Cation Transport in *Escherichia coli*

V. *Regulation of cation content*

WOLFGANG EPSTEIN and STANLEY G. SCHULTZ

From the Biophysical Laboratory, Harvard Medical School, Boston

ABSTRACT Measurement of cellular K and Na concentrations in growing *Escherichia coli* indicates that the osmolality of the medium is a major determinant of the cell K concentration. In contrast, the cell Na concentration is independent of the medium osmolality and is largely dependent on the Na concentration of the medium. Sudden changes in the osmolality of the medium lead to rapid changes in K content. Washing the cells with solutions of lower osmolality results in a very rapid loss of K, which is greater in more dilute and in cold solutions. A sudden increase in the osmolality of the growth medium produces a rapid uptake of K by a mechanism whose rate is a saturable function of the K concentration of the medium and which appears to involve an exchange of K for cellular H.

In previous studies of this series it has been shown that the ionic composition of *Escherichia coli* is (*a*) constant in the logarithmic phase of growth, (*b*) changes as the pH declines in the stationary phase, and (*c*) returns rapidly to the cation content typical of the logarithmic phase of growth when the stationary phase cells are resuspended in fresh medium (1, 2). In the present study we have sought further evidence on the role of K in this organism, and the mechanism of its movement, by investigating some of the factors which determine the K and Na contents of exponentially growing cells. The results indicate that the osmolality of the medium is a major determinant of the K content of these cells, and that abrupt changes in this osmolality result in rapid changes in the intracellular concentration of this cation.

METHODS

Media

The standard medium used in these studies was composed of: Na_2HPO_4 56 mM, NaH_2PO_4 14 mM, NaCl 10 mM, NH_4Cl 10 mM, KCl 2.5 mM, Na_2SO_4 1 mM, $MgSO_4$ 0.4 mM, $FeCl_3$ 1.5 μM, $CuSO_4$ 0.2 μM, $(NH_4)MoO_4$ 0.15 μM, and glucose 55 mM. After autoclaving, this medium had an osmolality of 260 to 270 mOsM and a pH of 7.15 to 7.20. Media of greater osmolality, prepared by the addition of NaCl, sucrose,

or glucose, will be referred to as hypertonic. Media of lower osmolality, referred to as hypotonic, were prepared by dilution of the phosphate, NaCl, and Na_2SO_4 of the standard medium; other ingredients were kept at the concentrations indicated above. In experiments with other substrates, glycerol (25 mM) or sodium lactate (30 mM) replaced glucose in the standard medium. Glucose was added as an osmotic agent only to those media in which it was already present as substrate. For studies at different pH's the ratio of monobasic to dibasic phosphate was changed without altering the total phosphate concentration. Sterilization was usually carried out by autoclaving. Glucose and sucrose were autoclaved separately from the other ingredients. Since significant hydrolysis occurs when sucrose is autoclaved, media which contained sucrose in the presence of glycerol or lactate as substrate, were sterilized by filtration through 0.45 μ pore size membrane filters (Millipore Filter Corp.).

Bacteria

As in previous studies strain K-12 of *Escherichia coli* was used. Stock cultures of the organism were grown on agar-solidified standard medium and maintained by monthly transfers. For experiments in which the cells were to be grown with lactate or glycerol as substrate the organisms were adapted by at least 3 transfers on solid medium containing the desired substrate. The bacteria were grown at 37°C, aeration and agitation being provided by passing humidified air through the cultures. A few cultures with slow growth rates were incubated in cotton-stoppered flasks which were gently shaken. Growth was monitored by periodic reading of the turbidity at 610 mμ using either round tubes in a Coleman model 11 colorimeter or 1 cm cuvettes in a Beckman model B spectrophotometer.

Analyses

The analytical techniques made use of special centrifuge tubes similar to those previously described (Fig. 1 of reference 1) but with a bore of 0.76 mm in the capillary portion. The quantity of bacteria in a culture, as measured by the height of the column of bacteria in the tube, was expressed as a volume ratio or in terms of volume per cent; this measurement will be referred to as the cytocrit. The standard deviation of this measurement was 0.7 per cent of the average cytocrit in 8 sets of 4 replicate determinations using cultures whose cytocrits ranged from 0.1 to 0.2 per cent. The cytocrit was used to calculate the wet or dry weight of bacteria per milliliter, using values for the density of the pellet and dry to wet weight ratios obtained as described below.

In cultures with a cytocrit above 0.1 per cent it was possible to extrude a portion of the pellet onto a small tared aluminum pan. These pellets, whose wet weight ranged from 1.5 to 5 mg, were weighed, dried, and handled in a manner similar to that described for the larger pellets used in earlier studies (1): A quartz helix (Microchemical Specialties Co.) with a 10 mg capacity was used in weighing. Na and K analyses on extracted pellets and suitably diluted supernatants were performed using a modified Perkin-Elmer model 52-A flame photometer (2). The standard deviation of groups of 3 to 10 replicate K determinations averaged 2 per cent of the analyzed K content, while the Na analyses were more variable, with an average standard devia-

tion of 7 per cent (variance in approximately 200 analyses of each cation). Analyses of the P content of the pellets were performed by digestion with 70 per cent $HClO_4$ (3) followed by determination of PO_4 by the method of Fiske and Subbarow (4), with color stabilized by heating at 100°C for 10 minutes (5). The average difference of duplicates in 7 sets of paired pellet P analyses was 0.9 per cent.

Extracellular spaces in pellets were determined with I^{131}-labeled human serum albumin (RISA, Abbott Laboratories). The labeled albumin was dialyzed for 24 hours and then diluted with bovine serum albumin (Armour and Co.) before use. The dried pellets were counted in a well-scintillation counter to a statistical counting error of less than 2 per cent. Drying the pellets for 12 to 24 hours at 90 to 100°C did not lead to a measurable loss of I^{131}. The extracellular spaces were found to be 0.183 ± 0.003 milliliter per gram wet weight[1] in 42 determinations carried out in media of varying osmolality not containing sucrose. In medium containing 0.4 M sucrose (6 determinations) the space was 0.214 ± 0.005 ml/gm. Values of $[K]_i$, $[Na]_i$, and dry to wet weight ratios have been corrected for the quantity of K, Na, and water in the extracellular space of the pellets.[2] The assumptions and errors involved in the use of the I^{131} albumin spaces have been previously discussed (1).

Another method of studying the bacterial K content was devised using membrane filters. Gridded 0.8 or 0.45 μ pore size filters (Millipore) of 17 mm diameter were washed in 0.1 M NH_4Cl to remove traces of K, rinsed repeatedly in distilled water, and dried. In later experiments more reproducible results were obtained using unwashed filters and correcting for the K content of the filters. 0.8 μ pore size filters were used in most of the experiments since they allowed more rapid filtering. In some experiments, notably those involving exposure of the cells to hypertonic solutions, an erratic loss of cell K was observed when the 0.8 μ filters were used. In such experiments use of the 0.45 μ filters gave consistent results.

The filters were held by suction on a specially devised holder which obviated the use of a clamping device. The surface tension exerted by the grid lines on the filter prevented spread of the sample and wash beyond the central part of the filter. With the full suction of a water aspiration pump applied to the filter holder, an aliquot of the culture, usually 1 ml, was carefully delivered onto the filter in such a way that the wetted area was restricted by the grid lines to the central 9 by 9 mm area of the filter. The filter was washed twice, each time with 2 or 3 drops of wash solution. Unless otherwise stated, washing was carried out at room temperature (20 to 23°C) with a sucrose solution slightly hypertonic to the medium in which the cells were grown. Such a wash did not lead to loss of cell K, as shown under Results. For analysis the filters were placed in polyethylene vials, treated with 1 drop of cold redistilled nitric acid, and about 10 minutes later diluted with $LiSO_4$ solution for flame photometry. This analysis gave a measure of the total cell K content per milliliter of culture. $[K]_i$ was then calculated using a value for cell H_2O content determined from the cytocrit, the density of the bacterial pellet determined by $CuSO_4$ flotation (6), the water content of the bacterial pellet, and the extracellular space of the pellet. In

[1] All errors are expressed as the standard error of the mean.

[2] Symbols in brackets represent concentrations in millimoles per liter of medium or cell H_2O. The subscripts i and e refer to the cellular and extracellular compartments respectively.

practice, values of the pellet density, wet to dry weight ratio, and extracellular space determined in the medium under study were used, so that $[K]_i$ could be calculated from a measurement of cell K and the cytocrit. When these two were not determined simultaneously, the required cytocrit values were interpolated from a semilogarithmic plot of cytocrit *versus* time. The filter method was especially useful for studying both cultures with low cell densities as well as rapid changes in cell K content.

Rates of net K uptake measured in some of the experiments have been expressed in pmols/cm^2 sec., using a value for the bacterial membrane area of 6.8×10^4 cm^2 per ml of bacteria, obtained from photomicrographs of dried smears of the organisms (2).

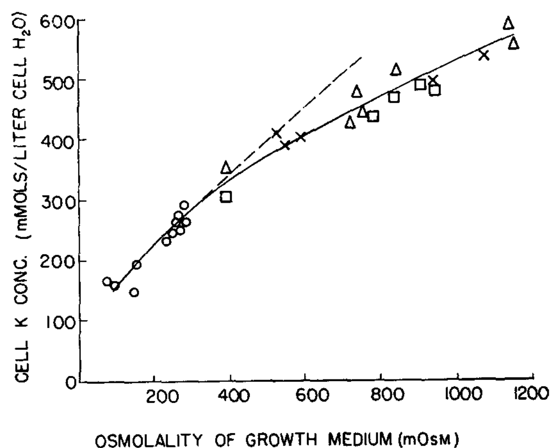

FIGURE 1. Effect of the osmolality of the growth medium on the $[K]_i$ of growing *E. coli*. Each point represents the average of 3 to 10 replicate determinations on cells grown with glucose as substrate. The symbols indicate the agent used to adjust the osmolality: ×, glucose; □, NaCl; △, sucrose; ○, no agent added, standard or dilute medium used. The pH ranged from 6.7 to 7.1. The dashed line is the tangent to the curve over the range of 100 to 400 mOsM.

Freezing points of culture supernatants were measured with the Aminco-Bowman apparatus. NaCl solutions were used as standards and the results were expressed in osmolal units using published values for NaCl solutions at 25°C (7). Since, as discussed under Results, bacteria are readily permeable to glycerol, the effective osmolality of media containing glycerol was approximated by subtracting 15 mOsM (the estimated glycerol concentration in the medium at the time of harvest) from the osmolality as measured by the freezing point depression.

pH was measured in all cultures using the Radiometer model 22 pH meter.

RESULTS AND DISCUSSION

K and Na Content

The effect of the osmolality of the growth medium on the K content of growing *E. coli*, using glucose as the substrate, is shown in Fig. 1. The osmolality is

clearly a major determinant of the K content of these organisms, with nearly a fourfold change in $[K]_i$ observed over the osmolality range studied. The substance used to adjust the osmolality does not appear to be important, since NaCl had the same effect as did osmotically equivalent concentrations of sucrose and glucose. The conclusion that these changes in K content are due to the osmotic pressure difference created by the agents used is supported by experiments with glycerol, which, when added in 0.3 M concentration to the standard medium containing glucose as substrate, had no effect on the $[K]_i$ of these bacteria. Glycerol is known to penetrate bacteria readily (8) and has been reported to be without effect on the K content of another Gram-negative organism, *Salmonella oranienburg* (9).

Growth in media of different osmolality has effects in addition to those on

TABLE I

EFFECT OF OSMOLALITY ON COMPOSITION AND GROWTH OF *E. coli**

Osmolality	Generation time	Dry weight/wet weight‡	$[K]_i$	K contents
mOsM	*min.*		*mmols/liter cell H_2O*	*mmols/gm dry weight*
148	66	0.247±0.002 (8)	150±2 (8)	0.457±0.007 (8)
270	51	0.285±0.003 (8)	246±2 (5)	0.617±0.007 (8)
539	63	0.297±0.002 (12)	399±2 (12)	0.945±0.006 (12)
938	86	0.326±0.002 (8)	495±2 (8)	1.02±0.01 (8)

* Errors are standard errors of the mean; the figure in parentheses indicates the number of determinations.

‡ These data refer to the dry-to-wet weight ratios of the cells. The data obtained using bacterial pellets have been corrected for extracellular water and solute.

K content. As shown in Table I there is an increase in the ratio of dry weight to wet weight, and thus a decrease in the cell water content, as the osmolality of the medium increases. Although this decrease in cell water accounts in part for the increase in $[K]_i$, most of the increase is due to an absolute increase in K content, as seen from the values in the last column of the table, expressed in millimoles per gram dry weight.

In contradistinction to the K content, the cell Na concentration is not dependent on the osmolality of the medium, but is determined by $[Na]_e$. Fig. 2 shows this relationship for cells grown on glucose. The points clustering about a $[Na]_e$ of 150 mM are representative of the many values in this range obtained using both normal media as well as media made hypertonic by addition of glucose or sucrose. Ninety-six determinations of the intracellular Na concentration in cells grown in media whose $[Na]_e$ was maintained essentially constant at 143 ± 1 mM but whose osmolalities ranged from 236 to 1154 mOsM gave an average $[Na]_i$ of 70 ± 1 mmols/liter cell water; the

average $[Na]_i$ from the 18 different media over the above range of osmolalities did not differ significantly from each other. The average ratio of intracellular to extracellular Na concentrations over this wide range of osmolalities is 0.49. The results shown in Fig. 2 are in good agreement with those previously reported using media isotonic with the normal growth medium in which $[Na]_e$ ranged from 0 to 120 mM (1). Table II shows the intracellular Na concentrations and contents in cells grown in media having constant $[Na]_e$.

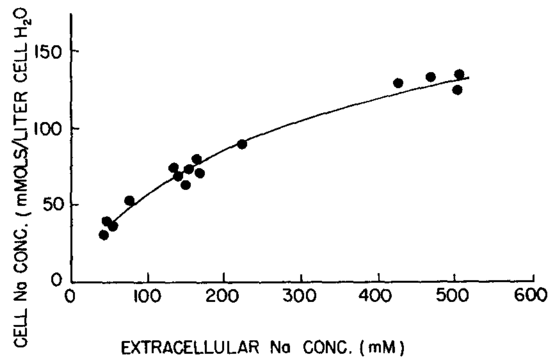

FIGURE 2. $[Na]_i$ as a function of $[Na]_e$. These results are from the same experiments for which values of $[K]_i$ are shown in Fig. 1.

TABLE II

EFFECT OF OSMOLALITY ON INTRACELLULAR Na IN *E. coli**

Osmolality	$[Na]_i$	Na content
mOsM	*mmols/liter cell water*	*mmols/gm dry weight*
270	67±2(8)	0.168±0.006(4)
539	65±2(12)	0.154±0.006(12)
938	69±2(8)	0.141±0.005(8)

* These values are from the same series of experiments shown in Table I. The extracellular Na concentration was 143 mM.

These data are from the same series of experiments shown in Table I. The 270 mOsM medium is the normal growth medium; the hypertonic media were the results of glucose or sucrose addition. As described above, the $[Na]_i$ does not differ significantly at the different osmolalities. However, since the cell water content decreases at higher osmolalities, there is a concomitant decrease in the cell Na content as shown in the last column of this table. Comparison of Tables I and II shows that in going from a medium whose osmolality is 270 mOsM to one of 938 mOsM the increase in K content is 0.40 mmol/gm dry weight whereas the cell Na content decreases by only 0.027 mmol/gm dry weight.

The cation content of *E. coli* is relatively insensitive to changes in growth

conditions other than osmolality and $[Na]_e$. Increasing $[K]_e$ from 2.5 to 27 mM produced a 3 per cent increase in $[K]_i$, in agreement with previous findings (1) that $[K]_i$ is essentially independent of $[K]_e$. The temperature of growth had no effect, since cells grown on glucose in the standard medium at 22 or 30°C had the same cation content as did cells grown at 37°C. Over the pH range from 6.3 to 7.5 in the lactate medium there was no change in $[K]_i$, while in the glucose medium K content averaged 6 per cent lower at pH 6.3 (2 determinations) and 12 per cent lower at pH 5.7 (7 determinations) compared with the K content in a medium of the same osmolality at pH 7.0.

Effects of Other Substrates

Studies carried out using glycerol or lactate as substrates with NaCl or sucrose as the osmotic agent showed that although the osmolality of the medium had a similar effect on cell K content, the $[K]_i$ observed in a medium of given osmolality with glycerol as substrate was slightly less than when glucose was the substrate. Lactate-grown cells had a considerably lower K content in hypertonic media than did glucose-grown cells; in an 850 mOsM medium the $[K]_i$ of lactate-grown cells was approximately 75 per cent of that observed when glucose was the substrate. Conversely, the $[Na]_i$ of glycerol- and lactate-grown cells was higher than that of the glucose-grown cells. In view of the existence of a K-Na exchange mechanism in *E. coli* (2), this reciprocal relationship between $[K]_i$ and $[Na]_i$ is not unexpected.

However, in spite of the quantitative differences among glucose-, glycerol-, and lactate-grown cells, the reasons for which are unknown, the effect of medium osmolality on $[K]_i$ is quite similar to that shown in Fig. 1 and thus appears to be independent of the substrate employed.

Rapid Changes in K Content

The variation of cell K content with the osmolality of the growth medium suggested that abrupt changes in the osmolality might have a rapid effect on K content. An acute loss of K was found to occur when the cells were suddenly exposed to a solution whose osmolality was lower than that in which they were grown. This procedure will be referred to as "downshock," the term used by Britten and McClure (10) in their studies of the effect of osmolality on amino acid pools in *E. coli.* In 14 experiments, cells were collected on filters and washed briefly on the filter with a sucrose or NaCl solution of chosen osmolality. When the osmolality of the wash solutions was somewhat lower than that of the medium in which the cells were grown there was a loss of K, as shown in the left part of Fig. 3. The loss of K was a transient and rapid phenomenon, since a wash as brief as 4 seconds removed as much K as did washes lasting as long as 45 seconds. The intercepts of the curves of Fig. 3 with the 100 per cent line suggest that at 37°C the osmolality of the

wash solution must be approximately 25 to 75 mOsm lower than that of the growth medium to produce a loss of K. The net loss of K becomes progressively greater as the wash solution becomes more dilute. Washing with more concentrated solutions than those shown in the figure produced no loss of K.

The temperature of the wash solution has a significant effect on the loss of K which results from downshock. A comparison of the effects of otherwise identical washes at 2 and at 37°C is shown in the right half of Fig. 3, typical

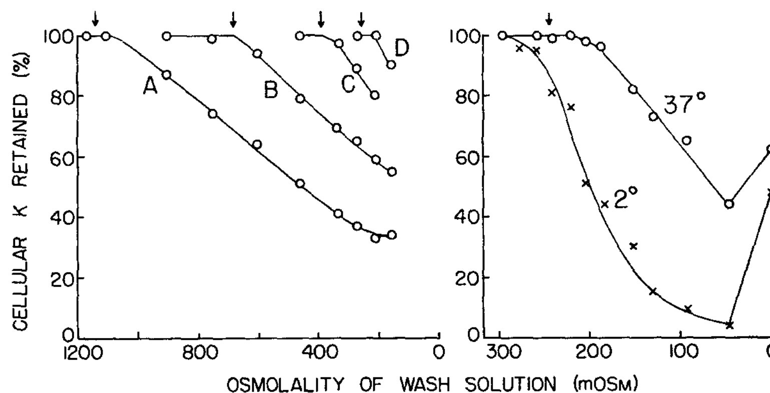

FIGURE 3. Left, retention of K after brief washing of *E. coli* with sucrose solutions of varying concentrations at 37°C. Each curve represents the results in a different medium; the arrow above each curve indicates the osmolality of the growth medium in that experiment. In *D* the standard medium was used; sucrose was added to the growth medium in the other experiments. Since the bacteria were growing, the cell K found after washing was divided by the total cell K at the time of sampling as determined from a semilogarithmic plot of cell K against time. Right, effect of the temperature of the wash solution on K retention. Bacteria from a culture growing at 37°C, whose osmolality is indicated by the arrow, were washed with NaCl solutions varying from 0 to 296 mOsm at either 37 or 2°C.

of 6 experiments of this type. Not only is considerably more K removed by the cold wash, but even an isosmotic wash at 2°C removed some K. It was further observed that dilute NaCl solutions produced a greater loss of K than did a wash with distilled water. This suggests that a sizeable fraction of cell anions is not removed by downshock, and, as a result, part of the cell K can only be removed by exchange with another cation.

Similar effects of downshock have been reported in investigations of the K content of *S. oranienburg* (11) and of amino acid pools in *E. coli* (10). Since a wide variety of substances has been reported to be removed by downshock, it appears that the loss is relatively non-specific, presumably occurring through small holes or enlarged pores produced by the tension imposed on the cell wall and membrane by the hydrostatic pressure which must develop within the cell in order to balance the difference in water activities across the mem-

brane. The influence of the temperature of the wash solution on the loss of cellular contents resulting from downshock does not appear to have been stressed previously. If our results on K retention are typical of the effects of downshock, a cold wash solution must be at least 50 mOsM hypertonic to the growth medium to avoid loss of cellular constituents. The effect of temperature may explain the observation of Kessel and Lubin that cold washes resulted in a loss of the proline pools of *E. coli* (12). The greater loss of cellular contents resulting from a wash with a cold solution may also explain the lethal effect of sudden chilling of Gram-negative organisms in dilute media (13).

Quite the opposite effect was observed when the cells were subjected to a

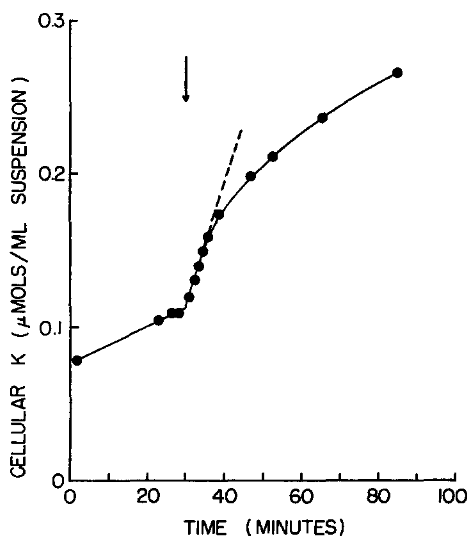

FIGURE 4. Effect of a sudden increase in osmolality on K content of *E. coli* growing in 1.2 mM K medium. At the arrow the osmolality was increased from 202 to 635 mOsM by adding a concentrated solution of glucose in growth medium. The K content of the pre-upshock samples has been multiplied by 0.8 to correct for the dilution produced by upshock.

sudden increase in the osmolality of the medium. Such a change, which will be referred to as "upshock," resulted in a rapid uptake of K by the cells. Upshock was produced by adding the desired osmotic solute as a concentrated solution in growth medium, thus changing the osmolality without changing the concentration of the other constituents of the medium. Glucose was used as the osmotic solute in most upshock experiments. Qualitatively similar effects were observed in a few experiments in which sucrose or NaCl was used as the osmotic solute. Fig. 4 shows the effect of upshock on cellular K content in one experiment, typical of the 39 upshock experiments performed. The increase in cell K content commenced within 10 sec. of upshock and the initial rate of this rapid K uptake was found to be a function of $[K]_e$. This dependence was studied by measuring the initial rate of K uptake produced by an identical degree of upshock in 11 otherwise identical media whose $[K]_e$ ranged from 0.17 to 8.0 mM. The initial rate was calculated from

the slope of the straight line fitted to the beginning of the rapid K uptake curve, as indicated by the dashed line in Fig. 4. This initial rate was corrected by subtracting the rate of net K uptake accompanying growth which was determined in each experiment from the rate of increase of cell K content prior to upshock. The results show that the initial rate of K uptake is a saturable function of $[K]_e$, as seen in Fig. 5. The maximum rate of K uptake, calculated from a Lineweaver-Burk plot of the data, is 10 pmols/cm² sec., and the uptake rate is half-maximal when the $[K]_e$ is 1 mM.

The dependence of K content on the osmolality of the medium suggests that K plays a major role in maintaining the internal osmolality of these bacteria,

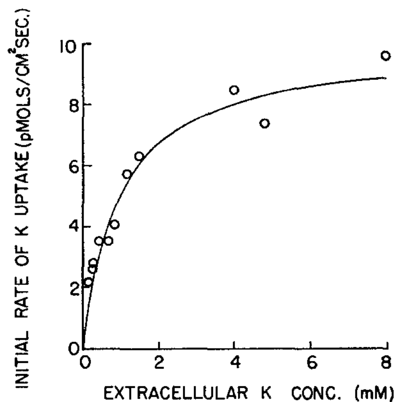

FIGURE 5. Initial rate of K uptake produced by upshock in 11 media whose $[K]_e$ ranged from 0.16 to 8.0 mM. The curve is the theoretical one for a process obeying Michaelis-Menten kinetics which has a maximal rate of 10 pmols cm² sec. and whose rate is half-maximal when $[K]_e$ is 1 mM. The experimental conditions were identical to those described in Fig. 4 except for the $[K]_e$.

and that cell K is in large measure osmotically active. Similar results, indicating a relationship between K content and medium osmolality, have been reported for *E. coli* by Ørskov (15) and for *S. oranienburg* by Christian (9). Both these authors measured K content after resuspending bacteria in a buffer which did not allow growth and whose osmolality was greater than that of the growth medium, so that their experiments indicate the effect of upshock on non-growing cells.

The uptake of K must be accompanied by the movement of another ion to preserve electroneutrality. Net K uptake in upshock cannot be attributed to an exchange of K for cellular Na, since there is little (see Tables I and II) change in cell Na content under these conditions. The possibility that K is taken up with PO_4 was excluded by measurements of the P contents of bacteria before and after upshock in one experiment similar to that shown in Fig. 4. The P content after upshock fell 0.03 mmol/gm dry weight while K content rose 0.40 mmol/gm dry weight. Furthermore, recent studies on the effect of medium osmolality on the intracellular Cl content, carried out in this laboratory (14), indicate that the increased $[K]_i$ cannot be balanced by this anion. Although no measurements were made to exclude the possibility that the

other anion in the medium, SO_4, accompanied the uptake of K, a quantitatively significant uptake of this anion is unlikely for reasons which have been previously discussed (2). Since it is unlikely that net K uptake is accompanied by the simultaneous uptake of anion(s) from the medium we must invoke the extrusion, by the cell, of a cation other than Na in order to satisfy the restriction of macroscopic electroneutrality. This cation must be available in large quantity and must be extruded at a sufficiently rapid rate to balance a rate of net K uptake which may be as high as 10 pmols/cm^2sec. It is well known that *E. coli* rapidly acidifies the growth medium by the secretion of the acid products of fermentative metabolism. We have shown that the rate of H secretion by dense suspensions of *E. coli* averages 17.3 pmols/cm^2sec. (2). It is difficult to measure the rate of H secretion in the light, highly buffered suspensions employed in these experiments. However, if the rate of H secretion in the present studies is only 60 per cent of that observed with dense suspensions, it would still suffice to account for the maximal rate of K uptake. Our previous demonstration of a K-H exchange mechanism capable of bringing about a rapid net uptake of K by *E. coli* (2) together with the above arguments suggests that a similar cation exchange process is responsible for K uptake following upshock.

It has been known for many years that exposure of non-growing Gram-negative bacteria to concentrated solutions of non-permeant solutes results in a decrease in cell volume with the resultant retraction of the plasma membrane from the rigid cell wall. This process, called *plasmolysis*, has been extensively employed for the study of the passive permeability of bacterial membranes to a wide variety of solutes (16). Ørskov was the first to demonstrate that plasmolysis of non-growing *E. coli*, resulting from exposure to concentrated NaCl or sucrose solutions, is rapidly reversed following the addition of K to the suspension (15). Thus the K taken up by the cells both in the present experiments and in those reported by Ørskov must not only be osmotically active but it must also increase the total intracellular osmolarity. If K were simply taken up by the cell in a one for one exchange with another osmotically active cation, deplasmolysis would not result. If, on the other hand, K were taken up in exchange for metabolically produced H which is either rapidly secreted or else bound within the cell by the high cellular buffer capacity, an increase in intracellular osmotic activity would result. The slope of the initial portion of the curve of $[K]_i$ *versus* osmolality (dashed line, Fig. 1) indicates that the $[K]_i$ increases approximately 100 mM for every 180 mM increase in medium osmolality. Assuming that the osmotic coefficient of cellular K is 0.9 (approximating that of KCl in free solution over the concentration range of 0.1 to 0.8 M (7)), this relationship indicates that the increase in $[K]_i$ raises the cellular osmolality by half as much as the increase in medium osmolality. Since each mole of K accumulated must be

associated with an equivalent of intracellular anion, the increase in cellular osmolality would be equal to the increase in medium osmolality if the anions accumulated with K were univalent and osmotically active. If the mechanism of K uptake is in fact an exchange for cellular H, the anions normally secreted with H, such as acetate, formate, and lactate, may well be the ones that are retained. Over the lower range of osmolalities, the variation in cell osmolality which would be produced by the accumulation of K and accompanying univalent anions, agrees well with the observation that cellular osmolality parallels that of the medium (17). In media of high osmolality the increase in $[K]_i$ is considerably less than half of the increase in osmolality. Although those other solutes which contribute to the maintenance of the internal osmolality in these concentrated media have not been identified, it is quite possible that many neutral molecules such as proline (10) participate significantly in this process.

CONCLUSIONS

The present results indicate that:

1. Under a variety of conditions including those suitable for optimal growth, the osmolality of the growth medium is a major determinant of the cell K content.
2. The growing cell responds to abrupt changes in the surrounding osmolality with rapid changes in cell K content in the direction necessary to minimize the osmotic difference.
3. The conclusion that the bulk of the intracellular K in *E. coli* exists in an unbound, osmotically active form, though not directly established, is strongly suggested.

The ability of *E. coli* to maintain its internal osmotic activity equal to, or somewhat greater than that of the surrounding environment, through regulation of its cell K content, is of profound functional significance for an organism which may be subjected to a wide range of growth conditions. It assures that the plasma membrane will be in close contact with the surrounding cell wall; a relationship which is essential for cell wall synthesis and bacterial division. Weiden (18) has demonstrated that, in a K-limited medium, growth of *E. coli* ceases only after virtually all the medium K has been depleted, at which time the ratio of intracellular to extracellular K concentrations approximates 10^6 to 1. This highly efficient K transport mechanism is ideally suited for the purpose of osmoregulation in an organism which is not very exacting about the media in which it will grow.

The loss of cell K which results from downshock is accompanied by an efflux of other intracellular constituents and a depletion of intracellular amino acid pools (19, 10). This process therefore appears to be relatively non-specific and is probably related to the loss of intracellular constituents which is

observed when growing cells are suspended in distilled water (19). The mechanism by which the intracellular K concentration is increased following upshock, as the result of net K transport against a large concentration difference, remains unknown. One possibility is that exposure to hypertonic media results in a decreased passive efflux or "leak" of K secondary to a decrease in the passive permeability of the membrane to K. If part of the K efflux is the result of restricted diffusion through aqueous channels or "pores" whose volume and linear dimensions decrease with increasing external osmolality (*i.e.* they behave like osmometers), an increase in the osmolality of the growth medium, in the presence of constant carrier-mediated K influx, would result in a new, increased steady-state intracellular K concentration. Although this model can explain the relationship between cell K concentration and medium osmolality in growing cells (Fig. 1), it does not satisfy the kinetics of K uptake following upshock (Fig. 4). We have previously demonstrated that under steady-state conditions, the K influx averages 1 to 2 pmols/cm^2sec. and is independent of the extracellular K concentration over the range of 0.06 to 14 mM (20, 21). The initial rate of net K uptake following upshock is a saturable function of the extracellular K concentration and is often more rapid than the above steady-state influx. Therefore a change in the efflux process alone cannot explain these results. Instead one must postulate that sudden exposure to hypertonic media activates a process of net K influx which is transient and appears to involve an exchange of K for intracellular H. Whether this influx process is mediated by an "osmotically sensitive" carrier system or whether it is the result of some other property of the membrane which is sensitive to membrane tension cannot be further clarified at this time.

Dr. Epstein is a Training Fellow of the Basic Sciences Research Training Program, United States Public Health Service (2G-466) of the Department of Medicine, New York University Medical Center and is at present on leave of absence from New York University.
Dr. Schultz was a Given Foundation-National Research Council Fellow in Academic Medicine at the time this work was performed.
The authors wish to express their appreciation to Dr. A. K. Solomon for his suggestions and comments.
This work was supported in part by the United States Atomic Energy Commission and the National Science Foundation.
Received for publication, February 12, 1965.

BIBLIOGRAPHY

1. SCHULTZ, S. G., and SOLOMON, A. K., *J. Gen. Physiol.*, 1961, **45,** 355.
2. SCHULTZ, S. G., EPSTEIN, W., and SOLOMON, A. K., *J. Gen. Physiol.*, 1963, **47,** 329.
3. KING, E. J., *Biochem. J.*, 1932, **26,** 292.
4. FISKE, C. H., and SUBBAROW, Y., *J. Biol. Chem.*, 1925, **66,** 375.
5. HORECKER, B. L., MA, T. S., and HAAS, E., *J. Biol. Chem.*, 1940, **136,** 775.

6. Phillips, R. A., Van Slyke, D. D., Hamilton, P. B., Dole, V. P., Emerson, K., Jr., and Archibald, R. M., *J. Biol. Chem.*, 1950, **183,** 305.
7. Robinson, R. A., and Stokes, R. H., Electrolyte Solutions, New York, Academic Press, Inc., 2nd edition, 1959.
8. Fischer, A., Vorlesungen über Bakterien, Jena, Fischer Verlag, 2nd edition, 1903.
9. Christian, J. H. B., *Australian J. Biol. Sc.*, 1955, **8,** 490.
10. Britten, R. J., and McClure, F. T., *Bact. Rev.*, 1962, **26,** 292.
11. Christian, J. H. B., *Australian J. Biol. Sc.*, 1958, **11,** 538.
12. Kessel, D., and Lubin, M., *Biochim. et Biophysica Acta*, 1962, **57,** 32.
13. Strange, R. E., and Dark, F. A., *J. Gen. Microbiol.*, 1962, **29,** 719.
14. Wittes, R., unpublished observations.
15. Ørskov, S. L., *Acta Path. Microbiol. Scand.*, 1948, **25,** 277.
16. Mitchell, P., and Moyle, J., *Discussions Faraday Soc.*, 1956, **21,** 258.
17. Knaysi, G., Elements of Bacterial Cytology, Ithaca, Comstock Publishing Co., 2nd edition, 1951.
18. Weiden, P., Senior Thesis, Harvard College, 1963.
19. Mitchell, P., and Moyle, J., *J. Gen. Microbiol.*, 1954, **10,** 533.
20. Schultz, S. G., Epstein, W., and Goldstein, D. A., *J. Gen. Physiol.*, 1962, **46,** 343.
21. Epstein, W., and Schultz, S. G., *J. Gen. Physiol.*, in press.