for K/K exchange by each of the systems. This aspect of the study was prompted by our earlier finding that the TrkA system requires both ATP and the protonmotive force in order to perform net K uptake (Rhoads and Epstein, 1977). This dual energy requirement is unique for bacterial transport, and suggested to us that one of these requirements represents a regulatory influence, whereas the other provides energy. Our results show that K/K exchange by the TrkA system requires ATP but not the protonmotive force, and are consistent with our model that the protonmotive force exerts a regulatory influence on the TrkA system such that high values of the protonmotive stimulate, while low values inhibit, the TrkA system.

METHODS

Bacterial Strains

The strains used in this work, all *Escherichia coli* K-12 F⁻, are listed in Table II with the K transport systems present in each and the relevant genotypes.

Media and Growth of Bacteria

Phosphate-buffered media, described earlier (Epstein and Kim, 1971), are referred to by K concentration in millimolar, e.g., K115 medium contains 115 mM K. K0 medium, to which no K is added, contains about 20 μ M contaminating K. Cells were grown at 37°C with 11 mM glucose as the sole carbon source. Derepression of the Kdp system was accomplished by transferring growing cells to K0 medium at 37°C until the cells were K limited for 1 h.

Transport Studies

Methods for transport measurements (Rhoads and Epstein, 1977), analysis of ⁴²K uptake (Epstein and Schultz, 1966) and osmotic upshock (Epstein and Schultz, 1965) have been described. Three buffers were used; all were pH 7.0 and contained 0.15 mM chloroam-

TABLE II
BACTERIAL STRAINS

Strain	K transport systems present	Relevant genotype	Reference
TK1001	TrkA TrkF	<i>kdpABC5 trkD1</i>	Rhoads et al. (1976)
TK1110	TrkD TrkF	<i>kdpABC5 trkA405</i>	Rhoads et al. (1977)
TK405m	TrkF	<i>kdpABC5 trkA405 trkD1</i>	Rhoads et al. (1977)
TK509*	Kdp TrkF	<i>trkA405 trkD1</i>	Rhoads and Epstein (1977)
TK510	Kdp TrkF	<i>trkA405 trkD1</i>	
TK1064	Kdp TrkA TrkF	<i>trkD1</i>	
TK1097	Kdp TrkA TrkF	<i>trkD1 unc</i>	Rhoads and Epstein (1977)

* This strain was called TAD 109 in Rhoads and Epstein (1977).

phenicol. Buffer A contained 75 mM Na-PO₄ and 0.4 mM MgSO₄, buffer B contained 75 mM K-PO₄ and 0.4 mM MgSO₄, and buffer C contained 110 mM Mg-maleate. Transport assays were conducted at 30°C. To study a single K transport system in relative isolation, a combination of suitably chosen mutations, growth conditions, and assay conditions was used as previously described (Rhoads and Epstein, 1977). For instance, the TrkD system is assayed in strain TK1110 which has both a *kdp* and a *trkA* mutation, and at K concentrations low enough that the TrkF system does not significantly contribute to the uptake. Uptake by the TrkD and TrkF systems was somewhat variable from experiment to experiment, so when a comparison was made between two modes of transport, both modes were measured on the same culture. Fluxes and cell K contents are expressed in units per gram dry weight, the latter determined from turbidity measurements at 610 nm with a Bausch and Lomb Spectronic 20 colorimeter (Bausch & Lomb Inc., Rochester, N. Y.) and a calibration curve.

Chemicals

⁴²KCl and L-[U-¹⁴C]glutamine were purchased from New England Nuclear (Boston, Mass.). L-[U-¹⁴C]Proline was purchased from Amersham/Searle Corp. (Arlington Heights, Ill.).

RESULTS

TrkA System

Steady-state K exchange mediated by the TrkA system exhibits saturation kinetics with respect to the external K concentration (Fig. 1A). This process has a V_{\max} of 110 $\mu\text{mol/g} \cdot \text{min}$ and a K_m of 0.5 mM K at 30°C. Both kinetic

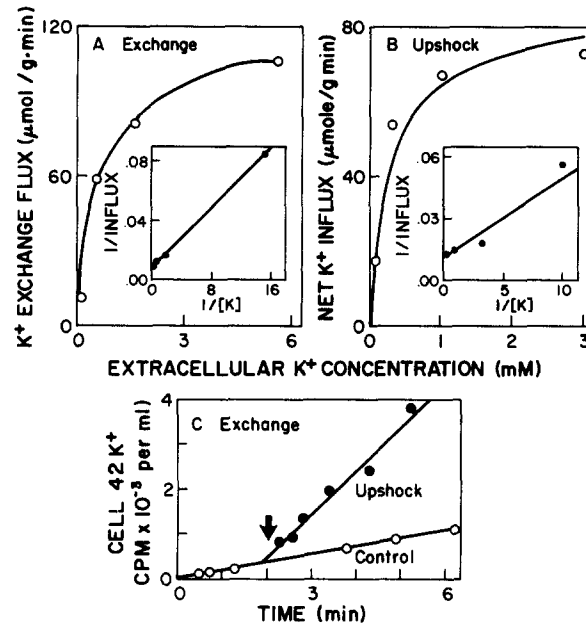


FIGURE 1. K fluxes mediated by the TrkA system. (A) Exchange flux. K115-grown strain TK1001 was suspended in buffer A containing 11 mM glucose and KCl at the indicated K concentration. The cells were allowed to equilibrate for 30 min before addition of the ^{42}K tracer to initiate the flux measurements. (B) Upshock-induced net uptake. Strain TK1001 was grown in K115 medium diluted 1:1 with H_2O and suspended in similarly diluted buffer A containing 11 mM glucose and the indicated amount of KCl. After 30 min net influx was initiated by the addition of 1/4 vol of diluted buffer A containing the same K concentration plus 2.5 M glucose. Cell K was measured chemically. Units for the double reciprocal plots are millimolar $^{-1}$ on the abscissa, and micromolar $^{-1}$ per gram per minute for the ordinate. (C) Unidirectional K influx during upshock. The experiment was performed as in B on K115-grown strain TK1064 at 1.1 mM KCl. ^{42}K tracer was added at zero time (\circ) and at the arrow an aliquot of the suspension was subjected to upshock (\bullet). The latter counts have been corrected for dilution. Control ^{42}K influx was 23 $\mu\text{mol/g} \cdot \text{min}$; after upshock, unidirectional ^{42}K influx was 116 $\mu\text{mol/g} \cdot \text{min}$ and net K influx was 92 $\mu\text{mol/g} \cdot \text{min}$.

parameters are less than those obtained for net K uptake by K-depleted cells (Table I); the V_{\max} is reduced fivefold and the K_m threefold. Temperature effects do not account for this difference because the TrkA system is relatively temperature independent between 30° and 37°C. In an experiment at 30°C with strain TK1001, net uptake had a V_{\max} of 500 $\mu\text{mol/g} \cdot \text{min}$ and a K_m of 1.5 mM.

In another experiment with strain TK1001, the exchange rate was $50 \mu\text{mol/g} \cdot \text{min}$ higher at 114 than at 14 mM K. This increase is due in part to the contribution of the TrkF system at higher K concentrations (see below), but mainly indicates that exchange by the TrkA system is not inhibited by high external K concentrations. Inasmuch as net K uptake was assayed in cells exposed to 2,4-dinitrophenol to deplete them of K, the possibility that treatment with dinitrophenol altered the TrkA system was ruled out by direct test. Cells of strain TK1001 were either assayed for K/K exchange at 1 mM K or depleted of K by dinitrophenol treatment. The K-depleted cells were divided into two portions. One portion was allowed to equilibrate with 1 mM K before the addition of a ^{42}K tracer while the other was equilibrated in the absence of K before addition of 1 mM K containing a ^{42}K tracer. The K-reloaded cells had a K influx of $52 \mu\text{mol/g} \cdot \text{min}$, close to the non-dinitrophenol-treated control value of $63 \mu\text{mol/g} \cdot \text{min}$, while net K influx into the K-depleted cells was $152 \mu\text{mol/g} \cdot \text{min}$, about threefold higher. Thus, K influx mediated by the TrkA system is reduced in K-replete cells regardless of a prior dinitrophenol treatment.

One line of evidence that K is an osmoregulatory solute in *E. coli* is that this bacterium is capable of rapidly increasing its intracellular K concentration in response to an increase in medium osmolarity (Epstein and Schultz, 1965, 1968). The majority of this response has been attributed to the TrkA system (Rhoads et al., 1976). Osmotic upshock-stimulated net uptake by the TrkA system is also a saturable function of K concentration. The experiment illustrated in Fig. 1B shows a K_m of 0.4 mM and a V_{max} of $90 \mu\text{mol/g} \cdot \text{min}$. Three strains, in which both the TrkA and the lower rate TrkD systems (see below) were present, had upshock-stimulated transport with K_m ranging from 0.3 to 0.6 mM and V_{max} from 75 to $109 \mu\text{mol/g} \cdot \text{min}$. The initial rate of net K influx catalyzed by this system is independent of the degree of upshock between osmolarity increases of 0.2-0.6 osmol, but the extent of K uptake increases with the change in osmolarity (data not shown). Our findings that K transport by K-replete cells, be it exchange or in response to upshock, is characterized by considerably lower K_m and V_{max} than uptake by K-depleted cells, indicate that intracellular K inhibits the TrkA system in a way to lower both K_m and V_{max} .

In the steady state, K influx and K efflux are by definition of equal magnitude. When the steady state is disturbed by osmotic upshock, a net influx component emerges. Kepes et al. (1976) have shown that for wild-type *E. coli*, upshock stimulates influx but efflux remains unchanged. This behavior was seen when the TrkA system was observed in isolation (Fig. 1C). The control ^{42}K uptake represents an exchange rate of $23 \mu\text{mol/g} \cdot \text{min}$. Osmotic upshock induced a net uptake rate of $92 \mu\text{mol/g} \cdot \text{min}$ while the unidirectional K influx rate increased to $116 \mu\text{mol/g} \cdot \text{min}$. The difference, $24 \mu\text{mol/g} \cdot \text{min}$, is the efflux which is the same as that in the control. This result indicates that the major effect of upshock on the TrkA system is to stimulate K influx.

Kdp System

Steady-state K flux measurements on the Kdp system yielded an unexpected result. This system mediates rapid K exchange at low external K concentrations

consistent with its high affinity, but the exchange is inhibited in high external K (Fig. 2A). The flux is reduced more than threefold when the external K is raised from 30 μM to 1 mM. No attempt was made to measure exchange by this system near its K_m for net uptake because large fluctuations in the external K would have been unavoidable. The Kdp system responded to an osmotic upshock at a low K concentration, but again, high external K inhibited this process (Fig. 2B). At 2 mM K net influx was reduced to less than half that at 0.2 mM K. Above 2 mM K the rate plateaus, but this is probably only apparent since at 20 mM the TrkF system begins to make a significant contribution (3.5 $\mu\text{mol/g} \cdot \text{min}$; see

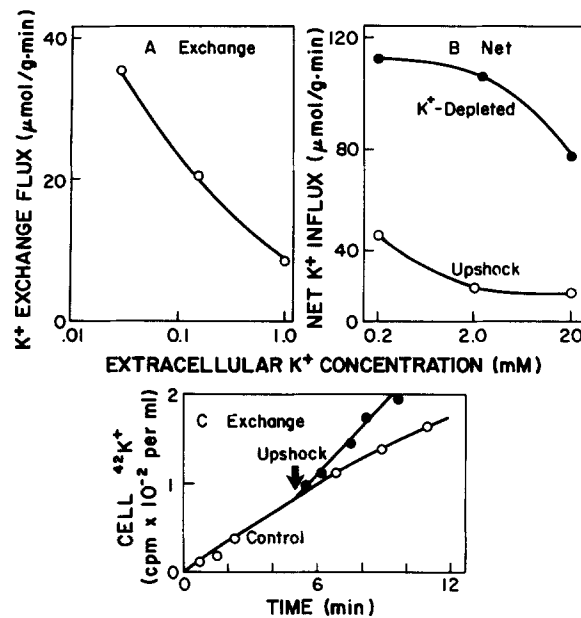


FIGURE 2. K fluxes mediated by the Kdp system. Protocols were identical to those described in the legend to Fig. 1. (A) K exchange flux in K-limited strain TK510. (B) Upshock-induced net uptake in K-limited strain TK509. Note the logarithmic scales of the abscissae. (C) Unidirectional K influx during upshock of K-limited strain TK510 at 0.4 mM KCl. (○) Control; (●) upshock. Control ^{42}K influx was 35 $\mu\text{mol/g} \cdot \text{min}$; after upshock, unidirectional ^{42}K influx was 74 $\mu\text{mol/g} \cdot \text{min}$ and net K influx was 38 $\mu\text{mol/g} \cdot \text{min}$.

Fig. 4B below). The shape of the curves of Fig. 4A and B suggest that inhibition may be negligible at low external K concentrations close to the 2 μM K_m of the Kdp system. Inhibition by external K, evident when transport is measured in K-replete cells as exchange or upshock-stimulated net uptake, is not seen at concentrations up to 10 mM when transport is measured as net K uptake in K-depleted cells (Rhoads et al., 1976). We infer that the Kdp system is inhibited by external K but only when intracellular K is high.

Unidirectional influx mediated by the Kdp system after osmotic upshock is shown in Fig. 2C. The control exchange rate was 35 $\mu\text{mol/g} \cdot \text{min}$ whereas after

upshock the net influx was $38 \mu\text{mol/g} \cdot \text{min}$ and the unidirectional influx was $74 \mu\text{mol/g} \cdot \text{min}$. Because the last quantity is equal to the sum of the other two, we conclude that for the Kdp system osmotic upshock stimulates influx and does not affect efflux.

TrkD System

The minor TrkD system exhibits an inhibition of K influx by internal K similar to that shown by the TrkA system. Inasmuch as fluxes by the TrkD system are sometimes variable from culture to culture, Fig. 3A shows the K-concentration dependence of both the steady-state exchange flux and the net K influx into K-depleted cells from a single culture of strain TK1110. For net K uptake the V_{max} is $60 \mu\text{mol/g} \cdot \text{min}$ and the K_m is 0.4 mM ; for exchange the values are $12 \mu\text{mol/g}$

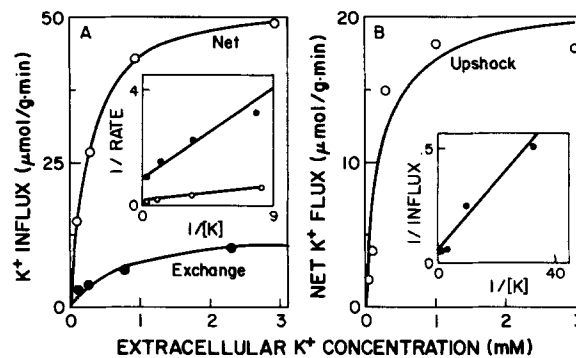


FIGURE 3. K fluxes mediated by the TrkD system. Exchange flux and upshock-induced net uptake were measured as described in the legend to Fig. 1. Net influx into K-depleted cells was measured on a portion of the culture used for the exchange measurement. Cells were subjected to a 30-min incubation at 37°C , suspended in K0 medium containing 10 mM dinitrophenol to deplete intracellular K. Depleted cells were suspended in buffer A plus 11 mM glucose at 30°C and allowed to equilibrate 15 min before the addition of KCl to the indicated concentration to initiate uptake. (A) K exchange flux in K-replete cells (\bullet) and net K influx into K-depleted cells (\circ). (B) Upshock-induced net influx. Units for the double reciprocal plots are the same as in Fig. 1.

$\cdot \text{min}$ and 0.6 mM . Low rates of transport by the TrkD system occur in response to upshock. The experiment of Fig. 3B shows a V_{max} of $22 \mu\text{mol/g} \cdot \text{min}$ and a K_m of 0.3 mM . Another experiment of this type indicated a V_{max} of $19 \mu\text{mol/g} \cdot \text{min}$ and a K_m of 0.25 mM . For the TrkD system high intracellular K reduces the V_{max} three- to fivefold, but has little if any effect on the K_m for transport.

TrkF System

Steady-state K flux mediated by the low affinity TrkF system is linearly dependent on the external K concentration, as is net uptake by this system (Fig. 4A). The linearity up to 70 mM suggests that, if the TrkF system is saturable, the K_m must be well above 0.5 M . Even this sluggish system is sensitive to osmotic

upshock; the induced net influx exhibits a nearly linear dependence on the external K concentration (Fig. 4B). The net influx into K-depleted cells from the same culture, shown for comparison, is lower than the upshock-induced net influx and appeared to be lower than the exchange influx. The latter effect suggested that the TrkF system exhibits exchange diffusion, so the experiment presented in Table III was performed to document this phenomenon. At both high and low external K concentrations, influx into K-replete cells, whether dinitrophenol-treated and K-reloaded or not dinitrophenol treated, is higher than K influx into K-depleted cells. We conclude that the TrkF system exhibits exchange diffusion.

Energy Requirement for K/K Exchange by the TrkA and Kdp Systems

To determine whether the energy requirement for K/K exchange was different from that for net K uptake, the two major K transport systems—the TrkA and

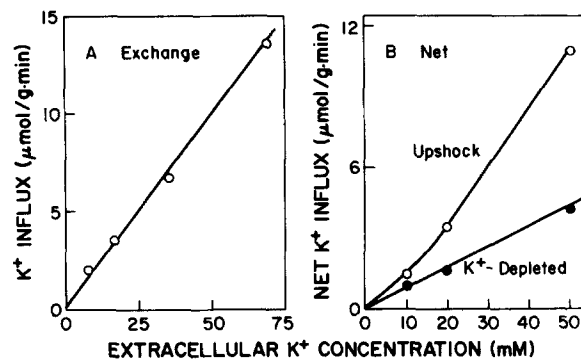


FIGURE 4. K fluxes mediated by the TrkF system. (A) K exchange flux was measured in K115-grown strain TK405m as described in the legend to Fig. 1 except that the appropriate K concentrations were achieved by mixing buffer A with buffer B. (B) Upshock-induced net influx in K-replete cells (○) and net K uptake by K-depleted cells (●). The former measurements were made on cells of strain TK405m grown in K115 diluted 1:1 with H₂O as described in the legend to Fig. 1, whereas the latter measurements were made on a portion of the same culture subjected to dinitrophenol treatment to deplete cells of K. K concentrations were achieved by mixing buffer A and buffer B, both diluted 1:1 with H₂O. Cell K was measured chemically.

the Kdp systems—were examined by methods similar to those used for the study of net K uptake (Rhoads and Epstein, 1977). The phosphate analogue arsenate prevents ATP synthesis whether made by substrate-level or by oxidative phosphorylation, yet allows formation of the protonmotive force (Klein and Boyer, 1972). Thus, the ATP requirement of a transport system can be observed in the presence of a protonmotive force. In *unc* mutant strains, the protonmotive force and ATP are not interconvertible because the energy-transducing Ca, Mg-ATPase which performs this conversion is defective (Berger, 1973). ATP must be formed through fermentation and, in the presence of either the terminal oxidase inhibitor CN⁻ or the uncoupler dinitrophenol, no protonmotive force is

generated. The protonmotive force-driven proline system and the ATP-driven glutamine system were used as controls to monitor the effects of the energy transduction inhibitors.

Table IV shows that K/K exchange mediated by both the TrkA and the Kdp systems is very sensitive to the effects of arsenate under conditions where high intracellular K concentrations were maintained with little or no net flux (lines 1a and 2a). Arsenate at 0.5 mM concentration reduced the TrkA flux by 98% and the Kdp flux by 95% of the control values whereas proline transport was inhibited by < 42% and glutamine transport was inhibited > 90%. We conclude from this result that the steady-state K flux mediated by the TrkA and the Kdp systems is dependent on ATP or another high-energy phosphate compound made from ATP.

TABLE III
EXCHANGE DIFFUSION BY THE TrkF SYSTEM

Experiment	Pretreatment		⁴² K influx	
	K-depleted	K concentration of equilibration buffer <i>mM</i>	K concentration <i>mM</i>	Rate $\mu\text{mol/g}\cdot\text{min}$
A	No	17	17	3.8
		64	64	9.1
B	Yes	0	14	1.1
		0	65	5.6
C	Yes	17	17	2.8
		75	75	13.1

⁴² K influx was measured in K115 grown cells of strain TK405m after the indicated pretreatment. In all experiments influx was measured at 30°C after 1 h of equilibration at 30°C in a mixture of buffers A and B with the indicated K concentration, and containing 11 mM glucose. In experiments B and C, the cells were first depleted of K by treatment with 10 mM dinitrophenol for 30 min at 37°C. In experiment C, the cells equilibrated with 17 mM K had attained approximately one-third their K content at the time the influx measurement was made.

Also shown in Table IV are the effects of CN⁻ and dinitrophenol on K/K exchange in an *unc* strain. The TrkA system exhibited a marked resistance to CN⁻, 70% of the TrkA-mediated flux and 63% of the glutamine uptake were retained, while proline uptake was reduced to < 10% of its control value. The TrkA exchange flux was less resistant to dinitrophenol than glutamine uptake, yet was more resistant than proline uptake (line 1b). At 1 mM dinitrophenol, 25% of TrkA-mediated exchange, 71% of glutamine uptake, and 9% of the proline uptake remained. In the presence of either inhibitor the cell K content was 70% of the control value. CN⁻ had a curious effect on K/K exchange by the Kdp system, stimulating the steady-state flux almost three-fold at 1 mM (line 2b). The increased K influx resulted in a 37% rise in the cell K content. Dinitrophenol reduced Kdp exchange to 30% while diminishing the cell K content to 80% of the control values (line 2b). In K-limited strain TK1097 45% of glutamine uptake was retained while proline uptake was reduced to 7% of its control value. The significant resistance to CN⁻ and the intermediate resistance

to dinitrophenol exhibited by the TrkA and the Kdp systems indicates that K/K exchange catalyzed by either system does not require a large protonmotive force.

The results presented here show that K/K exchange is an energy-dependent process. The energy requirement for the Kdp system is similar in the two modes studied—both net K uptake and K/K exchange depend on ATP but not on the protonmotive force. However, exchange by the TrkA system exhibits a less stringent energy requirement than does net uptake by this system. Net uptake is

TABLE IV
THE EFFECT OF ENERGY TRANSFER INHIBITORS ON K EXCHANGE BY
THE TrkA AND Kdp SYSTEMS*

	Cell K content‡	K exchange flux	Proline system	Glutamine system
	$\mu\text{mol/g}$		$\mu\text{mol/g}\cdot\text{min}$	
1. TrkA system				
(a) TK1064 (<i>unc</i> ⁺)§				
Control	370	55	4.6	3.0
AsO ₄ , 0.5 mM	327	1	2.7	0.14
(b) TK1097 (<i>unc</i> ⁻)§				
Control	480	18	1.3	2.5
CN ⁻ , 1 mM	342	13	0.12	1.6
Dinitrophenol, 1 mM	392	4.5	0.16	1.8
2. Kdp system				
(a) TK1064 (<i>unc</i> ⁺)				
Control	397	19	2.8	4.0
AsO ₄ , 0.5 mM	332	1	2.3	0.28
(b) TK1097 (<i>unc</i> ⁻)				
Control	484	30	3.4	3.4
CN ⁻ , 1 mM	667	86	0.22	1.6
Dinitrophenol, 1 mM	398	9	0.23	1.5

* The effect of arsenate was studied on cells suspended in buffer C containing 11 mM glucose and 1.0 mM KCl for the TrkA system or 0.1 mM KCl for the Kdp system. The effects of cyanide and dinitrophenol were studied on cells suspended in buffer A containing 22 mM glucose and 0.85 mM KCl for the TrkA system and 0.1 mM KCl for the Kdp system. The initial rates of uptake are given for proline and glutamine transport at 10 and 3 μM , respectively.

‡ This value remained constant (net efflux was $<2 \mu\text{mol/g}\cdot\text{min}$) during the course of the transport measurements.

§ Cells were grown in K115 medium.

|| Cells were K limited to derepress the Kdp system. K fluxes due to the TrkA system were calculated to be $<6 \mu\text{mol/g}\cdot\text{min}$ in the controls.

completely dependent on the presence of both ATP and the protonmotive force but exchange, although requiring ATP, is little if at all dependent on the protonmotive force.

DISCUSSION

The studies reported here provide further information on how K transport in *E. coli* is regulated. Earlier work showed that intracellular K concentration is determined primarily by the osmotic needs of the cell (Epstein and Schultz, 1965). The cell uses K as one of the major solutes whose concentration is so

regulated that the turgor pressure, the difference between internal and external osmotic pressure, remains relatively constant (Epstein and Schultz, 1968). The cell therefore must sense the turgor pressure and respond by regulating K transport. The consequences of lacking such regulation are deleterious at high external K concentrations where the V_{\max} for K transport is higher than the cell's K needs. Over-accumulation of K would increase turgor pressure beyond that which can be withstood by the cell wall, thereby resulting in leakage of cell solutes and possibly cell rupture. Inasmuch as all four systems studied have maximum rates exceeding those needed for growth (approximately 6 $\mu\text{mol/g}$ per min for cells with a doubling time of 60 min and cell K of 500 $\mu\text{mol/g}$), all systems must be regulated by osmotic signals. This expectation has been confirmed; we find that all four systems show stimulation of K transport by osmotic upshock.

Response to upshock involves stimulation of influx with no significant change in efflux (Figs. 1C and 2C). Our results, for the TrkA and Kdp systems, are identical to those reported by Kepes et al. (1976) in a wild-type strain in which most K transport is due to the TrkA system. A reduction in turgor pressure alters the function of at least two of the K transport systems, perhaps all four, by stimulating influx. Once cell K rises to the new equilibrium dictated by the higher medium osmolarity, influx falls to the control value and net K uptake ceases (Kepes et al., 1976).

The regulatory responses of the high rate systems to K can be explained as additional controls restraining activity to a range that can be adequately handled by osmotic regulatory mechanisms. Inhibition of the TrkA system by intracellular K allows cells to replete K rapidly, yet automatically reduce influx as cell K pools approach the desired level. Inhibition of the Kdp system by external K makes sense for a wild-type strain when both the TrkA and Kdp systems are present, such as after a period of K limitation. The Kdp system is progressively inhibited over the concentration range where the TrkA system becomes saturated. The net effect is to keep total K transport by both systems within reasonable limits. The TrkF system has such a low rate that no additional restraints are needed. Instead, it is stimulated when cell K is high, a feature of many transport systems and referred to as trans-stimulation or exchange diffusion.

By what mechanism do cells translate variations in turgor pressure into variations in K transport? One possibility is suggested by our observation that both ATP and the protonmotive force are required for net K uptake by the TrkA system (Rhoads and Epstein, 1977), whereas K/K exchange requires only ATP (Table IV). The protonmotive force is the sum of the electrical potential difference and the pH difference across the cell membrane. The total protonmotive force cannot vary a great deal during growth because it is a major energetic intermediate in bacteria and is usually close to equilibrium with the energy in ATP (Harold, 1977). If the TrkA system were regulated by only the electrical component of the protonmotive force, and increases in turgor pressure resulted in a larger pH difference and a smaller electrical potential difference, the needed regulation of the TrkA system would result. This type of regulation cannot apply to the Kdp system which is not dependent on the

protonmotive force, and seems instead to be somewhat stimulated under some conditions where the protonmotive force is low (Rhoads and Epstein, 1977; Table IV above).

Another possible mechanism involves direct control by the turgor pressure via a direct mechanical effect on K transport systems. A change in transmembrane pressure could produce conformational changes enhancing influx at low turgor pressure and inhibiting influx at high values of the turgor pressure. The pressure-sensitive component could be a K-specific channel with which several of the K transport systems interact. Components specific to each system would determine kinetic parameters and energy coupling, but all would utilize the K-specific channel and be affected by its conformation.

Both the Kdp and TrkA systems require ATP to perform exchange. Does this mean that exchange is energy consuming? We do not believe so. We suggest that the ATP requirement is for formation of a high-energy intermediate, perhaps a phosphorylated protein, but that no stoichiometric consumption of energy occurs. The Na/Na exchange activity of the Na/K transport system of animal cell plasma membranes has a similar ATP requirement. Reversible formation of a phosphoenzyme is postulated as necessary for Na translocation, but there is no net consumption of energy associated with the exchange (Garrahan and Glynn, 1967; for review, see Epstein, 1975).

In conclusion, we would like to reinterpret two earlier findings. The first concerns our observation that in a strain in which both the TrkD and Kdp systems should have been present, the kinetics of K uptake were solely those of the Kdp system (Rhoads et al., 1976). Our interpretation at the time, that the Kdp and TrkD systems interact, is probably incorrect. We have more recently noted that the activity of the TrkD system is sensitive to inactivation during energy starvation and K limitation. Inactivation probably accounts for the variable rate of the TrkD system from one experiment to the next, noted above under Methods. In the earlier experiments it is likely that the combination of K limitation to derepress the Kdp system followed by energy starvation to deplete cell K inactivated the TrkD system so that its contribution to K transport was negligible.

The other finding that has been puzzling is that K/K exchange was found to be independent of external K concentration over the range from 0.06 to 13.6 mM (Epstein and Schultz, 1966). We have here shown (Figs. 1-4) that none of the K transport systems exhibits K/K exchange that is constant with external K over this range. The older observations can be explained as a combination of two effects. First, the cells used were grown in low-K media which would have partially derepressed the Kdp system. Second, at higher K concentrations progressive inhibition of Kdp-mediated exchange probably compensated almost exactly for the increasing rate of K/K exchange mediated by the TrkA system. What we in fact observed was the ability of *E. coli* to use partial derepression of the Kdp system and its inhibition by external K to maintain constant K/K exchange rates over a wide range of external K concentrations.

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