ION TRANSPORT IN SINGLE CELL POPULATIONS

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The generality that K is the dominant intracellular cation holds as well for populations of single cells as for organized tissues such as nerve and muscle. This can be seen from Table I, which gives typical values of the ion concentration in several types of single cells whose usual environment contains a great deal of Na and very little K. The red cells of the dog are included as an example of a species in which the normal order is reversed and the usual sharp concentration differences across the membrane are absent. Although most of the cells in the Table are mammalian, the generality may also be applied to other types of cells, such as the bacterium, *Escherichia coli,* which is characterized by a particularly high intracellular K. Single-cell populations appear to contain appreciably more Cl than nerve and striated muscle, this ion making up 25% or more of the total cell anions. The remainder of the intracellular anions, whose amount is fixed by the triple restraints of electro-neutrality, osmotic balance, and Gibbs-Donnan steady state, consists almost entirely of non-diffusible components.

Strict osmotic requirements are imposed on cells bounded by distensible walls. The total cation content of the cells is regulated so that the intracellular diffusible ionic concentration is lower than extracellular by an amount which just balances the non-diffusible components. If this were not the case, osmotic pressures of several atmospheres would quickly develop and the cells would burst.

The permeability of cellular membranes to ions is markedly dependent upon the sign of the permeant ion. As a consequence, transmembrance diffusion potentials are produced which reach values of the order of 100 millivolts. Such potential differences can be measured accurately by intracellular microelectrodes in organized tissues. On the contrary, individual cells of the type included in Table I are as yet immune from direct measurement, so that potential differences are calculated from the distribution of a passively transported ion, commonly Cl. Since typical cellular membranes are only some 100 Å thick, each millivolt of potential difference corresponds to a field strength of 1000 volts/cm., a field which must surely exert an important influence on reactions in the neighborhood of the cell surface.

TABLE I
ION CONCENTRATIONS IN SOME SINGLE-CELL POPULATIONS

	K	Na	Cl	Reference
	mM/liter cell H_2O			
Red Cells				
man	130	16.8	78.8	(1, 2, 3)
dog	8.2	113	75.0	(4, 5)
Leucocytes*				
man	146	..	..	(6)
guinea pig	113	48	..	(7)
HeLa Cells	161	25	44	(8)
Ascites Tumor	134	50	64	(9)
E. Coli (K-12)	211	55	..	(10)

* Values given in mM/liter cells.

Evans and his colleagues have demonstrated that the genetic constitution of the animal sets the K concentration in the red cells of sheep and goats (11, 12, 13). Sheep from the Scottish highlands are characterized by red cells with a high K concentration, while those from the English lowlands have less than half as much K; this K concentration difference is determined by a single gene. Thus the ion transport mechanism, in common with all enzyme regulated processes, is seen to be subject to genetic control.

I should now like to discuss the details of ion transport in *E. coli,* a cell type in which it is possible to add the weapon of induced mutations to the armamentarium customarily employed in ion transport studies. Two important features of cellular architecture in this bacterium are illustrated in Figure 1, an electron microscope picture taken by Kellenberger and Ryter (14). The cytoplasm is bounded by a 60–80 Å membrane which is in turn encased within a 150 Å cell wall. This wall, whose major constituents are lipo-polysaccharides and proteins, provides structural stability and enables the bacterium to withstand osmotic pressures of many atmospheres, a feature which sets it off from the other single cell populations that have been discussed.

As a first step in our experiments on *E. coli,* which were carried out in collaboration with Dr. Stanley G. Schultz, bacterial pellets were obtained by centrifugation at 11,000 g in specially designed equipment. Figure 2 shows the extracellular space in these pellets, as evaluated both by inulin and albumin, and the water content as measured from the wet and dry weights of the pellets. Intracellular cation concentrations were determined in a flame photometer (15) on extracts prepared from the weighed pellets, and have been corrected for the ion content of the medium trapped in the pellet. The intracellular K and Na concentrations depend strikingly on the age of the culture, as shown in Figure 3. The decline in intracellular K is accompanied by a fall in pH as the cell population rapidly increases and the products of

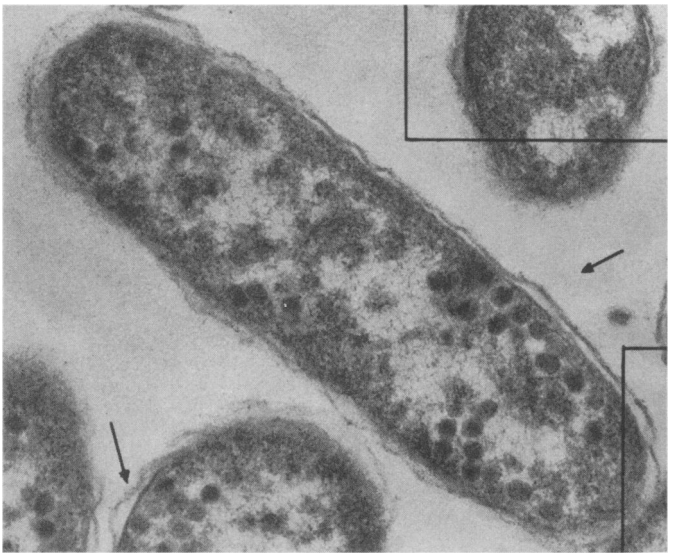

FIGURE 1 Electron micrograph of phage infected *E. coli* taken from Kellenberger and Ryter (14). Magnification 50,000 times.

bacterial metabolism accumulate in the medium. Control experiments show that the restoration of the pH to the normal value is sufficient to restore the cell's ability to maintain its initial high intracellular K concentration. After 48 hours, when the cells have stopped dividing and remained in the stationary phase for a long time, the preparation appears to have aged irreversibly since the cells are no longer able to restore the initial ion concentration even when suspended in fresh medium.

We next turned our attention to the relationship of intracellular cation concentration to extracellular environment. In the stationary phase, as shown in Figure 4, the cells cannot maintain an appreciable concentration difference across the cellular membrane. The K concentrations are identical inside and outside. The Na data may be fitted by a line which characterizes the equilibrium cation distribution for a potential difference of only 6 millivolts (cell negative). Since this potential difference is very slight, it would appear that the stationary state ion distribution is primarily passive for both ions. On the contrary, the logarithmic phase distributions are far from equilibrium. At an extracellular K concentration of 0.05 mM, the lowest studied, the cell is able to maintain a K concentration ratio of 3700 to 1. As the medium K concentration is increased, the intracellular K remains at essentially the same high level. By contrast, the intracellular Na concentration in the logarithmic phase is directly dependent on the extracellular concentration and is maintained at a considerably lower level than extracellular Na.

Cells harvested in the stationary phase are low in K and high in Na. When such cells are placed in fresh medium, the K rises rapidly and reaches its plateau value within an hour; meanwhile, the Na falls, as shown in Figure 5. From the optical density data in the top curve in Figure 5, it can be seen that K uptake does not de-

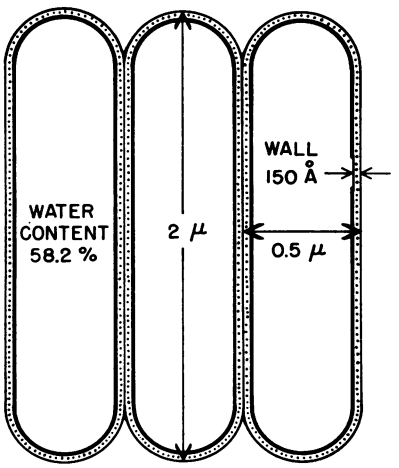

FIGURE 2 Water content, extracellular space and typical dimensions in *E. coli.*

EXTRACELLULAR SPACE (INULIN, ALBUMIN)
0.19 ± 0.01 ML. / G. WET WEIGHT

pend on an increase in the number of cells; nor indeed does it depend on protein synthesis, as shown by the control experiments on cells treated with chloramphenicol, a potent inhibitor of bacterial protein synthesis. The ability of K depleted cells to reaccumulate K is widespread. Thus, for example, cellular K rises and Na falls when cold stored red cells whose K has been depleted are incubated at 37° C. in the presence of glucose (16). These similarities in ion transport between dissimilar cell types lead to the inference that important general qualities characterize the mechanism of ion transport.

Metabolic energy is required for the uptake of K by *E. coli* as illustrated in Figure 6 which shows that cells without glucose cannot accumulate K normally. The fraction of K uptake which remains is attributed to small amounts of glucose transferred to the fresh culture with the unwashed cells or to intracellular energy stores which have not yet been consumed. The dependence on metabolic energy is confirmed by the experiments in which iodoacetate reduces K uptake virtually to zero. DNP (di-nitro-phenol) has a similar effect which indicates that high energy PO_4

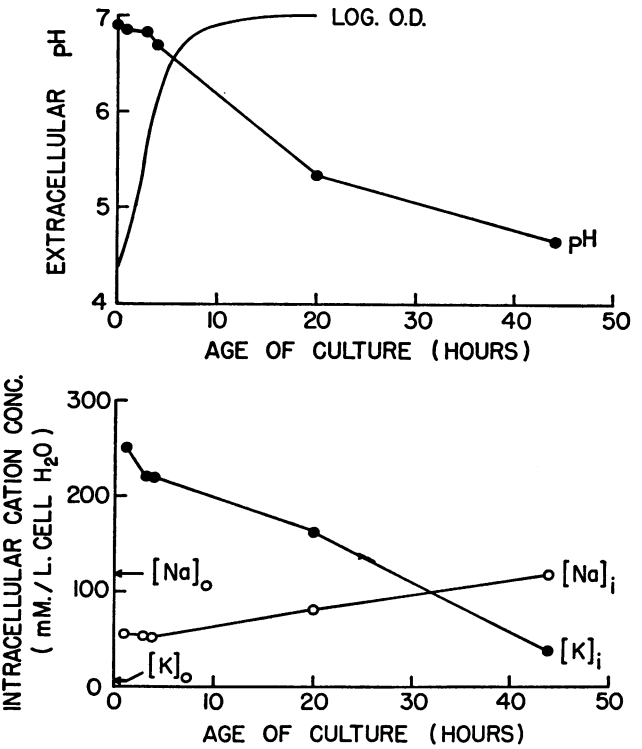

FIGURE 3 Effect of age of culture on cation concentration in *E. coli*. The upper figure gives optical density at 660 mμ, which shows the rate of cell division, and pH. The bottom figure gives intracellular cation concentrations. The arrows indicate the Na and K concentrations in the suspending medium.

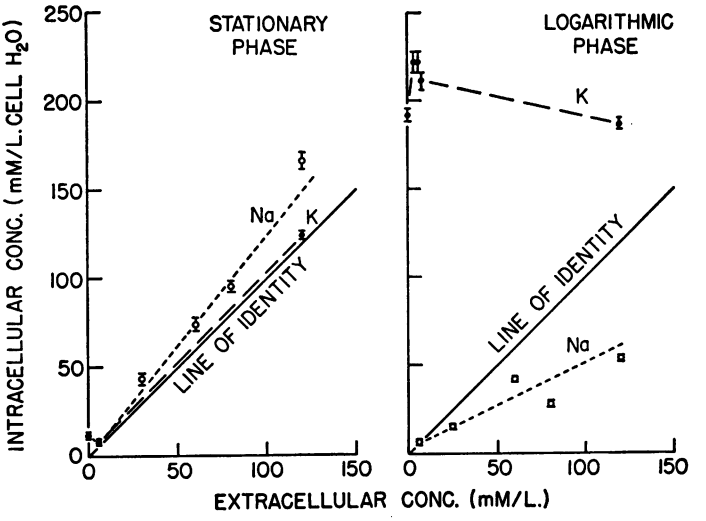

FIGURE 4 Intracellular Na and K concentrations in *E. coli* as functions of the Na and K concentrations in the growth medium.

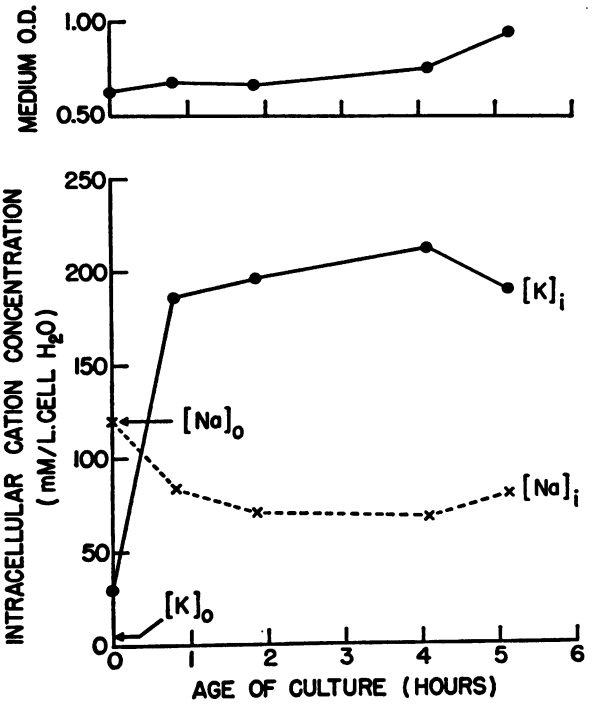

FIGURE 5 Intracellular cation concentrations during the initial 5 hours of growth when K poor, Na rich cells are incubated in a medium containing 5 mM K.

groups are essential to the process. These inhibitors produce comparable results on the extrusion of Na from the cell.

Active transport is evidenced when net transport of an ion can be shown to take place up an electrochemical potential gradient (17). In the logarithmic phase of growth, *E. coli* has the power simultaneously to accumulate K and to extrude Na, in both instances against a considerable concentration gradient. Although the potential difference across the *E. coli* membrane has not been measured, it is evident that active transport is required to move at least one of the two major cations in *E. coli* since they both bear the same charge and are transported in opposite directions. The two limiting cases are illustrated in Figure 7. Suppose the potential difference were such as to balance the Na concentration gradient exactly; the net transport of K inwards would have to be active since the combined electrical and chemical potential difference would come to 115 millivolts, a most significant barrier. A symmetrical argument may be made for Na in the case in which K is transported passively. This conclusion that at least one of the transport processes is active is in accord with the dependence of the ion transport system on metabolic energy, presumably mediated through high energy phosphate bonds.

Figure 7 also provides a quantitative approach for an investigation of the question of ion binding as a possible alternative mechanism leading to the transport of ions across cellular membranes, a suggestion put forward in the case of *E. coli* by Roberts and his colleagues (18, 19) and for muscle by Troshin (20) and Harris (21), among others. If Na transport across the membrane is passive, the interior of the cell will be 21 millivolts positive to the medium. Passive distribution of K between medium and cells would then require an intracellular K concentration of 2.3 mM. Thus, 99% of the K would have to be bound—specifically, 209 of the 211 mM K within the cell. Any binding of Na would only make the requirements for K more stringent.

Studies on the swelling and shrinking of protoplasts in our laboratory (22) have demonstrated that protoplasts isolated from cells in the logarithmic phase of growth reach a volume equal to that of the intact cells at a medium concentration equiva-

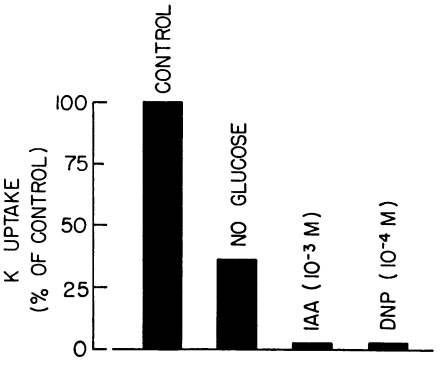

FIGURE 6 The effect of glucose deprivation and metabolic inhibitors on the net uptake of K in one hour following the incubation of K poor, Na rich cells in a medium containing 5 mM K.

lent to 300 mM NaCl, in agreement with values given by Mitchell and Moyle (23) and Knaysi (24). This figure also agrees well with the 280 mM total concentration of Na and K that we have measured during this phase of growth. If 209 mM of K were removed from participation in osmotic phenomena by binding, very large amounts of an as yet unidentified cation would have to be demonstrated within the cell. The expected absorption of small amounts of K on the membrane and in the cell wall does not, of course, affect the general nature of the conclusion that ion binding within the cell is not the primary explanation of K accumulation.

The argument may be put forward even more conclusively in the case of the red cell. Here the potential difference, though not yet measured surely, can be estimated from the distribution of Cl and HCO_3 as being 9 millivolts, cell negative. Passive distribution of K would require that 124 mM of the 130 mM within the cell must be bound. Since these cells are not contained within a rigid wall, their intracellular osmotic pressure is known to be equivalent to 155 mM Nacl. The total Na and K content is 142 mM which agrees well with the total osmolarity. If 124 mM of K were removed by binding, this would leave an imposing deficit to be accounted for by a wholly unknown cation, though the contents of this cell have now been

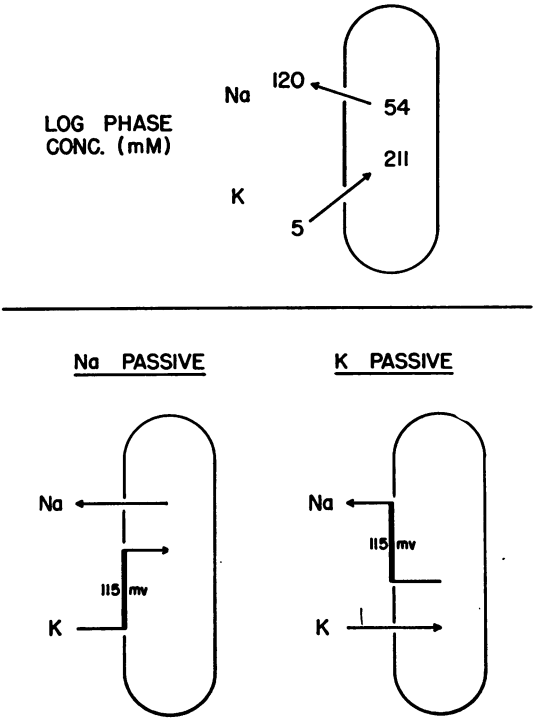

FIGURE 7 Schematic drawing demonstrating the active nature of the net ion transport for at least one cation.

thoroughly studied. Furthermore, the principal intracellular indiffusible compound, hemoglobin, present at a concentration of 7 mM, has been shown by Battley and Klotz (25) not to bind either Na or K. Recently, experiments to examine the question of red cells ion binding directly have been carried out by Goldstein (26) in our laboratory using electrodes constructed from the Na and K selective glasses developed by Eisenman, Rudin and Casby (27). Preliminary results indicate that the Na and K activity of concentrated human red cell hemolysates is not different from that in free solution of the same Na and K concentration. Thus, ion binding can not explain the accumulation of K within human red cells.

The question now arises as to whether the membrane which bounds the cell is also the seat of the active transport process. Experiments with the red cell are best suited to answer this question since it is possible to prepare red cell membranes which have been leached of their intracellular contents and yet remain osmotically stable. Such preparations of membranes, called ghosts, have been shown by Gardos (28) to be able to accumulate K provided ATP is present as the sole substrate. Similar findings have been made by Hoffman (29) on the extrusion of Na from human red cell ghosts. Hoffman finds that ATP may be replaced by other substrates only if ADP is present, so that ATP may be synthesized by the ghosts. It is characteristic of these ghost preparations that the ATP must be introduced during hemolysis, so that it is present within the cell. These findings lead not only to the conclusion that red cell ion transport may be driven by ATP alone, but also that ion transport is a property of the cellular membrane, independent of the normal constituents of the cytoplasm. Furthermore, in these systems, cytoplasmic ion binding has been totally excluded as a possible explanation of ion transport.

With the evidence already accumulated, it is possible to formulate a working hypothesis for ion transport in single cellular membranes, along lines previously put forward by many other authors (1, 30, 31). The hypothesis must account for the following features which are common to most single membrane transport systems:

1. An alkali cation is transported against an electrochemical potential gradient. This usually involves either the transport of K into the cell or Na out, or both.
2. The process is specific for the ion transported. Na is normally not carried by the K system and vice versa.
3. Cations can leak out of cells passively through pores in the membrane.
4. Exchange diffusion can take place. A labelled ion outside of the cell can exchange for an unlabelled one inside in a process that consumes essentially no energy.

The hypothesis shown diagrammatically in Figure 8 conforms to the above requirements. Species X and Y are confined to the membrane. Due to the lipoid nature of the membrane only the uncharged species, KX and Y, may diffuse through

the membrane, the charged species X^- being confined to the two membrane faces. All reactions are considered to be reversible.

At the outer face, K^+ combines with X^-, which has a specific affinity for K. The neutral molecule diffuses down its concentration gradient through the membrane until it reaches the inner face where the complex dissociates yielding K^+ to the cell contents. X^- is immediately metabolised to Y. The rapid disappearance of the carrier at the inner face is essential since otherwise the intracellular K would leak back as fast as it entered the cell. The particular counter-ion which is involved in the transfer of X^- to Y is unspecified. Y diffuses down its own concentration gradient to the outside face where it is remetabolised to X^-. The link to metabolic energy involves either the reaction $X^- \rightarrow Y$ at the inner face, or the converse reaction at the outer face.

Such a hypothesis conforms to the general requirements. It can transport ions up an electrochemical potential gradient using metabolic energy. The specificity is inherent in the reaction $K^+ + X^- \rightarrow KX$. The leak takes place through the pores in

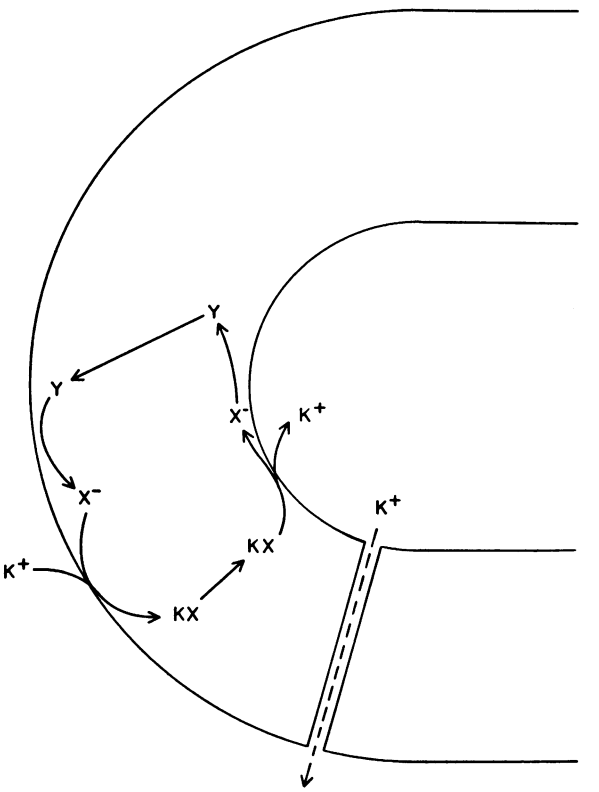

FIGURE 8 Schematic drawing of working hypothesis for ion transport in a single-cell membrane. The arrows have been drawn to indicate the directions of net movement; in the hypothesis the reactions are all considered reversible.

the cellular membrane which have been shown to have an equivalent pore radius in the range of 3–4 Å in tissues as diverse as red cells (32, 33, 34), nerve (35), intestine (36), HeLa cells (37), and *E. coli* (38). An equivalent pore radius of this dimension is large enough to allow passage of a hydrated K ion, whose rate of leak through the pore is probably determined by the charge barrier within the pore. Exchange diffusion can take place through the complex KX which is free to diffuse within the membrane fabric. Such a working hypothesis is valuable because it points out the two most important lacunae in our knowledge: the identity of the carrier molecule X, and the detailed mechanism by which metabolic energy is funneled into the process.

Although the nature of the counter ion that is returned when species Y moves from inside to outside the membrane is not specified in the present hypothesis, many arguments have been put forward that suggest it to be Na. The suggestion that Na and K transport are linked in mammalian red cells, put forward initially by Harris and Maizels (39), has been supported by a number of authors (16, 40). The most salient argument is that of Glynn (40) who has shown that, as the extracellular K is decreased, the energy-requiring K influx and Na efflux decrease in parallel, until, in the absence of extracellular K, Na efflux ceases. The experiments of Glynn suggest that there is a 1:1 linkage between the K influx and Na efflux, but other experiments, notably those of Post and Jolly (16) suggest that the ratio may be different from unity. Of course, a 1:1 ratio fits very well with the notion that Na is the counter ion for the return of Y to the outer face. A similar dependence of Na transport on K concentration has been shown in nerve and muscle (41, 42).

In this respect, the results with *E. coli* differ from those with red cells, for Na extrusion does not appear to be linked to K uptake. Thus, NaF, at a concentration of 30mM, inhibits Na extrusion completely, whereas it reduces K uptake by only 20%. Similarly, Na extrusion is essentially unchanged when the pH of the suspending medium is shifted from 4.5 to 8.0, whereas the same shift causes an 8 fold rise in the net K accumulated by the cells in a one hour period. The independence of movement of the two cations may be related to the high rate of acid production by *E. coli*. The efflux of H ions is much greater than would be required to balance K uptake exactly so that it would be possible for H to serve as counter ion for the bacterium. This would lead to the inference that the selectivity of the outward carrier to the counter ion is much less than the selectivity of the inward carrier to K, and might thus account in part for the varied ratios that have been observed between Na and K transport.

Though the identity of the carrier remains a mystery in spite of several ingenious suggestions (43, 44, 45, 46), progress has been made towards an understanding of the essential metabolic requirements. The experiments of Gardos (28) and Hoffman (29) demonstrate that ATP, in addition to those enzymes present in the cellular membrane, is sufficient to energize the transport mechanism in red cells. Further

support for the unique role of ATP comes from another source. Skou (47) has found that crab homogenates possess an ATP-ase activity that has many characteristics in common with the ion transport system. In particular, Skou's ATP-ase is inhibited by the cardiac glycoside, ouabain, which is a specific inhibitor of ion transport systems in red cells (48) and other systems. Furthermore, this nerve ATP-ase appears to be located in the membrane and is activated by Na and K. Post (49), Tosteson, Moulton and Blaustein (50, 51), and Dunham and Glynn (52), have investigated the properties of a red cell membrane ATP-ase which appears to have properties similar to those initially described by Skou. The concentrations of Na and K which give half maximum activation of this enzyme correspond very closely to the concentrations which give half maximum transport for each ion. The cardiac glycoside concentration which inhibits the ion transport by 50% also inhibits the ATP-ase activity by 50%. Most convincingly, Tosteson, Moulton and Blaustein have assayed the cardiac glycoside sensitive ATP-ase activity in sheep red cell membranes, using cells from both high and low K sheep (50). They have found that the membrane ATP-ase activity is quantitatively related to the ion pump in these cells. In the high K cells, the K influx pump runs 3–4 times faster than in the low K cells; the cardiac glycoside sensitive ATP-ase is also 3–4 times as active.

We have also studied K transport in *Pseudomonas,* a contaminant which inadvertently appeared on our plates during our search for an *E. coli* mutant with a defect in the ion transport system. K influx in *E. coli* and *Pseudomonas* has been compared in experiments in which cells harvested in the logarithmic phase of growth are suspended in a medium containing 5 mM K, a level which maintains the intracellular K concentration in the steady state. Figure 9 shows that K uptake by *Pseudomonas* is much less than that by *E. coli.* As shown in Figure 10, *Pseudomonas* in the early phase of growth in a 5 mM medium is able to maintain an intracellular K concentration less than half that of *E. coli,* possibly as a result of this lower K uptake. The observation that *Pseudomonas* loses its intracellular K less rapidly than

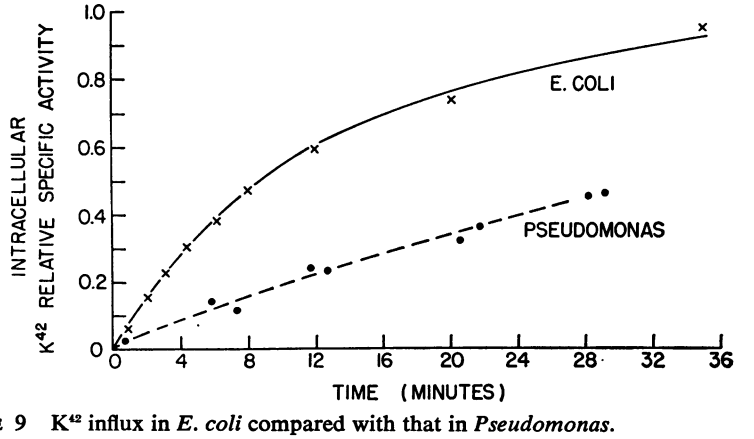

FIGURE 9 K^{42} influx in *E. coli* compared with that in *Pseudomonas.*

E. coli may be related to the lower rate of H production. The metabolic rates also differ, *Pseudomonas* having a division time of 110 minutes as compared to 60 minutes for *E. coli.*

Though the K influx is sharply reduced in *Pseudomonas,* it remains sufficient to keep the intracellular K above the extracellular K over the concentration range from 0.05 mM to 100 mM K, as shown in Figure. 11. The difference between these two bacteria is particularly marked at the lowest K concentration studied, 0.05 mM, where *Pseudomonas* is only able to reach an intracellular K concentration of 40 mM as compared to 187 mM for *E. coli.*

In the course of our studies of net K uptake by *E. coli,* we found that PO_4 is required, K uptake falling almost to zero in the absence of added PO_4, as shown in Figure 12. A similar situation does not obtain in human red cells, since normal K influx does not require extracellular PO_4. Molybdate, which Rothstein and Meier (53) have shown to inhibit all the surface phosphatases in yeast, also inhibits K influx in this bacterium, dropping it virtually to zero.

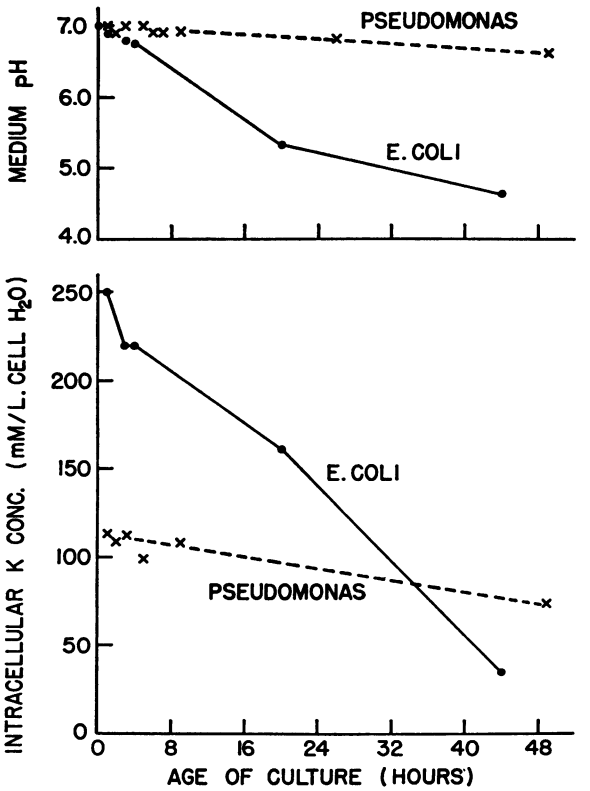

FIGURE 10 Comparison of intracellular K concentration in *E. coli* and *Pseudomonas.* The upper curve shows the changes in medium pH and the lower curve the K concentrations in the cell.

In view of these findings, it seemed appropriate to determine whether a cardiac glycoside sensitive ATP-ase was present in the bacterial membrane. When membrane fragments were obtained and concentrated by centrifugation, ATP-ase activity was demonstrated, as measured by the hydrolysis of ATP in the presence of Mg. The results presented in Figure 13 indicate that the enzyme activity falls to 78% of normal in the absence of K, a difference which is significant at the 99% level. Furthermore, the cardiac glycoside, ouabain, also inhibits the ATP-ase, dropping its activity to 70% of the control value. Molybdate suppresses all but 3% of the total

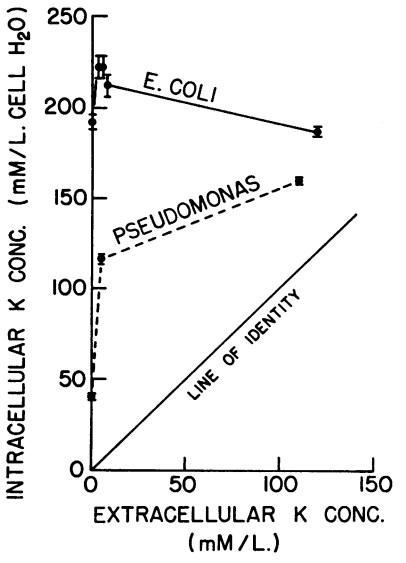

FIGURE 11 Comparison of *E. coli* and *Pseudomonas* with respect to the intracellular K concencentration during the logarithmic phase of growth as a function of the K concentration of the growth medium.

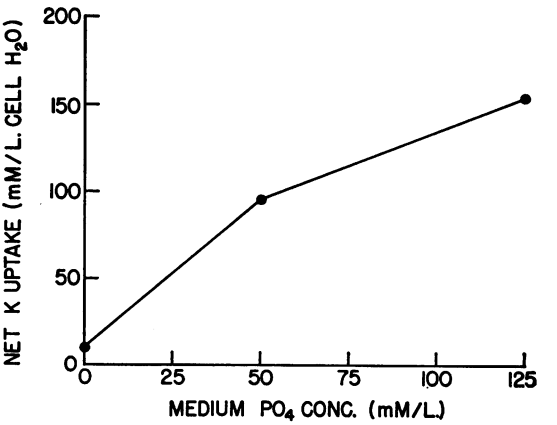

FIGURE 12 The effect of medium PO_4 concentration on net K uptake by *E. coli*, grown to the stationary phase in the presence of excess PO_4.

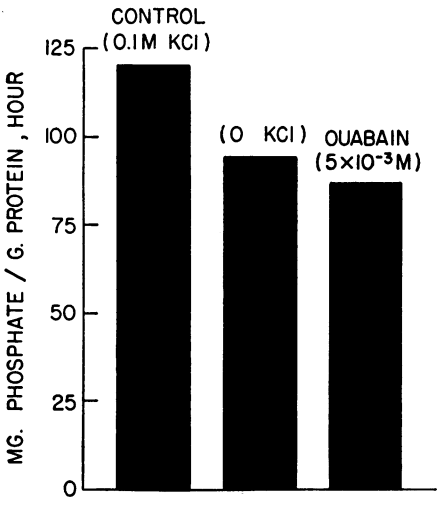

FIGURE 13 ATP-ase activity in bacterial membranes of *E. coli*.

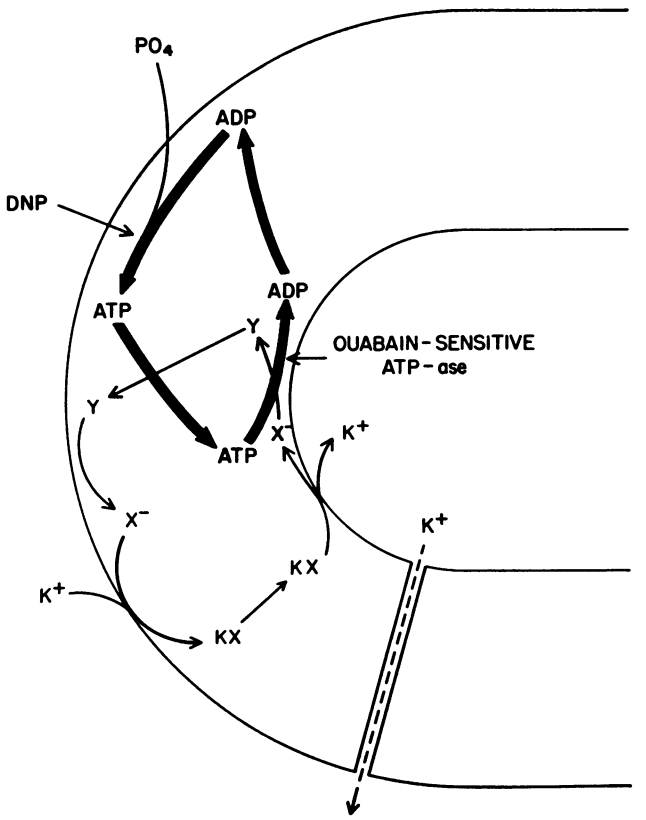

FIGURE 14 Working hypothesis for linkage of metabolism to cation transport in *E. coli*.

ATP-ase activity. These findings are consistent with the hypothesis that K transport in *E. coli* is dependent on ATP-ase activity.

On the basis of these findings in red cell and *E. coli,* we may now include ATP and ATP-ase in our working hypothesis for ion transport, shown in Figure 14. Skou (54) has shown that his preparation of cardiac glycoside sensitive ATP-ase is unable to incorporate inorganic PO_4 into ATP. Mitchell (55) has demonstrated that *S. aureus* is impermeable to PO_4 and that an active process, inhibited by DNP, must be invoked for the entrance of PO_4 into this bacterium; a similar situation obtains in *E. coli* (56). Human red cells are also impermeable to PO_4, which has been shown by Gerlach, Fleckenstein and Gross (57) to enter red cells through ATP, mediated by a singe precursor reaction which produces 1, 3 diphosphoglyceric acid. For these reasons, we have hypothesized that a DNP sensitive phosphorylation of ADP in the bacterium takes place at, or near, the outer border of the cell, and provides an important step in the route by which *E. coli* takes up PO_4. The apparently unique dependence in *E. coli* of net K uptake on medium PO_4 concentration may be related to the fact that the organism is metabolising very rapidly and thus may not have adequate PO_4 reserves.

The cardiac glycoside sensitive ATP-ase has been placed at the inner surface of the cellular membrane because experiments in red cell ghosts have shown that ATP must be within the cells in order to produce net cation movements, either uptake of K or extrusion of Na. A similar requirement exists in nerve, as shown by Caldwell, Hodgkin, Keynes and Shaw (58). Although Skou's enzyme does not catalyze the exchange of inorganic PO_4 with ATP, it does catalyze the exchange of P^{32} between ADP^{32} and ATP, presumably handing on the PO_4 to an unknown organic acceptor, which in our scheme would lead to the production of Y. A particular advantage of coupling the driving system directly to the $X^- \rightarrow Y$ reaction is the evident necessity for a rapid reaction at this point in order to minimize the leakage of K by back reactions. The observation that the glycoside sensitive ATP-ase in red cells is half-activated by a K concentration which half activates the inward transport of K might lead to the inference that the activity of this ATP-ase depends upon the K concentration within the membrane. Caldwell and Keynes (59) have shown that ouabain is ineffective when injected into the interior of the squid giant axon. This observation does not militate against the present hypothesis, in which the ouabain sensitive step is inside the membrane, because the experiments of Caldwell and Keyes do not rule out the possibility that ouabain becomes ineffective due to binding with other axoplasm components. Furthermore, the very high intracellular K concentration of 360 mM in squid giant axon would compete most effectively with ouabain, since the action of this drug in red cells has been shown to diminish by about 50% (60) when the K concentration is raised from 4 mM to 13 mM. The suggestion that ion transport in squid axon is coupled with two separate phosphorylations, one on each face of the membrane, has been discussed by Caldwell, Hodgkin,

Keynes and Shaw (58) and put in a cyclic form basically similar to the present hypothesis by Keynes (61). In view of the indications that phospholipides may be involved in cation transport (46, 62) it would not seem unlikely for the glycoside-sensitive ATP-ase to be engaged in phospholipide metabolism.

The present working hypothesis has been assembled from evidence supplied from many different living systems. Experiments must now be designed to determine whether these inferences are sound, and whether these reactions do indeed take place in *E. coli*. The temptation to generalize upon the unity of nature is always present; it would be most exciting if the basic ion transport mechanism were demonstrated to be a general property of living organisms.

REFERENCES

1. SOLOMON, A. K., J. Gen. Physiol., 1952, **36,** 57.
2. GOLD, G. L., and SOLOMON, A. K., J. Gen. Physiol., 1955, **38,** 389.
3. HASTINGS, A. B., SENDROY, J., JR., MCINTOSH, J. F., and VAN SLYKE, D. D., J. Biol. Chem., 1928, **79,** 193.
4. FRAZIER, H. S., SICULAR, A., and SOLOMON, A. K., J. Gen. Physiol., 1954, **37,** 631.
5. EICHELBERGER, L., FETCHER, E. S., JR., GEILING, E. M. K., and VOS, B. J., JR., J. Biol. Chem., 1940, **133,** 145.
6. SOLOMON, A. K., and GOLD, G. L., J. Gen. Physiol., 1955, **38,** 371.
7. SOLOMON, A. K., and EVANS, J. L., unpublished observations.
8. WICKSON, M., and SOLOMON, A. K., 5th Ann. Meeting Biophysic. Soc., 1961, Abstract SA 7.
9. HEMPLING, H. G., J. Gen. Physiol., 1958, **41,** 565.
10. SCHULTZ, S. G., and SOLOMON, A. K., J. Gen. Physiol., in press.
11. EVANS, J. V., HARRIS, H., and WARREN, F. L., Proc. Roy. Soc. London, Series B, 1958, **149,** 249.
12. EVANS, J. V., and PHILLIPSON, A. T., J. Physiol., 1957, **139,** 87.
13. EVANS, J. V., Nature, 1954, **174,** 931.
14. KELLENBERGER, E., and RYTER, A., J. Biophysic. and Biochem. Cytol., 1958, **4,** 323.
15. SOLOMON, A. K., and CATON, D. C., Anal. Chem., 1955, **27,** 1849.
16. POST, R. L., and JOLLY, P. C., Biochim. et Biophysica Acta, 1957, **25,** 118.
17. ROSENBERG, T., Acta Chem. Scand., 1948, **2,** 14.
18. COWIE, D. B., ROBERTS, R. B., and ROBERTS, I. Z., J. Cell. and Comp. Physiol., 1949, **34,** 243.
19. ROBERTS, R. B., ABELSON, P. H., COWIE, D. B., BOLTON, E. T., and BRITTEN, R. J., Studies of Biosynthesis in Escherichia Coli (2nd Printing), Washington, Carnegie Institution, Publication No. 607, 1957.
20. TROSHIN, A. S., Membrane Transport and Metabolism—edited by A. Kleinzeller and A. Kotyk, Prague, Czechoslovak Academy of Sciences, 1961, 45.
21. HARRIS, E. J., in "The Method of Isotopic Tracers Applied to the Study of Active Ion Transport", Ier Colloque de Biologie de Saclay, New York, Pergamon Press, 1958, 28.
22. SCHULTZ, S. G., BRUBAKER, R., and SOLOMON, A. K., unpublished observations.
23. MITCHELL, P., and MOYLE, J., Symp. Soc. Gen. Microbiol., 1956, **6,** 150.
24. KNAYSI, G., Elements of Bacterial Cytology, 2nd edition, New York, Comstock, 1951.
25. BATTLEY, E. H., and KLOTZ, I. M., Biol. Bull., 1951, **101,** 215.
26. GOLDSTEIN, D. A., private communication.
27. EISENMAN, G., RUDIN, D. O., and CASBY, J. U., Science, 1957, **126,** 831.
28. GARDOS, G., Acta Physiol. Acad. Sc. Hung., 1954, **6,** 191.
29. HOFFMAN, J. F., Fed. Proc. 1960, **19,** 127.

30. TOSTESON, D. C., in "Electrolytes in Biological Systems," edited by A. M. Shanes, Washington, D. C., American Physiological Society, 1955, 123.
31. HEINZ, E., and DURBIN, R. P., J. Gen. Physiol., 1957, **41,** 101.
32. SOLOMON, A. K., Proceedings of the First National Biophysics Conference, edited by H. Quastler and H. J. Morowitz, New Haven, Yale University Press, 1959, 314.
33. GOLDSTEIN, D. A., and SOLOMON, A. K., J. Gen. Physiol., 1960, **44,** 1.
34. GIEBEL, O., and PASSOW, H., Arch. ges. Physiol., 1960, **271,** 378.
35. VILLEGAS, R., and BARNOLA, F. V., J. Gen. Physiol., 1961, **44,** 963.
36. LINDEMANN, B., and SOLOMON, A. K., 5th Ann. Meeting Biophysic. Soc., 1960, Abstract SA3.
37. WICKSON, M., private communication.
38. GOLDSTEIN, D. A., and SCHULTZ, S. G., private communication.
39. HARRIS, E. J., and MAIZELS, M., J. Physiol., 1951, **113,** 506.
40. GLYNN, I. M., in "The Method of Isotopic Tracers Applied to the Study of Active Ion Transport", ler Colloque de Biologie de Saclay, New York, Pergamon Press, 1958, 46.
41. KEYNES, R. D., Proc. Roy. Soc., London, Series B, 1954, **142,** 359.
42. STEINBACH, H. B., Proc. Nat. Acad. Sc., 1952, **38,** 451.
43. CHRISTENSEN, H. N., Science, 1955, **122,** 1087.
44. SOLOMON, A. K., LIONETTI, F., and CURRAN, P. F., Nature, 1956, **178,** 582.
45. KIRSCHNER, L. B., J. Gen. Physiol., 1958, **42,** 231.
46. HOKIN, L. E., and HOKIN, M. R., Symposium on Membrane Transport and Metabolism, edited by A. Kleinzeller and A. Kotyk, Prague, Czechoslovak Academy of Sciences, 1961, 204.
47. SKOU, J. C., Membrane Transport and Metabolism, edited by A. Kleinzeller and A. Kotyk, Prague, Czechoslovak Academy of Sciences, 1961, 224.
48. VON SCHATZMANN, H.-J., Helv. Physiol. et Pharmacol. Acta, 1953, **11,** 346.
49. POST, R. L., and ALBRIGHT, C. O., Symposium on Membrane Transport and Metabolism, edited by A. Kleinzeller and A. Kotyk, Prague, Czechoslovak Academy of Sciences, 1961, 115.
50. TOSTESON, D. C., MOULTON, R. H., and BLAUSTEIN, M., Fed. Proc., 1960, **19,** 128.
51. TOSTESON, D. C., BLAUSTEIN, M. P., and MOULTON, R. H., Fed. Proc., 1961, **20,** 138.
52. DUNHAM, E. T., and GLYNN, I. M., J. Physiol., 1961, **156,** 274.
53. ROTHSTEIN, A., and MEIER, R., J. Cell. and Comp. Physiol., 1949, **34,** 97.
54. SKOU, J. C., Biochim. et Biophysica Acta, 1960, **42,** 6.
55. MITCHELL, P., Symp. Soc. Exp. Biol., 1954, **8,** 254.
56. MITCHELL, P., Biochem. Soc. Symp., 1959, **16,** 73.
57. GERLACH, E., FLECKENSTEIN, A., and GROSS, E., Arch. ges. Physiol., 1958, **266,** 528.
58. CALDWELL, P. C., HODGKIN, A. L., KEYNES, R. D., and SHAW, T. I., J. Physiol., 1960, **152,** 561.
59. CALDWELL, P. C. and KEYNES, R. D., J. Physiol., 1959, **148,** 8P.
60. SOLOMON, A. K., GILL, T. J., III, and GOLD, G. L., J. Gen. Physiol., 1956, **40,** 327.
61. KEYNES, R. D., Private communication.
62. HOKIN, L. E., and HOKIN, M. R., Nature, 1961, **189,** 836.