# BIOLOGICAL ION EXCHANGER RESINS

## III. MOLECULAR INTERPRETATION OF CELLULAR ION EXCHANGE

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ABSTRACT The cell is presented as a biological ion exchanger resin. The similarities between ion accumulating cells and ion exchanger resins are correlated. The kinetic characteristics of biological ion exchange are shown to be amenable to analysis by a model commonly used for ion exchanger resins. The theories of ion exchange equilibria currently in use with ion exchanger resins are reviewed with their suitability for adaptation to biological ion exchange in mind.

### INTRODUCTION

It has previously been suggested (1–4) that the ion accumulating properties of living cells, and bacteria in particular, can be explained with a certain simplicity if the cell is imagined as an ion exchanger resin, i.e., a biological ion exchanger resin.

In this paper, the similarities between ion accumulating cells and ion exchanger resins will be correlated. The data indicating that ion exchange events extend beyond the surface membrane to the polyelectrolytes making up the fabric of the cell are discussed.

Throughout nature, wherever it has been investigated, ion exchange reactions typical of the ion accumulating properties of living cells are a dominating feature of the chemistry of natural materials. The ion exchange properties of organic fertilizers, clays, glauconites, zeolites, and humic acids have been extensively studied (5–14) and constitute a major feature of the chemistry of soils. The cation and anion exchanges occurring in the fixed charge matrix of rocks, lignitic and bituminous coals, and pitch have occupied an important role in geochemistry (10, 15–17). In industrial chemistry, ion exchange materials can be said to be virtually indispensable. They have contributed to ion exchanger glasses in glass electrodes (18). They have provided the ion exchange resins that catalyze organic reactions that recover precious metals from industrial wastes, that decontaminate water coolants in nuclear reactors, that "soften" water for domestic use, and that purify saline water for the growing public needs of expanding populations.

In short, ion exchanger resins are virtually of ubiquitous occurrence in nature. Yet the line is drawn arbitrarily at contemporary biology. It is natural, therefore, to consider extending the concept of the ion exchanger resin to the living cell.

In traditional "membrane theory" only the cell membrane ("pump" or "carrier" mechanism) is believed to participate in the ion exchange events that characterize ion transport. However, the compiled charge profile (19) indicates that the great majority of the ion exchange sites of the cell do not reside in the membrane. Instead, 78% (1146/1461, Table IV *a* [19]) of the ions available for pairing with potassium are fixed charge groups on intracellular macromolecules such as nucleic acid and protein. Photographic evidence of the cytoplasmic abundance of this polyelectrolyte fixed charge can be seen in the electron micrograph of *Escherichia coli* (Fig. 1). The most evident region of the cytoplasm, for example, is the ribosome layer, a dense zone of polyelectrolyte consisting of nucleic acid–protein particles. This polyelectrolyte region occupies 63.3% of the cytoplasmic cross-sectional area. Deoxyribonucleic acid (DNA) occupies the remainder. Consequently, treating the cell as a membranous sac of aqueous protein and electrolyte, as is commonly done in classical "membrane theory," does not constitute a realistic approximation of the *natural state* of a living cell.

Furthermore, recent evidence provided by nuclear magnetic resonance (NMR) raises other questions about the adequacy of this view. NMR measurements of water in muscle by Bratton et al. (20) and by Hazlewood et al. (21), of $D_2O$ in brain and kidney by Cope (22), of water in frog sciatic nerve by Fritz and Swift (23, 24), and of water in *E. coli* (25) have all demonstrated marked shortening in NMR relaxation times and led to the conclusion that cell water is highly organized, a conclusion more consistent with the electron micrographic evidence presented above than with the classical view of the cell as a simple aqueous solution within a membranous sac.

In addition, calculations from Debye-Huckel theory (paper II [reference 25]) assuming 1.0 molal of fixed charge suggest that strong water polarizing forces are distributed over the 4–8 A hydration atmospheres solvating intracellular charge, indicating serious departure of properties such as the dielectric from simple aqueous behavior.

Other conflicts between traditional "membrane theory" and experiment exist. Investigators have reported that insufficient energy is available from intermediate metabolism to drive the postulated "membrane pumps" (26–28).[1] Also, significant "gradients" of intracellular potassium (76:1) are accumulated by aqueous suspensions of *E. coli* when the cell membranes have been removed altogether (19). Furthermore, the chemical analysis (paper I [reference 19]) indicates that 80% of the time potassium is paired with a fixed charge site on a macromolecule making membrane pumps unnecessary for maintaining concentration "gradients."

NMR studies corroborate this conclusion. Marked departure from aqueous behavior has been observed in the "relaxation" rate of cellular $^{39}K$ (29). Increased relaxation rates indicate that intracellular potassium is experiencing electric field

[1] Damadian, R., and L. Minkoff. 1971. Manuscript in preparation.

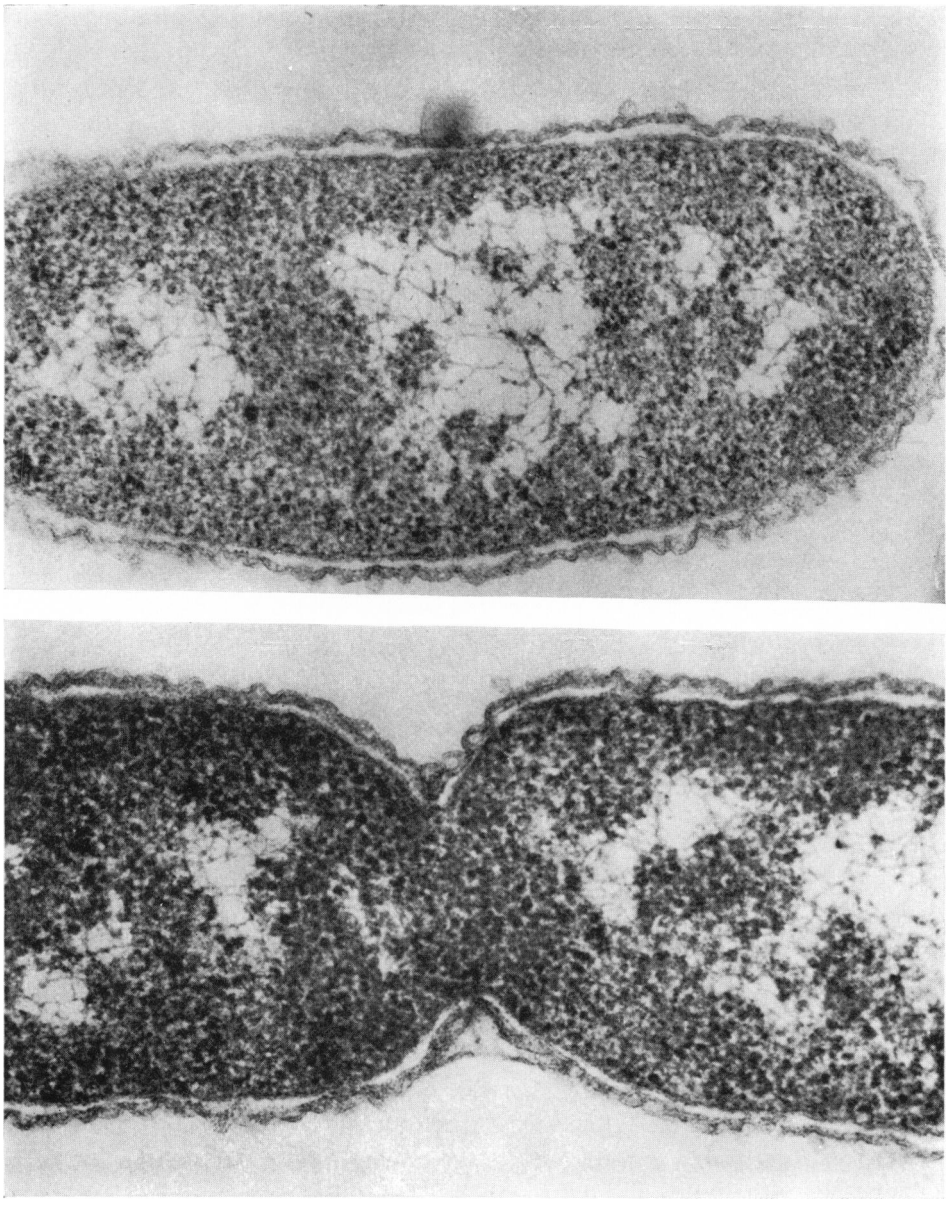

FIGURE 1 Electron micrograph of *E. coli*. × 90,000.

gradients (30, 31) as would occur in the vicinity of fixed charge sites and in highly structured cell water (32). Similarly, the relaxation times ($T_1$ and $T_2$) of intracellular Na are markedly shortened indicating that cell Na is bound (33, 34) rather than freely dissolved in aqueous. Moreover, a specific instance of cross-correlation between ion

exchanger resins and cells was obtained by demonstration of close quantitative agreement between the spin-spin relaxation time ($T_2$) of Na in muscle, brain, and kidney (0.75–0.90 msec) and in Dowex 50 (Dow Chemical Co., Midland, Mich., 1.35 msec) resin beads (33). Relaxation times of aqueous Na and Na in biological tissue, on the other hand, differed by an order of magnitude.

## METHODS

### *Bacteria and Growth Media*

The experiments were performed utilizing *E. coli* strain CBH (1). The bacteria were cultivated as described in the preceding papers of this series. *E. coli* (strain RD-2) and CBH were grown in medium NaA (1) containing 0.05% Vitamin-Free Casamino Acids (Difco Laboratories, Detroit, Mich.), 1% glucose, and the desired amount of potassium as the chloride salt.

### *Isotope Exchange*

The cells were resuspended (0.3 mg dried cells/ml) in medium NaA containing 1% glucose and equilibrated for 1 hr at 37°C before tracer ($^{42}$KCl, Cambridge Nuclear Corp., Iso/Serv Div., Cambridge, Mass.) was added. Flux values were determined by methods described in detail in a previous publication (1).

### *Swelling*

Bacteria harvested in the logarithmic phase of growth were depleted of potassium by methods described in paper I of this series (19). Change in packed cell volume during potassium accumulation was determined in cytocrit tubes. Use was made of an optical comparator to measure pellet volume (35).

## RESULTS AND DISCUSSION

### *The Kinetics of Biological Ion Exchanger Resins*

Implicit in the membrane "pump" model is the assumption that the rate-limiting process in cell transport kinetics can uniformly be assigned to surface transfer rather than diffusion within the bulk phase of the cell. By applying the kinetic theory of ion exchanger resins to cell transport kinetics, it is possible to make a quantitative distinction between these alternatives. In resin beads, two potential rate determining steps can be separated: (*a*) the rate of diffusion of mobile ions within the ion exchanger itself (particle diffusion), and (*b*) the rate of diffusion of mobile ions in the film adhering to the bead (film diffusion). Whether the kinetics of the exchange are under particle diffusion control or film diffusion control can be determined by isotopic exchange experiments (36). When the exchange is rate-limited at the surface, the half time, $t_{1/2}$, to the attainment of isotopic equilibrium exhibits dependence on both the ionic concentration in the medium and in the resin and is given by the relation

$$t_{1/2} = 0.23 \frac{r_0 \overline{C}}{\overline{DC}}, \qquad (1)$$

(36, p. 264) where $r_0$ is the bead radius, $\delta$ the film thickness, $D$ the diffusion coefficient in the film, and $\bar{C}$ and $C$ the ion concentrations in the resin and external solution respectively. When the exchange is under particle diffusion control, the half time, $t_{1/2}$, is governed by

$$t_{1/2} = 0.030 \frac{r_0^2}{\bar{D}}, \tag{2}$$

(36, p. 261) and is independent of solute concentration altogether. ($r_0$ is the bead radius and $\bar{D}$ the diffusion coefficient within the bead.) Furthermore criteria have been derived for predicting the nature of the rate-determining step (36, p. 255):

$$\frac{X\bar{D}\delta}{CDr_0}\left(5 + 2\,K_{A/B}\right) \ll 1 \text{ particle diffusion control,} \tag{3}$$

$$\frac{X\bar{D}}{CDr_0}\left(5 + 2\,K_{A/B}\right) \gg 1 \text{ film diffusion,} \tag{4}$$

where $X$ is the concentration of fixed ionic groups, $C$ the concentration of the solution, $\bar{D}$ the diffusion coefficient in the ion exchanger, $D$ the diffusion coefficient in the film, $r_0$ the bead radius, $\delta$ the film thickness, $K_{A/B}$ the selectivity coefficient for the monovalent ion exchange A $\leftrightarrow$ B.

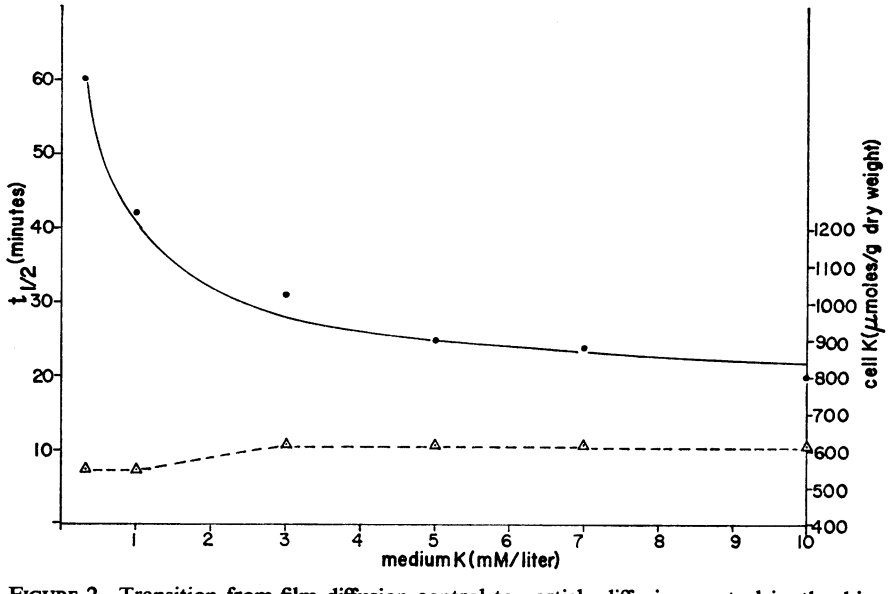


FIGURE 2 Transition from film diffusion control to particle diffusion control in the biological ion exchanger resin. Variation of $t_{1/2}$ for $^{42}K \leftrightarrow {}^{39}K$ isotope exchange with medium potassium concentration. The tracer exchange was carried out in medium NaA containing 1% glucose and lacking histidine to maintain a steady state with respect to cell population. Methods used for carrying out and calculating the tracer flux have been described in detail in previous publications (1, 2).

By equations 3 and 4, film diffusion control is favored as solution concentration $C$ decreases. Particle diffusion control is favored as solution concentration increases.

Application of these rate laws of ion exchange to the biological ion exchanger provides a fresh perspective upon which to base the kinetic analysis of the ion exchange processes in biological systems. In isotope exchange experiments ($^{42}K \leftrightarrow {}^{39}K$) in *E. coli*, carried out with cells that were at equilibrium except for isotopic distribution of potassium, the half time to tracer equilibration, $t_{1/2}$, was evaluated as a function of potassium concentration in the external medium. Since the ionic concentration within the biological resin $\bar{C}$ is essentially constant (dashed plot in Fig. 2) variation of $t_{1/2}$ in equation 1 reflects chiefly variation in external potassium concentration. Fig. 2 demonstrates the marked dependence of $t_{1/2}$ on external concentration at low medium concentrations ($<3$ mM). At higher concentrations, however, $t_{1/2}$ is relatively insensitive to changes in external concentration. This result suggests that in the concentration regions where marked dependence of $t_{1/2}$ on external concentration is exhibited the exchange is under film diffusion control, i.e. rate-limited at surface pene-

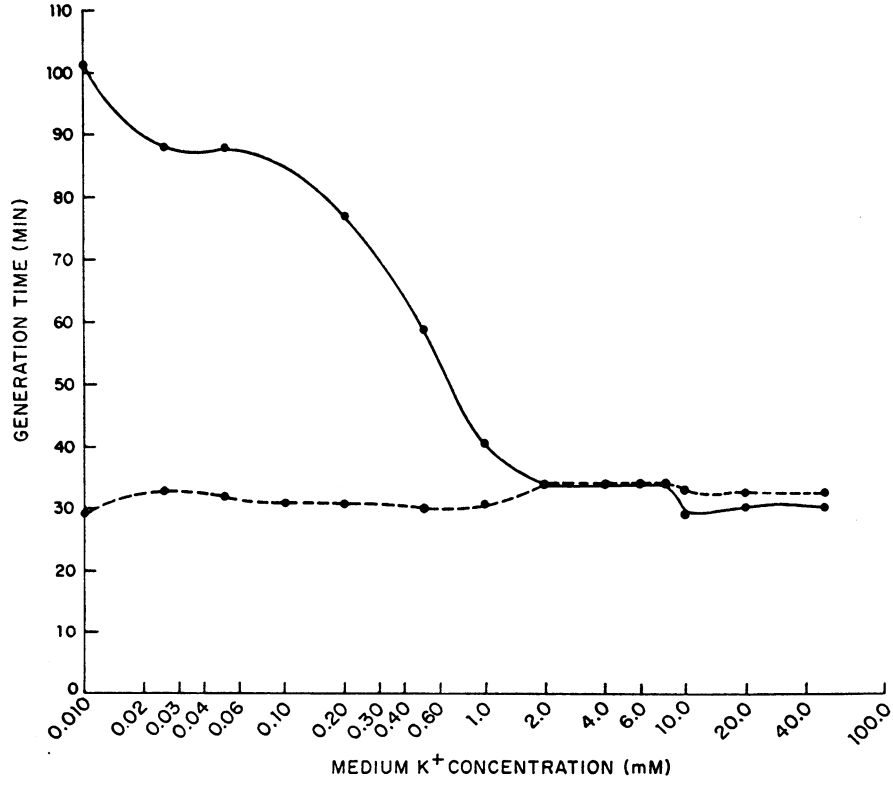


FIGURE 3 Variation of doubling time in parent strain *E. coli* (CBH) and a K deficient mutant of *E. coli* (RD-2). Bacteria grown in medium NaA (1) supplemented with desired amount of KCl, 0.05% Vitamin-Free Casamino Acids and 1% glucose.

tration of the bacteria, whereas at higher concentrations (>3 mM) the exchange is predominantly under the influence of particle diffusion control, i.e., rate-limited principally by diffusion within the bulk region of the cell and not at the surface. This conclusion is difficult to reconcile with a membrane-mediated transfer mechanism since it is essential to classical "membrane theory" that the rate-limiting step is at the surface layer (37). Transition from particle diffusion control to film diffusion control in *E. coli* as concentration decreases corresponds to observed behavior in ion exchange beads as expressed in equations 3 and 4. The same observation has been made by Ling (38) in frog sartorius muscle using a slightly different quantitative formulation. K exchange can be separated into a bulk-phase limited exchange occurring at high external concentrations, and a surface-limited exchange at low concentrations.

This kinetic model provides an explanation of the behavior of potassium transport–deficient mutants of *E. coli* (1). Comparative plots of the growth rate of the K-deficient mutant (RD-2) and parent strain (CBH) as a function of medium potassium concentration (Fig. 3) indicate that the mutant does not exhibit its defect until extracellular K concentrations below 2 mM are reached. It has always been unclear why the mutant defect appears at this particular concentration when large concentration "gradients" exist at medium K concentrations well above 3 mM (cell K 220 millimoles/liter). The emergence of the mutant's defect at 3 mM can now be related to the position of transition from film diffusion control to particle diffusion control in *E. coli*. The transport defect in the mutant does not manifest itself until the kinetics are under surface diffusion control, and suggests that the defect in the mutant resides in the chemistry of its surface layer.

### *The Ion Exchanger Resin Model*

A typical ion exchanger resin is formed by a series of interconnecting chains that cross-link the polymer backbone of the resin (Fig. 4). The charged matrix is elastic and can expand or contract in conjunction with the changes in swelling pressure that accompany ion exchange. The degree of cross-linking determines the average width of the mesh ("pore") which can vary from a few angstroms in highly cross-linked resins to 100 A in weakly cross-linked resins fully swollen with solvent (36).

Quantitative formulations of ion exchange equilibria that take into account these macroscopic properties of bulk phase ion exchangers as well as their molecular determinants have been proposed (38–42). Of these, the one due to Ling (38 *a*, *b*) is the most suitable for biological systems, since in addition to the coulomb interactions of the associated ion pairs considered in each of these theories, Ling's theory includes contributions from dipole interaction energies due to solvent as well as London dispersion forces and Born repulsions.

*E. coli* as a biological ion exchanger resin is represented schematically in Fig. 4. The polyelectrolytes, chiefly nucleic acid and protein (e.g., ribosomes, soluble protein, DNA, and RNA) in which 80% (19) of the ion exchange capacity of the cell

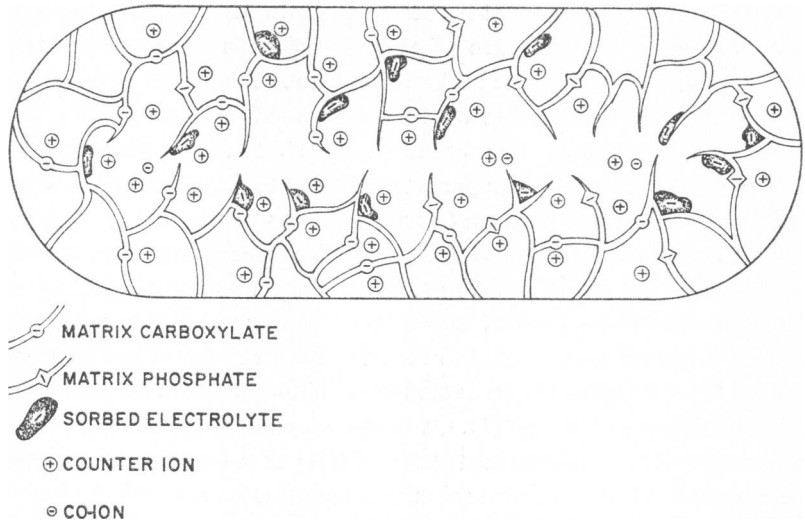


FIGURE 4 Structure of a biological ion exchanger resin (schematic).

resides, constitute the matrix that contains the fixed charge ion exchange sites. The bifunctional character of the exchanger with respect to cation exchange as observed in the K $\rightleftarrows$ Na ion exchange isotherm (paper I [reference 19]) is represented by two classes of cation exchange sites, phosphate (designated by a diamond) principally from nucleic acid, and carboxylate (designated by a circle) from cell protein. Each contributes approximately 50% of the ion exchange capacity of the cell (paper I [reference 19]).

Cross-linkages in the condensed region of the cytoplasm would occur in the form of intermolecular salt bridges between oppositely charged polyelectrolyte groups (19, 38) and as hydrophobic interactions between nonpolar groups of biopolymers. Since there is no reason, a priori, to expect any significant amount of covalent cross-linking *between* polyelectrolyte molecules, the cross-linking would be highly fluid and random,[2] with linkages forming and reforming according to changes in cell hydration and ionic strength. Under these conditions the degree of cross-linking would vary throughout the course of intermediate metabolism as intermediate metabolites such as organic acids and sugar phosphates accumulate and serve as salt bridging agents between fixed charge groups on the exchanger backbone or compete for solvent with them. The degree of cross-linking in turn determines the average mesh width ("pore size") of the biological resin and thus the swelling ability of the biological resin and the mobilities of the counter ions within the resin. The latter determines the rate of ion exchange and the electrical conductivity of the resin.

[2] It is essential to note in this context that ion exchanger resins range in structure from liquid extraction exchangers to dense crystalline solids (36) and do not necessarily imply a rigid covalently linked matrix commonly associated with standard commercial polystyrenes.

Mobile counter ions are shown as univalent cations associating with the matrix charges. In addition several mobile cations are shown paired with mobile anions (inorganic anions). The pairs are shown occupying the "free water" (water not totally committed to solvation of intracellular fixed charge) space and in *E. coli* make a relatively small contribution to the total charge profile since inorganic anions (e.g., $HCO_3^-$, $Cl^-$, $SO_4^=$, etc.) were not present in significant amounts in *E. coli* (Table IV of paper I [reference 19]) cultivated in medium KA.

Also indicated as molecules (shaded) adherent to the ion exchanger backbone of the biological resin are the sorbed metabolites that pool in the cell during the course of intermediate metabolism (paper I [reference 19] Table IV *b*). Sorption of metabolites to the biological exchanger is analogous to the sorptive phenomena in synthetic ion exchanger resins (43–47). In resins, the attachment of solute molecules to the ion exchanger by mechanisms other than electrostatic interactions with the ion exchange sites are considered to result from intermolecular forces such as London or dipole-dipole interactions between the solute molecule and the exchanger backbone. The result is a tendency for the solute molecule to be squeezed onto the phase boundary between the ion exchanger matrix and the solution with consequent attachment of the solute to the exchanger.

Sorbed solutes constitute a central feature of the biological ion exchanger resin and form the basis for the substrate-dependent, "active transport" phase of cation accumulation (paper I [reference 19] Fig. 1) in traditional "membrane theory." Sorption of a number of solutes in biologic tissues has been demonstrated by Ling (48–50). Glucose has been shown to fit a simple Langmuir adsorption isotherm.

"Swelling," internal molality, and dependence of ion selectivity on osmotic strength and temperature are other major properties of ion exchanger resins common to ion accumulating cells. Swelling is a central feature, for example, in Gregor's theory (39) of ion exchange equilibria. In the quantitative formulation of this model

$$\ln K_{A/B} = \frac{\Pi}{RT} (| z_A | v_B - | z_B | v_A),$$

where $z$ is the ionic valence and $v$ the ionic molar volume. Major dependence of the selectivity coefficient $K_{A/B}$ is attributed to the physical action of swelling pressure ($\Pi$), that develops during the ion exchange event. The swelling pressure is treated as a force that exerts preference for the least hydrated ion. Hence the dependence of the selectivity coefficient $K_{A/B}$ on osmotic pressure ($\Pi$) (25). Significant expansion of the cell or swelling also accompanies the biological ion exchange process. An increase of 12.3% in cell volume is associated with the K accumulation reaction (Table I).

Swelling in synthetic ion exchanger resins provides example of swelling in response to differences in osmotic pressure ($\Pi$) in a system where a surface-limiting membrane does not exist. Previously, swelling in biological systems was regarded as prima facie

TABLE I

SWELLING ACCOMPANYING POTASSIUM ACCUMULATION IN *E. COLI*

| K | Cytocrit |
|---|---|
| *mmoles/liter* | $\left(\frac{cm^3\ cells \times 10^2}{cm^3\ suspension}\right)$ |
| 166 ± 4.0 | 0.114 ± 0.001 (SEM) |
| 247 ± 0.0 | 0.122 ± 0.001 |
| 251 ± 0.0 | 0.128 ± 0.000 |

Bacteria harvested during the logarithmic phase of growth were K depleted by methods described in previous publication (1). Simultaneous measurements of cytocrit and potassium uptake were then conducted utilizing methods described in reference 1.

evidence that the membrane, not the bulk properties of the matrix, regulated cell hydration.

The importance of internal molality in determining ion exchange equilibria in resins has been demonstrated in the NMR studies of Reichenberg and Lawrenson (51), DeVilliers and Parrish (52), and Frankel (53). Chemical shift of internal water protons have been shown to be proportional to the internal molality of the exchanger. Biological ion exchangers fit well in this respect. The molality of fixed charge groups in cells, 1.0 molal, is typical of a condensed system and in the range of most types of synthetic ion exchanger resins (F. Helfferich, 36). From the studies of Pepper et al. (54) relating internal molality to resin cross-linking, *E. coli* is similar in internal molality to a sulfonated polystyrene resin that is 2.5% cross-linked.

The ion exchanger resin model explains two cardinal features of biological ion transport: accumulation and selection. Using membrane models, it has proved difficult to devise a satisfactory mechanism for coupling the energy requirements of the "pump" to a mechanism for sensing the intracellular cation concentration and maintaining it constant. *E. coli*, for example, maintains an intracellular concentration of 200 mM at all external concentrations from 0.02 mM to 100 mM (Fig. 2). In an ion exchanger, on the other hand, the relatively fixed ion exchange *capacity* of the cell maintains a fixed counter ion concentration regardless of external concentration.

With respect to selectivity, no membrane proteins have been isolated (4) that are capable of alkali cation selectivity during competition equilibrium dialysis and a quantitative theory of membrane selectivity is not available. Selectivity in ion exchanger resins, however, is well-known (36) and quantitative theories have been developed to explain the data (38–42).

Furthermore, it provides a rational basis for the explanation of physiological effects of other alkali cations that are difficult to explain in terms of "membrane-situated pumps." The alkali cations Li and Rb, for example, have been reported to

alter neuroexcitability. $Li_2CO_3$ has been shown to calm patients afflicted with manic-depressive states during manic phases of their disease. Rb on the contrary heightens neuroexcitability (55). These effects can be related to the differences in the effects of these two ions on water structure. Li, the smallest of the alkali cations, has the greatest tendency to hydrate and order the structure of water in its hydration shell. Consequently, it is generally classified as a "structure maker" (56) (i.e., it increases the macroscopic viscosity of the bulk solution). Evidence of this can be inferred from the magnetic resonance relaxation data in Table III of the preceding paper (25). Conversely Rb, by its greater ionic size, has much less tendency to hydrate and tends to break water structure (i.e., decrease its bulk viscosity) (56). A similar explanation can be made for the embryological effect of Li.[3]

The concept of the cell as a biological ion exchanger resin provides a useful physical model for structuring new experiments. It served as a basis for predicting that cancerous tissue would be characterized by an increase in the amount of free water when studied by NMR (57).

*The suitability of an ion exchanger resin model for biological transport phenomena can be summarized as follows:*

—Ion exchanger resins are of ubiquitous occurrence in natural chemistry. The division at contemporary biology seems arbitrary when it is known that cells contain an extensive internal polyelectrolyte matrix of protein and nucleic acid.

—Recent NMR studies (20–25) have all concluded that cell water is not a simple aqueous solution in agreement with the calculations from Debye-Huckel theory implying strong water-polarizing forces on cell water.

—Quantitative analysis of *E. coli* indicates that 80% of cell potassium is paired with fixed charge on macromolecules rendering "membrane pumps" superfluous for the maintenance of "concentration gradients."

—Metabolic energy available to operate "membrane pumps" is insufficient (26–28).

—Excellent quantitative agreement exists between the magnetic resonance relaxation times of $^{23}Na$ in resin beads and $^{23}Na$ in cells, whereas a 10-fold difference exists between the relaxation rate of cellular and aqueous Na. Preliminary NMR studies of $^{39}K$ suggest that cell potassium is experiencing electric field gradients from fixed charge sites.

—The fixed ion exchange *capacity* of the biological resin explains the constancy of intracellular potassium, with varying external concentration. With the membrane model, it is difficult to devise a mechanism for sensing the intracellular cation concentration and maintaining it constant.

—A physical concept for structuring new experiments is provided. The correlation of internal water structure with alkali cation selectively provided a basis for predicting the NMR properties of water in cancer tissue.

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[3] In embryology Li is noted for its tendency to shift the development potentialities of the developing embryo towards mesodermal differentiation and consequently is considered a vegetalizing agent.

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