A VARIABLE STOICHIOMETRY MODEL FOR pH HOMEOSTASIS IN BACTERIA

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ABSTRACT The composition of the proton-motive force of a hypothetical bacterial cell of wide pH tolerance is analyzed according to a model whereby the electron transport chain and various proton-linked sodium and potassium ion transporting modes are responsible for the development of the membrane potential and the chemical potentials of the three cations. Simultaneous use of two or more modes employing the same metal cation, but at a different stoichiometric ratio with respect to protons, produces nonintegral stoichiometry; the modes could represent either different devices or different states of a single device. Cycling of the cation, driven by proton-motive force, results. The relative conductances of the various modes are postulated to be pH-dependent. The pattern of potentials that results is qualitatively in accord with current knowledge and may reflect the mechanism of pH homeostasis in bacteria. The membrane potential is outwardly directed (positive inside) at extremely acid pH, becoming inwardly directed as the pH increases; the pH gradient across the membrane is large and inwardly directed (alkaline inside) at acid pH, becoming smaller and eventually inverting at alkaline pH values; the transmembrane potassium gradient is outwardly directed (high concentration inside) at all pH values; the transmembrane sodium gradient is inwardly directed at all pH values, following the pH gradient from acid through neutral pH, but then diverging at alkaline pH.

INTRODUCTION

Individual species of bacteria have to tolerate considerable variation in the pH of their environment (pH_{ex}), and among bacterial species from acidophiles through neutrophiles to alkalophiles, the range of pH_{ex} that is successfully dealt with is enormous (18). Although cytoplasmic pH (pH_{in}) is not maintained entirely constant, it varies far less than pH_{ex}, i.e., there is substantial pH homeostasis (2). As a consequence, pH_{in} is alkaline relative to an acidic environment, and acidic relative to an alkaline environment. Since the magnitude of the proton-motive force ($\Delta\tilde{\mu}_{H^+}$) is relatively independent of pH_{ex}, its composition ($\Delta\psi$ vs. ΔpH) has to change drastically to give such pH homeostasis (16, 36). At acidic pH_{ex}, there is a large, inwardly directed ΔpH and a small or even inverted $\Delta\psi$ (18, 23, 37); at neutral pH_{ex}, there is a small, inwardly directed ΔpH and a moderately large, inwardly directed $\Delta\psi$ (25); at alkaline pH_{ex}, there is an inverted ΔpH and a large, inwardly directed $\Delta\psi$ (11, 18, 22).

The primary means of H^+ extrusion is the electron transport chain (in the case of respiring organisms) or the H^+-ATPase (in the case of fermenting organisms). Both devices transport only protons across the membrane, and by themselves would, because of the relative ease of developing potential energy electrically compared with osmotically, generate almost exclusively $\Delta\psi$ and a negligible ΔpH. How then is the variable composition of $\Delta\tilde{\mu}_{H^+}$ achieved?

There is by now a considerable body of evidence to suggest that monovalent metal cations, specifically Na^+ and K^+, are involved. Since bacteria maintain an inwardly directed ΔpNa (5, 11, 13, 19, 20, 27, 34) and an outwardly directed ΔpK (8, 12, 29, 33, 35), the roles of these cations are likely to be quite different. It has been recognized that electrogenic uptake of K^+, with or without H^+, would compensate for charge extrusion by the electron transport chain and thus permit the development of ΔpH without excessive $\Delta\psi$; and that electrogenic antiport of Na^+ and H^+ would be a means of generating an inverted ΔpH. Suggestive evidence has been presented for the existence in bacteria of modes for H^+/K^+ symport (1, 17, 33), H^+ uniport (35), $-H^+/K^+$ antiport (4), $-H^+/Na^+$ antiport (28), and $-2H^+/Na^+$ antiport (20, 22). In the order just given, these would generate a progression of compositions of $\Delta\tilde{\mu}_{H^+}$, as we show below.

Although it has been suggested that these modes are used, it is not usually stated which are operating, in what combination, or with what conductances and consequences. For example, it has been suggested (26) that, below some specified value of pH_{ex}, H^+/Na^+ antiport is electroneutral ($-H^+/Na^+$), while above some other specified value it is electrogenic ($-2H^+/Na^+$). The manner of operation at intermediate pH_{ex}, when presumably both stoichiometries are in use, is usually not considered; neither are the consequences of using modes for K^+ and Na^+ simultaneously.

In a recent paper (6), we discussed the consequences of the simultaneous use of two different stoichiometric modes for transport of the same molecule, say Na^+, driven by the same energy source, say $\Delta\tilde{\mu}_{H^+}$. The different stoichiometries could derive either from use of different physical devices or from variation of the stoichiometry of a single device; we use the term "mode" to refer to either case. The principal conclusions were that, when both modes were at steady state, there would be a nonintegral apparent stoichiometry, a futile cycling of the transported molecule, and concomitant dissipation of the energy source.

Here we apply these concepts to the specific context of H^+ and monovalent metal cation transport in bacteria, and relate them to the phenomenon of pH homeostasis. We present a simple model in which the potentials developed by a bacterial cell derive only from the electron transport chain and whatever cation-transporting modes are operative. The treatment progresses through consideration of (*a*) the electron transport chain alone, then the electron transport chain in concert with (*b*) one cation-transporting mode, (*c*) two modes for the same cation, (*d*) one mode each for two different cations, and finally (*e*) two modes each for two different cations. Despite its simplicity, the treatment gives a remarkably satisfactory description of the manner in which the composition of $\Delta\tilde{\mu}_{H^+}$ and the magnitude of the other ion potentials is observed to behave.

QUANTITATIVE CALCULATIONS

We make a number of simplifying assumptions in order to focus on the issue of variable stoichiometry and pH homeostasis:

(*a*) The redox potential available to the electron transport chain is constant.

(*b*) $\Delta\tilde{\mu}_{H^+}$ is virtually at equilibrium with the redox potential. This is equivalent to assuming that fluxes through other modes are not large enough to load down the primary energy source of the cell. In reality, $\Delta\tilde{\mu}_{H^+}$ decreases somewhat as pH_{ex} becomes more alkaline (6, 36).

(*c*) Only modes for transporting H^+, Na^+, and K^+ are considered. Cation transport systems linked to ATP hydrolysis, such as the *Kdp* system of *E. coli* (9), are not considered explicitly; however, since the process of ATP synthesis is driven by $\Delta\tilde{\mu}_{H^+}$, a transporter such as *Kdp* may be viewed as an indirectly driven H^+/K^+ symport.

Transport devices for inorganic anions such as Cl^- are excluded because of the absence of any experimental evidence that they are important in pH regulation in bacteria. If H^+/cation transport modes are the principal means by which the bacterial cell regulates the composition of $\Delta\tilde{\mu}_{H^+}$, they probably have a high capacity compared with other transport modes (e.g., H^+/carboxylic acid transport or Na^+/sugar transport) that employ H^+ or other charged species; such modes will alter to some degree the composition of $\Delta\tilde{\mu}_{H^+}$, but they will not affect the general conclusions. (Note that a mode that uses H^+ and no other charged species will constitute a load on the electron transport chain but will not directly affect the composition of $\Delta\tilde{\mu}_{H^+}$).

(*d*) Chemical potentials are expressed in terms of concentrations, i.e., all activity coefficients are assumed to equal unity.

(*e*) Initially, all potentials ($\Delta\psi$, ΔpH, ΔpNa, and ΔpK) other than the redox potential are zero. The specified modes are then "turned on" and the system allowed to approach its position of equilibrium or steady state, which is determined solely by these modes. In other words, there are no unspecified leaks.

(*f*) The extracellular compartment is infinite and so its composition is unaffected by ion movements across the cell membrane.

(*g*) The pH buffering capacity of the cytoplasm is constant and the Na^+ and K^+ buffering capacities are zero. This would be a poor assumption if pH_{in} were to vary extensively; it is true that the variation is considerable if one compares acidophiles and alkalophiles, but within any one organism pH homeostasis ensures that only a rather limited variation occurs.

(*h*) All modes operate at a rate (i.e., generate a flux J) proportional to the appropriate net thermodynamic driving force Φ, the constant of proportionality being the phenomenological coefficient, or conductance, g; this is the linear force-flux assumption of nonequilibrium thermodynamics. Deviations from linearity, which undoubtedly occur, will affect the quantitative but not the qualitative conclusions derived from the model.

Calculation of Energy Terms

Let the stoichiometry for mode M for a cation Ξ^+ be $n_{M,\Xi}$ (with a positive value referring to outward flux). For example, if mode M is electroneutral antiport of H^+ with Na^+, then $n_{M,H} = -1$, $n_{M,Na} = 1$ and $n_{M,K} = 0$. The number of ions transferred by each mode is the product of its stoichiometry $n_{M,\Xi}$ and the number of times x_M it turns over.

It should be clearly recognized in all that follows that the calculation of the number of times a mode turns over is simply a book-keeping procedure for establishing what the final condition of equilibrium or steady state will be. It says nothing about kinetics. The actual manner and rate at which this final condition is attained (e.g., fast development of the $\Delta\psi$ component of $\Delta\tilde{\mu}_{H^+}$ followed by interchange of this for ΔpH via metal cation movements [14]) cannot be prescribed without knowledge of the numbers of devices and their conductances.

All energy terms are calculated in p units, where a concentration ratio of 10 represents a chemical (osmotic) potential of 1 p unit. For a monovalent cation at 25°C, a $\Delta\psi$ of -59 mV is equivalent to 1 p unit. An inwardly directed potential, expressed in p units, is defined as positive. The constant value of $\Delta\tilde{\mu}_{H^+}$, equal to the redox potential

available to the electron transport chain, is arbitrarily chosen to be 3 p units.

For simplicity, and in the absence of any generally agreed upon value for the H^+/e^- stoichiometric ratio of the electron transport chain, we set $n_{ET,H} = 1$, i.e., we define the elementary event in terms of the extrusion of one H^+ rather than the transport of an electron pair. The number of turnovers[1] calculated in this way will accordingly be greater than the actual number undergone by the chain.

Calculation of Membrane Potential ($\Delta\psi$). The membrane potential developed by mode M after x_M turnovers is given by

$$\Delta\psi = x_M(n_{M,H} + n_{M,K} + n_{M,Na})F/N_0C, \quad (1)$$

where F is Faraday's constant, N_0 is Avogadro's number, and C is the total capacitance of the cell membrane. Using a specific membrane capacitance of 10^{-14} F μm^{-2} (15) and a membrane area of 5 μm^2, $C = 5 \times 10^{-14}$ F and $F/N_0C = 5.4 \times 10^{-5}$ p units (ionic charge)$^{-1}$.

Calculation of Proton Chemical Potential (ΔpH). The proton chemical potential developed by mode M after x_M turnovers is given by

$$\Delta pH = x_M n_{M,H}/BN_0V, \quad (2)$$

where B is the buffering capacity, and V is the cytoplasmic volume of a bacterial cell. We make the simplifying assumption that B is pH-independent and has a value of 100 mM (pH unit)$^{-1}$ (2). Using $V = 10^{-15}$ 1, $1/BN_0V = 1.7 \times 10^{-8}$ p units $(H^+)^{-1}$.

Calculation of Na^+ and K^+ Chemical Potentials (ΔpNa and ΔpK). For a metal cation Ξ, the chemical potential developed by mode M after x_M turnovers is given by

$$\Delta p\Xi = \log [c_{0,\Xi}/(c_{0,\Xi} - x_M n_{M,\Xi}/N_0V)], \quad (3)$$

where $c_{0,\Xi}$ is the external (and initial cytoplasmic) concentration of Ξ^+. The values for $c_{0,Na}$ and $c_{0,K}$ are chosen to be 100 and 10 mM, respectively.

The values chosen above for the various boundary conditions are arbitrary though reasonable ones, chosen for illustrative purposes.

Equilibrium Reached by the Electron Transport Chain Alone

The equilibrium achieved by the electron transport chain (ET) results in a $\Delta\tilde{\mu}_{H^+} = \Delta\psi + pH$ of 3 p units. Substitution for $\Delta\psi$ and ΔpH, in terms of the energy expressions given above and the numbers of cycles x_{ET} that the electron transport chain has executed, yields

$$\Delta\tilde{\mu}_{H^+} = x_{ET}\, n_{ET,H}\, (F/N_0C + 1/BN_0V), \quad (4)$$

which after substituting constants yields $x_{ET} = 55{,}200$.[2] The resultant $\Delta\tilde{\mu}_{H^+}$ composition is $\Delta\psi = 2.999$, $\Delta pH = 9 \times 10^{-4}$ (Table I). The heavy bias toward $\Delta\psi$ occurs because the electrical capacitance of the membrane is small compared with the pH buffering capacity of the cytoplasm.

TABLE I
COMPOSITION OF $\Delta\tilde{\mu}_{H^+}$ AND CATION POTENTIAL PRODUCED BY THE ELECTRON TRANSPORT CHAIN AND ONE CATION-TRANSPORTING MODE

Cation-transporting mode	$\Delta\psi$	ΔpH	ΔpNa	ΔpK
		p units		
None	~3	9×10^{-4}	NA	NA
H^+/Na^+ symport	−2.21	5.21	−0.79	NA
H^+/K^+ symport	−1.35	4.35	NA	−1.65
Na^+ uniport	0.54	2.46	−0.54	NA
K^+ uniport	1.26	1.74	NA	−1.26
$-H^+/Na^+$ antiport	~3	3×10^{-4}	3×10^{-4}	NA
$-H^+/K^+$ antiport	~3	8×10^{-4}	NA	8×10^{-4}
$-2H^+/Na^+$ antiport	3.99	−0.99	2.01	NA
$-2H^+/K^+$ antiport	3.10	−0.10	NA	2.90

Note the following characteristics of the table: (*a*) $\Delta\psi$ and ΔpH in all cases sum to the constant value of $\Delta\tilde{\mu}_{H^+} = 3$; (*b*) the values of $\Delta\psi$, ΔpH, and $\Delta p\Xi$ for the cation are equilibrium values for that mode (e.g., for 1:1 symport $2\Delta\psi + \Delta pH + \Delta p\Xi = 0$); (*c*) there is a progressive shift to inwardly directed $\Delta\psi$ and outwardly directed ΔpH in going from symport to antiport modes; (*d*) the range of compositions of $\Delta\tilde{\mu}_{H^+}$ generated by use of Na^+-transporting modes are more extreme than by use of K^+-transporting modes. (*e*) NA, not applicable.

Equilibria Reached by the Electron Transport Chain and One Cation-Transporting Mode

The equilibrium equation for the electron transport chain, after substitution for $\Delta\psi$ and ΔpH in terms of the numbers of turnovers executed by the electron transport chain ET and a single mode (mode 1) for a cation Ξ^+, is

$$\Delta\tilde{\mu}_{H^+} = \Delta\psi + \Delta pH = [x_{ET}\, n_{ET,H} + x_1(n_{1,H} + n_{1,\Xi})]\, F/N_0C + (x_{ET}\, n_{ET,H} + x_1 n_{1,H})/BN_0V = 3 \quad (5)$$

and the corresponding equilibrium equation for mode 1 is

$$n_{1,H}\Delta\tilde{\mu}_{H^+} + n_{1,\Xi}\Delta\tilde{\mu}_{\Xi^+} = (n_{1,H} + n_{1,\Xi})\Delta\psi + n_{1,H}\Delta pH + n_{1,\Xi}\Delta p\Xi = 0, \quad (6)$$

where $\Delta\psi$ and ΔpH can be expanded as they were in Eq. 5 and $\Delta p\Xi$ is expanded according to Eq. 3. A sample

[1] We use the slightly cumbersome term number of turnovers to distinguish it from the term turnover number (which is really a turnover rate) as used in enzyme kinetics.

[2] Here and subsequently, the turnover numbers generated by the calculation have been rounded to the nearest hundred.

calculation using these equations is outlined in the next section and the results for others, including symport, uniport, and antiport modes, are provided in Table I.

Electron Transport and a 1:1 H^+/K^+ Symport Mode. For a 1:1 H^+/K^+ symport mode, $n_{1,H} = -1$, $n_{1,K} = -1$. Substituting these and other constants into Eqs. 5 and 6 gives

$$(x_{ET} - 2x_1) \times 5.4 \times 10^{-5} + (x_{ET} - x_1) \times 1.7 \times 10^{-8} = 3 \quad (7)$$

$$-2(x_{ET} - 2x_1) \times 5.4 \times 10^{-5} - (x_{ET} - x_1) \times 1.7 \times 10^{-8} - \log [10^{-2}/(10^{-2} + x_1/6.02 \times 10^8)] = 0. \quad (8)$$

x_{ET} and x_1 are found by (*a*) using Eq. 7 to obtain an explicit expression for x_1 in terms of x_{ET}, (*b*) substituting for x_1 in Eq. 8 to give an implicit (mixed logarithmic-linear) equation in x_{ET}, and then (*c*) using an iterative procedure (e.g., reference 7) to evalute x_{ET}.

Such calculations indicate that after 524,114,400 H^+ extrusion turnovers of the electron-transport chain and 262,069,600 uptake turnovers of the symport mode (net H^+ extrusion of 262,044,800, net charge uptake 24,800), the modes would have reached equilibrium (Table I), with $\Delta\tilde{\mu}_{H^+}$ consisting of a large, inwardly directed ΔpH of 4.35 units and a $\Delta\psi$ of −1.35 units (i.e., positive inside), and symport equilibrium achieved with a ΔpK of −1.65 units (i.e., K^+ concentration in the cytoplasm about 45-fold higher than outside). Note the large extent of H^+ extrusion and K^+ uptake needed to reach a substantial ΔpH and ΔpK, and the much smaller net charge uptake needed to reach a substantial $\Delta\psi$.

Electron Transport and Other Examples of Cation-Transporting Modes. Similar calculations may be carried out for other transporting modes for K^+ and Na^+ (Table I). As the stoichiometric ratio shifts from H^+/cation symport to H^+/cation antiport, the composition of $\Delta\tilde{\mu}_{H^+}$ shifts to an increasing inwardly directed $\Delta\psi$ and an increasing outwardly directed (acid-inside) ΔpH.

Note that the perturbation that a cation mode of given stoichiometry makes to the simple electron transport equilibrium is in all cases greater for Na^+-transporting modes than for K^+-transporting modes. This is a consequence of the chosen boundary conditions—the higher initial Na^+ concentration requires more turnovers before equilibrium is reached.

Interpretation of the Patterns of $\Delta\tilde{\mu}_{H^+}$ Composition. It is worth examining how the composition of $\Delta\tilde{\mu}_{H^+}$ (Table I) is determined by the stoichiometry of the cation-transporting mode used. We can do this by initially permitting only the electron transport chain to conduct and reach equilibrium, and then permitting a given cation-transporting mode to conduct and examine the perturbation that it causes.

The cation-transporting mode will initially be far from equilibrium. Operating with a H^+ stoichiometry of $n_{1,H}$ and a cation stoichiometry of $n_{1,\Xi}$, it will have a H^+ uptake per turnover $h = -n_{1,H}$ and a net charge uptake $z = -(n_{1,H} + n_{1,\Xi})$ (e.g., a 1:1 symport mode would have $h = 1$ and $z = 2$; a −2:1 antiport mode would have $h = 2$ and $z = 1$). For all devices other than a pure H^+ transport mode such as the electron transport chain, there is thus an imbalance between transfer of H^+ and charge, with cation symport and uniport modes favoring charge (class A modes) and antiport modes favoring H^+ (class B modes).

As the cation-transporting mode begins to conduct, if the electron-transport chain were to extrude exactly h H^+ per turnover of the cation-transporting mode, ΔpH would remain constant. However, the electron transport chain cannot operate in this fashion, because with class A modes the cell would become depolarized (which violates the assumption that the electron transport chain operates close to equilibrium and maintains a constant $\Delta\tilde{\mu}_{H^+}$), while with class B modes it would become hyperpolarized (which is thermodynamically forbidden since the electron transport chain was already close to equilibrium).

Nor can the electron transport chain extrude z H^+ per turnover of the cation-transporting mode to maintain $\Delta\psi$ constant, because with class A modes the cell would increase its ΔpH (which is thermodynamically forbidden) and with class B modes it would decrease its ΔpH (which violates the assumption of constant $\Delta\tilde{\mu}_{H^+}$).

The electron transport chain can, however, continue to operate close to equilibrium by transporting ν H^+ per cycle of the cation-transporting mode, with $h < \nu < z$ (in the case of class A modes) or $h > \nu > z$ (in the case of class B modes), such that the changes in $\Delta\psi$ and ΔpH compensate for each other, with the magnitude of $\Delta\tilde{\mu}_{H^+}$ remaining constant, but its composition progressively shifting until the cation-transporting mode too has reached equilibrium. For class A modes, the shift would be towards increased ΔpH and decreased $\Delta\psi$, while for class B modes it would be the reverse.

Steady States Reached by the Electron Transport Chain and Two Modes for the Same Cation

Whereas the electron-transport chain and a single cation-transporting mode can, in the idealized model we are considering, both reach equilibrium, this cannot occur if there are two modes of different stoichiometry for the same cation Ξ^+, except in the trivial case where all potentials are zero. Instead, the cation-transporting modes come to steady state, with the cation having a nonzero cyclic flux, but a zero net flux, through them (6). The cyclic flux is driven by a net flux of H^+ through these modes, balanced by an ongoing H^+ flux through the electron-transport chain. Thus the latter also comes to a steady state rather than to equilibrium; however, the assumption of high capacity (see assumption *b* [above]) enables us to consider it to be effectively at equilibrium.

The two cation-transporting modes have a differential H^+ and cation stoichiometry that is a multiple of the H^+ stoichiometry of the electron transport chain. This permits the numbers of ions transported by any one of the three modes to be expressed as a linear combination of the numbers transported by the other two. Thus, for example, one turnover of the electron transport chain is equivalent to one inward turnover of a cation uniport mode plus one outward turnover of a 1:1 H^+/cation symport mode. To bring the system to steady state, turnovers of only two of the three modes need be invoked; we have chosen the two cation-transporting modes in our calculations.[3]

The effective equilibrium of the electron transport chain is, after substitution for $\Delta\psi$ and ΔpH, given by

$$\Delta\tilde{\mu}_H = [x_1(n_{1,H} + n_{1,\Xi}) + x_2(n_{2,H} + n_{2,\Xi})]F/N_0C + (x_1n_{1,H} + x_2n_{2,H})/BN_0V = 3. \quad (9)$$

The steady-state constraint on Ξ^+ is that its net flux, J_Ξ, through modes 1 and 2 must be zero. The component fluxes, $J_{1,\Xi}$ and $J_{2,\Xi}$, may be expressed (see assumption *h* [above]) in terms of the driving forces Φ_1 and Φ_2 and the corresponding conductances g_1 and g_2. The net flux equation then becomes

$$\begin{aligned} J_\Xi &= J_{1,\Xi} + J_{2,\Xi} = g_1\Phi_1 + g_2\Phi_2 \quad (10)\\ &= g_1(n_{1,H}\Delta\tilde{\mu}_{H^+} + n_{1,\Xi}\Delta\tilde{\mu}_\Xi) \\ &\quad + g_2(n_{2,H}\Delta\tilde{\mu}_{H^+} + n_{2,\Xi}\Delta\tilde{\mu}_{\Xi^+}) = 0, \end{aligned}$$

where the potentials can be expanded as before. The solution of these equations for one example of a pair of transport modes for the same cation is outlined below.

Electron Transport, a 1:1 H^+/K^+ Symport Mode and a 0:1 H^+/K^+ Uniport Mode. For 1:1 H^+/K^+ symport and 0:1 H^+/K^+ uniport modes, $n_{1,H} = -1$, $n_{1,K} = -1$, $n_{2,H} = 0$, $n_{2,K} = -1$. Substituting these and other constants into Eqs. 9 and 10 yields

$$-(2x_1 + x_2) \times 5.4 \times 10^{-5} - x_1 \times 1.7 \times 10^{-8} = 3 \quad (11)$$

$$g_1(2\Delta\psi + \Delta\text{pH} + \Delta\text{pK}) + g_2(\Delta\psi + \Delta\text{pK}) = 0. \quad (12)$$

Rearranging Eq. 12 gives

$$-(2g_1 + g_2)(2x_1 + x_2) \times 5.4 \times 10^{-5} - g_1x_1 \times 1.7 \times 10^{-8} + (g_1 + g_2)\log\{10^{-2}/[10^{-2} + (x_1 + x_2)/6.02 \times 10^8]\} = 0. \quad (13)$$

There are thus two unknowns (the number of turnovers for the two modes) and two constraints (electron transport equilibrium and steady state of the metal cation). These two equations may then be solved for any specified values of the conductances g_1 and g_2. The composition of $\Delta\tilde{\mu}_{H^+}$ and the magnitude of ΔpK does not depend on the absolute values of the conductances, only their relative values. We could use the concept of fractional conductance $g_f = g_1/(g_1 + g_2)$; at $g_f = 1$ and 0 the system is identical to operation of the symport alone and the uniport alone, respectively. However, more illuminating than fractional conductance is the concept of apparent stoichiometry (6), which we describe next.

$\Delta\tilde{\mu}_{H^+}$ Composition as a Function of Apparent Stoichiometry. The steady-state flux equation for the two cation modes (Eq. 10) may be rearranged to the form

$$[(g_1n_{1,H} + g_2n_{2,H})/(g_1n_{1,\Xi} + g_2n_{2,\Xi})]\Delta\tilde{\mu}_{H^+} + \Delta\tilde{\mu}_{\Xi^+} = 0. \quad (14)$$

We may regard the coefficient $(g_1n_{1,H} + g_2n_{2,H})/(g_1n_{1,\Xi} + g_2n_{2,\Xi})$ as the apparent stoichiometric ratio, n_{app}, of the real process involving two modes operating at steady state, so that

$$n_{app}\Delta\tilde{\mu}_{H^+} + \Delta\tilde{\mu}_{\Xi^+} = 0. \quad (15)$$

This equation, however, is also of the correct form to describe the equilibrium of an imaginary single mode M for H^+-linked cation transport operating with an actual stoichiometric ratio of n_{app} ($n_{M,H} = n_{app}n_{M,\Xi}$). Since the value of n_{app} will (in general) be nonintegral and nonrational, this imaginary mode cannot correspond to a physically attainable single mode (ions are not subdivisible!). It is nonetheless a convenient quantity that makes it possible to plot the composition of $\Delta\tilde{\mu}_{H^+}$ over any arbitrary range of stoichiometric ratios, without considering explicitly which pairs of modes give rise to it.

Such plots are given for K^+ and Na^+ in Fig. 1, *A* and *B*, respectively. The composition of $\Delta\tilde{\mu}_{H^+}$ is seen to progressively shift from an outwardly directed $\Delta\psi$ and an inwardly directed ΔpH to the reverse, as n_{app} goes from positive (symport) values to negative (antiport) ones. This is the type of behavior observed experimentally as the external pH of the cell goes from acid to alkaline values (cf. Introduction). The values of $\Delta\psi$ and ΔpH are linearly dependent on n_{app} throughout a substantial part of their range, for the following reason: Eq. 15 can be written as

$$n_{app}\Delta\tilde{\mu}_{H^+} + \Delta\psi + \Delta p\Xi = 0 \quad (16)$$

and $\Delta p\Xi$ is quite resistant to change as a result of uptake, i.e., it takes a large number of Ξ^+ transfers to increase the internal concentration appreciably (note the shallow slope of the cation potentials at $n_{app} > 0$ in Fig. 1, especially that for Na^+ (Fig. 1 *B*).

As the cytoplasm becomes appreciably depleted of cation, however, the ΔpH that can be developed reaches a limit (Fig. 1, *A* and *B* at large negative values of n_{app}). A higher initial concentration would relieve that limit (hence

[3]Recall that this is simply a book-keeping procedure, and that the steady state attained is independent of the path. In the real-life situation of a cell becoming energized, the path employed in reaching the steady state would of course be one that was both energetically and kinetically feasible.

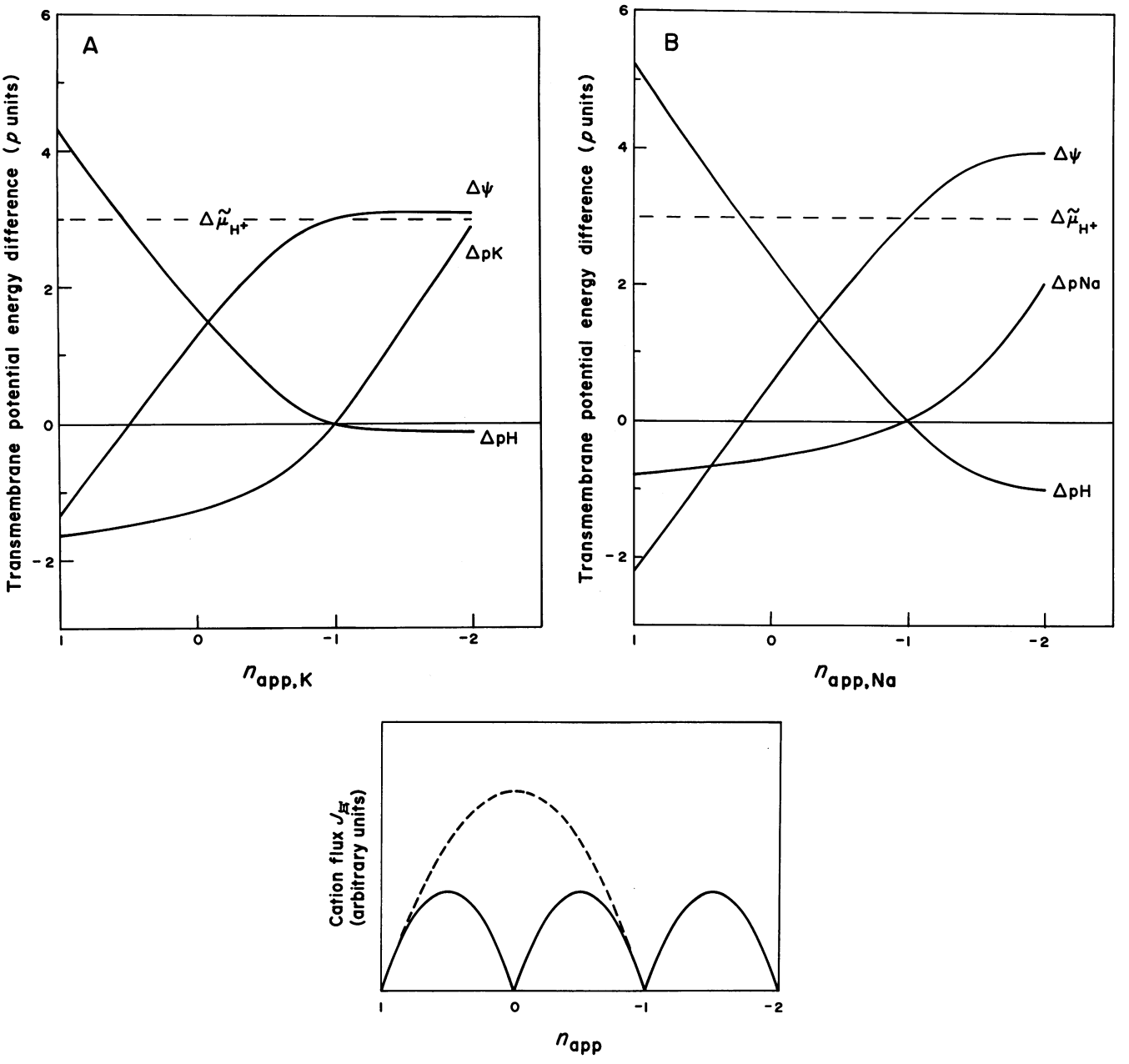

FIGURE 1 (*A* and *B*) Values of $\Delta\psi$, ΔpH, and $\Delta p\Xi$ produced by varying the apparent stoichiometric ratio n_{app} of a set of two or more modes for transporting a given cation Ξ^+ (K^+ in *A*, Na^+ in *B*), with primary H^+ extrusion occurring by means of the electron transport chain. Values for integral n_{app} correspond to the equilibrium potential values that would be generated by a single cation-transporting mode (see Table I). The horizontal line at a potential of zero indicates the polarity of any given potential, and the dashed line at a potential of 3 p units indicates the constant value of $\Delta\tilde{\mu}_{H^+} = \Delta\psi + \Delta$pH. (*C*) Cation fluxes produced under the above conditions where (*solid lines*) pairs of modes of adjacent stoichiometry are responsible for the observed value of n_{app} and (*dashed line*) an example of where nonadjacent stoichiometries are responsible; for simplicity the combined conductances of the two modes have been assumed constant.

it is reached later by Na^+ extrusion than by K^+ extrusion, cf. Fig. 1, *B* and *A*); an alternative means of avoiding the limit would be to recycle the cation electroneutrally through some non-H^+ mode, as has been noted by others (2, 3).

Ion Fluxes Through the Pairwise Modes. At steady state, the zero net flux of cation through a pair of modes actually consists of a cyclic flux between them (cf. reference 6). Thus the flux through mode 1 is

$$J_{1,\Xi} = g_1(n_{1,H}\Delta\tilde{\mu}_{H^+} + n_{1,\Xi}\Delta\tilde{\mu}_{\Xi^+}) \quad (17)$$

and (Eq. 10) is equal and opposite to the flux through mode 2:

$$J_{2,\Xi} = g_2(n_{2,H}\Delta\tilde{\mu}_{H^+} + n_{2,\Xi}\Delta\tilde{\mu}_{\Xi^+}). \quad (18)$$

In the simple case where the combined conductance

$(g_1 + g_2)$ is assumed constant, the flux is found to be parabolic, with zero flux at $g_1 = 0$ and $g_2 = 0$ (which are equilibrium positions) and maximum flux at $g_1 = g_2$.

If the values of n_{app} in Fig. 1, *A* and *B* derive from pairwise use of integral modes with adjacent stoichiometries (for example, $-1 > n_{app} > -2$ to be achieved by use of −1:1 and −2:1 antiport modes) the resultant fluxes form a scalloped pattern (Fig. 1 *C*). However, n_{app} need not derive from adjacent stoichiometries—any two that span n_{app} will do. For example, the range $1 > n_{app} > -1$ could be achieved with a 1:1 symport mode and a −1:1 antiport mode, although at the cost of a larger cyclic flux (Fig. 1 *C*, dashed line).

Use of More Than Two Modes for the Same Cation. The above treatment can easily be extended to more than two modes of transport of the same cation, for example, the simultaneous use of Na^+ uniport, −1:1 H^+/Na^+ antiport, and −2:1 H^+/Na^+ antiport modes. At steady state, the two extreme modes would operate in opposite directions while the intermediate mode could operate in either direction or not at all, depending on whether its stoichiometry was greater than, smaller than, or equal to the n_{app} of the system as a whole.

Equilibria for Modes for Different Cations

The analysis so far has been restricted to modes that employ the same cation. Although this produces a satisfactory pattern of $\Delta\tilde{\mu}_{H^+}$ composition, it does so in an unrealistic fashion with respect to the cation used. If we choose Na^+, it is accumulated at $n_{app} > -1$ (reaching a value of > 600 mM at $n_{app} = 1$) in conflict with the observation that Na^+ is invariably extruded by bacteria. If we choose K^+, it is extruded at $n_{app} < -1$ (reaching an extremely low cytoplasmic level of 10 μM at $n_{app} = -2$) in conflict with the observation that K^+ is invariably accumulated by bacteria, and with the fact that there are cytoplasmic functions, including protein synthesis (21) that require K^+ at relatively high concentrations. The use of modes for only one cation in the model is therefore unsatisfactory, and we now extend it to include simultaneous use of modes for both Na^+ and K^+.

We first treat the case where the modes for the two cations are of fixed stoichiometry. Because the cations are different, the electron transport chain and both modes can be at equilibrium even when $\Delta\tilde{\mu}_{H^+}$ is nonzero. There are thus three unknowns in the calculation (the numbers of turnovers undergone by the three modes) and three constraints (the equilibria). For the electron transport chain the equilibrium equation is

$$\Delta\tilde{\mu}_{H^+} = [x_{ET}\, n_{ET,H} + x_1(n_{1,H} + n_{1,K}) + x_2(n_{2,H} + n_{2,Na})]\, F/N_0C + (x_{ET}\, n_{ET,H} + x_1 n_{1,H} + x_2 n_{2,H})/BN_0V = 3, \quad (20)$$

and the corresponding equilibrium equations for modes 1 and 2 are

$$n_{1,H}\Delta\tilde{\mu}_{H^+} + n_{1,K}\Delta\tilde{\mu}_{K^+} = (n_{1,H} + n_{1,K})\Delta\psi + n_{1,H}\Delta pH + n_{1,K}\Delta pK = 0 \quad (21)$$

$$n_{2,H}\Delta\tilde{\mu}_{H^+} + n_{2,Na}\Delta\tilde{\mu}_{Na^+} = (n_{2,H} + n_{2,Na})\Delta\psi + n_{2,H}\Delta pH + n_{2,Na}\Delta pNa = 0, \quad (22)$$

where the potentials can be expanded as before in terms of the numbers of turnovers of the three modes.

The equilibrium equation for the electron transport chain is used to express the numbers of turnovers used by any one mode, say x_{ET} for the electron transport chain, in terms of the numbers of turnovers x_1 and x_2 used by the other two, and this value is then substituted into the equilibrium equations for the two cation-transporting modes. Both of these equations are implicit (mixed logarithmic-linear) in form; their solution may be found by a double iteration procedure.

TABLE II
COMPOSITION OF $\Delta\tilde{\mu}_{H^+}$ AND CATION POTENTIALS PRODUCED BY THE ELECTRON TRANSPORT CHAIN AND CATION-TRANSPORTING MODES FOR Na^+ AND K^+

Cation-transporting modes	Δψ	ΔpH	ΔpNa	ΔpK
		p units		
H^+/Na^+ symport dominant				
Over H^+/K^+ symport	−2.24	5.24	−0.76	−0.76
Over K^+ uniport	−2.20	5.20	−0.80	2.20
Over $-H^+/K^+$ antiport	−2.20	5.20	−0.80	5.20
Over $-2H^+/K^+$ antiport	−2.20	5.20	−0.80	8.20
H^+/K^+ symport dominant				
Over Na^+ uniport	−1.27	4.27	1.27	−1.73
Over $-H^+/Na^+$ antiport	−1.27	4.27	4.27	−1.73
Over $-2H^+/Na^+$ antiport	−1.27	4.27	7.27	−1.73
Na^+ uniport dominant				
Over K^+ uniport	0.51	2.49	−0.51	−0.51
Over $-H^+/K^+$ antiport	0.55	2.45	−0.55	2.45
Over $-2H^+/K^+$ antiport	0.55	2.45	−0.55	5.45
K^+ uniport dominant				
Over $-H^+/Na^+$ antiport	1.42	1.58	1.58	−1.42
Over $-2H^+/Na^+$ antiport	1.43	1.57	4.57	−1.43
$-H^+/Na^+$ antiport dominant				
Over $-H^+/K^+$ antiport	~3	3×10^{-4}	3×10^{-4}	3×10^{-4}
$-2H^+/Na^+$ antiporter dominant				
Over $-2H^+/K^+$ antiport	4.09	−1.09	1.91	1.91
No clear dominance				
$-H^+/Na^+$ and $-2H^+/K^+$ antiports	3.03	−0.03	−0.03	2.97
$-2H^+/Na^+$ and $-H^+/K^+$ antiports	3.65	−0.65	2.35	−0.65

The comments to Table I apply here also. The mode given in italics is considered in combination with each of the modes listed immediately below; the potentials given are for both modes in use simultaneously. Note that the composition of $\Delta\tilde{\mu}_{H^+}$ corresponds rather closely to that given in Table I for the italicized mode, i.e., that mode dominates the composition when both modes are in use simultaneously.

The resulting potentials for a variety of pairs of modes (Table II) indicate a definite hierarchy of dominance of the modes, the more dominant of a pair of modes effectively determining the composition of $\Delta\tilde{\mu}_{H^+}$ and hence the gradient of the cation that is being transported by the other mode (cf. compositions in Table I, where only one cation-transporting mode is in use). The hierarchy takes the following form: (*a*) Wherever the two modes have the same stoichiometry, the Na^+ mode dominates the K^+ mode. (*b*) A symport mode dominates a uniport or antiport mode, regardless of the cations used in the two modes. (*c*) A uniport mode dominates an antiport mode, regardless of the cations used in the two modes. The dominance of Na^+ over K^+ is a consequence of its higher initial concentration—ΔpNa is less readily perturbed than ΔpK. The hierarchy symport > uniport > antiport may be understood in terms of the charge:H^+ (*z*:*h*) ratio; symport contributes more to charge movement than do uniport or antiport, and charge movement more readily perturbs $\Delta\psi$ than H^+ movement perturbs ΔpH.

Steady States Reached by Pairwise Modes for Each of Two Cations

The above treatment for two cations provides results only for a single integral stoichiometry for each cation. A more general treatment would allow the stoichiometries to vary. Since the use of two modes for a given cation is equivalent to the use of a single imaginary mode of nonintegral stoichiometry (see above), an equilibrium calculation based on the use of two such imaginary modes, one for each cation, yields the same potentials as would a calculation based on the use of two pairs of real modes, one pair for each cation.

Such an analysis would provide values for $\Delta\tilde{\mu}_{H^+}$ composition and ionic potentials, as continuous functions of two variables: the apparent H^+/cation stoichiometric ratios $n_{app,K}$ and $n_{app,Na}$. Instead of generating those functions in their entirety, we have chosen to generate a subset where $n_{app,Na}$ and $n_{app,K}$ co-vary in a manner that might roughly reflect the behavior of a hypothetical "wide-range" organism that can tolerate pH_{ex} from quite acidic to quite alkaline values. We provide this organism with a H^+/K^+ symport mode, a K^+ uniport mode, a $-H^+/K^+$ antiport mode, a $-H^+/Na^+$ antiport mode, and a $-2H^+/Na^+$ antiport mode, the modes to be regulated in the following manner (cf. abscissa to Fig. 2). For K^+: range 1, H^+/K^+ symport progressively shifting to K^+ uniport; ranges 2 and 3, K^+ uniport shifting to $-H^+/K^+$ antiport; range 4, $-H^+/K^+$ antiport only throughout. For Na^+: ranges 1 and 2, $-H^+/Na^+$ antiport only throughout; ranges 3 and 4, $-H^+/Na^+$ antiport shifting to $-2H^+/Na^+$ antiport. The reasons for choosing the above ranges will become clearer after the results have been presented and discussed.

The results of the calculations are shown in Fig. 2 *A*. In range 1, the H^+/K^+ symport and K^+ uniport modes dominate the composition of $\Delta\tilde{\mu}_{H^+}$, so that it closely resembles that for K^+ modes alone in the range $1 > n_{app,K} > 0$ (Fig. 2, *B* cf. Fig. 1 *A*); the existence of the $-H^+/Na^+$ antiport mode makes essentially no difference, and ΔpNa simply follows the value of ΔpH dictated by the K^+ modes. The K^+ modes continue to dominate in range 2, with the composition of $\Delta\tilde{\mu}_{H^+}$ resembling that for K^+ modes alone in the range $0 > n_{app,K} > -0.5$ (Fig. 2 *B* cf. Fig. 1 *A*). In ranges 3 and 4, as the effect of the K^+ modes get weaker (approaching that of electroneutral antiport) and the effect of the Na^+ modes gets stronger, the pattern of ΔpH versus $\Delta\psi$ continues, but with the actual values more closely resembling those for Na^+ modes alone in the range $-1 > n_{app,Na} > -2$ (Fig. 2, *B* cf. Fig. 1 *B*). Furthermore (Fig. 2 *A*), ΔpNa now begins to break its parallel relationship with ΔpH and starts to rise, again resembling the behavior of Na^+ modes alone (Fig. 1 *B*). In range 4, the pattern of ΔpH versus $\Delta\psi$ determined predominantly by the Na^+ modes saturates because of Na^+ starvation.

Where only one mode for a given cation is active ($-H^+/Na^+$ antiport in ranges 1 and 2, $-H^+/K^+$ antiport in range 4), it is at equilibrium and contributes no flux. Where more than one mode is active, there is a cyclic flux of the cation and a net flux of H^+ through these modes.

DISCUSSION

The treatment given above is simple enough to be tractable and readily understandable, yet realistic enough to give a good indication of how variable stoichiometry of cation-transporting modes would contribute to determination of the composition of $\Delta\tilde{\mu}_{H^+}$ and to pH homeostasis in the complexity of a real cell. Provided the major underlying assumption is correct—that cation transport modes of different stoichiometries are major contributors to the balance of charge and H^+ movements across the cell membrane—then oversimplifying assumptions such as linear force-flux relations or constant buffering capacity will affect only the quantitative and not the qualitative picture that has been presented.

Mechanism of Change in Stoichiometry

Two broad classes of mechanism may be envisaged, one involving mass action effects on the occupancy of multiple binding sites for transport, and the other involving allosteric effectors that place the mode in an active or inactive mode for a given stoichiometry.

The former mechanism seems wholly unsatisfactory: a H^+/Na^+ antiport with two H^+ sites, for example, would be more likely to operate in the −2:1 mode at acid pH and the −1:1 mode at alkaline pH, which is the opposite of the desired usage. Furthermore, it is hard to imagine mass action providing the necessary sensitivity for pH homeostasis.

We think an allosteric mechanism, probably one with positive cooperativity, is much more likely. The mechanism

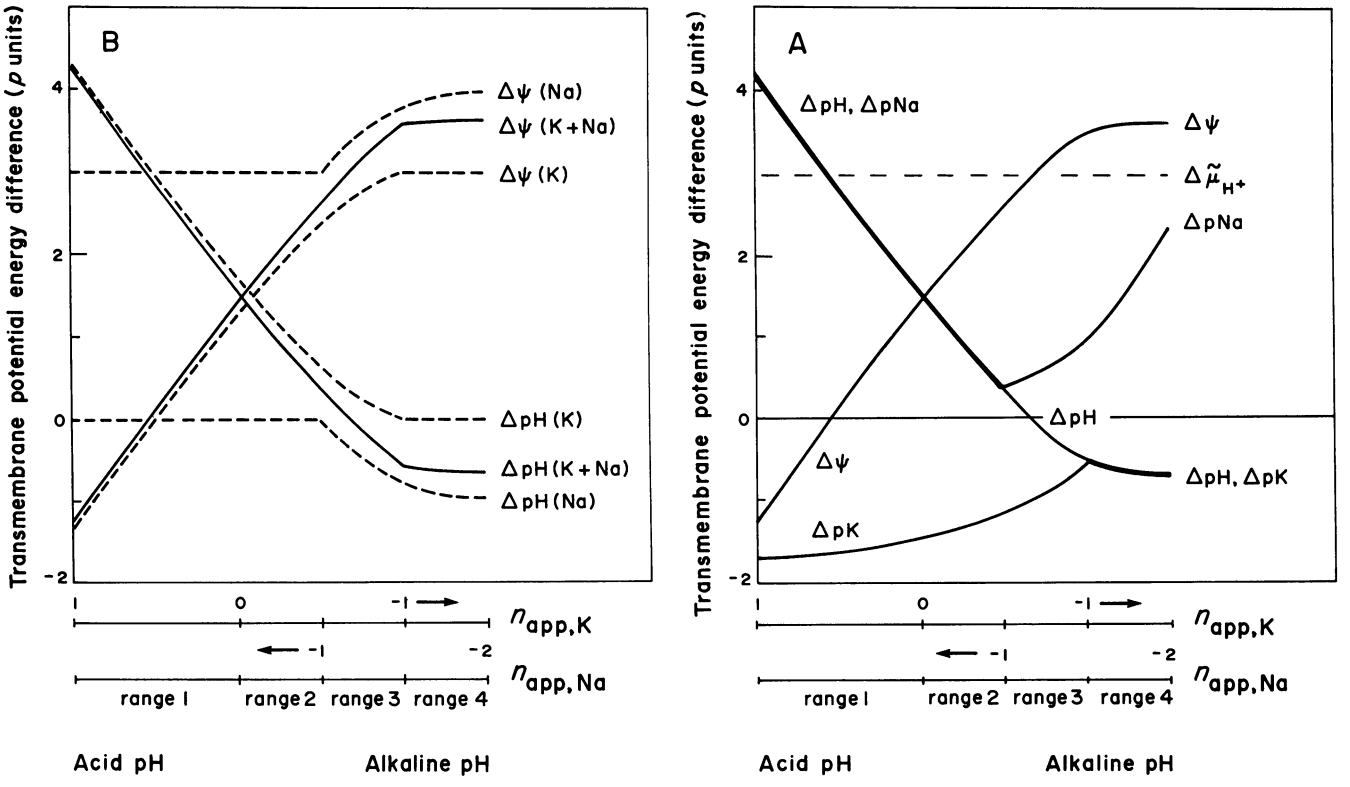

FIGURE 2 (*A*) Values of $\Delta\psi$, ΔpH, ΔpK, and ΔpNa that result from simultaneous use of two modes for each of two cations K^+ and Na^+ (as indicated on the abscissa), together with primary H^+ extrusion by means of the electron transport chain. The apparent stoichiometries, $n_{app,K}$ and $n_{app,Na}$, of the pairwise modes provide the scale for the abscissa, and fall into the ranges shown; the abscissa may also be regarded as a rough representation of the cytoplasmic pH over a narrow range or the external pH over a larger range (see the text for explanation and discussion). In contrast to Fig. 1, *A* and *B*, the cation chemical potentials ΔpK and ΔpNa maintain the observed polarity (outward and inward, respectively) throughout. (*B*) The values of the $\Delta\psi$ and ΔpH components of the proton-motive force $\Delta\tilde{\mu}_{H^+}$ that would exist at the indicated values of n_{app} (*a*) if modes for both cations were present (*solid lines*, cf. Fig. 2 *A*) and (*b*) if only transporting modes for one cation were present (*dashed lines*, cf. Fig. 1, *A* and *B*). Note the resemblance of the composition of $\Delta\tilde{\mu}_{H^+}$ in case (*a*) to that in case (*b*) for K^+ modes alone in ranges 1 and 2 and (to a lesser degree) for Na^+ modes alone in ranges 3 and 4.

could be used to activate and deactivate distinct physical devices (e.g., a H^+/K^+ symporter encoded by one gene and a K^+ uniporter encoded by another gene); the activation/deactivation would not need to be all or none, but could consist of a graded control of conductance. Alternatively, the mechanism could cause the switching of a single device from one stoichiometric mode to another. The latter mechanism would have the advantage of automatically providing a redistribution of conductance, whereas the former would require separate regulation of both devices.

The Regulatory Signal

If the variation of stoichiometry is allosterically determined, what is the regulatory signal? The most likely candidates are pH_{ex}, ΔpH, or pH_{in}. One might be tempted to suggest pH_{ex}, since this varies greatly and is therefore potentially a signal of considerable size; however, it is possible to have different pH_{in} values for the same pH_{ex} value, depending on such factors as carbon source (with the possibility of generation or consumption of scalar protons), availability of oxygen, etc., and so it is not easy to see how pH_{ex} itself would be useful as the direct signal for pH homeostasis. A similar criticism applies to ΔpH as the signal. We therefore think that pH_{in} itself is likely to be the signal; there is some experimental evidence in support of this hypothesis (10, 22, 24). In respiring *E. coli*, pH_{in} varies by ~0.1 unit per unit change in pH_{ex} (31) and so the signal, though small, would not be absurdly so.

Mechanism of pH Homeostasis

Let us use the hypothesis that pH_{in} is the signal and consider the response of a cell to a sudden decrease in pH_{ex}, when previously the cell was at steady state, for example at $n_{app,K} = -0.5$ and $n_{app,Na} = -1$ (i.e., at the boundary between ranges 2 and 3 in Fig. 2). The immediate effect would be an increase in ΔpH and $\Delta\tilde{\mu}_{H^+}$. Next there would be a net influx of H^+ through the electron transport chain to restore it to equilibrium. This would cause a decrease in ΔpH (and also $\Delta\psi$) and hence a lowering of pH_{in}, to a value below the eventual stable value; for example, if the original value of pH_{in} were 7.5, it might fall transiently to 7.1 before regulating at 7.4 (cf. references 6 and 30). At $pH_{in} = 7.1$, regulation of the K^+ modes would move towards higher $n_{app,K}$ values, with the result that the composition of $\Delta\tilde{\mu}_{H^+}$

would begin to shift in favor of ΔpH. pH_{in} would therefore start to rise, and $n_{app,K}$ to fall. At the point when pH_{in} and $n_{app,K}$ had reached consistent values in terms of pH_{ex} and the steady-state composition of $\Delta\tilde{\mu}_{H^+}$, the cell would have adapted to the new environment, and pH homeostasis have been achieved. Similar arguments may be constructed for changes of pH_{ex} towards alkaline values, in which case the K^+ mode would shift toward a $-1{:}1$ stoichiometry and the Na^+ mode toward a $-2{:}1$ stoichiometry.

If values of n_{app} for Na^+ and K^+ modes are determined by pH_{in}, the abscissa of Fig. 2 may alternatively be thought of as a pH_{in} scale. This would cover a relatively narrow pH range, suggesting the need for cooperativity in the regulation process. The abscissa may also be thought of in terms of the pH_{ex} values, covering a much larger range, that would result in a given pH_{in}. Roughly speaking, the subranges might be as follows: range 1, pH_{ex} from 3 to 6 (the acidophile range); range 2, pH_{ex} from 6 to 7, and range 3, pH_{ex} from 7 to 8 (the neutrophile range); and range 4 (perhaps supplemented by recycling of Na^+ [2]), pH_{ex} from 8 to 10 (the alkalophile range).

CONCLUSIONS

The model described here provides a conceptual framework for thinking about the phenomenon of pH homeostasis in bacteria. It builds on a variety of ideas and observations in the literature, notably the role of $\Delta\tilde{\mu}_{H^+}$ as the central energy currency of the cell, and its role in development of K^+ and Na^+ gradients; of H^+/K^+ symport and K^+ uniport in pH regulation at acidic pH_{ex}; and of electroneutral and electrogenic H^+/Na^+ antiport in pH regulation at alkaline pH_{ex}.

It contains explicitly the concept of nonintegral stoichiometry deriving from the simultaneous use of two or more modes for the same cation, assumes that the devices using these modes are primarily responsible for the composition of $\Delta\tilde{\mu}_{H^+}$, and that the conductances of these modes are regulated, probably by pH_{in} itself.

It makes one definite prediction, namely, that at most pH values there ought to be K^+ or Na^+ cycling, even in the absence of "leaks" or other modes requirng $\Delta\tilde{\mu}_{K^+}$ or $\Delta\tilde{\mu}_{Na^+}$ as their energy source; related to this cycling, it predicts that there should be an ongoing H^+ flux whose magnitude should be pH-dependent. It might be possible to measure the latter via respiration rates, if other loads on the electron transport chain could be kept at a minimum. Further testing of the model should be possible as the molecular identities of the cation transporting devices are established.

Finally, we note that although the cycling of cations may be regarded as "futile" in the sense that it is an energy expenditure for the bacterium, it would be worthwhile if it enabled such an important physiological parameter as cytoplasmic pH to be regulated.

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