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Preface

The fifth volume of *Current Topics in Membranes and Transport* presents chapters dealing with the interaction of solutes and membrane proteins, as exemplified by galactoside transport (Boos), and its energetic aspects in amino acid transport (Heinz). Three chapters treat electrolyte transport, in bacteria (Harold and Altendorf), in renal and bladder cells (Brodsky and Schilb), and in the intestine (Schultz and Curran). The final chapter, by Tasaki and Carbone, reviews the experimental basis for the macromolecular hypothesis of nerve excitation.

We believe these reviews conform to our editorial policy of not shunning controversy. We therefore hope they will contribute to our understanding of the molecular basis of biological transport.

FELIX BRONNER
ARNOST KLEINZELLER

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The Editors wish to thank Mark Solomon for his photograph of Aharon Katzir-Katchalsky which appeared in Volume 4.

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Cation Transport in Bacteria: K^+, Na^+, and H^+

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I. INTRODUCTION

The internal ionic milieu of bacteria generally differs radically from that of the medium. As a rule, K^+ is by far the most abundant cytoplasmic cation, even though Na^+ may predominate in the environment. Even when this generalization must be qualified, as is the case for halophilic bacteria, it remains true that a high internal concentration of K^+ is required for growth. K^+ is probably an essential nutrient for all bacteria (some species accept Rb^+ as a substitute for K^+), whereas a requirement for Na^+ is seen only occasionally. This universal preference for K^+ poses fundamental questions: How do bacteria selectively extract K^+ from an environment generally far richer in Na^+? And why must they do so?

The study of cation transport in bacteria is still emerging from the descriptive phase. The literature leaves little doubt that ion translocation is mediated by specific transport systems located in the cytoplasmic membrane, and records numerous attempts to define these by kinetic criteria. It also seems clear that ion transport in bacteria does not involve the familiar Na^+,K^+-dependent ATPase of mammalian membranes. Beyond this, attempts to define the molecular nature of the transport catalysts and their relationship to metabolic pathways rely as much upon conjecture as upon established fact. This article draws freely upon concepts and techniques originating outside the bacterial world, and especially upon Peter Mitchell's chemiosmotic hypothesis. Our purpose is not to provide a comprehensive survey of the literature, but rather to construct a framework on which to hang present and future experimental data. In choosing this course we are well aware that the devil lurks in the details; but we also share Bacon's conviction (Kuhn, 1970) that truth emerges more readily from error than from confusion.

II. THE ION BALANCE OF BACTERIAL CELLS

The principle of electroneutrality dictates that the electrical charges of cellular cations must at all times be balanced by an equivalent amount of anions. Any imbalance generates an electrical potential, hence a force that tends to restore overall electrical neutrality. Ignoring, for the time being, the ionic imbalance that underlies bioelectrical potentials, we can anticipate that any change in the amount of cellular ion will be accompanied by ion shifts of opposite sign such that overall electroneutrality is preserved. This section is concerned with the balance of the charge account and with the physical state of the cytoplasmic ions.

A. Cations and Anions

From the K^+ content of the cells and the internal water space, the cytoplasmic K^+ concentration is estimated to be near 0.2 *N* in *Escherichia coli*, 0.4 *N* in *Streptococcus faecalis*, and a prodigious 5 *N* in certain halophilic bacteria (Table I). Which anions balance the positive charges? Tempest (1969) has recently summarized his extensive studies on the composition of bacteria growing in a chemostat. In *Aerobacter aerogenes*, under conditions such that K^+ limits the rate of growth, nucleic acid phosphorus accounted for a large part of the cellular K^+ and Mg^{2+}. Cations and nucleic acids varied in parallel as a function of the growth rate, leading to the conclusion that much of the cellular K^+ is associated with ribosomes. That K^+ is required for protein synthesis is of course well known (Section VI, A). In *Bacillus subtilis*, phosphate groups of nucleic and teichoic acids are the chief anionic residues, replaced under some conditions by the carboxyl groups of teichuronic acid (Tempest, 1969). In exponentially growing *S. faecalis*, the total content of K^+ or Rb^+ was nearly equivalent to the total phosphorus of nucleic acids and phospholipids (Harold and Baarda, 1967a). Clearly, much of the cellular K^+ is electrically balanced by the anionic groups of macromolecules.

The only comprehensive analysis of a bacterial ion balance known to us is due to Damadian (1971a), from whose work Table II is drawn. The data refer to cells harvested during the exponential phase of growth (K^+

TABLE I

CATION CONTENT OF SELECTED BACTERIA[a]

	Streptococcus faecalis[c]		*Escherichia coli*[d]		*Halobacterium*[e]	
Cation[b]	Stationary	Exponential	Stationary	Exponential	Stationary	Exponential
K^+	220	560	10	220	3700–4000	3700–4000
Na^+	250	5	180	80	500–700	1600–2100
H^+	100 (pH_i near 5)	— (pH_i near 7)	—	—	—	—
Cl^-	—	—	—	—	2300–2900	3200–4000

[a] Values given are concentrations in millimoles per liter of cell water.

[b] H^+ refers to titratable acidity (Harold and Papineau, 1972a).

[c] Data from Zarlengo and Schultz, 1966 and unpublished experiments in this laboratory.

[d] Data from Schultz *et al.* (1962a).

[e] Data from Ginzburg *et al.* (1970), for an unidentified species.

TABLE II

ELECTROSTATIC BALANCE OF IONS IN *Escherichia coli*[a,b]

	Exponential phase cells, K^+ form (μeq/gm dry weight)	Alkali-treated cells, Na^+ form (μeq/gm dry weight)
Anionic residues		
Phospholipid phosphate	144	144
Nucleic acid phosphate	624	624
Soluble phosphate esters	112	80
Inorganic phosphate	29	29
Protein carboxylate	522	522
Organic acid carboxylate	128	39
Amino acid carboxylate	52	23
Other anions	8	0
Total anionic residues	1619	1461
Cationic residues		
K^+	550	17
Na^+	0	160
NH_4^+	50	72
Mg^{2+}	142	282
Other inorganic cations	70	19
Protein amine	752	752
Amino acid amine	55	19
Phospholipid amine	134	134
Total cationic residues	1753	1454

[a] Data from Damadian (1971a), with kind permission.

[b] The K^+ cells were harvested during growth and analyzed. To replace K^+ by Na^+ the cells were subjected to repeated treatment with alkali.

cells); part was subjected to alkali treatment so as to replace K^+ by Na^+ (Na^+ cells). The omission of polyamines from the analysis is regrettable, but the data make it clear that a large fraction of the cellular K^+ or Na^+ must be paired with anionic groups of macromolecules, both phosphate and carboxylate. Only about a quarter of the anionic groups comes from diffusible metabolites. This leads Damadian (1971a) to regard bacterial cells as a mixed-function cation-exchange resin, a concept to whose implications we shall return.

In growing cells the internal pH is not too far from neutrality, and H^+ makes a minor contribution to ion stoichiometry. This is often not true

for cells harvested during the stationary phase of growth from media acidified by the products of metabolism. In such cells H^+ may make up a substantial part of the cation complement (Table I); the H^+ is expelled, and replaced by K^+, when the cells are allowed to metabolize.

B. Physical State of Cytoplasmic Cations

In an earlier era of cell physiology, it was quite widely held that the capacity of cells to accumulate various nutrients could be accounted for by the binding, or sorption, of small molecules to specific sites on the macromolecular matrix of the cytoplasm. According to Ling (1965, 1969), who has presented the most sophisticated treatment of this conception, both pool size and selectivity are determined by specific association of solutes with binding sites, and the membrane does not constitute a significant permeability barrier to small molecules. Today, this view of cellular structure has little currency among students of microbial physiology. The accumulated evidence of two decades (see Rothstein, 1959; Epstein and Schultz, 1967; Harold, 1972) leaves little doubt that cytoplasmic solutes are in general osmotically active, and that their entry into the cell is controlled by specific transport systems which reside in the plasma membrane. But the case of cations is a somewhat special one since, as noted in Section II, A, they are to a large extent paired with macromolecular anions. It is thus appropriate to reconsider the mobility of cytoplasmic cations, their contribution to the osmotic pressure, and the specificity of their association with anionic groups.

The osmotic pressure of bacteria was originally measured by Mitchell and Moyle (1956), both by plasmolysis and by allowing cell pastes to equilibrate with sucrose solutions of known vapor pressure. The conclusion that the cytoplasm of *Staphylococcus aureus* is near 1 osmolal requires most of the small solutes to be osmotically active; K^+, a major component, is by implication among these. More direct evidence comes from the studies of Epstein and Schultz (1967) on the relationship of K^+ content to osmotic pressure in *Escherichia coli*. We consider this matter in Section V, I; here we note only that the K^+ content of growing cells increased as a function of medium osmolarity; on the assumption that the extra K^+ is neutralized by a diffusible anion, and that both are osmotically active, there was good quantitative correspondence up to ca. 400 milliosmolal. Plasmolysis of the cells induced by addition of glucose was reversed under conditions that allowed the cells to accumulate K^+ from the medium. These results, too, could be accounted for on the assumption that the K^+ taken up is osmotically active.

In a recent article, Marquis and Carstensen (1973) addressed themselves directly to the state of cations in *S. faecalis* and *Micrococcus lysodeikticus* by measuring both high-frequency electrical conductivity and osmotic characteristics. The electrical conductivity measurements yielded values of 0.90 and 0.68 mho per meter for *S. faecalis* and *M. lysodeikticus*, respectively; these values are only about a third of the conductivity predicted from the ion content of the two cell types, taking K^+ to be the main current-conducting ion. The discrepancy was resolved through studies on the conductivity of suspensions of cells whose membranes had been damaged with butanol or by freezing and thawing; the conductivities of dilute suspensions were in good agreement with expectations, but those of concentrated suspensions were progressively less, extrapolating to conductivities near those found for intact cells. These investigators therefore concluded that the relatively low conductivity of intact cells reflects the behavior of electrolytes in a concentrated mixture of small ions and cell polymers of various sizes. However, the ions were osmotically active both when the cell was intact and after disruption; indeed, the internal osmolality estimated from the plasmolysis threshold was somewhat higher than that calculated from the solute content. Overall, then, it appears that small cytoplasmic ions are free to move in an electrical field, albeit with reduced mobility. Both the high viscosity and the proximity of charged macromolecules may contribute to the restraints on cation mobility (Marquis and Carstensen, 1973). But there is no need to invoke tight binding or "sorption" of the ions to cellular polymers.

Another nondestructive technique to shed light on the physical state of cations is nuclear magnetic resonance (NMR). Studies with mammalian tissues, which are not reviewed here (Ling and Cope, 1969; Cope, 1970; Czeisler *et al.*, 1970), led to the conclusion that a large fraction of Na^+ in muscle, brain, and kidney is complexed, behaving like Na^+ associated with macromolecules of an ion-exchange resin. By analogy, at least, the same may be true for K^+, but this cannot be verified directly for most bacteria because of the weakness of the signal from K^+. Only with *Halobacterium*, which contains as much as 5 *M* K^+, were Cope and Damadian (1970) able to detect $^{39}K^+$ signals; they inferred from their results that much of the K^+ is either complexed by fixed charges, or else solvated in semicrystalline water. Indeed, there is considerable evidence that cytoplasmic water has a structure more ordered than that of external water, and this also depends on the ionic composition of the cells (Wiggins, 1971; Damadian *et al.*, 1971). It is thus quite possible that the solvent properties of cellular water are not quite the same as those of ordinary water, which could have important consequences for the state of cellular ions.

The question of physical state is posed most sharply by the halophiles

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