Cation Transport in *Escherichia coli*

VII. *Potassium requirement for phosphate uptake*

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ABSTRACT When *Escherichia coli* K-12 is grown in media containing limiting amounts of K, growth continues normally until all the extracellular K has been consumed. Thereafter the rates of growth, glucose consumption, and oxygen consumption decrease progressively, and the cell contents of K and P fall. These changes, referred to as K limitation, are all reversed by the addition of K. By specifically altering the ionic composition of the cells it was shown that these metabolic disturbances are not due to changes in the cell content of K or Na, but are directly related to the absence of K from the extracellular medium. The cell pool of inorganic P and the uptake of PO_4 from the medium are low in K-limited cells and are immediately stimulated by the addition of K, suggesting that the primary effect of K limitation is to inhibit PO_4 uptake. All the metabolic effects of K limitation can be attributed to inhibition of PO_4 uptake. The requirement of extracellular K for PO_4 uptake may be due to a coupling between the uptake of K and PO_4.

The requirement of microorganisms for K has been related to the role of this cation in several metabolic processes. K activates numerous cell enzymes including those engaged in protein synthesis (1, 2) and acts as an osmotic solute for the cell (3). When *Escherichia coli* is grown in media in which K is the limiting component, growth ceases some hours after the exhaustion of medium K even though cell K content is still quite high. Such cells, referred to as "K-limited," show marked changes in their composition and metabolism. These changes cannot be interpreted in terms of known functions of K and thus are evidence for an additional role for K in cell metabolism. In this paper the alterations of the K-limited state will be described and evidence linking these alterations to interference with PO_4 uptake will be presented. A preliminary report of this work has appeared (4).

METHODS

Media

Escherichia coli, strain K-12, was used throughout these investigations. The methods of storage, inoculation, and growth have previously been described (3). Two media

were employed: a PO_4 medium containing Na_2HPO_4, 56 mM; NaH_2PO_4, 14 mM; NH_4Cl, 10 mM; Na_2SO_4, 1 mM; and $MgSO_4$, 0.4 mM; and, a Tris–maleic acid medium (TMA)[1] (suggested by Dr. Elmer Pfefferkorn) containing Tris, 100 mM; maleic acid, 100 mM; NaOH to a final pH of 7.4 (approximately 105 mM); Na_2HPO_4, 0.5 to 2.0 mM; and NH_4Cl, Na_2SO_4, and $MgSO_4$ as in the PO_4 medium. A few measurements of $^{32}PO_4$ uptake and of cell PO_4 and ATP pools were made in a Tris medium in which 100 mM Tris adjusted to pH 7.4 with HCl replaced the Tris, maleic acid, and NaOH of the TMA medium. Since growth in this medium is not exponential after a cytocrit of 0.07% is reached it was not employed in the later phases of this investigation. KCl was added to each medium to obtain the desired [K]. Glycerol, 110 mM, was used as the carbon source except for experiments in the PO_4 medium, in which glucose, 55 mM, or lactate (as the Na salt), 50 mM, was also used. In experiments in the PO_4 medium, the carbon source and the medium were autoclaved separately and then mixed aseptically. All concentrations are those of the final media.

The pH of the bacterial suspension was usually between 6.7 and 7.1 and in all cases was between 6.5 and 7.1. All experiments were carried out at 37°C.

Analytic Techniques

Methods used for the determination of optical density, bacterial cytocrits, cell water content, pellet density, cell cation concentrations, total cell P, and pH have been described previously (3, 5, 6). The ratio of intracellular to extracellular [K] in K-limited cultures was determined by growing the bacteria in ^{42}K-labeled medium, filtering a sample of the culture through a membrane filter (Millipore, 0.45 μ pore size), and counting both the filtrate and the filtered cells. Cell and extracellular K concentrations were calculated using the specific activity of ^{42}K determined from samples of the total suspension.

Oxygen consumption was determined in a closed chamber using a Clark electrode (7). The electrode was calibrated in buffer solutions equilibrated with 100% N_2, 9.9% O_2–90.1% N_2, and air, and the output was recorded directly on a Varian G-11 strip chart recorder.

Glucose uptake was determined from the disappearance of glucose from the medium. Samples of the suspension were filtered through a membrane filter and glucose in the filtrate was analyzed by a glucose oxidase assay (Glucostat, Worthington Corp., Columbus, Ohio).

PO_4 Analyses

Total cell P content near the time of K limitation was determined in medium containing $^{32}PO_4$. Cells in 1 ml samples of the culture were collected on membrane filters and washed three times with two drops of 0.5 M sucrose. The filters were glued to aluminum planchets, dried, and counted in an automatic flow counter to a statistical error of 1%.

[1] Abbreviations used are: TMA, Tris–maleic acid medium as described in the text; cytocrit, volume fraction of bacteria in the suspensions; [K] and [Na], concentration of K and Na; 7'P, 7 min hydrolyzable P.

PO_4 uptake was measured by adding $^{32}PO_4$ to an unlabeled culture, collecting samples, and counting as described above. The uptake of isotope is a valid measure of net uptake only if the rate of PO_4 efflux is small compared to the rate of influx. The magnitude of the PO_4 efflux can be ascertained by comparing the increase of PO_4 determined chemically with that determined by the uptake of $^{32}PO_4$; the difference between these two measurements is the efflux. In growing cells the rate of P uptake is 11.7 ± 0.8 μmoles/g-min calculated from the P content of the cells and their doubling time; the rate calculated from $^{32}PO_4$ uptake in three experiments was 11.2 ± 0.3 μmoles/g-min. 3 hr after K limitation, P uptake is 1.0 ± 0.2 μmole/g-min (4 experiments) based on chemical determinations and 1.3 ± 0.2 μmoles/g-min based on $^{32}PO_4$ uptake (12 experiments). The good agreement between the two measures of P uptake indicates that efflux is small and that the use of $^{32}PO_4$ to measure net P uptake is valid. Similar findings have been reported for a Gram-positive bacterium (8, 9) and a yeast (10).

Cell P_i and 7′P were determined by collecting 10 ml samples of suspensions on a membrane filter, washing twice with 0.5 M sucrose, and extracting with ice cold 1 N HCl. The filters were scraped, the suspension centrifuged at 4°C, and the supernatant removed for PO_4 determination. Part of the supernatant was heated for 7 min at 100°C; 7′P was calculated as the difference between the PO_4 content of this hydrolyzed sample and the PO_4 content of the unheated supernatant. PO_4 was determined by the method of Fiske and Subbarow (11) using the isobutanol extraction modification of Berenblum and Chain (12). In some of the early experiments 1 N $HClO_4$ or 5% trichloroacetic acid was used in place of 1 N HCl.

In later experiments assays of cell PO_4 pools were performed on cells grown in medium labeled with $^{32}PO_4$. When PO_4 had to be added in the course of an experiment, as in experiments with resuspended cells, the added PO_4 was always of the same specific activity as that of the culture in which the cells were grown. The PO_4 analyses were carried to the point of extraction with isobutanol; portions of the isobutanol layer were dried on planchets and then counted in an automatic flow counter to a statistical error of 1%.

K-limited cells in K-free, PO_4-free medium were obtained by growing cells in TMA medium containing 1 mM PO_4 to a K-limited stationary phase. The cells were collected by centrifugation at room temperature, the container rinsed, and then the pellet resuspended in a volume of K-free, PO_4-free medium equal to the original volume of the suspension. The resuspended bacteria were allowed to equilibrate for 1 hr at 37°C with aeration before an experiment was begun. Such cells showed considerably lower levels of the pools of P_i and 7′P as well as less scatter of the results from one experiment to the next when compared with unresuspended cells. At the end of the equilibration period, the extracellular PO_4 concentration, determined by both chemical and radioactive tracer techniques, was less than 6 μM.

Cell ATP was determined with the firefly lucerifin-luciferase system (Sigma) (13) on Tris-neutralized samples collected and extracted with 1 N HCl as for PO_4 determinations.

Cell composition is expressed in terms of moles per gram dry weight or per liter cell water.

RESULTS

Limitation

During the growth phase of the culture prior to K limitation the optical density, bacterial cytocrit, and total cell K per milliliter suspension all increase exponentially at the same rate (Fig. 1). Since the cytocrit and total cell K per

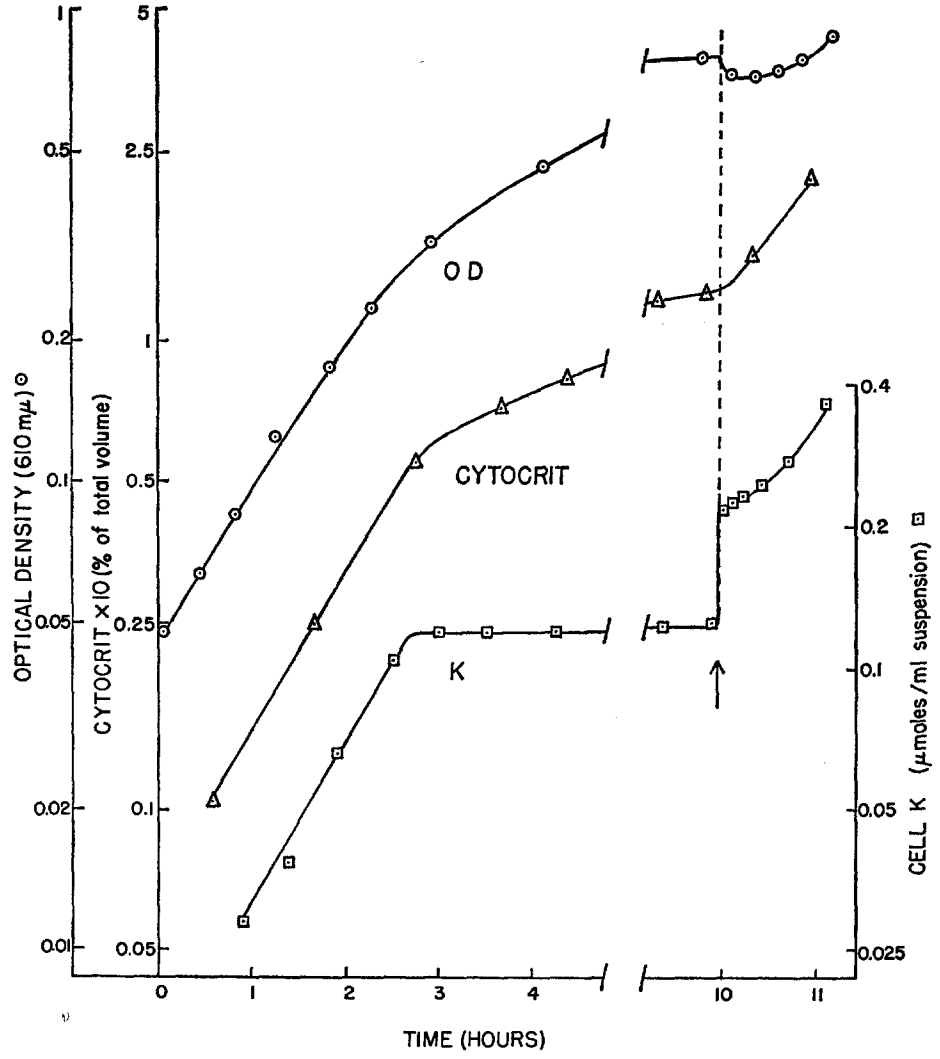

FIGURE 1. Growth and K content of *Escherichia coli* before, during, and after K limitation. The bacteria were grown in PO_4-glucose medium containing 0.09 mM added KCl. The inflection of the cell K curve near 3 hr is due to exhaustion of K in the medium. At the arrow KCl, to a final concentration of 1 mM, was added.

milliliter suspension increase at the same rate, the cell [K] remains constant during this phase. The growth rate of *E. coli*, strain K-12, is independent of the medium [K] provided that extracellular K is not exhausted. This is in contrast to the situation in *E. coli*, strain B (14, 15), and *Aerobacter aerogenes* (16), in which the growth rate is diminished at low extracellular [K].

After exhaustion of extracellular K the growth rate decreases progressively over a period of several hours (Fig. 1). For the first hour or two the rate is approximately linear and may correspond to the linear growth of *E. coli* B in K-free medium reported by Roberts and Roberts (17). By 4 hr after K limita-

TABLE I

COMPOSITION OF GROWING AND K-LIMITED *ESCHERICHIA COLI*

Medium	State	Doubling time	Cell water	[K]	[Na]	[K] + [Na]	K + Na	P
		min	*g/g*	*mM*	*mM*	*mM*	*μmoles/g*	*μmoles/g*
PO_4	Growing	55±1 (10)	2.74±0.02 (6)	255±5 (6)	82±5 (6)	337±7 (6)	925±20 (6)	940±30 (4)
	K-limited		1.98±0.03 (12)	181±9 (12)	169±10 (12)	350±13 (12)	695±30 (12)	710±10 (4)
	Resumed growth*	105±7 (6)	2.50±0.08 (6)	250±4 (7)	83±1 (7)	333±4 (7)	830±30 (6)	x
TMA	Growing	64±4 (7)	x	285±3 (7)	x	x	x	950±10 (2)
	K-limited		2.02±0.01 (6)	175±7 (8)	x	x	x	710±10 (10)
	Resumed growth*	141±16 (3)	x	272±10 (8)	x	x	x	750±5 (5)

P determinations in both media and cation determinations in PO_4 medium were performed on bacterial pellets; cell K analyses in TMA medium were performed on samples collected on filters. Errors are standard errors, number of determinations is indicated in parentheses.

x, not measured.

* Resumed growth values are determined 30 min after the addition of K.

tion the growth rate has fallen markedly to a 6% increase in cytocrit per hour, and by 8 hr growth is a negligible 2% increase per hour.

The composition of cells which have been K-limited for 4 to 8 hr is compared with that of growing cells in Table I. During this interval cell content of water, K, Na, and P remains nearly constant. Several features should be noted:

1. Cell [K] is decreased in the K-limited state as a result of the increase in total cell volume while total K content remains constant (Fig. 1). In the high PO_4 medium, cell [Na] rises as the [K] falls so that the sum of cell [K] and [Na] remains essentially constant (Fig. 2).
2. There is a decrease in cell water content during K limitation. Since the

sum of cell [K] and [Na] remains essentially constant, the total cation content per gram decreases as shown in column 8.

3. K limitation is associated with a decrease in cell P content.

The K-limited cell is capable of maintaining a concentration ratio for K that is extremely large. In 10 determinations in two different K-limited cultures,

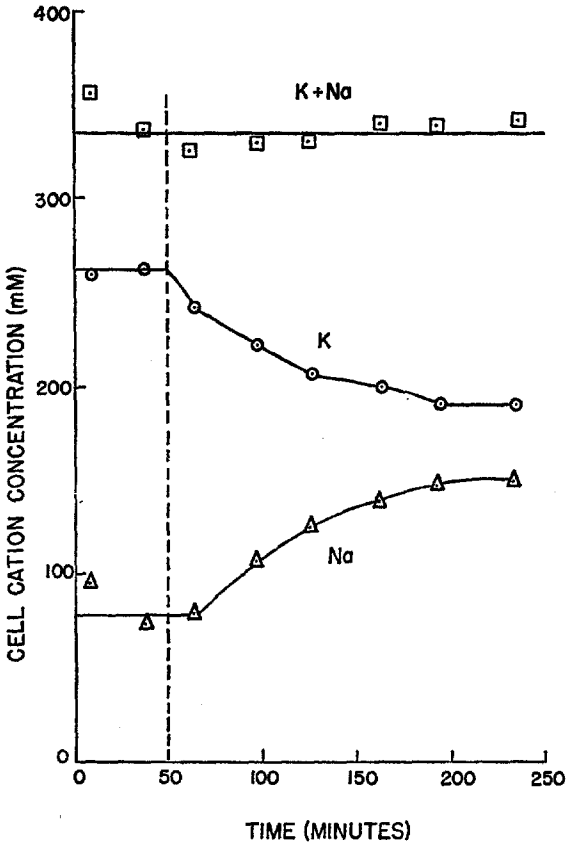

FIGURE 2. Cell [K] and [Na] in growth and in K limitation. The dashed line indicates the time of onset of K limitation. PO_4-glucose medium containing 0.09 mM added KCl was used. Each point is the average of duplicate analyses.

medium [K] ranged from 0.07 to 1.1 μM, resulting in ratios of cell to medium [K] ranging from 0.2×10^6 to 2.6×10^6, with a mean of $(1.1 \pm 0.3) \times 10^6$. Since contamination of the filtrate with relatively few cells would result in erroneously high values for medium [K], the higher ratios should be more reliable than the lower ones. Hence, the mean is probably a minimum estimate of the concentration ratio of K that these bacteria can maintain. In the light of this large concentration ratio, it is of some interest to note that the minimum energy required to transport a mole of K against a concentra-

tion ratio of 10^6 and an electrical potential difference of −29 mv (18), is of the order of 8000 cal, approximately the amount of energy available from the hydrolysis of one mole of ATP (19).

Reversal of K limitation

The addition of excess K to cells that have been K-limited for 4 to 8 hr results in a rapid reversal of the cation alterations and a prompt resumption of growth. The cell K concentration rises within 1 min to the level found in growing cells. This response is unaffected by chloramphenicol (50 μg/ml, added 60 min before K) and occurs in both the phosphate and TMA media with either glucose, glycerol, or lactate as carbon source. In the PO_4 medium, the uptake of K is closely paralleled by a loss of cell Na, the principal medium cation, when both are expressed in terms of cell concentrations (Table I). Both the cell [K] and [Na] then remain essentially constant and equal to the levels found in growing cells. The growth rate of K-limited cultures to which K has been added is approximately half the original growth rate under these conditions. However, if the period of K limitation is reduced to 3–4 hr at 30°C, growth at the original rate resumes immediately after the addition of K (20).

The characteristics of the K-limited state and its reversal are similar under anaerobic conditions. Growth after the exhaustion of extracellular K is, however, slightly less inhibited in anaerobic cultures than it is in aerobic cultures, suggesting that aerobic conditions place additional stress upon the K requirement of these bacteria.

Glucose Uptake and Oxygen Consumption

K limitation is associated with decreases in the rates of glucose uptake and oxygen consumption by the cells. These phenomena are most readily studied by examining the effects of addition of K to cultures which have been K-limited for 4–8 hr. Within this interval, the metabolic stimulation of K addition is not affected by the duration of K limitation. The rate of glucose uptake by aerobic K-limited cells is increased threefold by the addition of K, as shown in Table II. The rates after the addition of K have been corrected for the increase in bacterial mass of about 10% which occurs during the determination of glucose uptake. Similar results are obtained in the presence of chloramphenicol (50 μg/ml, added 60 min before K) indicating that the increased rate of glucose uptake is not dependent upon the resumption of protein synthesis.

The rate of glucose uptake under aerobic conditions in the presence of extracellular K is 77% of the anaerobic rate. However, during K limitation, glucose uptake in the aerobic state is only 34% of the rate under anaerobiosis (Table II). Thus, the inhibitory effect of respiration on glucose uptake, which is only

TABLE II
GLUCOSE UPTAKE

Medium	Aerobic		Anaerobic	
	K-limited	After addition of K	K-limited	After addition of K
	μmoles/g-min			
PO_4*	60±5(7)‡	180±5(5)	175±10(3)	235±10(2)
PO_4 + chloramphenicol	60±5(3)	155±5(2)	120(1)	—
TMA	50±5(2)	150±5(2)	—	—

* Rate of glucose uptake during aerobic growth is 190 ± 5 μmoles/g-min.
‡ All values are expressed to the nearest 5 μmoles/g − min. Errors are the standard error of three or more determinations, average error of two determinations. Number of determinations is indicated in parentheses.

slight when the cells are suspended in a high PO_4 medium containing K, is augmented by K limitation.

The rapidity of the response of K-limited cells to K addition is best demonstrated by the oxygen consumption. As illustrated in Fig. 3, an increase in oxygen comsumption could be demonstrated as early as 5 sec after the addition of K, a time interval comparable to the lag due to mixing and electrode response. Within 45 sec the oxygen consumption reaches a constant rate which is ap-

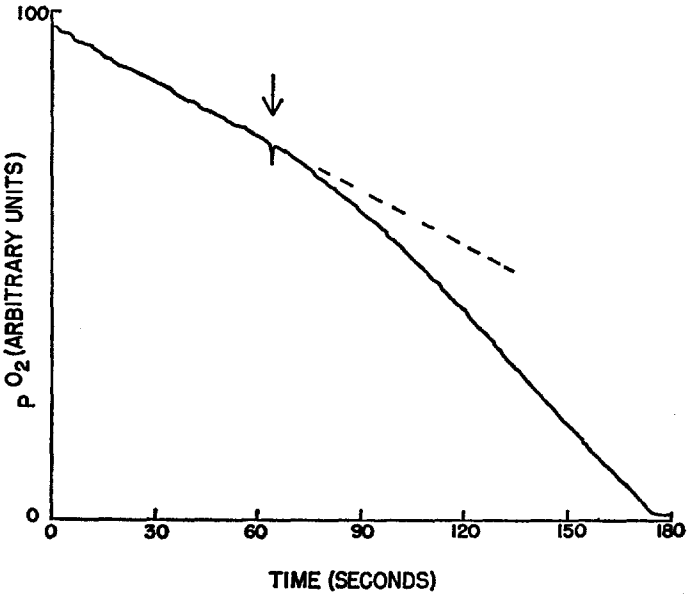

FIGURE 3. Effect of K on the oxygen consumption of K-limited cells. Cells were grown in PO_4-glucose medium containing 0.09 mM added KCl. At the arrow KCl, to a final concentration of 1 mM, was added. The curve is drawn from a polarographic tracing.

proximately twice that before the addition of K. This effect was seen in both the PO_4 and TMA media, and with the variety of carbon sources listed in Table III.

Roles of Cell K and Na in K Limitation

Since K limitation is accompanied by a decline in cell K and, in the PO_4 medium, an increase in cell Na, one might attribute the inhibition of growth and metabolism to these changes in cell ion composition. To examine this possibility, K limitation was carried out in three media which differed with respect to osmolality and Na concentration. We have previously demonstrated that cell [Na] is a function of extracellular [Na] over a wide range, and that cell

TABLE III
OXYGEN CONSUMPTION

Medium	Substrate	Before K	After K
		μmoles/g-min	
PO_4	Glucose (5)*	115±10‡	215±15
	Glucose + chloramphenicol (2)	140±10	270±10
	Glycerol (3)	90±5	175±5
	Lactate (3)	180±5	260±10
TMA	Glucose (2)	125±10	280±5
	Glycerol (3)	135±10	295±15

* Number of determinations.
‡ Values have been rounded off to the nearest 5 μmoles/g-min. Errors are standard errors when three or more determinations were made and average errors when two determinations were made.

[K] increases with increasing osmolality of the growth medium (3). Thus, use of these media permitted us to vary cell [Na] and [K] independently. The results of these experiments are given in Table IV. In each instance, K limitation was accompanied by a decrease in the rates of growth and oxygen consumption. However, cells which were K-limited in the hypertonic medium had higher cell [K] than cells growing normally in the standard media (Table I). Further, cells K-limited in the standard medium always had cell [K] greater than 160 mM, while in sufficiently hypotonic media cells grow exponentially with a cell [K] of 150 mM (Table I of reference 3). Cells which were K-limited in low Na medium had lower cell [Na] than cells growing normally in the standard or high Na media.

Thus, the metabolic consequences of K limitation cannot be attributed to a fall in cell [K] below a critical value necessary for sustaining normal processes, nor to an inhibitory effect of an absolute increase in cell [Na]; the metabolic alterations appear to be independent of the absolute values of cell [Na] and

[K]. Further, the sum of [K] and [Na] in cells K-limited in the PO_4 medium does not differ significantly from that of cells growing normally in this medium. Assuming that the osmotic activity of cell Na is approximately equal to that of K, these results suggest that the effects of K limitation cannot be attributed to alterations in the intracellular osmotic activity or ionic strength. These observations clearly suggest that changes in intracellular [K] and/or [Na] are not responsible for the effects of K limitation and that the absence of extracellular K per se must be implicated.

TABLE IV

K LIMITATION IN DIFFERENT MEDIA

Medium			Cells			
			K limitation		Resumed growth*	
Type‡	Effective osmolality§	[Na]	[K]	[Na]	[K]	[Na]
	milliosmols	*mM*	*mM*	*mM*	*mM*	*mM*
Hypertonic	730	164	305	207	415	117
High Na	510	255	170	217	247	142
Low Na	225	12	148	13	202	14

Each value is the average of duplicate determinations.

* Resumed growth values are determined 30 min after the addition of K.

‡ Hypertonic medium is PO_4 medium, glucose as substrate, and 0.4 M sucrose. High Na medium is PO_4 medium and 100 mM Na lactate as substrate. Low Na medium is TMA medium from which maleic acid and NaOH are omitted, and the pH of the medium is brought to pH 7.4 with HCl; substrate is glycerol.

§ Effective osmolality is the osmolality of the medium calculated from the concentrations of those constituents which do not readily permeate the bacterial membrane.

K Limitation and PO_4 Uptake

The results of a typical experiment in which cell K and P were measured in cells growing in low K medium are shown in Fig. 4. Within 5 min of the time that extracellular K is depleted, the curve of cell P content inflects sharply and the rate of net PO_4 uptake falls to a very low value. This inhibition of PO_4 uptake occurs at a time when the growth rate has fallen by less than 15% and before there is a substantial fall in cell [K].

To study the interaction between K limitation and PO_4 uptake more closely we turned to the reversal of K limitation. If inhibition of PO_4 uptake is the mechanism whereby K limitation interferes with growth and metabolism, stimulation of PO_4 uptake should be an early event in the reversal of K limitation by the addition of K. Studying reversal has the added advantage of convenience in that the time of reversal can be chosen, while the time of K limitation in an experiment such as that of Fig. 4 can be determined with certainty only after completion of the experiment. The effect of reversal of K limitation

on PO_4 uptake is shown in Fig. 5. Within a few seconds after the addition of K there is a very rapid uptake of PO_4 for 1–2 min followed by an approach to a constant rate of PO_4 uptake. In 12 experiments the average rate of PO_4 uptake of 1.3 ± 0.2 μmoles/g-min rose to 6.5 ± 0.5 μmoles/g-min after the addition of

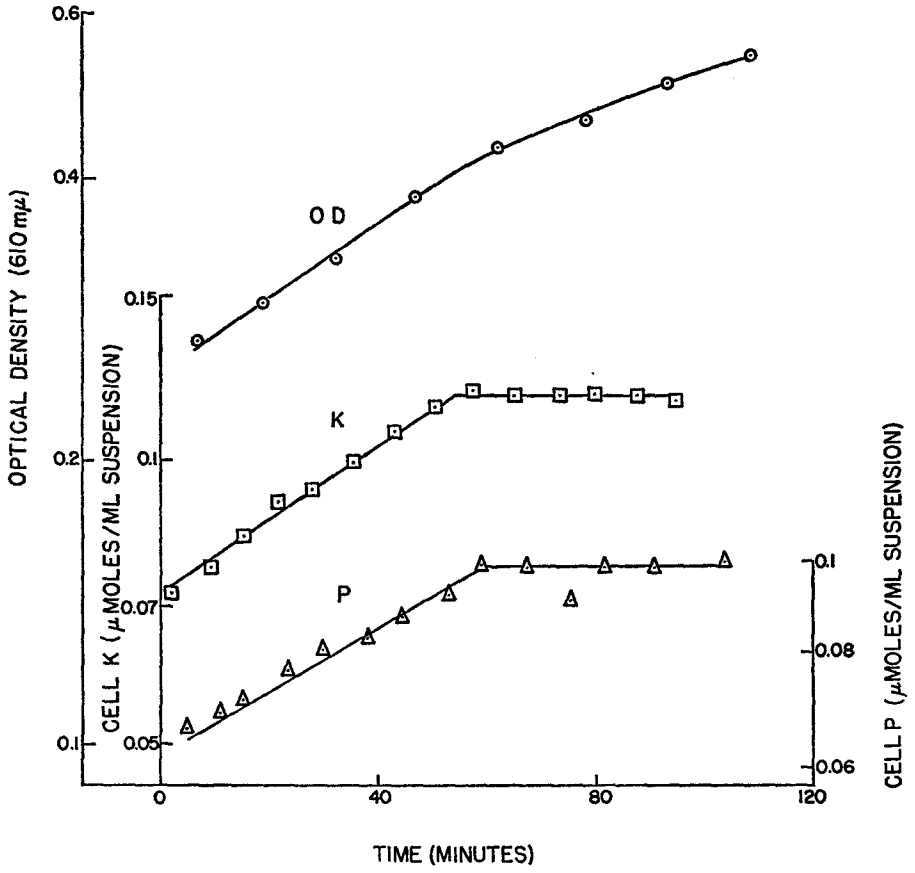

FIGURE 4. Cell P and K during onset of K limitation. The inflection in the cell K curve near 60 min is due to exhaustion of extracellular K. Cells were grown in TMA-glycerol medium with 0.1 mM added KCl.

K. The average amount of PO_4 taken up in the 1.5 min after the addition of K was 30 ± 2 μmoles/g.

The rate of PO_4 uptake in growing cells after the reversal of K limitation is only 6.5 μmoles/g-min, while the rate in growing cells before K limitation is 11.7 μmoles/g-min. This difference indicates that the addition of K does not fully reverse the K-limited state. These reversed cells also have a slower growth rate than do cells prior to K limitation (doubling time of 141 ± 16 as against 64 ± 4 min) and their total P content as long as 1½ hr after reversal (750 ±

5 μmoles/g) is lower than that found in growing cells prior to K limitation (950 $\pm$ 10 μmoles/g). We presume that this incomplete return to normal growth conditions is a result of the long periods (4–8 hr) of K limitation

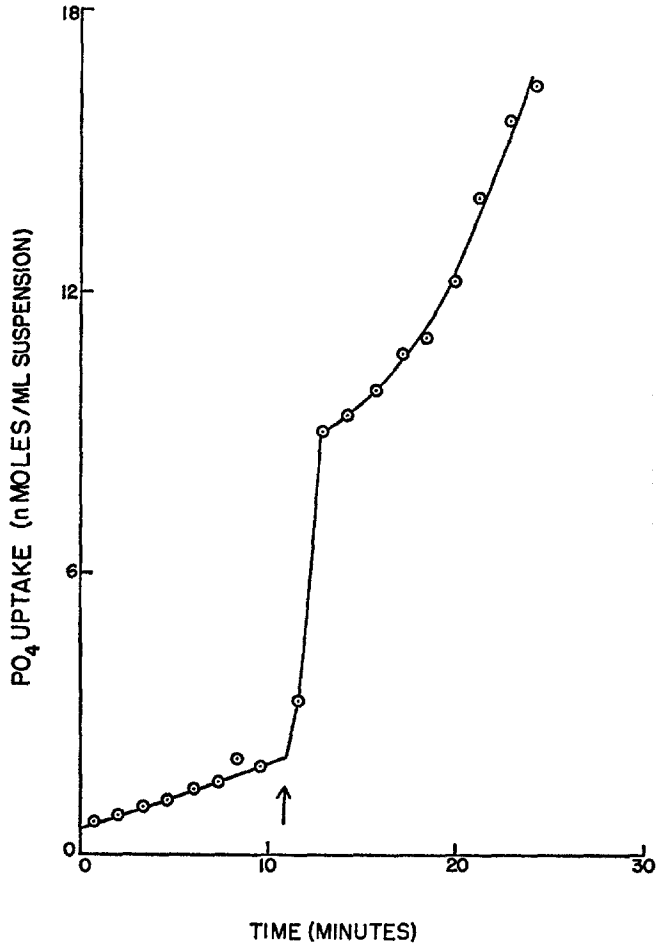

FIGURE 5. Effect of K on $^{32}PO_4$ uptake. Cells were K-limited by growing them in TMA-glycerol medium with 0.1 mM added KCl and 1.0 mM unlabeled PO_4. At time 0, $^{32}PO_4$ was added. At the arrow KCl, to a final concentration of 1 mM, was added. The values plotted on the ordinate are calculated using the specific activity of $^{32}PO_4$ in the medium.

during which irreversible or only slowly reversible changes may have occurred in a sizable fraction of the cells. Shorter periods of K limitation are followed by a rapid return to a normal rate of growth (20) and presumably to normal P content and rates of P uptake. But since short periods of K limitation lead to less pronounced alterations in cell composition, and thus smaller changes on reversal, we have used cells that have been K-limited for rather long periods even though the reversal is, in terms of growth and P uptake, incomplete.

Having demonstrated that K causes an immediate stimulation of PO_4 uptake in K-limited cells, we examined the effect of PO_4 on K uptake. After several hours of K limitation, cells were resuspended in K-free, PO_4-free me-

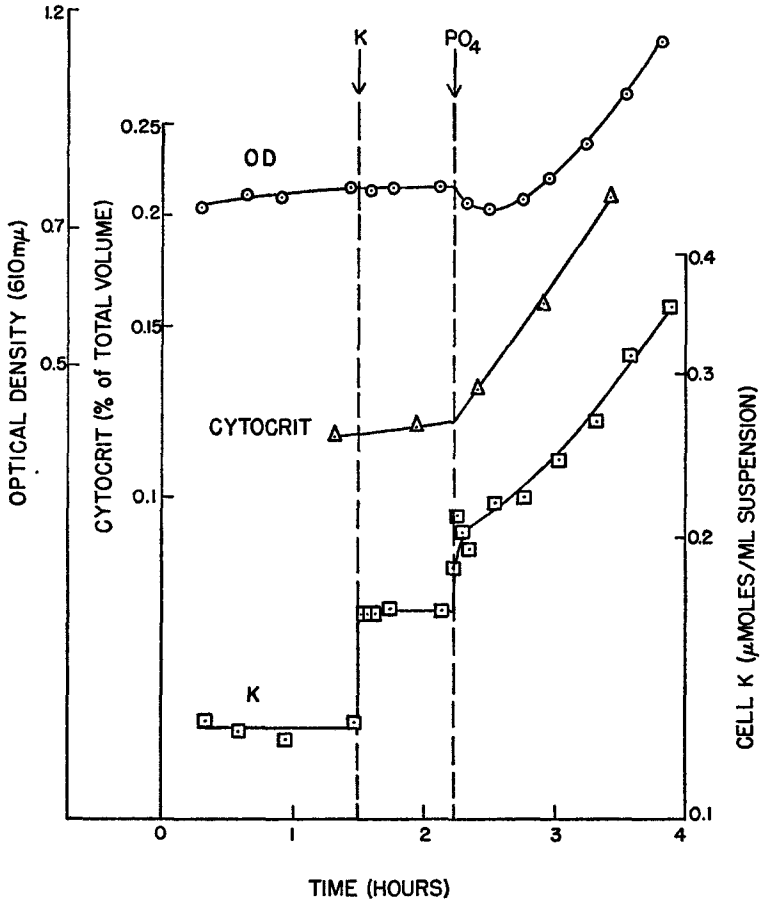

FIGURE 6. Effects of K and PO_4 on resuspended K-limited cells. Cells were grown in TMA-glycerol medium containing 0.1 mM added KCl. After several hours of K limitation, cells were resuspended in K-free, PO_4-free medium and equilibrated for 1 hr with aeration. At the first arrow KCl, to a final concentration of 1 mM, was added. At the second arrow PO_4, to a final concentration of 1 mM, was added.

dium. As shown in Fig. 6, the addition of K results in a rapid uptake of K. In nine such experiments the average uptake was 120 ± 10 μmoles/g, raising the cell [K] from 183 ± 5 to 245 ± 7 mM. At the same time oxygen consumption increased for several minutes and then fell again to approach the base line rate in 10 or 15 min (Fig. 7). The subsequent addition of PO_4 resulted in the resumption of growth and in a sustained rise in oxygen consumption to a rate 2.1 times the initial value. In addition, there was a rapid uptake of 60 ±

5 μmoles/g (three experiments) of K within the 3 min following the addition of PO_4, resulting in an increase of cell [K] to 275 ± 5 mM. Thus, addition of K alone to cells in a K-free, PO_4-free medium results in a considerable uptake of K. However, the cells take up the additional amount of K necessary to return their [K] to the level found in growing cells only after PO_4 is added, and only then is there a sustained increase in oxygen consumption

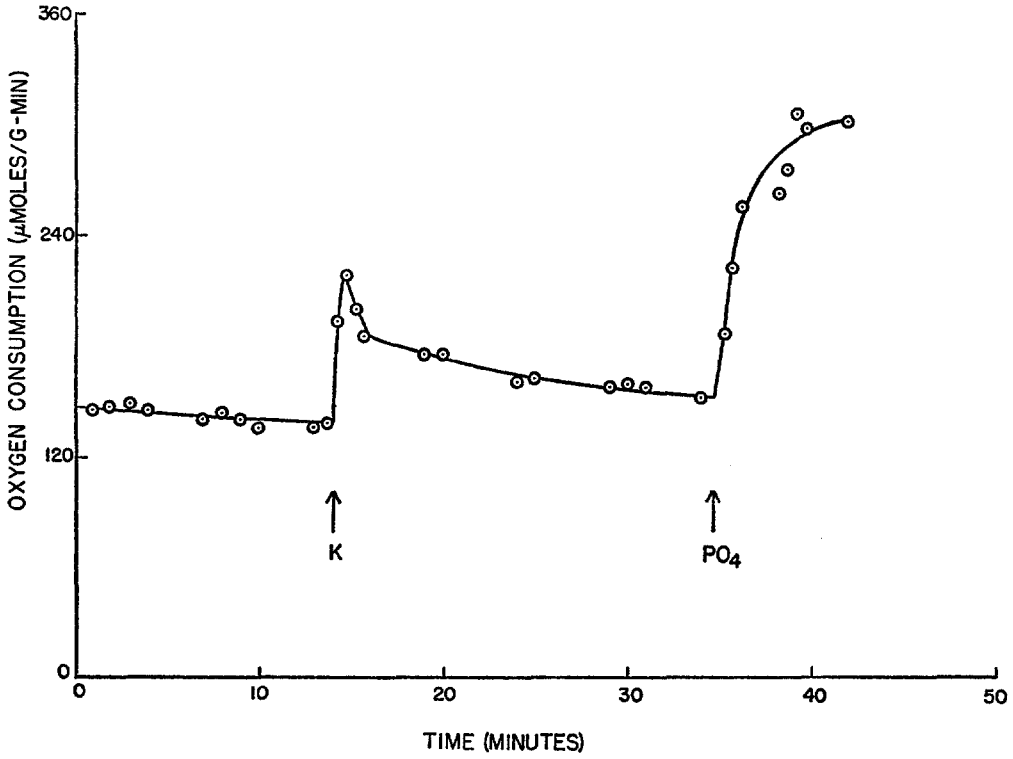

FIGURE 7. Effect of K and PO_4 on oxygen consumption of resuspended K-limited cells. Experimental details are as for Fig. 6.

PO_4 Pools

The above data suggest a direct effect of extracellular K on the uptake of PO_4, but do not permit localization of this effect to a specific stage of PO_4 metabolism. Thus K limitation could block some step in the utilization of PO_4 beyond the entry step, and the increased uptake seen after reversal could be secondary to the removal of this block. To investigate this possibility, the cell pools of P_i and 7'P were measured before and after reversal of K limitation. The first step in the utilization of medium PO_4 appears to be the transport of PO_4 across the membrane into the cell P_i pool (8). If a step after the transport of PO_4 were blocked by K limitation the cell pool of P_i should be elevated, and

should fall after the addition of K. On the other hand, if transport is inhibited, reversal should result in a rise of P_i. In 12 experiments P_i rose from 18 ± 1 μmoles/g during K limitation to 24 ± 1 μmoles/g after the addition of K ($P < 0.001$). 7′P, measured in eight of these experiments, was 28 ± 2 μmoles/g during K limitation and 27 ± 3 μmoles/g after the addition of K. These changes show that the reversal of K limitation is associated with a rise in the P_i pool, a finding consistent with inhibition of transport but not with inhibition of a later step in PO_4 utilization. The absence of a significant decrease in

TABLE V

EFFECT OF K AND PO_4 ON RESUSPENDED K-LIMITED CELLS

Conditions	P_i	7′P	K
		μmoles/g	
A. K added first			
Control, (K-free, PO_4-free)	4.0±0.5	13.9±0.3	355±10
After K addition	3.7±0.5	12.3±0.5	475±15
After PO_4 addition	23.3±0.6	30±1	530±10
B. PO_4 added first			
Control, (K-free, PO_4-free)	4.4±0.5	16.1±0.8	320±5
After PO_4 addition	11.5±0.2	25.3±0.7	335±10
After K addition	20.1±0.5	29.9±0.7	540±10

The additions indicated were carried out in sequence from top to bottom. Values of P_i and 7′P represent the averages of six to eight analyses carried out in two separate experiments. The analyses were made using $^{32}PO_4$-labeled cultures grown in TMA-glycerol medium. Both K and PO_4 were added to a final concentration of 1 mM. Values for K are from similar experiments carried out separately from those in which P_i and 7′P were measured. The results are final values measured 4 to 15 min after each addition. Errors are standard errors of the mean.

7′P rules out the possibility that the rise in P_i is due to breakdown of labile phosphorylated compounds.

Similar results were obtained in experiments in which K and PO_4 were sequentially added to K-limited cells which were resuspended in K-free, PO_4-free medium. These results, summarized in Table V, indicate that although the addition of either K or PO_4 alone results in partial repletion of intracellular K and P respectively, normal intracellular levels are achieved only when *both* K and PO_4 are present in the medium.

Requirement for Extracellular K

The requirement for both K and PO_4 in the reversal of K limitation has been demonstrated, and it has been shown that the uptake of each of these ions is incomplete in the absence of the other. Rothstein (21) has reported a somewhat similar situation in yeast in which only limited amounts of PO_4 can be

taken up without concomitant cation uptake. However, yeast cells preexposed to K can take up PO_4 at an enhanced rate in the absence of extracellular K, suggesting that a direct action of extracellular K on the PO_4 uptake process is not involved. The results presented in Table VI indicate that this is not the situation in K-limited *E. coli*. K was added to K-limited cells resuspended in K-free, PO_4-free medium. These cells, which had replenished approximately two-thirds of their cell K deficit, were then resuspended again in K-free, PO_4-free medium. While the addition of PO_4 does result in the uptake of some PO_4, the amount is not much greater than that taken up by cells which have not replenished the major portion of their K deficit. The subsequent addition of excess K results in a further rapid increase in both P_i and 7'P. These results, together with the data of Fig. 5, indicate that extracellular K must be present to allow K-limited cells to transport sufficient PO_4 to increase cell P_i and 7'P

TABLE VI

P_i AND 7'P POOLS IN K-PRELOADED CELLS

Conditions	P_i	7'P	K
		μmoles/g	
Control, (K-free, PO_4-free)	—	—	270±5
After K addition followed by resuspension in K-free, PO_4-free medium	2.5±0.1	13±2	425±10
After PO_4 addition	14±2	32±2	455±15
After K addition	25±2	39±3	610±10

Experimental details are as indicated for Table V, except that analyses of K content were carried out in the same experiments in which the PO_4 pools were measured.

pools to levels necessary for growth and normal metabolism; previous replenishment of most of the cell K is not sufficient.

ATP Pools

The 7'P pool consists of acid-labile PO_4 groups from several cell constituents including ATP (22). In view of the importance of ATP in cell metabolism, the effect of reversal of K limitation on ATP levels was studied. In contrast to what was found for K, P_i, and 7'P, there was no immediate increase in ATP content. Rather, ATP content began to increase only ½–4 min after the addition of K and reached a final plateau value some 8–20 min later (Table VII). The fact that measured ATP levels did not increase until some minutes after the rise in 7'P was essentially complete suggests that the low ATP levels in K limitation are not due solely to conversion to ADP. Instead it would appear that K limitation results in a relative decrease in cell ATP without greatly altering the ratio of ATP to ADP. The rise seen in Table VII may reflect synthesis of the nucleoside moiety, a process that would be expected to be much slower than either phosphorylation of ADP or uptake of P_i.

Metabolic Control

It was noted above that the addition of K to cells resuspended in K-free, PO_4-free medium resulted in a transient increase in oxygen consumption. Fig. 7 shows the time course of this effect in a typical experiment. Oxygen consumption rose rapidly, only to decline approximately 1 min after the addition of K. Within 12 min, oxygen consumption dropped to a rate 15–25% above the base line rate in four experiments and to the base line rate in three experiments. 100 ± 3 μmoles/g (seven experiments) of extra oxygen are utilized in the 1½ min after the addition of K; the amount of K taken up by the cells in the same

TABLE VII

CELL ATP IN K LIMITATION AND AFTER THE ADDITION OF K

Medium		ATP	
		K limitation	After K*
		μmoles/g	
TMA-glycerol	(3)‡	4.3±0.8	6.4±1.4§
Tris-Cl-glycerol	(3)	3.9±0.4	6.7±0.5
PO_4-glucose	(1)	3.6	6.2

Errors are standard errors of the mean.

* Values after K were measured 8 to 20 min after the addition of K when cell ATP had reached a stable plateau and are corrected for growth subsequent to the addition of K.

‡ The number in parentheses refers to the number of separate experiments carried out in that medium. In each experiment two to three determinations of ATP were carried out.

§ In each of these three experiments a significant rise in ATP occurred, ranging from 1.2 to 3.9 μmoles/g.

time interval is 120 ± 10 μmoles/g. Addition of PO_4 after K resulted in a somewhat slower rise in oxygen consumption, which reached its final value within 4 min and was sustained as growth resumed. Stimulation of oxygen consumption related to the active transport of beta-galactosides in *E. coli* (23) and the stimulation of glycolysis when K-poor, Na-rich *Streptococcus fecalis* take up K (24) have been reported. In both of these instances close agreement between calculated ATP production rates and substrate transport rates was demonstrated. The increase in oxygen consumption following the addition of K to cells suspended in K-free, PO_4-free medium may be another example of the stimulation of metabolism by a situation in which increased demands on the energy output of the cells are made.

CONCLUSIONS

The question posed by the initial observations in this study was: why are bacterial growth, glucose uptake, and oxygen consumption markedly in-

hibited by K limitation in spite of the fact that cell K concentration under these conditions is still quite high? The results of our studies indicate that the metabolic consequences of K limitation are not dependent on the levels of intracellular K and/or Na, but rather are due to the depletion of extracellular K per se. An alternative conclusion is that cell K is compartmentalized and that K limitation is the result of the depletion of an important intracellular K compartment. Such a compartment (for example the plasma membrane) would have to be subject to rapid depletion and repletion of K in response to changes in the extracellular medium. The present methods cannot distinguish between such a compartment and the extracellular medium itself.

A quite different effect of K starvation in *E. coli* has been studied by Lubin and Ennis (2). These workers found that protein synthesis is selectively inhibited when mutants of *E. coli* B are depleted of most of their cell K. The effect described by Lubin and Ennis differs from the effects of K limitation described in this paper in that the organisms we have studied never become markedly depleted of cell K. The lowest cell K concentration we have observed (160 mM) is still sufficient to allow protein synthesis to proceed at more than 90% of the normal rate, based on the data of Lubin and Ennis. Thus, although K appears to be required for protein synthesis, this is not the explanation for the growth inhibition observed when wild-type *E. coli* K-12 deplete the growth medium of K.

The inhibition of growth and metabolism of K-limited cells can be attributed to inhibition of PO_4 uptake. A decreased P_i pool should result in an inhibition of growth. There is abundant evidence implicating P_i in the regulation of glucose and oxygen consumption in a variety of nonbacterial systems (25, 26). Although similar evidence in bacteria is lacking, the stimulation of oxygen consumption by the addition of K to K-free, PO_4-free cells shown above, and the data of Kepes (23) and Zarlengo and Schultz (24) suggest that some metabolic regulation does exist in bacteria. If P_i plays a major role in the regulation of bacterial metabolism, the decreased oxygen and glucose consumption observed in K-limited cells would be an expected consequence of the decreased P_i pool in these cells.

Glucose and glycogen stores in *E. coli* are relatively low (27), so that sizable changes in glucose uptake over the 60 min periods used for the data of Table II must reflect similar changes in the rate at which glucose is consumed. The inhibition of glucose uptake by respiration may therefore be considered as an example of the Pasteur effect. The mechanism of the Pasteur effect is incompletely understood at present, but there is little doubt that P_i plays an important role in this regulatory process (28, 29). In *E. coli* a Pasteur effect is present in low PO_4 medium and is abolished by the addition of 60 mM PO_4 (30). The absence of a Pasteur effect in cells suspended in high PO_4 medium presumably reflects a high cell P_i pool; in a low PO_4 medium the cell P_i pool is

lower and a Pasteur effect is demonstrable. Our finding that K-limited cells show a sizable Pasteur effect in high PO_4 medium is consistent with a low cell P_i pool due to impaired PO_4 uptake.

Thus, the impaired PO_4 uptake in K-limited cells provides a *sufficient* explanation for all the changes observed in K limitation; no additional primary effects of K *need* be invoked. On the other hand, our data in no way exclude the possibility that depletion of extracellular K directly affects other vital cell processes in addition to PO_4 uptake.

The nature of the K requirement for PO_4 uptake is not clear. One possibility is that K is required at the outer surface of the cell membrane for a reaction necessary for PO_4 uptake. If such is the sole function of extracellular K one would not expect PO_4 uptake to have any effect on the uptake of K. But, since part of the K uptake by K-limited cells is PO_4-dependent (Fig. 6 and Table V), some coupling between the movements of these two ions is more likely. A stoichiometric relation between the rapid K-dependent PO_4 uptake and part of the K uptake is suggested by our data. In TMA medium the rapid component of the K-dependent PO_4 is 30 μmoles/g, while the rapid PO_4-dependent K uptake is 60 μmoles/g. If PO_4 is taken up with the same net charge that it carries in solution at pH 7, the PO_4 uptake amounts to 50 μeq/g. This approximates the charge carried by the PO_4-dependent K uptake, and suggests that the coupling of K and PO_4 uptake is a coupling of charge movement resulting in an electrically neutral net movement. On the other hand, the agreement between the charge transfer due to PO_4 uptake and that due to K uptake could be fortuitous. Further studies of the transport of K and PO_4 are necessary before the nature of their interaction can be clarified.

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