Cation Transport in *Escherichia coli*

III. Potassium fluxes in the steady-state

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**ABSTRACT** The present study is concerned with the measurement of the unidirectional K flux in *E. coli*. Methods are described by means of which a fairly dense suspension of cells may be maintained in a well defined steady-state with respect to the intracellular K concentration and the pH of the medium. The kinetics of K$^{+}$ exchange under these conditions are consistent with the presence of a single intracellular K compartment with a unidirectional K flux of 1 pmol/(cm$^2$ sec.). This rate is independent of the extracellular K concentration over the range studied. The simultaneous rate of H secretion averages 16 pmols/(cm$^2$ sec.) indicating that in the steady-state the efflux of metabolically produced H is not linked mole for mole to K movement.

Previous studies have shown that during the logarithmic phase of growth the intracellular K concentration in *E. coli* is much greater than that in the growth medium, and that K uptake by this organism requires a supply of metabolic energy and is almost certainly an active process taking place against an electrochemical potential gradient (1, 2). The present study is concerned with the measurement of the unidirectional K flux in cells harvested during the logarithmic phase of growth and maintained in a well defined steady-state with respect to the intracellular K concentration and the pH of the medium. In addition, the interrelationships of K flux, H secretion, and glucose uptake have been investigated.

**METHODS**

*Escherichia coli*, strain K-12, was used throughout these experiments. The methods of storage, growth, and harvesting of the organisms, as well as the methods used for determining intracellular cation concentrations and bacterial cytocrits have previously been described in detail (1). The growth and experimental media contained: NaH$_2$PO$_4$, 22 mM; Na$_2$HPO$_4$, 40 mM; (NH$_4$)$_2$SO$_4$, 8 mM; Na$_3$ citrate, 5 mM; MgSO$_4$, 10 mM; and glucose, 0.6M.
0.4 mM; and glucose, 55 mM. The desired extracellular K concentration was obtained by the addition of KCl.

Cells were harvested in the logarithmic phase of growth after overnight cultivation in either a 2.5 or a 5 mM K medium. The cells were then resuspended in the chamber shown in Fig. 1 in 50 to 70 ml of prewarmed medium containing 20 μg/ml chloramphenicol. The chamber and resuspension medium were preequilibrated at 37°C for at least 1 hour prior to resuspension of the organisms, and the experiments were carried out in a warm room at that temperature. The cell suspension was stirred with a magnetic stirrer.

Preliminary studies had shown that a 2 per cent by volume suspension of E. coli would rapidly acidify the suspension medium with the pH falling from 7.0 to 5.5 in 30 minutes. To prevent this pH shift, the pH of the suspension was continuously monitored with a Radiometer (model 22) pH meter whose output was connected to a pH-stat constructed in this laboratory. The latter device consists of an on-off relay which activates a magnetic valve. When the output of the pH meter falls below a preset value, the valve opens and allows alkali to flow from a calibrated buret into
the suspension. In this way the pH of the suspension was automatically maintained between 6.95 and 7.05 for prolonged periods. The alkali input was immediately adjacent to the magnetic stirring bar, assuring rapid mixing, and the volume of the periodic additions was determined to 0.01 ml. By employing concentrated alkali solutions the total volume added during a 35 minute period was limited to 3 to 5 per cent of the initial volume of the suspension. The calomel electrode used in the pH-stat was a fiber plug type selected so that the diffusion of KCl during the course of the experiment was negligible.

When 1 M NaOH was used in the pH-stat, the cells were resuspended in medium which was diluted by 20 per cent so that the initial extracellular Na concentration was 92 mM. This was done to prevent an inordinately high final extracellular Na concentration due to the gradual addition of 35 to 50 mmols/(1 suspension) NaOH. In order to rule out the possibility that the K flux was influenced by this increase in the extracellular Na concentration, several experiments were performed using 1 M tris (tris(hydroxymethyl)aminomethane, Sigma) as the alkali, with the extracellular Na remaining constant at 115 mM. The K flux under these conditions did not differ significantly from that observed when NaOH was used as the neutralizing agent.

Following resuspension, the organisms were equilibrated in the chamber for a period of 12 to 15 minutes. During this period the rate at which alkali was added to the suspension was recorded. Control studies have shown that the rates of glucose uptake and H secretion by the cells are constant over the experimental period from 0.5 minute after resuspension. Thus, the rate of H secretion may be calculated from a knowledge of the total volume of the suspension, the bacterial cytocrit, and the rate of alkali addition during this 12 to 15 minute period. At the end of this period an aliquot of suspension was removed and rapidly cooled to 4°C for duplicate determinations of the initial cytocrit and intracellular cation concentrations. Using the methods which have been described previously (1) duplicate cytocrits agreed to within 1 per cent and duplicate determinations of the intracellular K concentration agreed to within 3 per cent. A tracer quantity of K42 was then rapidly injected into the suspension. Control studies have shown that complete mixing is achieved by 15 seconds after the addition of the tracer. Sampling of the suspension was begun at 30 seconds, and repeated periodically during the 30 minute experiment. The sampling procedure was as follows. A 1 to 2 ml aliquot of the suspension was withdrawn into the syringe (Fig. 1) and then, by means of a three way valve, extruded through a Swinney filter apparatus containing a glass fiber prefiler and a 0.8 μ pore size membrane filter (Millipore, Bedford, Massachusetts). The clear filtrate was collected in the glass chamber above, and then transferred to test tubes. The time of sampling is taken as the moment when filtrate is first observed to enter the glass collecting chamber. Using this method, samples of supernatant solution may be obtained at 20 to 30 second intervals. At the completion of the experiment, an aliquot of the suspension was rapidly cooled to 4°C for duplicate determinations of the final cytocrit and intracellular cation concentrations.

0.1 to 0.25 ml aliquots of the sample filtrates were assayed for K42 in a well-type crystal scintillation counter (Nuclear Chicago, model 182). The counted aliquots were then appropriately diluted and the K concentration was determined by flame
photometry using either the flame photometer of Solomon and Caton (3) or the Perkin-Elmer model 52-A. In this way the specific activity of K\textsuperscript{42}, \( \rho_2^* \), in each filtrate was determined directly, thus eliminating the necessity for correcting for the dilution of the suspension due to the periodic addition of alkali.

The specific activity of K\textsuperscript{42} in the total suspension, which represents the equilibrium specific activity, \( \rho_*^* \), of the system, was obtained by assaying an aliquot of the total suspension for K\textsuperscript{42} in the well-type scintillation counter, and then digesting with redistilled concentrated HNO\textsubscript{3} by heating to dryness in an oven at 105°C. The dried residue was dissolved in distilled water and the K content of the aliquot was determined by flame photometry.

In three experiments, glucose concentrations of the filtrate were determined using the glucose oxidase method (glucostat, Worthington Biochemicals) (4).

**KINETICS**

Assuming that the intracellular water and the suspending medium behave as two well mixed compartments, and that K\textsuperscript{42} is an ideal tracer for potassium, the following equation may be used to describe the exchange of K across the bacterial cell membrane:

\[
d\frac{P_2}{dt} = nA (\Phi_{12} \rho_1^* - \Phi_{21} \rho_2^*)
\]

where the subscripts 1 and 2 represent the intracellular and extracellular compartments respectively; \( P \) = the total radioactivity in the compartment in counts per minute; \( \rho_*^* \) = the specific activity of K\textsuperscript{42} in the compartment in counts per minute per millimole; \( n \) = the total number of bacteria present; \( A \) = the area of each bacterium in square centimeters; \( \Phi_{12} \) = the flux of K from the cell interior to the extracellular medium in millimoles per square centimeters minute; and \( t \) = time in minutes.

Since, in the steady-state, \( \Phi_{12} \) and \( \Phi_{21} \) are constant and equal, and the compartment volumes are constant, we may further define:

\[
\Phi_{12} = k_{12}S_1 \quad \text{and} \quad \Phi_{21} = k_{21}S_2
\]

where \( s \) = the concentration of K in the compartment in millimoles per liter; \( S \) = the total K content of the compartment in millimoles; \( V \) = the water volume of the compartment in liters; and the \( k \)’s are constants with units of cm min\textsuperscript{-1}. Since at \( t = 0 \), \( \rho_*^* = \rho_1^* = \rho_2^* = 0 \), the solution of equation (1) is

\[
\ln \left[ \frac{\rho_2^*}{\rho_2^*_{\infty}} - 1 \right] = -(1 + \beta)ak_{12} t + \ln \beta
\]

(4)
where $\beta = S_1/S_2$ and $\alpha = nA/V_1$. The latter may also be expressed as

$$\alpha = \frac{nA}{V_1} \frac{V_4}{V_3} = \alpha'/V_{\text{att}} \quad (5)$$

where $V_i$ is the total volume of bacteria in the system; $\alpha' = \text{the area to volume ratio of one bacterium}$; and $V_{\text{att}} = \text{the ratio of the volume of intracellular H}_2\text{O in one bacterium to the total volume of one bacterium}$.

*Evaluation of $\alpha$*

*E. coli* is a rod-shaped organism which may be geometrically approximated by a cylinder with hemispherical ends. Thus the area to volume ratio may be calculated from a knowledge of the over-all length and width. The average dimensions of cells grown under the conditions used in these experiments were determined from photomicrographs of dried smears and were $0.52 \pm 0.01$ (width) and $1.58 \pm 0.03 \mu$ (length), giving a value of $8.6 \mu^{-1}$ for $\alpha'$. In arriving at this value two uncertainties are immediately apparent. First, it is now well established that the permeability barrier to the passage of small molecules and ions in bacteria is the cytoplasmic membrane immediately underlying the rigid, porous cell wall (5). The thickness of this cell wall, determined by electron microscopy varies between 0.01 and 0.02 $\mu$ depending upon the age of the culture, and the methods used to fix and prepare the bacterial sections. Thus even if the over-all dimensions of the bacterium are known the ratio of membrane area to intracellular volume remains uncertain. The use of the over-all dimensions in the present study is supported by the fact that the extracellular space of the bacterial pellets, $0.19 \text{ml/gm}$ wet weight (1), was determined with C$^{14}$-inulin and I$^{131}$-albumin both of which probably do not penetrate the cell wall (7). Second, the data presented by Luria (8) suggest that the dimensions obtained from photomicrographs of dried smears may be as much as 50 per cent smaller than those of the living organism. Thus the true flux is greater than the calculated flux, possibly by as much as a factor of 2. However, since the same conversion factor has been used throughout this study, the interrelationships of the K, H, and glucose fluxes are not affected by this uncertainty.

The value of $V_{\text{att}}$ may be calculated from the values of the wet and dry weights, the extracellular space, and the density of the bacterial pellet. The latter was determined both by the copper sulfate method (9) and from the densities of a suspension of known cytocrit and of the supernatant solution, which were separately determined using a standard 50 ml pycnometer. The value of $1.06 \pm 0.00 \text{gm/cm}^3$ thus obtained is in good agreement with the reported value of 1.1 for the bacterial cell in the absence of trapped extracellular medium (8). Using a dry-to-wet weight ratio of 0.23 and an extra-
cellular space of 0.19 ml/gm wet weight (1), the ratio of the intracellular water volume to the total cell volume, \( V_{eff} \), may be shown to be 0.77 (density of water assumed to be 1.00). The value of \( \alpha \) which is equal to \( \alpha' / V_{eff} \), is thus \( 1.1 \times 10^4 \) cm\(^{-1}\).

**Evaluation of \( \beta \)**

An examination of Equation 4 reveals that \( \beta \), the ratio of the total intracellular \( K \) to the total extracellular \( K \) may be determined graphically from the intercept of a plot of \( \ln \left[ \frac{P_x^*}{P_e^*} - 1 \right] \) against \( t \). \( \beta \) may also be calculated independently from a knowledge of the intracellular and extracellular \( K \) concentrations and of the ratio of the intracellular to extracellular water volumes. The ratio of \( \beta \) determined analytically, to \( \beta \) determined graphically, is thus a measure of the internal consistency of the methods employed. In the ten steady-state experiments to be reported this ratio was 0.99 ± 0.06 (sd).

**RESULTS AND DISCUSSION**

**Steady-State K Fluxes**

The criteria for the maintenance of a steady-state were that the volumes and \( K \) concentrations of the intracellular and extracellular compartments remained constant within narrow limits during the experimental period. The increase in the volume of the extracellular compartment resulting from the periodic addition of alkali to the suspension was limited to 3 to 5 per cent. A comparison of the initial and final cytocrits after correction for this dilution revealed an average 3 per cent decline in the bacterial volume. This decline could not be correlated with the type or amount of alkali added to the suspension. The ratio of the initial to final dry to wet weight ratios of the bacterial pellets averaged 1.00 ± 0.02 (sd).

The average intracellular \( K \) concentration was 203 ± 7 mmols/liter cell \( H_2O \), and the ratio of the initial to final intracellular \( K \) concentrations averaged 0.97 ± 0.06 (sd). Experiments in which the intracellular \( K \) concentration changed by more than 10 per cent have not been included in this report. Since the elapsed time between the determinations of the initial and final intracellular \( K \) concentrations (35 minutes) is sufficient for 80 to 90 per cent exchange of intracellular \( K \), the average net change of 3 per cent represents a small departure from the steady-state.

The results of a typical experiment are shown in Fig. 2; the experimental details are given in the legend. The curve is a linear plot of Equation 4 using the values of \( \beta \) and \( k_{13} \) obtained from a semilogarithmic plot of the experimental data. The good agreement of the experimental points with the theoretical curve, and the fact that by 38 minutes 94 per cent of the chemically
determined intracellular K had exchanged with the tracer, indicate that the kinetics of K\textsuperscript{41} influx are consistent with a single intracellular K compartment in \textit{E. coli}.

The K flux was determined in the presence of extracellular K concentrations varying from 1.3 to 5.2 mM (average, 3.2 mM). The average unidirectional K flux (ten experiments) was 1.0 ± 0.1 pmols/(cm\(^2\) sec.), and was independent of the extracellular K concentration over the range studied. This flux is equivalent to 6.6 ± 0.3 mmols/(1 cell H\(_2\)O min.).

It should be noted that in these experiments the cell densities averaged 2 per cent by volume, approximately 10 times the cell density of the exponentially growing cultures from which the bacteria were harvested. Under these conditions the rate of oxygen utilization by the cells greatly exceeds the rate of oxygen transfer from the atmosphere to the suspension, even with rapid stirring. Thus the chemical energy required by the cells for active transport and other endergonic processes is derived, primarily, through anaerobic metabolic pathways. To determine whether the degree of aerobiosis has a significant effect on the magnitude of the observed K fluxes, duplicate experiments were performed in which the bacterial suspensions were vigorously bubbled with either 50 per cent O\(_2\)-50 per cent N\(_2\) or 100 per cent N\(_2\). The K flux observed when 100 per cent N\(_2\) was used did not differ significantly from that observed with 50 per cent O\(_2\).
Previous studies of $K^{4+}$ uptake by microorganisms (10–13) have indicated that the equilibration of the intracellular $K$ with the tracer is a rapid process. These studies are based entirely on the distribution of the radioactive tracer. Thus, in the absence of chemical determinations of the intracellular $K$ concentrations, one cannot distinguish between $K^{4+}$ uptake resulting from a steady-state exchange process, and that which would result from a net uptake of $K$ by the cells. This distinction is of particular importance since, although the kinetics of $K^{4+}$ exchange in the steady-state may be described by a single exponential, the kinetics of net $K$ uptake by $K$-depleted cells are characterized by a very rapid initial uptake followed by a slower influx process (14).

It is of further interest to note that the average $K$ flux observed in these studies is of the same order of magnitude as that observed in a wide variety of animal cells (15). The rapidity with which intracellular $K$ equilibrates with extracellular tracer is thus a consequence of the large surface to volume ratio of this organism rather than the result of a highly permeable cell membrane. The findings of Fricke and coworkers (16) that $E. coli$ possesses a low conductance membrane with electrical properties similar to those found in many biological membranes are consistent with this conclusion.
**H Secretion and Glucose Uptake**

The rate of H secretion may be calculated from the rate of addition of alkali required to maintain the pH of the suspension constant, the volume of the suspension, the bacterial cytoerit, and \( \alpha' \). In eight experiments in which 1 M NaOH was employed as the neutralizing agent, the net H efflux averaged 16 ± 1 pmols/(cm\(^2\) sec.) or 106 ± 11 mmols/(1 cell H\(_2\)O min.). It should be noted that the method employed is valid only if the secreted organic acids have pK values well below the pH at which the system is maintained; that is, the method is based on the assumption that the buffer value of the medium, at the stated pH, does not change significantly during the course of the experiment. Since the major acid products of glucose fermentation by *E. coli* (formic, lactic, acetic, and succinic acids) are all at least 98 per cent dissociated at pH 7.0, their contribution to the buffer value of the medium is negligible. To confirm this, titration curves were obtained on both fresh growth medium and old growth medium obtained from a 48 hour stationary phase culture. As is shown in Fig. 3 the slopes of the titration curves of fresh and old media do not differ significantly at pH 7.0. The buffer value of the old medium is greater than that of the fresh medium over the pH range of

![Diagram](image-url)
4.8 to 6.0 as would be expected from the accumulation of the above organic acids.

The results of an experiment in which glucose uptake and H secretion were simultaneously determined on cells harvested in the logarithmic phase of growth and resuspended in fresh growth medium at pH 7.0 are shown in Fig. 4. The details of the experiment are given in the legend. In three similar experiments the glucose uptake averaged 7.0 ± 0.4 pmols/(cm² sec.). The ratio of H secretion to glucose uptake of 2.3 ± 0.4 (SD) is not significantly different from the values of 2.5 to 2.9 for glucose fermentation by E. coli reported in the literature (17, 18).

Conway and O'Malley (19) and Rothstein and Enns (20) have demonstrated that in the course of glucose fermentation by yeast net K uptake is balanced by a nearly equivalent secretion of H ions. In E. coli two observations suggest the presence of a K-H exchange mechanism similar to that described for yeast. The first is that K uptake during fermentation is accompanied by an acidification of the growth medium (1, 21), and the second is that K uptake is inhibited by a low extracellular pH (1, 14). Indeed, experiments with K-depleted cells have indicated that a K-H exchange mechanism operates during the process of net K uptake by these cells (14). During the steady-state however, as indicated by the present results, the rate of H efflux is 16 times the rate of K influx. Thus although a small fraction of the H efflux (less than 7 per cent) may be coupled mole for mole to K influx, the bulk of the metabolically produced H is not linked to K movement. Furthermore, since in these experiments there is no net uptake of cations by the cells, H secretion must be accompanied by an equivalent secretion of anions. Stokes (17) and Blackwood et al. (18) have demonstrated that a total of 2.5 to 2.9 moles of lactate, acetate, formate, and succinate are produced per mole of glucose fermented by E. coli. Our finding that the ratio of H secretion to glucose uptake was 2.3 is thus consistent with the conclusion that the H secreted was accompanied by the organic anions arising from glucose fermentation, and a net efflux of other intracellular anions such as PO₄ or SO₄ need not be invoked.

Dr. Schultz is a Given Foundation-National Research Council Fellow in Academic Medicine.
Dr. Epstein is a Training Fellow of the Basic Science Research Training Program, United States Public Health Service (2G-466), and is at present on leave of absence from New York University-Bellevue Medical Center.
Dr. Goldstein is a Research Fellow of the Helen Hay Whitney Foundation.
The authors are grateful to Dr. A. K. Solomon for his encouragement and advice during the course of this study, and for his critical reading of the manuscript.
The authors also acknowledge the assistance of Dr. G. Majno, of the Department of Pathology, and of Mr. B. Pearson in obtaining the photomicrographs; and of Messrs. R. E. Dooley, W. Kazolias, E. Gunders, and B. Corrow in the construction and maintenance of the equipment.
This work was supported in part by the United States Atomic Energy Commission and the National Science Foundation.
Received for publication, June 8, 1962.
BIBLIOGRAPHY