BIOLOGICAL ION EXCHANGER RESINS

I. Quantitative Electrostatic Correspondence of Fixed Charge and Mobile Counter Ion

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ABSTRACT Utilizing *Escherichia coli* as the prototype of an ion-accumulating cell, the ion exchange isotherm is introduced as a concise method of characterizing biological ion exchange events. The ion exchange isotherm for the alkali cation exchange, K ↔ Na, is described. The total charge profile of this bacterium is compiled and compared for bacteria in the Na form and in the K form. Macromolecule fixed charge was found to provide 80% of the counter ions that pair with potassium. Therefore, in its physiological state, 80% of the cell potassium in *E. coli* is associated with an ion exchange site on a macromolecule. The primary cation exchange sites are found to be about equally divided between carboxylate and phosphate sites indicating that *E. coli* is a bifunctional resin with respect to cation exchange. During substrate-dependent cation accumulation ("active transport"), phosphate esters and organic acids were shown to accumulate. One may conclude that the role of intermediate metabolism in "active transport" is to increase the ion exchange capacity of the biological resin by the production of charged metabolites that sorb to the framework of the resin.

INTRODUCTION

Spreading research interest in the chemistry of ion exchanger materials in the last twenty years has proven especially productive. The combined results of empirical approaches and theory have led to a comprehensive and consistent picture of the principal determinants in ion exchange and the subject may now be considered to be rich in qualitative and quantitative detail (1). The extension of this knowledge to biological ion exchange phenomena suggests a replacement of the traditional membrane "pump" mechanism for cellular ion accumulation by a model for the cell as an ion exchanger resin (2). The consideration on which the present paper was based was the need for a quantitative description of the total charge profile of a living cell to provide a basis on which to begin the study of biological ion ex-

changer resins. *E. coli* has been chosen as the prototype cell of the biological ion exchanger resin.

MATERIALS AND METHODS

Bacteria and Growth Media

E. coli strain CBH (3), a histidine auxotroph of *E. coli* B, was used in these studies. The histidine requirement served as a convenient method for arresting growth in suspensions where steady-state populations were desired. 2-ml aliquots of stock culture were maintained at −120°C in a Revco ULT freezer (Revco, Inc., Scientific and Industrial Div., West Columbia, S.C.) and the bacteria were routinely grown from the inocula in medium KA (3) containing 0.05% Vitamin-Free Casamino Acids (Difco Laboratories, Detroit, Mich.) and 1% glucose, and unless stated otherwise were harvested when the turbidity of the culture reached 0.400 OD 620.

Potassium Depletion: Conversion of E. coli to the Na Form

Bacteria harvested in the logarithmic phase of growth (K-rich cells) were resuspended in medium NaA (3) that was adjusted to pH 9.2 with NaOH and supplemented with 0.014 M NaCl. The final suspension containing 1 mg dry weight of bacteria/ml was incubated with gentle shaking for 15 min at 37°C and centrifuged. Two to three repetitions of the alkaline incubation were usually necessary before total K depletion was achieved.

Equilibrium Dialysis and the Ion Exchange Isotherm

K-depleted cells were washed twice with 400 mM sucrose and unless indicated otherwise were resuspended to a final concentration of 32 mg dry weight/ml in dialyzing medium minus alkali cations 1-ml aliquots were placed in short lengths of 10 mm VisKing tubing (Will Scientific Inc., Bronx, N.Y.), securely tied, and dialyzed to equilibrium at 22°C. Care was taken to establish the exact position of equilibrium by a series of time-dependent studies.

Phosphate Esters, Nucleic Acid Phosphate, and Phospholipid

Bacteria for these experiments were prepared by cultivating them in the medium described above to which $^{32}P_i$ as $H_3{}^{32}PO_4$ (New England Nuclear, Boston, Mass.) had been added to a final specific activity of 0.75 μCi/mmole PO_4. Bacteria in the logarithmic phase of growth were harvested, washed twice in 0.400 M sucrose, centrifuged, and the pellet suspended in 4 ml cold (0°C) 5% trichloroacetic acid (TCA) and allowed to stand for 30 min with intermittent agitation at 0°C.

The cells were then centrifuged and the TCA extract decanted for later measurement in a liquid scintillation spectrometer. This extract constitutes the phosphate ester fraction reported in Table IV after correction for inorganic phosphate.

The acid-insoluble residue was extracted first in 75% alcohol (30 min at 45°C) and then with an alcohol-ether solvent composed of 1 part 75% alcohol and 1 part diethyl ether. These extracts were discarded.

The alcohol-ether insoluble material was resuspended in 4 ml of 5% TCA and placed on a boiling water bath for 30 min to extract nucleic acids. After centrifugation, the supernatant was removed and counted for determination of nucleic acid phosphate.

Phospholipid ^{32}P was determined on separate bacterial pellets cultivated and washed as described above. The pellets were extracted overnight at 60°C in chloroform-methanol (2:1)

and the extract was removed and evaporated to dryness. The dried extract was taken up in 2 ml of chloroform and 0.1 g of silicic acid was added. The silicic acid was then removed by centrifugation and washed twice with petroleum ether and twice with 0.5 ml methanol. The methanol extract was taken up quantitatively in Bray's solution and counted.

^{32}P activity was determined in a Picker Nuclear Liquimat 220 (Picker X-Ray Corp., White Plains, N.Y.) equipped with a radium external standard to detect variation in quenching. The scintillator mixture contained 0.1 ml of sample, 0.75 ml ethanol, and 1.0 ml hyamine hydroxide dissolved in 10 ml of toluene scintillate (5 g 2,5-diphenyloxazole [PPO], 100 mg 1,4-bis-[2-(5-phenyloxazolyl)]benzene [POPOP]/liter toluene).

Inorganic phosphate values for the determination of growth medium specific activity and P_i content of the cold TCA extracts were obtained by the method of Fiske and SubbaRow (4).

Mg, Ca, Cl, HCO_3, Fe, NH_4

Mg and Ca in concentrated nitric acid extracts of bacteria were measured in an atomic absorption flame photometer. The Cl content of dried bacterial pellets was measured by potentiometric titration (5) and HCO_3 was determined in a Van Slyke manometer on samples from growing cultures that were collected and centrifuged under mineral oil. NH_4 and Fe analyses were obtained on bacteria that were separated from suspending medium by high speed centrifugation. The surface of the pellet was washed free of medium by overlaying it with 0.4 M sucrose. Knowledge of the trapped plasma (3) and analysis of the suspending medium permitted correction of the pellet values for trapped medium. The bacteria were crushed in a French Press (6000 psi, American Instrument Co., Inc., Silver Spring, Md.) at 0°C. The fragmented suspension maintained throughout at 0°C was analyzed immediately. The content of NH_4 in the fragmented suspensions was determined by the method of Chaney and Marback (6). Fe was determined in an autoanalyzer by the method of Young and Hicks (7).

Bacterial Dry Weight, K, Na, and Calculation of Solute Concentrations

The methods for quantitative measurement of bacterial dry weight, conversion of analytical data for *E. coli* to molal concentrations and the techniques for determining cell K and Na have been described in detail in a previous publication (3).

RESULTS AND DISCUSSION

Resolution of Bacterial Ion Exchange into Two Phases: Fixed Charge Associations Not Requiring Metabolism and Fixed Charge Associations Resulting from Intermediate Metabolism

The segregation of selective K accumulation in *E. coli* into two stages is illustrated in Fig. 1. Stage I occurs in the absence of an energy source (8) and is activated by the addition of potassium alone. Stage II is initiated by substrate and is the traditional metabolism-dependent phase of "active transport."

The accumulation of K during stage I occurred in the absence of metabolic activity and was confirmed by the failure of NaF to inhibit the accumulation ($K_{K/Na}$ unchanged), the lack of significant $^{32}P_i$ incorporation by selective cells ($K_{K/Na}$ 1.8) and the lack of significant acidification of the medium after 5 hr of accumulation (Table I). Despite the presence of glucose as an energy source, preference for po-

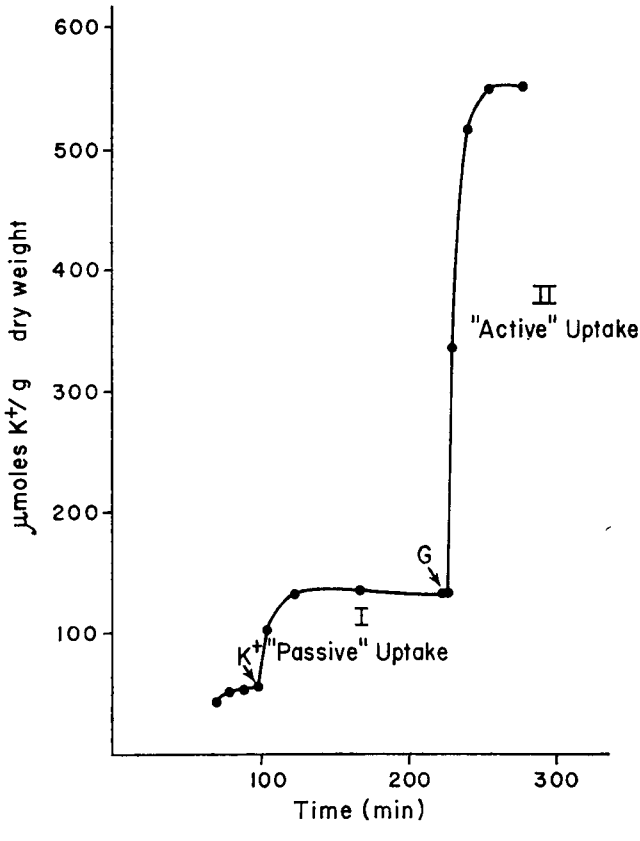

FIGURE 1 Potassium uptake in *E. coli*. Reaction mixture: medium NaA, 0.3 mg/ml (dry weight) K-depleted *E. coli* CBH. K added as KCl to final concentration 0.005 M. Glucose (G) added to a final concentration of 1%.

tassium over sodium was not exhibited (Table I *b* iii) even though substantial metabolic activity was evidenced by $^{32}P_i$ incorporation (8000 cpm/ml), thus demonstrating that intermediate metabolism per se is not a sufficient condition for selective accumulation ($K_{K/Na} = 1.0$). A molality requirement for the external solution exists (9).

The separation of potassium accumulation during stage I from accumulation during stage II provided the opportunity to disconnect cellular selectivity and accumulation from intermediate metabolism, permitting specific study of the ion exchanger resin characteristics of whole cells.

Potassium Associations with Macromolecules in the Absence of Intermediate Metabolism

Equilibrium dialysis and subcellular fractionation of cells from stage I made it possible to determine the chemical nature of the groups serving as counter ions

TABLE I

ABSENCE OF INTERMEDIATE METABOLISM DURING STAGE I SELECTIVE POTASSIUM ACCUMULATION

(*a*) NaF inhibition of stage I selective accumulation*

Medium composition	$K_{K/Na}$‡
3 mM KCl, 3 mM NaCl, 400 mM sucrose	1.66
10 mM KCl, 10 mM NaF, 400 mM sucrose	1.61

(*b*) ^{32}P incorporation in stage I selective accumulation*§

	Medium composition	$K_{K/Na}$	Cell phase	Medium
			(*cpm/ml*)	(*cpm/ml*)
(i)	3 mM KCl, 3 mM NaCl	1.0	3500	2800
(ii)	3 mM KCl, 3 mM NaCl, 400 mM sucrose	1.8	2800	2800
(iii)	3 mM KCl, 3 mM NaCl, 27.5 mM glucose	1.03	8000	2800

(*c*) pH changes during stage I selective accumulation in unbuffered thick bacterial suspensions (75 mg dry weight/ml)||

Time	Medium composition	μmoles K/ g dry wt	Suspension pH
min			
0	150 mM NaCl, 3.3 mM KCl	68	7.1
225	150 mM NaCl, 3.3 mM KCl	285	7.05

* Measurements *a* and *b* made in bacterial suspensions (30 mg dry weight/ml) after dialysis against medium of indicated composition.

‡Selectivity coefficient for K ↔ Na exchange. Precise definition appears in the section dealing with the ion exchange isotherm.

§ In *b*, dialyzing medium contained $^{32}P_i$ as $H_2{}^{32}P\,O_4$ in addition to the solutes indicated.

|| In *c*, instead of equilibrium dialysis, pH and cell K were measured directly on thick bacterial suspensions that were allowed to incubate for 5 hr at 22°C.

for potassium during stage I accumulation. The results of the subcellular fractionations appear in Table II. Suspensions of fragmented and intact cells bound potassium to the same extent when dialyzed to equilibrium in 1 mM KCl (pH 6.5) (rows 2 and 3). Since the exclusion limit of the dialyzing membrane is 10,000, it indicates that stage I potassium atoms are mobile counter ions for fixed charge sites on macromolecules.[1] The accumulation represents a quantitative exchange of Na for K (Table II, footnote *). It was also concluded that the potassium-fixing activity of the fragmented suspension was not affected by a low speed centrifugation

[1] Chloroform-methanol extraction does not alter the binding activity of the fragmented suspension excluding significant K-binding contribution from phospholipids.

TABLE II

BINDING BY INTACT CELLS AND SUBCELLULAR FRACTIONS

Row No.	Fraction	1 mM KCl			3.0 mM KCl–100 mM NaCl			
		Sac K concn	μmoles K/g dry wt cells	Cell K concn	Sac K concn	μmoles K/g dry wt cells	Cell K concn	Sac Na concn
		mmoles/liter		*mmoles/liter*	*mmoles/liter*		*mmoles/liter*	*mmoles/liter*
1	Medium	1.00 ±0.01			3.00 ±0.06			100.0 ±1.0
2	Intact cells	7.08 ±0.25	206 ±7.1*	83.1 ±2.9	8.30 ±0.12	183 ±2.6	73.9 ±1.05	99.0 ±0.9
3	Fragmented cells	6.48 ±0.20	189 ±5.9*	76.2 ±2.4	3.90 ±0.05	31 ±0.40	15.73 ±0.16	106.0 ±1.0
4	Supernatant‡	6.70 ±0.1	196 ±2.9	79.2 ±3.2				
5	Pellet‡	(0.43 ±0.01)§	14.8 ±0.034	5.98 ±0.014				
6	Supernatant‖	4.36 ±0.20	116.0 ±5.33	46.8 ±2.2				
7	Pellet‖	2.58 ±0.03	54.4 ±6.32	21.9 ±2.5				

Cells harvested during the logarithmic phase of growth in medium KA were K-depleted and fractured as described in the text. Fractions derived from bacterial suspensions containing 29 mg dry weight/ml were dialyzed in VisKing tubing for 3 hr at 20°C against 1 mM KCl (pH 6.0) or against an aqueous mixture of Na and K salts (3 mM KCl–100 mM NaCl, pH 6.0). The K uptake in these preparations took place in the absence of metabolism. Incubation at 0°C and in the presence of 50 mM potassium azide did not alter the result and no acidification of the unbuffered medium occurred during the incubation. The values are the means of three measurements, ± the standard error of the mean.

* Na values for intact and fragmented suspensions before dialysis were 180 μmoles/g dry weight. After dialysis against 1.0 mM KCl, they were 1.9 and 2.9 μmoles/g dry weight respectively.

‡ 20 min centrifugation at 17,000 *g*.

§ Potassium concentration of the fragmented suspension due to binding by cell wall and membrane fragments. After equilibrium dialysis, an aliquot of the broken cell suspension was centrifuged at 17,000 *g* for 20 min. The pellet was resuspended in the same volume of H_2O and the potassium concentration was determined by flame photometry.

‖ 2-hr centrifugation at 100,000 *g*.

(17,000 *g*) that removed all membrane and surface layer fragments (Table II, row 4). Furthermore, the observed concentration difference between the two dialyzing compartments did not require membrane-situated "pumps" to maintain the difference. Pumps, therefore, are superfluous for the accumulation of at least one-third of the total complement of cell potassium (189 μmoles/g dry weight) indicating that the macromolecule fraction of the cell maintains an "apparent concentration gradient" of 76:1 (Table II). Table III contains the contributions to potassium

TABLE III

COMPARATIVE BINDING OF POTASSIUM BY CELLS, SUBCELLULAR FRACTIONS, AND PROTEIN FRACTIONS

Cell or fraction	Total protein	Potassium bound	Specific activity
	g	*μmoles*	*μmoles K/ g protein*
Intact cells	2.00	400	204
Fragmented cells	2.00	373	187
Subcellular fractions			
Supernate*	1.51	336	222
Particulate*	0.384	45	117
Total	1.894	381	
$(NH_4)_2SO_4$ fractionation of supernate (% saturation)			
19.4	0.470	69.0	147
30.0	0.194	32.0	166
40.0	0.183	27.0	145
55.0	0.460	79.0	171
80.0	0.090	19.0	206
100.0	0.000	0.0	
Total	1.397	226.0	

Results of equilibrium dialysis against aqueous KCl (1 mM) of protein fractions obtained by differential centrifugation and $(NH_4)_2SO_4$ salt precipitation. The ammonium sulfate fractionation was performed at 0°C. Salt was added to a continuously stirred mixture. Bacteria (3.22 g, dry weight) were grown in medium KA and harvested during the logarithmic phase of growth. Bound K determined by equilibrium dialysis of the indicated fractions in 1 mM KCl is the difference of medium and dialysis sac potassium concentrations and is reported as the amount bound by the entire fraction.
* 2 hr centrifugation at 100,000 *g*.

binding by the cell's proteins. Recovery totals establish that the cellular proteins have all been accounted for. The data indicate that all protein fractions participate in potassium binding with relatively little variation in specific activity between fractions.

Potassium Binding and pH (Fig. 2)

The first major increment in potassium binding, 220 μmoles/g dry weight, occurs in the pH region from 3.5 to 7.0, the titration region for protein carboxylate (10) and the primary acid dissociations of phosphate. The larger increment in potassium binding (250–500 μmoles/g dry weight) occurring in the alkaline region of the titration curve is attributed mainly to deprotonation of the ε-amino groups (pK 9.8)

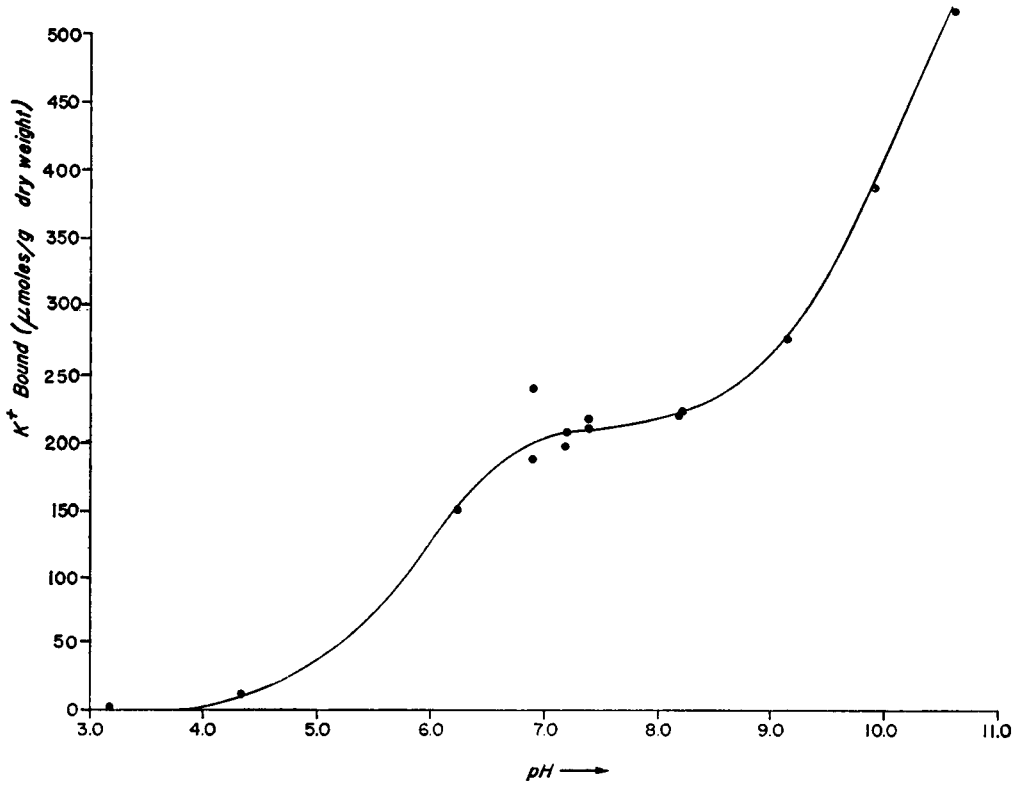

FIGURE 2 Variation of potassium binding in *E. coli* with pH. Equilibrium dialysis of aqueous suspensions (30 mg dry weight/ml) of K-depleted *E. coli* at 22°C against aqueous KCl (3 mM) adjusted to desired pH with HCl or KOH.

of the lysine residues (10) of cell proteins. Imidazole, phenol, and sulfhydryl amino acids make relatively small contributions to charge (Tables IV *a*, IV *b*, and VI). The contribution of the guanidino amine of arginine, whose acid dissociation is at pH 12.48, is considered minimal in this titration region (pH 3.0–11.0) as it was in the titrations of bovine serum albumin by Tanford et al. (10).

While the alkaline increment in potassium binding by stage I cells could be attributed simply to an increase in net charge resulting from deprotonation of amines as commonly assumed in the Linderstrøm-Lang theory for proteins in dilute solution, an alternative explanation, particularly for macromolecules in condensed environments (11), is the possible existence of salt linkages between the carboxylic groups and cationic nitrogen on cell proteins. By this mechanism, the increment in potassium binding at alkaline pH would be due to "unmasking" of carboxylate by deprotonation of amines in amine-carboxylate pairs. In particular, the concept requires intermolecular bridging between intracellular macromolecules arising from the closeness of approximation of oppositely charged carboxylate and amine groups

on neighboring proteins. It is an important consideration since the conceivable presence of intracellular salt linkages implies a powerful motive force for the molecular ordering of cell structure.

This postulate, considered in detail by Steinhardt and Zaiser (12) to explain the lack of reactivity ("masking") of certain ϵ-amino groups in native horse pseudoglobulin and other proteins, and supported by Tanford et al. (10) in the case of bovine serum albumin, is a central feature in the association-induction hypothesis (11) of Ling.

The Ion Exchange Isotherm for the Biological Ion Exchanger Resin

Ion exchange equilibria are commonly characterized by the ion exchange isotherm (Fig. 3). In principle, the isotherm graphically specifies all possible experimental conditions at a given temperature, any specific set of experimental conditions corresponding to one point on the isotherm surface, e.g. the ratio of areas set off by perpendiculars through any point on the isotherm surface (I and II in Fig. 3) is the selectivity coefficient, $K_{A/B}$, for that point. The ion exchange isotherm is an equally concise representation for biological ion exchange equilibria and characterizes the ionic composition of the exchanger (e.g., the cell) as a function of the experimental conditions. Since the results of the experiments in the preceding section indicated that at least some of the mobile cations were exchanging at fixed

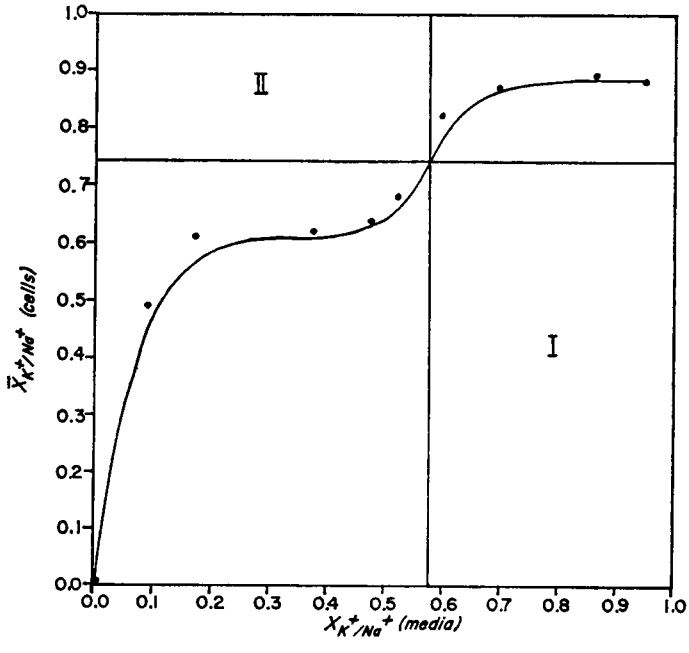

FIGURE 3 K ↔ Na ion exchange isotherm for *E. coli*. Equilibrium exchange conducted in 0.4 M sucrose containing the chloride salts of Na and K at a total normality of 0.01 N.

charge sites on macromolecules, it is useful to introduce the ion exchange isotherm at this point to begin the characterization of cellular ion exchange equilibria.

X and $\bar{X}$ are the equivalent ionic fraction of the solution and endocytic (intracellular) phase respectively and are defined as usual by

$$X_A = \frac{z_A m_A}{\sum_i z_i m_i},$$

where m represents molality. We define also the *rational selectivity coefficient* used in these papers that characterizes the ion exchange equilibrium

$$R \cdot z_A B + z_B A \rightleftharpoons R \cdot z_B A + z_A B,$$

where z_i is the valence of the ith species, A and B are the mobile counter ions, and R designates resin counterion complex.

The rational selectivity coefficient[2] for this exchange is given by

$$K_{A/B} = \frac{\bar{X}_A^{|z_B|} X_B^{|z_A|}}{\bar{X}_B^{|z_A|} X_A^{|z_B|}}.$$

The absolute value of the valence is used so that $K_{A/B} > 1$ corresponds to preference for A regardless of the sign of the valence.

The ion exchange isotherm for $K \leftrightarrow Na$ exchange in *E. coli* (stage I cells) appears in Fig. 3. The exchange was carried out on cells initially in the Na form (K-depleted cell) in an external solution with a total normality of 0.01 N. The biphasic shape of the isotherm is typical of a bifunctional cation exchanger (1), and is consistent with the conclusions from the analytical data (see Tables IV–VI) that two distinct ionic groups, PO_4^{3-} and COO^- function as the principal exchange sites for cation exchange in cells. Accordingly, it can be said that *with respect to cations, the biological resin can be considered to be a bifunctional cation exchanger.* The failure of the isotherm to rise to unity at uniform medium ionic composition indicates that a small inaccessible Na fraction exists that does not participate in exchange.

The Ionic Species of E. coli and Their Quantitative Electrostatic Correspondence

The electrostatic correspondence of counter ion and fixed charge species in *E. coli* are presented in Tables IV *a* and IV *b*. Table IV *a* contains the charge profile of *E. coli* in which all the mobile alkali cations have been replaced by Na. Table IV *b* compiles the charge configuration of *E. coli* after conversion of the biological resin

[2] Note that the ion exchange isotherm conveniently specifies the rational selectivity coefficient for every point on the isotherm. It is given by the ratio of the areas of the rectangles (indicated by Roman numerals) that converge at a point on the isotherm surface.

from the Na form to the K form by the intermediate metabolism of glucose. The quantitative electrostatic correspondence or charge stoichiometry agreed to within 7.8% for the K form of the cell and was in nearly exact agreement for the Na form.

In addition to containing two fixed groups, phosphate and carboxylate, as exchange sites for mobile cations, the biological resin also contains a significant amount of protonated amines from protein amino acids. The guanidino groups of arginine and the ϵ-amines of lysine, for example, provide fixed charge sites for exchange of mobile anions (Tables IV *a*, IV *b*, and VI). The combined anion exchange capacity of these groups in *E. coli* is 752 μmoles/g dry weight. At pH's below their acid dissociation, the amine groups of the nucleic acid bases guanine (3.2), cytidine (4.5), etc., would contribute significantly to the anion exchange capacity of cells. At the near-neutrality of most physiological events, however, they are not a material consideration. The composite biological resin therefore can be regarded as a "mixed bed" resin capable of both cation and anion exchange.

The data in Tables IV–VIII conform to the practice of characterizing the capacity of an ion exchange resin by its "scientific weight capacity" (μeq/g dry weight) a number that is invariant with hydration and, therefore, a characteristic constant of the material. The molal equivalents of the weight capacity, fixed charge concentration quantities that are more convenient for theoretical treatment of ion exchange phenomena, appear in parentheses beside the weight capacity. The molalities were calculated making use of the ratio of bacterial dry weight to intracellular water (2.48 ± 0.014 mg/g) obtained previously (3) and are corrected for swelling changes that accompany K accumulation (2). The biological ion exchanger resin is approximately 1.0 molal in fixed charge groups containing approximately 0.6 molal in anionic fixed charge sites and 0.4 molal in cationic fixed charge sites (Tables IV *a* and IV *b*). By way of comparison with commercial ion exchanger resins, the internal molality of *E. coli* is similar to the internal molality of sulfonated polystyrenes (Dowex 50) that are 2% cross-linked (13).

The capacity of the macromolecule fraction for cation exchange is 1290 μeq/g of dried cells comprised of 144 μeq of phospholipid phosphate, 624 μeq of nucleic acid phosphate, and 522 μeq of protein side group carboxylate (Tables IV *a* and IV *b*).

The macromolecule fixed charge from nucleic acid phosphate, protein amino acid carboxylate, and phospholipid phosphate provide the great majority, 1290/1461 and 1290/1619, of the anions available as counter ions for potassium (Tables IV *a* and IV *b*). Furthermore, since there is no evidence to date that suggests ion pair associations of potassium with specific cell counter ions–the molar association energies compiled in Table VII *a*, for example, reveal little distinction between potassium phosphate and potassium carboxylate associations–statistical distribution of potassium associations among cell counter ions would require that *8 times out of 10, potassium would be associated with an ion exchange site on a macromolecule.*

TABLE IV

(*a*) QUANTITATIVE ELECTROSTATIC CORRESPONDENCE AND THE ION PAIRS OF *E. COLI* IN THE Na FORM (K-DEPLETED)

(μeq/g dry weight)*

Anionic residues			Cationic residues		
Phosphate			Divalent cation		
Phospholipid phosphate	144	(66)‡	Mg^{++}	282	(129)
Nucleic acid phosphate§	624	(286)	Ca^{++}	17	(8)
Phosphate ester‖	80	(37)	Fe^{++}	2	(0.9)
Inorganic phosphate	29	(13)			
Total phosphate	877	(402)	Total divalent cation	301	(138)
Inorganic			Alkali cation		
$SO_4^{=}$¶	—		Na^+	160	(74)
Cl^-	—		K^+	17	(8)
			NH_4^+	72	(33)
Carboxylate			Total alkali cation	249	(115)
Organic acid carboxylate					
Acetate	12	(6)			
Pyruvate	—		Amines		
Succinate	—		*Protein amine*		
α-ketoglutarate	—		Imidazole	14	(6)
Malate	—		Guanidino	335	(154)
Lactate	—		Lysine	403	(185)
Fumarate	—		Total	752	(345)
Formate	—				
HCO_3^-	27	(12)	*Free amino acid amine*	19	(9)
Total	39	(18)	*Phospholipid amine*‡‡	134	(62)
			Total amine	904	(416)
Protein-carboxylate					
Aspartate	193	(89)			
Glutamate	329	(151)			
Total	522	(240)			
Free amino acid carboxylate	23	(11)			
Total carboxylate	584	(268)			
Total anion	1461	(670)	Total cation	1454	(669)

Summary of the ion exchange capacities of Na form *E. coli*	Anionic	Cationic	Internal molality (total)
Ion exchange capacity of macromolecule fraction	1290 (595)	886 (407)	1002
Ion exchange capacity of the sorbed metabolite fraction (inorganic ions excluded)	142 (66)	19 (9)	75
Total ion exchange capacity	1432 (661)	904 (416)	1077

Fragmented cell suspensions as well as intact cells do not release any Mg or Ca when dialyzed against solutions containing alkali cations (Table V) but lacking a carbon source. On the other hand, the addition of substrate and the onset of intermediate metabolism results in a net release of 89 μeq of divalent cation/g of dried bacteria (Table VIII). This is the result of a decrease in Mg from 282 to 142 μeq during potassium enrichment coupled with a gain in Ca from 17 to 68. Magnesium, therefore, is firmly bound to the ion exchange sites on nondialyzable macromolecules and does not exchange for monovalent alkali cation. The result is in keeping with the general axiom of ion exchange that for purely electrostatic reasons the ion exchanger prefers the counter ion of higher valence (14–24).

Ion Pairing and Associations

The possibility of specific ion pairing within the biological exchanger was explored in Tables VII *a* and VII *b*. Molar free energies (ΔG^0) calculated for a variety of ion pairs indicate that the strongest associations exist between divalent ions and nucleotide phosphate suggesting that, on the average, divalent metal ions (Mg in particular) have a greater tendency to pair with nucleotide and nucleic acid phosphate in the cell than with carboxylate. This result is consistent with the well-known tendency of Mg to appear as Mg nucleate during nucleic acid separations.

Additional supporting evidence for the tendency for Mg to pair with nucleic acid phosphate was obtained experimentally by comparing the K and Mg analyses of nucleic acid–enriched cell fractions obtained from the pellets of fragmented suspensions after high speed centrifugation. The coefficient for the partitioning of Mg in the nucleic acid–enriched fraction, 1.41 (Table VII *b*), demonstrated a preference of Mg for the nucleic acid fraction relative to monovalent alkali cation.

Beyond this, the ion-pairing orders cannot be reduced further. The association energies do not help distinguish between alkali cation phosphate associations and alkali cation carboxylate associations (Table VII *a*). In fact, the association ener-

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Data summarizes analyses made on *E. coli* B (strain CBH) after K depletion.
Internal molality is expressed as the total fixed charge molality to facilitate interfacing with the literature of ion exchange resins.
* Calculation of equivalents based on dissociations at pH 7.0, pH of the bacterial suspensions at time of analysis.
‡ Numbers in parentheses: molal equivalent of the weight capacity—meq/kg cell H_2O.
§ 5% of total nucleic acid phosphate was assumed to be as end groups (secondary phosphate dissociation 6.0 [39]).
‖ Phosphate ester equivalents estimated conservatively (1 eq/mole phosphate).
¶ Estimated from the data of Roberts et al. (40).
** Dashes indicate compound was undetectable.
‡‡ Estimate made on basis of 93% of phospholipid in *E. coli* existing as phosphatidylethanolamine (33, 34).

TABLE IV

(*b*) QUANTITATIVE ELECTROSTATIC CORRESPONDENCE AND THE ION PAIRS OF *E. COLI* IN THE K FORM (K-RICH)

(μeq/g dry weight)*

Anionic residues			Cationic residues		
Phosphate			Divalent cation		
Phospholipid phosphate	144	(58)‡	Mg^{++}	142	(57)
Nucleic acid phosphate§	624	(251)	Ca^{++}	68	(27)
Phosphate ester‖	112	(45)	Fe^{++}	2	(0.8)
Inorganic phosphate	29	(12)			
Total phosphate	909	(366)	Total divalent cation	212	(85)
			Alkali cation		
Inorganic anions			Na^{+}	—	(—)**
$SO_4^{=}$¶	6	(2)	K^{+}	550	(222)
Cl^{-}	2	(1)	NH_4^{+}	50	(20)
Total inorganic anions	8	(3)	Total alkali cation	600	(242)
Carboxylate			Amines		
Organic acid carboxylate			*Protein amine*		
Acetate	18	(7)	Imidazole	14	(6)
Pyruvate	36	(15)	Guanidino	335	(135)
Succinate	16	(6)	Lysine	403	(163)
α-ketoglutarate	10	(4)			
Malate	20	(8)	Total	752	(304)
Lactate	3	(1)			
Fumarate	3	(1)	*Free amino acid amine*	55	(21)
Formate	7	(3)			
HCO_3^{-}	15	(6)	*Phospholipid amine*‡‡	134	(54)
Total	128	(52)	Total amine	941	(380)
Amino acid carboxylate					
Aspartate	193	(78)			
Glutamate	329	(132)			
Total	522	(210)			
Free amino acid carboxylate	52	(22)			
Total carboxylate	701	(284)			
Total anion	1619	(652)	Total cation	1753	(707)

Summary of the ion exchange capacities of K form *E. coli*	Anionic	Cationic	Internal molality (total)
Ion exchange capacity of macromolecule fraction	1290 (520)	886 (358)	878
Ion exchange capacity of the sorbed metabolite fraction (inorganic ions excluded)	292 (118)	55 (21)	139
Total ion exchange capacity	1582 (638)	941 (379)	1017

TABLE V

THE EFFECT OF EQUILIBRIUM DIALYSIS AGAINST MONOVALENT CATIONS ON THE BINDING OF DIVALENT CATIONS BY MACROMOLECULES

	Before dialysis		After dialysis	
	Mg^{++}	Ca^{++}	Mg^{++}	Ca^{++}
	μeq/g dry wt		*μeq/g dry wt*	
Intact cells	282	12.6	282	16.6
Fragmented cells	278	16.0	280	33.2

Suspensions of intact cells and fragmented bacteria (30 mg dry weight/ml) that had been K-depleted were dialyzed against a solution containing 400 mM sucrose, 3 mM NaCl, and 3 mM KCl at 22°C for 3 hr.

gies available for alkali cation carboxylate and phosphate salts are similar enough to suggest that the alkali cations distribute without significant preference among the carboxylate and phosphate exchange sites within the cell.

Sorbed Electrolytes: Carboxylic Acids, Amino Acids, Phosphate Esters, and Miscellaneous Inorganic Ions

In addition to a fixed charge framework and mobile counter ions, naturally occurring and synthetic ion exchangers normally contain a solute fraction that has attached to the exchanger framework by sorption (25) rather than by ion exchange (Fig. 5, paper III [reference 2]). London forces and dipole-dipole interactions, molecular interaction forces associated with the attachment of nonpolar and polar molecules (25–29) to the exchanger backbone, result in the tendency of hydrocarbon groups to be squeezed out of polar solutions onto the phase boundary between the exchanger matrix and the solution. The result is a distribution coefficient

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Data summarizes analyses made on *E. coli* B (strain CBH) harvested during the logarithmic phase of growth in medium KA.

Internal molality represents total fixed charge molality.

* Calculation of equivalents based on dissociations at pH 7.0, pH of the bacterial suspensions at time of analysis.

‡ Numbers in parentheses: molal equivalent of the weight capacity—meq/kg cell H_2O.

§ 5% of total nucleic acid phosphate was assumed to be as end groups (secondary phosphate dissociation 6.0 [39]).

‖ Phosphate ester equivalents estimated conservatively (1 eq/mole phosphate).

¶ Estimated from the data of Roberts et al. (40).

** Dashes indicate compound was undetectable.

‡‡ Estimate made on basis of 93% of phospholipid in *E. coli* existing as phosphatidylethanolamine (33, 34).

TABLE VI

AMINO ACID COMPOSITION OF K-RICH AND K-DEPLETED *E. COLI*; PROTEIN AMINO ACIDS AND FREE AMINO ACID POOLS

Amino acid	Protein amino acids of K form cell and Na form cell	Free amino acids of Na form cell	Free amino acids of Na form cell		Free amino acids of K form cell	Free amino acids of K form cell	
			COO^-	RNH_3^+		COO^-	RNH_3^+
	μmoles/g dry wt	*μmoles/g dry wt*	*μeq/g*	*μeq/g*	*μmoles/g dry wt*	*μeq/g*	*μeq/g*
Half cystine	87 ± 5*	—					
Aspartic acid	193 ± 27	3.7	6.44		2.7	5.2	
Asparagine	310 ± 10	3.0	3.00	6.73	2.23	2.23	4.93
Glutamic acid	329 ± 15	1.5	3.0	2.6	0.78	1.56	1.30
Glutamine	194 ± 10	1.1	1.1	1.1	0.52	0.52	0.52
Threonine	285 ± 10	trace	—	—	trace	—	—
Serine	247 ± 11	trace	—	—	trace	—	—
Proline	188 ± 17	trace	—	—	4.4	4.4	4.4
Alanine	542 ± 33	3.0	3.0	3.0	13.0	13.0	13.0
Glycine	569 ± 61	1.4	1.4	1.4	2.7	2.7	2.7
Valine	387 ± 19	1.8	1.8	1.8	6.8	6.8	6.8
Methionine	120 ± 10	—	—	—	—	—	—
Isoleucine	266 ± 20	1.0	1.0	1.0	2.0	4.0	4.0
Leucine	431 ± 21	1.0	1.0	1.0	2.4	2.4	2.4
Tyrosine	182 ± 8	0.97	0.97	0.97	0.96	1.92	1.92
Phenylalanine	163 ± 11	0.66	0.66	0.66	0.96	1.92	1.92
Lysine	403 ± 22	trace	—	—	6.0	6.0	12.0
Histidine	151 ± 10	trace	—	—	trace	—	—
Arginine	335 ± 15		—	—	1.7	1.7	3.4
Totals		19.16	23.39	19.16	47	51.6	55.4

Protein amino acids were measured in bacteria collected at 0.600 OD 620 in the logarithmic phase of growth, washed twice in distilled water, and dried to constant weight at 60°C. Free amino acids were determined on TCA (5%) extracts of French Press-crushed suspensions (30 mg dry weight/ml). Care was taken not to remove free amino acids by washing procedures. Instead, corrections were made by amino acid analysis of the extracellular fluid trapped in centrifuged bacterial pellets, and they proved negligible. Amino acid analyses were performed by Dr. Fred Wagner, Department of Biochemistry, University of Nebraska in Lincoln, by ligand exchange chromatography on the Hitachi-Perkin-Elmer KLA-3B analyzer (Hitachi Ltd., Tokyo, Japan; Perkin-Elmer Corp., Instrument Div., Norwalk, Conn.) utilizing hydrolysis and chromatography techniques described elsewhere (41, 42 *a*). Amino acid amides were determined on lyophilized samples of *E. coli* by the methylation and borohydride reduction procedure of Wilcox (42 *b*).

* Standard error of mean.

TABLE VII

(*a*) CALCULATED ASSOCIATION ENERGIES (ΔG^0) FOR PAIRING BETWEEN MOBILE CATIONS AND BIOLOGICAL ANIONS (AT 298°K)

	Mg^{++}		Ca^{++}		K^+		Na^+		Arginine	
	Value	(Ref.)	Value	(Ref.)	Value	(Ref.)	Value	(Ref.)	Value	(Ref.)
	kcal/mole		*kcal/mole*		*kcal/mole*		*kcal/mole*		*kcal/mole*	
Carboxylates										
Citrate	4.37	(43)	4.37	(43)						
Oxalate	4.65	(43)	4.07	(43)						
Acetate			1.06	(48)						
Amino acid carboxylate										
Glycine	4.695	(43)	1.95	(43)						
Proline	5.46	(44)								
Tryptophane	5.46	(44)								
Glycylglycine	1.5	(44)								
Protein carboxylate										
Calisano protein			4.1	(44)						
Albumin			1.36–1.77	(45)						
β-lactoglobulin							2.97–3.18	(45)		
Myosin					3.14–3.96	(50)	3.55–4.37	(50)		
Phosphates										
Adenosine triphosphate	6.77	(46)			3.14	(51)				
Nucleic acid (DNA)	7.22	(47)	4.78	(49)					3.01	(52)

for the partitioning between aqueous and matrix phase that increases as the molecular weight of the solute (25, 28, 29). The partition coefficient in ion exchanger resins, for example, increase progressively along the sequence acetic acid, propionic acid, *n*-butyric acid.

Typically, sorbed solutes are removed from the exchanger by washing with solvent. Similarly, biological ion exchangers contain a solute fraction readily removed by washing with solvent and analogous to the sorbed solute fraction in resins. Pools of intracellular metabolites such as carboxylic acids, amino acids, and phosphate esters constitute such a fraction (30–32). Accordingly, it is convenient to refer to organic and inorganic electrolytes in this pool of metabolites as sorbed electrolytes.

The principal organic acids that accumulate in K-rich *E. coli* are acetate, pyruvate, succinate, and malate (Fig. 4). The combined total of all the organic acids and bicarbonate is 128.7 μeq/g of dried cells. The organic acid metabolites accumu-

TABLE VII

(*b*) RELATIVE ASSOCIATION OF K^+ AND Mg^{++} WITH NUCLEIC ACID CELL FRACTIONS

	K^+	Mg^{++}
	μeq/g dried cells	*μeq/g dried cells*
Supernatant*	116.0	189
Pellet*	54.4	125
Partition coefficient for partitioning of Mg^{++} between nucleic acid–rich pellet and aqueous supernatant‡	1.41	

Suspensions of K-depleted bacteria (30 mg dry wt/ml) were fragmented in the French press and fractionated by differential centrifugation.

* Supernatant and pellet from 2 hr of centrifugation at 100,000 *g*.

‡ Partition coefficient determined as

$$\frac{[Mg]_{pellet} \cdot [K]_{sup}}{[Mg]_{sup} \cdot [K]_{pellet}}.$$

late within the cell as products of intermediate metabolism and are reduced to trace concentrations during K depletion washes (Tables IV *a*, IV *b*, and VIII).

A small amount of free amino acids also accumulate as products of intermediate metabolism and are removed by washing. K-rich *E. coli* contain 47 μmoles of free amino acids/g dried cells contributing 54.6 anionic μeq/g and 55.4 cationic μeq/g to the sorbed electrolyte pool whereas K-depleted *E. coli* contain 28.4 anionic μeq and 19.2 cationic μeq/g (Tables IV *a*, IV *b*, and VI).

Similar results were obtained for the phosphate ester pools. Significant amounts of phosphorylated metabolite were removed during the K-depleting washes reducing the pool size from 112 to 80 μeq/g. The pool was reconstituted during substrate-stimulated K accumulation. The resulting change in the ion exchange capacity of the cell (Tables IV *a* and IV *b*) from sorption of electrolytic metabolite is from 142 μeq/g in the Na form of the cell to 292 μeq/g in the potassium-enriched cell. The increase of 150 μeq is the sum of an increase of 32 μeq in phosphate esters, 89 μeq in carboxylic acids, and 29 μeq in free amino acid carboxylate.

Substrate-dependent potassium accumulation (stage II "active transport," Fig. 1) can thus be summed up as the result of two effects of intermediate metabolism:

(*a*) *Metabolism increases the ion exchange capacity of the biological resin by the accumulation (sorption) of charged metabolites (phosphate esters, carboxylic acids, and amino acids) that then require electrical neutralization by additional mobile cation*, and

TABLE VIII

CHANGES IN THE ION CHARACTER OF *E. COLI* DURING POTASSIUM ENRICHMENT BY METABOLIZABLE SUBSTRATE

	K^+-depleted cells Fixed and labile counter ion pools	K^+-rich cells Fixed and labile counter ion pools	Δ
	μeq/g dry wt	*μeq/g dry wt*	*μeq/g dry wt*
(*a*) Anionic			
Phosphates	877	909	32
Phospholipid phosphate	144	144	—
Nucleic acid phosphate	624	624	—
Phosphate esters	80	112	32
Inorganic phosphate	29	29	—
Carboxylates	645	763	118
Organic acid carboxylate	39	128	89
Acetate	12.5	18	5.5
Pyruvate	—	36.2	36.2
Succinate	—	16.2	16.2
α-ketoglutarate	—	9.85	9.85
Malate	—	20.30	20.30
Lactate	—	2.90	2.90
Fumarate	—	2.90	2.90
Formate	—	7.25	7.25
Bicarbonate	27	15.00	−12.00
Protein amino acid carboxylate	522	522	—
Aspartate	193	193	—
Glutamate	329	329	—
Free amino acid carboxylate	23	52	29
Inorganic			
Sulfate		6	6
Chloride		2	2
(*b*) Cationic			
Alkali cations	249	600	351
Sodium	160	—	−160
Potassium	17	550	533
Ammonium	72	50	−22
Divalent cations	301	212	−89
Magnesium	282	142	−140
Calcium	17	68.2	51.2
Iron	2	2	—
Amines	905	941	36
Protein amino acid amine	752	752	—
Histidine (imidazole)	14	14	—
Lysine	403	403	—
Arginine	335	335	—
Phospholipid amine	134	134	—
Free amino acid amine	19	55	36

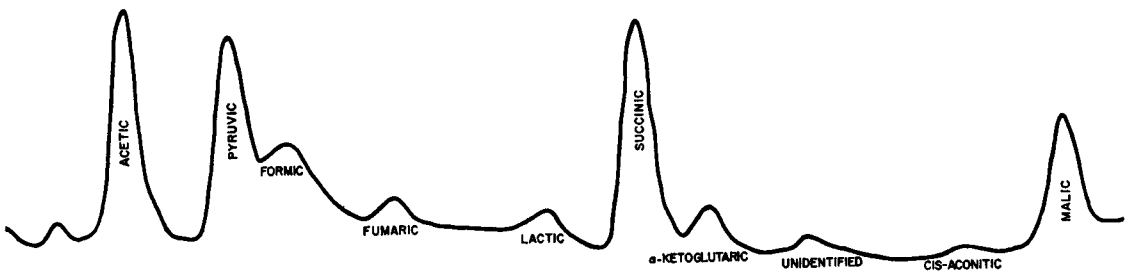

FIGURE 4 Chromatogram of intracellular organic acids in *E. coli*. Bacteria harvested in the logarithmic phase of growth were immediately immersed together with Millipore filter (0.45 μ, Millipore Corporation, Bedford, Mass.) in a sufficient volume of ice water to produce a concentration of 12 mg dried bacteria/ml. The suspension was crushed in a French Press (6000 psi) at 0°C, sulfuric acid added to a final concentration of 0.1 N, and 0.5 ml of acidified suspension added directly to a hydrated silica gel column. Organic acids were chromatographed by Dr. L. Kesner using an automated partition chromatography analyzer developed by him in 1965 (35–38).

(*b*) *Metabolism initiates the exchange of divalent cation, principally Mg, for K at cellular charge sites,* an exchange that does not occur in the absence of substrate (Table V).

Miscellaneous Inorganic Ions (Sulfate, Chloride, Iron)

The cell concentration of chloride, sulfate, and iron were negligible in these studies. Chloride and sulfate were totally removed by washing. Cell iron remained unchanged.

Macromolecule Fraction

The macromolecule components of the biological ion exchanger, phospholipid, nucleic acid, and protein are invariant with washing and potassium accumulation (Tables IV *a* and IV *b*). If the phospholipid fraction is regarded as electrically internally compensated[3] and not contributing any net charge to the ion exchanger matrix, as in the equilibrium exchange experiments (Table II, row 5), it is possible to *summarize E. coli as a bacterial cation exchanger resin whose functional groups are approximately half phosphate (624 μeq/g) and half carboxylate (522 μeq/g).*

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[3] 93% of phospholipid in *E. coli* is phosphatidylethanolamine (33, 34) and is isoelectric at neutral pH.

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