their entropy production, accompanying selectivity, is

$$\sigma(\xi_0) \cdot h = R \sum_j \mathcal{J}_j z_j \alpha_j(0)[V - V_j]$$

$$\sigma(\xi_1) \cdot h = R \sum_j \mathcal{J}_j z_j \alpha_j(1)[V - V_j]. \quad (70)$$

Clearly, if transport is downhill (i.e., $V - V_j$ and J_j have the same sign; Tables 1 and 2), and the selectivities $\alpha_j(\cdot)$ are positive, the entropy production in the selectivity filters is positive, as it must be, if the selectivity filters are to be strictly dissipative and local, as we suppose in this theory.

The situation is somewhat more complex if several species are present and one species, say k, moves uphill through the selectivity filter (say, the one at $x = 0$) while another species, say m, moves downhill through it. Then (see Tables 1 and 2), $V - V_k$ and J_k have opposite signs. The uphill flux of k is accompanied by, of course, negative entropy production $\sigma_k(0) < 0$ for that component in that selectivity filter. In a locally dissipative system like the selectivity filters envisioned here, negative entropy production by one ion k can occur only when it is balanced (at that location) by the positive entropy $\sigma_m(0) > 0$ generated by another ion m, moving downhill, at that location. Thus, uphill transport can occur in a dissipative selectivity filter, if, but only if, the selectivities $\alpha_j(0)$ (describing the selectivity filter at $x = 0$) are constrained so that the total entropy production there $\sigma(0)$ is positive. The same discussion applies separately to the entropy production $\sigma(1)$ (and its components $\sigma_k(1)$ and $\sigma_m(1)$), at the other selectivity filter, at $x = 1$; in a locally dissipative system, like that considered in this paper, a decrease in entropy at one location cannot be balanced by an increase at another location.

In the PNP channel, flux coupling occurs because of dissipative processes in each of the selectivity filters. In these filters the selectivity parameters of different ions (say, $\alpha_m(1)$ and $\alpha_k(1)$ or $\alpha_m(0)$ and $\alpha_k(0)$) are constants, not varying with experimental conditions, not coupled to one another in any way. This description of selectivity is compatible with thermodynamics provided the selectivities $\alpha_j(0)$ at one end of the channel and the set $\alpha_j(1)$ at the other both satisfy thermodynamic constraints:

Thermodynamic Constraint

$$\sum_j \mathcal{J}_j z_j \alpha_j(0)[V - V_j] \geq 0$$

$$\sum_j \mathcal{J}_j z_j \alpha_j(1)[V - V_j] \geq 0. \quad (71)$$

These constraints are required in addition to the thermostatic constraints on the phase-boundary potentials $\delta_j(0)$ and $\delta_j(1)$, described in text (Eq. 30). It is not difficult to choose selectivities that satisfy both constraints, as do the selectivities used in the calculations reported in Results. The need for these constraints, in addition to the differential equations and boundary conditions of the PNP model itself, is not a weakness of the model. In the analysis of many nonequilibrium systems, thermodynamics is needed to impose "constraints on the transport coefficients by predicting that they must obey a certain number of inequalities" (Balian, Ref. 63, p. 267).

The PNP model does not specify the physical mechanism by which the entropy consumed by uphill transport of one ion in one selectivity filter is coupled to the entropy production produced there by the downhill movement of other ions. Indeed, it seems clear that the physical processes described by the PNP differential equations cannot account for that coupling of entropy consumption and production by themselves, without boundary conditions like Eq. 31. On the other hand, the underlying processes are probably closely related to interactions found in free solution or gases in which the flux of one species is driven by the driving force of another, usually described by Onsager reciprocal relations or generalized diffusion coefficients D_{jk} (see pp. 540, 715–717 of Hirschfelder et al., Ref. 64, for the analogous treatment of gases). Such interactions can be expected at the ends of channels, where massive dehydration must be balanced by massive re-solvation, with small imbalances having large effects on permeation, selectivity, and coupling.

APPENDIX: UNIDIRECTIONAL AND TRACER FLUXES

The unidirectional fluxes defined in this paper (e.g., Eq. 22) were theoretical constructs until radioactive tracers became available. Since then, however, hundreds if not thousands of papers have used the net flux of tracer (measured when tracer is present in significant amounts on only one side of a membrane) as an estimate of the unidirectional flux of the main species through channels (2, 3) or transporters (1, 11, 65). In the present context, channel transport would be expected in systems with $\gamma_j > 1$ and mediated transport in systems with $\gamma_j \leq 1$.

Tracer fluxes reported in the literature flow, of course, through macroscopic numbers ($\sim 10^5$) of channels or transporters because the flux of ions through a single channel or transporter protein is much too small to be measured directly, by chemical or isotope counting techniques. The electrical charge carried by ions through a single channel is, however, large enough to measure—indeed that current is now measured routinely in hundreds of laboratories every day with the patch clamp method—but the charge moving through other transport proteins is expected to be invisible in the background noise of presently available patch-clamp amplifiers (see Wang, Tang, and Eisenberg, Ref. 66, for a possible exception).

Unidirectional flux through gated channels or transporters

A macroscopic tracer flux can be interpreted as a unidirectional flux through a single channel or transport molecule only if the number of open channels through which the flux flows does not change as experimental and biological conditions are manipulated. That is to say, the area through which the flux flows must remain constant; experimental interventions used to manipulate the system and fluxes must not change gating.

Measurements of currents through single open channels have shown that many experimental manipulations modify the gating of channels, without changing the current flowing through a single open channel. Measurements of current through single transport proteins (other than channels) are rare, and measurements of tracer flux nonexistent, but their scarcity represents a technological and historical constraint rather than a biological phenomenon. Our present inability to observe gating in most transport proteins does not mean gating is absent in these molecules.

Indeed, one must suspect that transport proteins are able to transport ions only a certain fraction of the time, just like channels; one must suspect that they are sometimes active, sometimes inactive, just like channels. If so, the current through them, when they are open (i.e., active), may be much larger than commonly supposed. In fact, the usual estimates of flux through the transport protein would have to be divided by the fraction of time the protein is active.[3] In that case, experimental interventions might change macroscopic unidirectional flux by changing the fraction of time the transport protein is transporting, as well as by changing the amount of transport when the protein is active. Without a description of such "gating" phenomena, no theory, certainly not that presented here, will be able to describe isotope fluxes through macroscopic numbers of channels observed experimentally and their variation with ionic and electrical conditions.

Unidirectional flux estimated by the net flux of tracer

It is perhaps worthwhile presenting a careful analysis of the usual physiological method of estimating unidirectional fluxes, because our colleagues in the physical and mathematical sciences seem unaware of the method and

[3] The fraction of time a single protein molecule is active equals the probability of any one molecule being open in a patch of membrane (about 0.1 in many cases) divided by the number of those proteins in that patch (say 20,000 in one case of interest) (66).

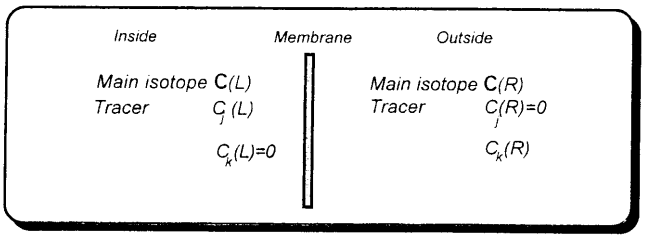

FIGURE 8 Typical set-up for measuring unidirectional isotope flux. Note the concentration of main isotope (nonradioactive) is $\mathbf{C}(L)$ written without a subscript. The concentration of the j isotope, confined to the left, is $C_j(L)$; the concentration of the k isotope, confined to the right, is $C_k(R)$.

are unconvinced of its power by the derivations and arguments we have located for them in the physiological literature.

Consider a set-up for measuring tracer flux (Fig. 8) in which three different isotopes of the same ion species are present. The concentration of the main isotope on the left is $\mathbf{C}(L)$, written without a subscript in bold face, and the concentration of one tracer (also on the left) is denoted by a subscript j, namely $C_j(L)$. The concentration of the main isotope on the right is $\mathbf{C}(R)$, written without a subscript, and the concentration of a different tracer (on the right), is denoted by a subscript k, namely $C_k(R)$. Each tracer is confined to one side of the channel: the concentration of one tracer $C_j(R)$ on the right is kept insignificant, as is the concentration of the other tracer $C_k(L)$ on the left. That is to say, the *trans* concentrations of tracer are negligible compared to the concentrations of tracer on the *cis* side:

$$C_j(L) \ll \mathbf{C}(L); \qquad C_k(R) \ll \mathbf{C}(R). \tag{72}$$

Because the isotopes are indistinguishable except for their radioactivity, all functions, variables, and parameters except concentration and flux are the same for all species. In particular, one set of functions $\Phi(x)$, $\beta(0)$, $\beta(1)$, etc. describe all the isotopes.

The unidirectional fluxes of the main species are written in this Appendix without subscript and in bold face, using Eq. (47):

$$\text{Unidirectional efflux} = \mathbf{J}(L \to R) = \mathbf{C}(L) \cdot \frac{\beta(0; \cdot)\exp\{zV\}}{\int_0^1 \exp\{z\Phi(\varsigma)\}d\varsigma} \tag{73}$$

$$\text{Unidirectional influx} = \mathbf{J}(R \to L) = \mathbf{C}(R) \cdot \frac{\beta(1; \cdot)}{\int_0^1 \exp\{z\Phi(\varsigma)\}d\varsigma}. \tag{74}$$

The unidirectional and net flux $J_j(\cdot)$ of the tracer species j are written with subscripts. Then,

$$\text{Tracer influx} = J_j(R \to L) = 0, \quad \text{because } \textit{trans} \text{ concentration } C_j(R) = 0. \tag{75}$$

$$\text{Tracer efflux} = J_j(L \to R) = C_j(L) \cdot \frac{\beta(0; \cdot)\exp\{zV\}}{\int_0^1 \exp\{z\Phi(\varsigma)\}\, d\varsigma} = J_j\,(\text{net}). \tag{76}$$

The unidirectional and net flux $J_k(\cdot)$ of the other tracer species k are also written with subscripts. Then,

$$\text{Tracer influx} = J_k(R \to L) = C_k(R) \cdot \frac{\beta(1; \cdot)}{\int_0^1 \exp\{z\mathbf{C}(L)\Phi(\varsigma)\}\, d\varsigma} = J_k\,(\text{net})$$

$$\text{Tracer efflux} = J_k(R \to L) = 0$$

because *trans* concentration $C_k(L) = 0$.

The result is that each unidirectional flux $\mathbf{J}$ of the main species is proportional to the unidirectional flux of one of the tracer species, which is also the net flux J_j of that tracer isotope.

$$\mathbf{J}(L \to R) = \left\{\frac{\mathbf{C}(L)}{C_j(L)}\right\} \cdot J_j(L \to R) = \left\{\frac{\mathbf{C}(L)}{C_j(L)}\right\} \cdot J_j\,(\text{net})$$

$$\mathbf{J}(R \to L) = \left\{\frac{\mathbf{C}(R)}{C_k(R)}\right\} \cdot J_k(R \to L) = \left\{\frac{\mathbf{C}(R)}{C_k(R)}\right\} \cdot J_k\,(\text{net})$$

The proportionality constants, shown in the big braces, are the reciprocal of the specific activity of each isotope on the *cis* side and can easily be estimated experimentally as the ratio of the concentration of the main species to the number of radioactive disintegrations (of a particular isotope) observed in a unit time on the *cis* side. (On the *trans* side the specific activity is negligible, that is, "zero," because the concentration of isotope is kept negligible, compared to that on the *cis* side, by the experimental apparatus and protocol.) In this way, measurement of the net flux of isotope allows easy estimation of the unidirectional fluxes of the main species.

The derivation presented here uses the PNP theory for the sake of clarity and specificity. It can easily be generalized to any theory of ionic motion, provided

(a) the concentration (in moles/liter) of all tracers is negligible compared to the main species;
(b) the tracer species is chemically and physically indistinguishable from the main species (except for its radioactive disintegrations);
(c) the concentration of isotope on the *trans* side of the channel is negligible; and
(d) the functionals describing unidirectional influx and efflux (analogous to the right-hand sides of Eqs. 73 and 74) are zero when the *cis* concentrations are zero, i.e., when $\mathbf{C}(L) = 0$ and $\mathbf{C}(R) = 0$, respectively.

Then, the functionals (analogous to those on the right-hand sides of Eqs. 74 and 77) are equal and so the result, namely Eqs. 79 and 80, is established.

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