Cation Transport in *Escherichia coli*

I. Intracellular Na and K concentrations and net cation movement

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ABSTRACT Methods have been developed to study the intracellular Na and K concentrations in *E. coli*, strain K-12. These intracellular cation concentrations have been shown to be functions of the extracellular cation concentrations and the age of the bacterial culture. During the early logarithmic phase of growth, the intracellular K concentration greatly exceeds that of the external medium, whereas the intracellular Na concentration is lower than that of the growth medium. As the age of the culture increases, the intracellular K concentration falls and the intracellular Na concentration rises, changes which are related to the fall in the pH of the medium and to the accumulation of the products of bacterial metabolism. When stationary phase cells, which are rich in Na and poor in K, are resuspended in fresh growth medium, there is a rapid reaccumulation of K and extrusion of Na. These processes represent oppositely directed net ion movements against concentration gradients, and have been shown to be dependent upon the presence of an intact metabolic energy supply.

A mutant form of the bacterium *Escherichia coli* which is unable to transport K at the normal rate has been isolated (1). In order to determine the nature of this induced defect, it has been necessary to characterize the cation transport system in the wild-type bacterium. The present report is concerned with the intracellular Na and K concentrations in the wild-type *E. coli* and the relationship between bacterial metabolism and the transport of the cations.

The studies of Na and K metabolism previously carried out in *E. coli* (2, 3), *B. lactis aerogenes* (4), *A. faecalis* (5), and several marine bacteria (6) have depended either on indirect measures of the Na and K content of the bacterium or on the distribution of radioactive isotopes. The present communication describes methods for the determination of intracellular Na and K concentrations from direct measurements on the cells. The maintenance of these intracellular cation concentrations is shown to be related to the age of the culture and to be dependent on metabolic sources of energy.

METHODS

Bacteria

Escherichia coli, strain K-12, kindly provided by Dr. B. Davis, was used throughout the experiments. The organisms were maintained until use by monthly transfers on nutrient agar (Difco) plates and stored at 4°C. The basic media used in these studies are given in Table I. The zero K medium was prepared by a mol for mol substitution of Na phosphate for the K phosphate in Davis' medium A (7). The salt solutions and glucose solutions were autoclaved separately, then mixed aseptically. The organisms were transferred directly from the stock plates into 500 ml of medium contained in 1 liter flasks and grown overnight at 37°C with agitation. Unless otherwise indicated, the 5 mM K medium was used for this initial cultivation. The bacteria were harvested in the early stationary phase after approximately 18 hours of growth, by centrifugation at 1,400 × *g* for 25 minutes at room temperature.

TABLE I

COMPOSITION OF GROWTH MEDIA

Compound	Medium A	Zero K medium	5 mM K medium
	mM	*mM*	*mM*
KH_2PO_4	22	0	0
K_2HPO_4	40	0	0
NaH_2PO_4	0	22	22
Na_2HPO_4	0	40	40
$(NH_4)_2SO_4$	8	8	8
Na citrate	5	5	5
$MgSO_4$	0.4	0.4	0.4
KCl	0	0	5
Glucose	55	55	55

Experimental Procedure

The sedimented organisms were then inoculated into 2 to 4 liters of medium contained in 6 or 12 liter Florence flasks. These cultures were grown at 37°C with vigorous shaking. At desired intervals 250 to 500 ml. aliquots were withdrawn aseptically and concentrated 50 to 100-fold by centrifugation at room temperature. The volume fractions of bacteria in the resulting suspensions were 1 to 3 per cent. These suspensions were then aspirated into the specially designed centrifuge tubes described in Fig. 1. Centrifugation results in a densely packed column of cells with a sharp horizontal meniscus. The volume of the cell column could be determined with a precision of ± 0.02 microliter using an optical comparator. The latter consists of a movable stage which activates a machinist's dial gauge. The centrifuge tube is placed on the stage, and the bottom and top of the cell column are successively brought into focus

under a fixed cross-hair. The distance traversed by the stage can be read with an accuracy of 1 part in 500. Using this method, cytocrits of the same suspension centrifuged simultaneously agreed to within 0.1 per cent and, when centrifuged successively, agreed to within 0.5 per cent.

After centrifugation, the teflon plug was removed and a pellet of varying length was easily extruded from the open capillary tip onto a preweighed aluminum foil pan by the application of air pressure at the opposite end of the tube. The column of cells generally had a total height of 2 to 3 cm and consisted of three fairly distinct layers. The layer immediately above the teflon plug consisted of heavy debris and pigmented cells. It was rarely greater than 1 mm in height and was discarded prior

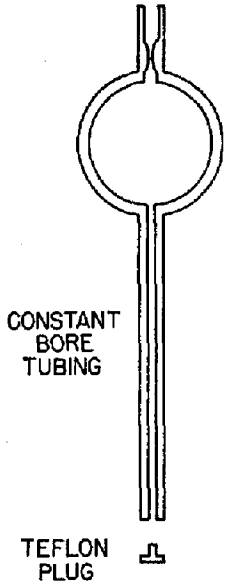

FIGURE 1. *E. coli* cytocrit tube. These tubes have total volumes of 2.5 to 3.0 cm^3 and have been calibrated with mercury. The shank of the centrifuge tube is precision bore capillary tubing (Fischer and Porter) having an inner diameter of 1.28 mm and a capacity of 60 μl. This capacity represents 2 to 3 per cent of the total capacity of the tube. The tube is filled up to the constriction in the neck by aspirating the bacterial suspension into the capillary opening. The capillary opening is then sealed with a teflon plug, and the suspension is centrifuged at 11,000 $\times$ *g* for 25 minutes at 2°C in a specially constructed, horizontal rotor designed to accomodate these tubes (constructed to specification by Laboratory Associates, Inc.).

to the extrusion onto the aluminum pan. The uppermost layer, usually 1 to 2 mm in height, was more translucent than the main cell layer and probably consisted of light debris from disrupted cells. The upper and lower layers were frequently absent in young cultures and reached their greatest heights in very old cultures. When care is taken not to extrude the uppermost 1 cm of column, the possible error due to diffusion at the upper boundary of the extruded pellet will be less than 0.2 per cent in 1 hour (8).

Water Content

The total water content of the extruded pellet was measured using two misco quartz helix microbalances with capacities of 20 and 50 mg. These helices are linear over the range employed and can be read with an accuracy of $\pm$ 0.02 mg, or less than $\pm$ 1 per cent of the pellet weight. To correct for surface evaporation during the time

elapsed from the extrusion of the pellet to the reading of the wet weight, this time period was measured and a second reading of the wet weight was made after a subsequent equal interval of time. Assuming a constant rate of evaporation over the total period measured (usually 1 minute), the initial wet weight may be appropriately corrected. The pellet was then dried for 12 hours at 110°C and reweighed. Drying for an additional 48 hours resulted in an additional weight loss of less than 1 per cent. The wet weights of the pellets were generally between 10 and 20 mg and the dry weights between 2 and 5 mg.

Extracellular Space

The trapped extracellular spaces of the pellets were measured with both C^{14}-inulin and I^{131}-labeled albumin. The I^{131}-albumin was dialyzed against isotonic KI solution for 24 hours at 2°C before use. In each case an appropriate amount of the tracer was added directly to the growth medium prior to the final centrifugation. For the C^{14}-inulin determinations, the pellets, after drying, were extracted into a volume of tracer-free growth medium for 48 hours. The extract and supernatant fluid were assayed in a flow counter as previously described by Page and Solomon (9). All samples had counts greater than ten times background, and self-absorption could be neglected over the range counted. The I^{131}-albumin spaces were determined in a well-type scintillation counter prior to drying to avoid losses by sublimation.

Sodium and Potassium Determinations

Following the determinations of the dry weights, the pellets and albuminum pans were extracted in 10 ml of 0.1 M NH_4 oxalate solution for 48 hours at room temperature. The extraction was carried out in acid-cleaned vycor test tubes capped with parafilm. Control experiments disclosed no evaporation during the 48 hour period. The presence of the NH_4 ion, which could displace minute amounts of Na or K bound to the dry pellet, resulted in greater recoveries of Na and K than had been obtained by extraction in distilled water, or by ashing with concentrated nitric acid and subsequent dilution with water. The extraction process was checked by ashing the extracted pellets; no residual K or Na was detected. All cation determinations were made using the flame photometer of Solomon and Caton (10) and were corrected for the trace amounts of Na and K present in the NH_4 oxalate solution.

Intracellular ion concentrations were calculated using an equation of the following type:

$$[K]_i = \frac{K_t - \theta W_w [K]_o}{[W_w(1 - \theta) - W_d]}$$

in which $[K]_i$ = the intracellular concentration of K in millimoles per liter intracellular water, $[K]_o$ = the concentration of K in the medium in millimoles per liter, K_t = the total K in the extruded pellet in micromoles, W_w = wet weight of the pellet in grams, θ = milliliters of trapped medium per gram wet weight (density assumed 1.0), and W_d = dry weight of the pellet in grams.

Using these methods, duplicate determinations of the intracellular ion concentrations usually agreed to within 3 per cent in the case of K and within 8 per cent in the case of Na, and the values were independent of the volume of the extruded column. The relatively poorer agreement for Na probably reflects the ease of contamination with Na as well as small variations in the volume of the trapped space with varying pellet sizes.

It is assumed that the intracellular Na and K are uniformly dissolved in the measured intracellular water. This assumption, although generally accepted in the case of animal cells, requires further clarification in the case of bacteria. *E. coli* may be geometrically represented by a cylinder with hemispherical ends, 0.5 μ in diameter and 2 μ in over-all length (11). The protoplast membrane is surrounded by a porous cell wall, approximately 15 mμ thick, which constitutes 20 to 30 per cent of the dry weight (12) and 13 per cent of the total volume of the cell. Mitchell and Moyle (13) have shown that disruption of the bacterial membrane of *S. aureus*, under conditions which left the cell wall intact, allowed nucleotides and coenzymes to escape. The cell wall, on the other hand, was impermeable to dextran (molecular weight of 10,000) and serum albumin. These results indicate that the trapped space measured in *E. coli* with C^{14}-inulin or I^{131}-albumin may not include water within the interstices of the cell wall. This would mean that the cell water could be overestimated by as much as 13 per cent, and our calculated intracellular K concentrations could be erroneously low by as much as 13 per cent. The exact correction would depend on the K and water content of the cell wall. Since the degree of hydration of the cell wall is not known, and some ionic interaction with the highly charged polymers which constitute the cell wall (12) is likely, no correction for this factor has been attempted. We shall consider "intracellular" to refer to the region bounded by the inulin or albumin space.

pH measurements were made with the Radiometer model 22 pH meter to an accuracy of $\pm$ 0.02 pH unit. Optical densities were determined at 660 mμ using the Beckman model B spectrophotometer and optically matched cuvettes.

RESULTS AND DISCUSSION

Extracellular Space

The dry to wet weight ratio, based on 100 determinations using cells in all stages of growth, was 22.8 $\pm$ 0.2 per cent (standard error) giving a total water content of 77.2 $\pm$ 0.2 per cent of the wet weight. These values are independent of the size of the extruded pellet.

The extracellular space measured with C^{14}-inulin (11 determinations) was 0.18 $\pm$ 0.01 ml/gm wet weight. The I^{131}-albumin space (6 determinations) was 0.20 $\pm$ 0.01 ml/gm wet weight. These two values do not differ significantly and the combined value of 0.19 $\pm$ 0.01 ml/gm wet weight has been used in this study. Spaces determined immediately after the addition of tracer and after an interval of 35 minutes did not differ significantly, showing that both inulin and albumin remained extracellular during our analysis.

Intracellular Na and K Concentrations

During the early logarithmic phase of growth the intracellular K concentration in *E. coli* exceeds that of the external medium, whereas the intracellular Na concentration is less than that of the external medium, a feature characteristic of many cells.

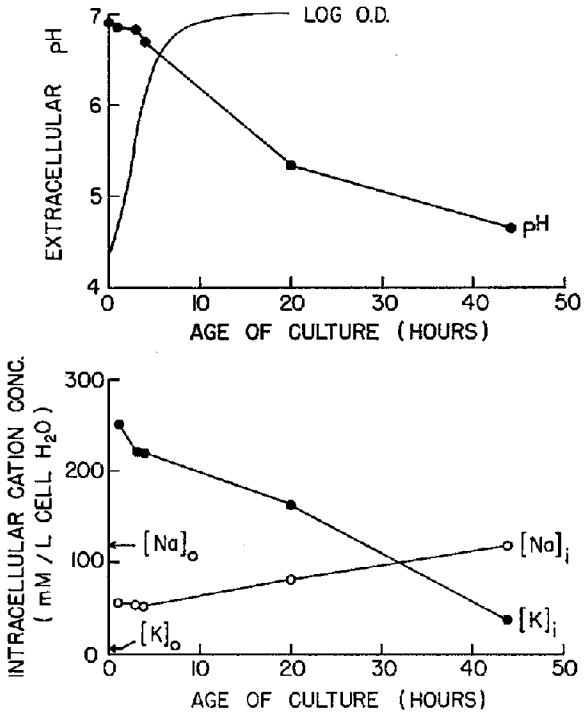

FIGURE 2. The intracellular K and Na concentrations as functions of the age of the culture. The pH and optical density (O.D.) of the medium are also shown as functions of time. The extracellular cation concentrations are indicated by $[Na]_o$ and $[K]_o$.

The intracellular K concentration has been studied in external K concentrations from 0.050 mM to 120 mM. Intermediate external K concentrations were achieved by the addition of KCl to the zero K medium. However, the zero K medium was not entirely free of K because of trace contamination of commercially available reagents. The extracellular K concentration remained constant throughout the experiments since the volume of the medium was essentially infinite.

As shown in Fig. 2, the intracellular K concentration is at its highest level during the early logarithmic phase of growth. The average K concentration in 60 determinations during the first 10 hours is 211 ± 3 mM. This high intra-

cellular K concentration, which is achieved only during the early phase of growth, may reach a value 3600 times greater than the medium K; it appears to be essentially independent of the extracellular K concentration over the 2000-fold range studied.

During the late logarithmic and stationary phases of growth, the intracellular K concentration falls, as does the pH of the medium. Experiments in which determinations were made after 72 hours of growth reveal that the decline in the K concentration continues until the values approach the extracellular concentration. Curves similar to that shown in Fig. 2 have been observed in 10 different experiments employing growth media with K concentrations ranging from 0.050 to 120 mM.

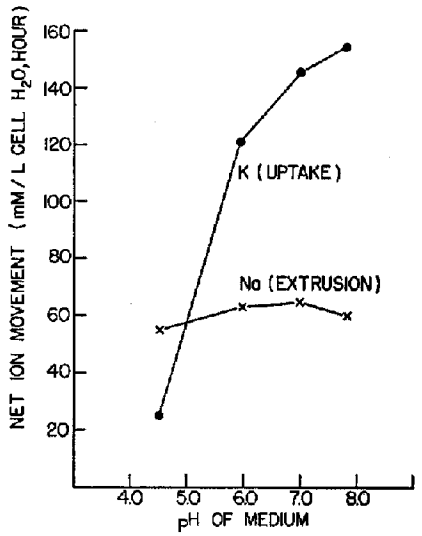

FIGURE 3. The effect of the initial pH of the growth medium on net K uptake and net Na extrusion.

A typical plot of the intracellular Na concentration as a function of the age of the culture is also shown in Fig. 2. During the logarithmic phase of growth the intracellular Na concentration is less than the 120 mM Na of the medium (5 mM K). As the age of the culture increases, the intracellular Na concentration rises, until, by the late stationary phase it reaches the extracellular concentration, and in some cases exceeds it.

Growth in media containing an excess of glucose is accompanied by an acidification of the medium due to the release of the small organic acids which are the products of bacterial fermentations (14). During the early logarithmic phase of growth, this acidification proceeds slowly because of both the buffering action of the medium and the availability of oxidative metabolic pathways. In the late logarithmic and stationary phases of growth acidifica-

tion of the medium occurs rapidly as the buffer capacity of the medium is exceeded and the dense bacterial suspension can no longer be adequately oxygenated.

Since the changes in intracellular cation concentrations are accompanied by concomitant changes in the pH of the medium, it seemed desirable to carry out experiments to see whether the pH drop or the accumulation of metabolic products in the medium caused the cation shift. Fig. 3 shows the effect of pH on net ion movement following suspension of K-poor, Na-rich cells from the same parent culture in 5 mM K medium for 1 hour. The media were adjusted to the desired pH by the addition of small amounts of HCl or NH_4OH. In 4 experiments, although net K uptake was markedly inhibited by a pH of 4.5, net Na extrusion was essentially independent of the initial pH, the net extrusion at pH 4.5 being slightly less than that at pH 7.

TABLE II

EFFECT OF PRODUCTS OF BACTERIAL GROWTH ON NET K UPTAKE AND NA EXTRUSION

	pH	Relative net K uptake (1 hr.)	Relative net Na extrusion (1 hr.)
Fresh medium	7.0	1.0	1.0
Old medium	7.2	0.9	1.0
	4.5	0	0
Fresh medium	4.2	0	0.9
Fresh medium and 0.03 M K formate	7.0	1.0	0.9
	4.5	−0.1	−0.1

A similar effect of pH on the uptake of K^{42} by *B. lactis aerogenes* has been reported by Eddy and Hinshelwood (4). Leibowitz and Kupermintz (15) have also observed an increase in extracellular K concentration during the course of glucose fermentation by *E. coli*.

The top three rows in Table II give the results of duplicate experiments investigating the influence of the age of the medium on the pH effect. Three 250 ml aliquots of a 48 hour culture were harvested by centrifugation. The cells in one aliquot were resuspended in fresh 5 mM K medium. The supernatant of a second aliquot was adjusted to pH 7.2 by the addition of concentrated NH_4OH; the third remained at pH 4.5. The cells were resuspended in these media and harvested for analysis after 1 hour of incubation at 37°C. The data show that restoration of the old medium to pH 7.2 is sufficient to restore the net K uptake and net Na extrusion almost to control values. The effect on K uptake appears to be solely dependent on pH, the age of the medium being unimportant.

In the case of Na, Table II shows that both an old medium and a lowered

pH are required to inhibit Na extrusion; neither one inhibits alone. This suggests that inhibition may be caused by one or more of the products of bacterial growth in the presence of a low pH. Dagley, Dawes, and Foster (14), and Gale and Epps (16) have shown that Na formate, in concentrations approximating that found in stationary phase cultures, markedly inhibits growth of *E. coli* when the initial pH of the growth medium is below 6.0. Bacterial growth was not affected by the same concentration of formate in neutral media. The last two rows in Table II show that 0.03 M K formate depresses both net K uptake and Na extrusion in fresh media when the pH is lowered to 4.5. Thus, K formate at low pH could account for the observed inhibition of Na extrusion. The pH dependence of this inhibitory process may simply reflect the ability of the small, neutral formic acid molecule to

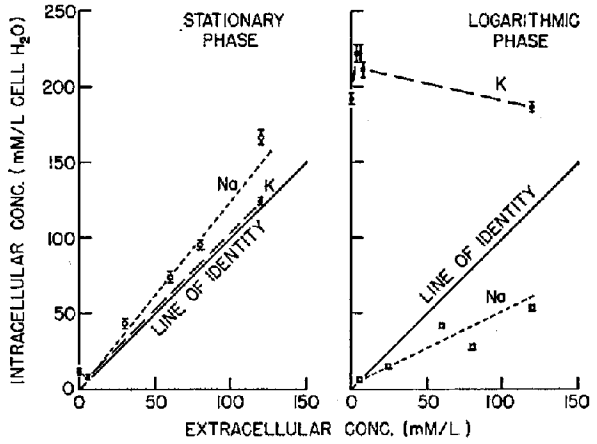

FIGURE 4. The intracellular concentrations of K and Na in the stationary and logarithmic phases of growth as functions of the extracellular cation concentrations. The vertical bars represent standard errors of the mean.

diffuse into the cell. The formate ion, on the other hand, can enter the cell only in exchange for another anion, or when accompanied by a cation. Mitchell and Moyle (17) have shown that protoplasts of *E. coli* are osmotically stable in solutions of K acetate at neutral pH, whereas the bacterial membrane is permeable to the neutral acetic acid molecule.

Effect of Extracellular Cation Concentration

Fig. 4 shows the intracellular Na and K concentrations during the stationary and logarithmic phases of growth as functions of the Na and K concentrations in the growth medium. These figures are typical of results obtained in 19 experiments. In the stationary phase, the intracellular K concentration is

closely equal to the extracellular concentration; the intracellular Na concentration slightly exceeds the extracellular. The relatively good agreement with the line of identity suggests that the distribution is primarily passive in the stationary phase, if the potential difference across the cellular membrane is small; the points in the case of Na are fitted, as shown in Fig. 4, by a line drawn for passive distribution in a cell whose intracellular potential is 6 mv negative to the medium.

It has already been pointed out that the intracellular K concentration in the logarithmic phase may be many times greater than, and is essentially independent of, the extracellular K concentration. On the other hand, the intracellular Na concentration is much lower than, and directly dependent on, the extracellular Na concentration. If the potential difference is of the order of magnitude required to account for the stationary phase concentration, the intracellular concentrations in the logarithmic phase represent a considerable perturbation, very possibly the result of an active process.

Previous investigations of Na and K transport in bacteria have yielded somewhat conflicting results regarding the intracellular Na concentrations. Roberts and coworkers (2) studied the distribution of Na^{24} in *E. coli* (strain B) and concluded that the membrane is freely permeable to Na and that the intracellular Na concentration is equal to that of the external medium. Krebs and coworkers (5) arrived at a similar conclusion from their studies on *A. faecalis*. Further, MacLeod and Onofrey (6) found that the intracellular Na concentration in marine bacteria averages 80 to 95 per cent of that in the growth medium. All these results are compatible with our observations on intracellular Na concentrations during the stationary phase.

Net Na and K Movement

Cells harvested in the stationary phase are poor in K and rich in Na. When these cells are resuspended in fresh medium, they rapidly accumulate K and extrude Na. Fig. 5 shows the results of a typical experiment in which a heavy inoculum of cells from a 48 hour culture was suspended in fresh 5 mM K medium at 37°C. In measurements during the 1st hour after resuspension (16 experiments) the intracellular K concentration rose from 23 ± 3 mM to 174 ± 6 mM whereas the intracellular Na concentration fell from 143 ± 6 mM to 77 ± 4 mM. This rapid increase in intracellular K and decrease in Na occurred before any significant change in the optical density of the culture could be detected, and hence cannot be attributed to a resumption of growth and division on the part of the cells. To confirm this point, duplicate experiments were carried out in which either a nitrogen source was removed from the growth medium or chloramphenicol was added in a concentration (50 μg/ml) which has been shown to inhibit protein synthesis (18). In both

cases there was no effect on net Na or K movement. After resuspension for 5 hours, the intracellular K concentration begins to fall, as does the pH of the medium. This is accompanied by the onset of a rise in the intracellular Na concentration. By 48 hours, the cycle has been completed, resulting in K-poor, Na-rich cells, not unlike the original inoculum.

The effect of glucose and of various metabolic inhibitors may be studied by measuring net ion movements after a 1 hour period of incubation at 37°C. Although preliminary experiments have shown that the true rate of ion transport is measured in minutes rather than in hours, 1 hour net movements

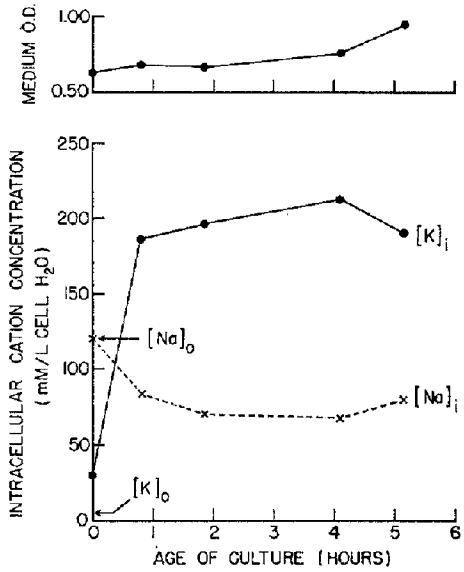

FIGURE 5. Net K uptake and Na extrusion following the resuspension of cells from a 48 hour old culture in 5 mM K medium. The optical density of the medium is also shown as a function of time. The pH of the medium fell from 7.0 to 6.6 during the time period shown.

are satisfactory as qualitative indices of relative ion movements. In the absence of added glucose, as shown on the left side of Fig. 6, both net K uptake and net Na extrusion are inhibited. The residual net movements may result from trace amounts of glucose transferred to the fresh culture with the unwashed cells or other energy stores which have not been depleted.

Fig. 6 also presents the results obtained with metabolic inhibitors. The cells were exposed to each of the inhibitors in the original growth medium for a period of 30 minutes prior to resuspension in fresh medium. Sodium iodoacetate (10^{-3} M) and 2-4-dinitrophenol (10^{-4} M) markedly inhibit K influx and Na efflux, reducing both processes to less than 5 per cent of the control. The failure of cyanide ion (10^{-3} M) to inhibit net K and Na movements may be

explained by the presence of a significant cyanide-insensitive metabolic pathway. Asnis, Vely, and Glick (19) have shown that the oxidations of succinate, formate, and lactate by a particulate fraction of sonic lysates of *E. coli* are only inhibited 40 to 70 per cent by 3×10^{-3} M KCN.

Since the two net flows are in opposite directions, a potential difference across the cell cannot cause both movements. A 210 mv potential difference would be necessary to make the peak inward K movement passive. Such an imposing potential difference would require an unusually effective pump to transport Na against both an electrical potential and chemical concentration

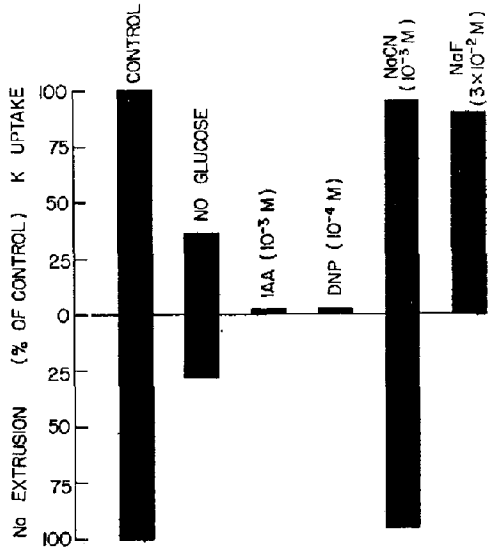

FIGURE 6. The effect of glucose deprivation and metabolic inhibitors on net Na and K movement. In each case, the height of the bar refers to an average of 2 experiments; except for NaF, in which there were 3. The results of these duplicate experiments agreed to within 5 per cent except in the case of glucose deprivation where residual cation transport varied between 15 and 40 per cent of the control value.

gradient. Alternatively, the 20 mv potential difference required to make the Na distribution passive would still require the K to surmount a considerable electrochemical potential barrier. Thus it seems likely that an active process is involved in at least one of these net movements. This conclusion is supported by the requirement for glucose and by the effect of the metabolic inhibitors, iodoacetate and DNP.

It is possible, however, that the high intracellular K concentration could result from intracellular K binding. If this were so, the present experiments would require, at least, that the K binding be under metabolic control. Such a system was initially proposed by Roberts and his colleagues (2) although

they have subsequently modified their views (20). This group initially considered the protoplast membrane of *E. coli* to be freely permeable to K. The intracellular K was believed to consist of two fractions: one in free solution in the intracellular water at a concentration equal to that in the external medium; and the other bound to non-diffusible intracellular compounds formed during carbohydrate metabolism. They further proposed that the number of binding sites available to K, and hence the total intracellular K, was under metabolic control (3). A similar theory has been proposed by Lester and Hechter (21) in an investigation of Rb uptake by *Neurospora crassa*.

The contrary evidence is inferential and rests mainly on evaluations of the intracellular osmolarity of *E. coli* by Mitchell and Moyle (22), Knaysi (23), and Schultz, Brubaker, and Solomon (24) which show that the internal osmolarity is of the order of 500 to 600 mOsM. These high values suggest that the bulk of low molecular weight intracellular solutes must exist in free solution. Mitchell and Moyle (22) have calculated that most of the trichloroacetic acid extractable material of *E. coli* and *S. aureus* must contribute to this internal osmotic pressure. In *E. coli*, the 280 mM sum of the intracellular Na and K concentrations, the major intracellular cations during the logarithmic phase of growth, accounts for approximately one-half of the observed internal osmolarity during this phase. Knaysi has demonstrated that the internal osmotic pressure in *E. coli* may reach 15 atmospheres in rapidly growing cells, whereas it is only 2 to 3 atmospheres in stationary phase cells (23).

The subject of ion binding has been recently reviewed by Ussing (25) who has concluded that the high intracellular K concentrations found in biological systems cannot be accounted for by a selective protoplasmic binding of K. Snell (26) has been unable to demonstrate Na or K binding by any of a series of organic phosphate esters, including glucose 1-PO_4, glycerophosphate, and ATP. On the basis of the present cation analyses, the requirements of an ion-binding hypothesis can be clearly formulated. As previously discussed, a passive distribution of Na during the early logarithmic phase of cell growth would require a transmembrane potential difference of 20 mv. If K were distributed passively in accordance with this potential difference, 99 per cent of the 211 mM would have to be bound. Binding of 209 mM of K seems excessive, particularly in view of Hinke's recent measurements (27) of the intracellular activities of Na and K in crab and lobster muscle. In these tissues, Hinke estimates the Na activity to be one-third of the Na concentration and the K activity to be one-half the K concentration. Calculations from Hinke's (28) observations of the Na and K activities in squid nerve axoplasm *in situ* indicate that the measured K activity coefficient is 0.60 at a K concentration of 0.37 M. This is not far from the figure of 0.67, the activity coefficient of an aqueous solution of KCl at the same concentration (29). Thus, Hinke's measurements are consistent with the view that most of the K is free, in agree-

ment with the prior observations of Hodgkin and Keynes (30). Consequently, we are inclined to the view that intracellular cation binding cannot account for the observed cation distribution in *E. coli*, and that active transport of either Na or K is very probable.

The reciprocal relationship between K and Na movements leads to an inquiry into the question of whether these fluxes are linked, as has been suggested for erythrocytes (31) and other tissues (32). Two lines of evidence suggest that this is not the case for *E. coli*. Thus, although net K uptake is inhibited in a fresh acid medium, Na extrusion is essentially unchanged. Furthermore, as shown in Fig. 6, net Na extrusion is completely inhibited by 30 mM NaF, although K uptake remains at 90 per cent of the control value. Thus the mechanisms for Na and K transport in *E. coli* may be varied independently. A lack of reciprocity between net Na and K fluxes has also been demonstrated in the mouse ascites tumor cell (33) and in the rabbit polymorphonuclear leukocyte (34).

In summary, the present experiments have shown that *E. coli* is capable of maintaining an intracellular K concentration many times greater than that in the external phase. The intracellular cation concentrations are functions of the age of the culture, the K concentration falling and the Na concentration rising as the culture age increases. These changes may be attributed, at least during the first 48 hours of incubation, to the fall in the medium pH and the accumulation of metabolic products, such as formate. When cells from an old medium are placed in a fresh medium, they accumulate K and extrude Na against large concentration gradients by processes which require metabolic energy. Though both net ion movements are against concentration gradients, the ions move in opposite directions, an observation which implies that at least one of the processes is active.

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