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# THE THERMOSTATICS AND THERMODYNAMICS OF COTRANSPORT

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**(1) The thermostatics of cotransport are reviewed. A static-head equilibrium state across a cotransport system, without leaks, is thought to occur when the electrochemical potential of the driven solute, B prevents net flow of the driving solute, A. For a symport this gives the relationship**

$$\left(\frac{A_1}{A_2}\right)^n = \left(\frac{B_2}{B_1}\right)$$ **and for an antiport** $$\left(\frac{A_1}{A_2}\right)^n = \left(\frac{B_1}{B_2}\right)$$

**Where $n$ is the stoichiometric coefficient, namely the number of moles of A transported per mole of B. (2) If either a symporter with a 2:1 stoichiometric coefficient and a 1:1 symporter, or alternatively, a 1:1 symporter and a 1:1 antiporter are placed in a series membrane array, then the predicted static-head equilibrium across the entire array conflicts with the zeroth law of thermodynamics. (3) There are two major reasons for this failure of cotransport theory; these are: (A) the thermostatic relationships derived shown in Point 1 are based on the assumption that the cotransport process takes place within a closed system. However, the membrane and the external reservoirs are open to the cotransported ligands. It follows that A and B in the external reservoirs can vary independently of the changes within the cotransport process. As no chemical reaction between A and B occurs in the external solutions, reactions within the membrane phase do not affect the equilibrium between the transported ligands in the open reservoirs. (B) It is assumed that the law of mass action can be applied to the cotransport chemical reactions within the membrane phase, without any allowance for the fact that these reactions occur within a 'small thermodynamic system'. Any proper analysis of the chemical potential of the transported intermediate must consider the effects of lower order ligand-carrier forms, which coexist and compete for space with the higher order cotransported forms on the binding matrix. If account is taken of this necessity, then a simple extension of the work of Hill and Kedem (1966) J. Theor. Biol. 10, 399–441 shows that: (a) the static-head equilibrium state cannot exist; (b) the stoichiometry of cotransport, whether symport, or antiport, does not affect the static-head distribution of cotransported ligands; (c) the hypothetical net charge of the transported ligand-carrier complex does not affect static-head equilibrium; (d) the only equilibrium state where there is zero net flow of both driving and driven transported ligand is at true equilibrium when the ligands are uniformly distributed across the membrane. (4) It is deduced that cotransport is not entirely an affinity-driven, but is partially an entropy-driven process. The stationary state distribution of driven ligand B depends on the affinity of the driving solute across the membrane, and is a power function of $q/\sqrt{L_{22}/L_{11}}$, where $|q|$, the degree of coupling is a measure of the tightness of coupling within the cotransporter is always less than 1 and $L_{22}$ and $L_{11}$ are the straight conductance coefficients of the driven and driving ligands across the cotransporter. The main differences between this and the conventional view are that (a) the flow ratio of driving to driven solute cannot be fixed and (b) accumulation of driven solute is not related to the 'mechanical stoichiometry', as determined by the flow ratio.**

## Introduction

The $Na^+$-gradient hypothesis, as proposed by Crane [1]; the chemiosmotic hypothesis, proposed by Mitchell [2–5] and the model for axonal $Na^+$-$Ca^{2+}$ exchange, proposed by Blaustein and Hodgkin [6], suggest that dissipation of the electrochemical potential gradient of one solute across a biological membrane can be utilized by cotransporting systems to energize the flow of other solutes.

## Brush-border cotransport

The sodium gradient energizes the uphill flow of sugars across the brush borders of intestinal and renal proximal tubule cells and into membrane vesicles made from isolated brush-border membranes, Crane [1].

The electrochemical potential difference of $Na^+$ across the small intestinal brush border consists of a 10-fold $Na^+$ activity gradient with a measured electrical potential difference of $-50$ mV. This should be capable of supporting a sugar flux ratio of 70 at maximum (11 kJ/mol). However, the observed ratio of mucosal-serosal flux: serosal-mucosal flux for $\beta$-methyl-D-glucoside across sheets of rabbit ileum in vitro is in the range 150–250, (14 kJ/mol) (Holman and Naftalin [7]). Reinvestigation of the stoichiometry of $Na^+$-dependent cotransport has revealed that 2 $Na^+$ equivalents are transported per 1 mol of sugar in chick intestinal cells (Kimmich [8,9]) and similarly a stoichiometry of 2 $Na^+$ : 1 sugar has also been found in rabbit proximal tubular brush-border membranes. (Turner and Moran [10], Kaunitz, Gunther and Wright [11]).

*Cotransport in single cells and vesicles*

Vidaver and Shepherd [12] were first to claim that multivalent carrier kinetics affect the accumulation of solute coupled to $Na^+$. They showed that glycine accumulation by pigeon erythrocytes is proportional to $(Na_0^+/Na_i^+)^2$ and suggested that one mole of glycine is cotransported with 2 equivalents of $Na^+$.

Similarly, Cockburn, Earnshaw and Eddy [13] have shown that glutamate ion is taken up by the yeast, *Saccharomyces* along with 3 protons (2 net positive charges). However, they only find a maximal 70-fold accumulation ratio of glutamate by the yeasts. This is surprisingly small considering the large potential of this system.

Using *Escherichia coli* membrane vesicles, Ramos and Kaback [15] have shown that downhill movement of protons into vesicles energizes accumulation of proline and lactose by the vesicles. At pH 7.5 a proton motive force of 50 mV (5 kJ/mol) can induce lactose, or proline accumulation up a gradient equivalent to 100 mV (10 kJ/mol). Based on a model by Rottenberg [16], Ramos and Kaback deduced the stoichiometry of cotransport by the symporter is 2 protons: 1 solute at pH 7.5.

*Squid axon*

Downhill flow of $Na^+$ into the squid axon is coupled to uphill movement of $Ca^{2+}$ out of the axon. However, the energy available from the $Na^+$ gradient across the squid axon is insufficient to energize uphill movement of $Ca^{2+}$ against its very large electrochemical potential. Blaustein and Hodgkin [6] suggested that if 3 $Na^+$ ions were coupled to the movement of 1 $Ca^{2+}$ ion, sufficient energy from the $Na^+$ gradient would be available to account for the steady-state equilibrium distribution of $Ca^{2+}$. More recently, DiPolo, et al. [14], found that the energy required for $Ca^{2+}$ extrusion is 41 kJ/mol and suggest that the stoichiometry required of $Na^+ : Ca^{2+}$ binding to the squid axon antiporter is 4 $Na^+$ : 1 $Ca^{2+}$.

## Cotransport by intracellular organelles

*Chromaffin granules*

Uptake of catecholamines into chromaffin granular ghosts is also coupled to downhill outflow of protons, (Njus, Knoth and Zallakian [17]). Epinephrine is accumulated by adrenal medullary cortical granules by 30000-fold. As the activity coefficient of epinephrine within the granules is only 0.33, the activity ratio is 10000-fold (24 kJ/mol). The pH gradient is 1.5 units (9 kJ/mol) and the membrane potential is 60 mV (6 kJ/mol). Accordingly if the stoichiometry of the proton: catecholamine antiporter were 2 : 1, the pH difference across the membrane would generate 18 kJ/mol, and since the net charge movement per cycle of the 2 : 1 antiporter is ($nH^+ - Z1 = 1$), the

total available energy from the electrochemical potential gradient of protons ($2 \times 9 + 1 \times 6 = 24$ kJ/mol) would be just sufficient to generate the observed very high accumulation of catecholamines.

## Mitochondrial chemiosmosis

The chemiosmotic hypothesis, as originally proposed by Mitchell [4,5] suggested that the driving force from the electrochemical potential difference of protons across the mitochondrial membrane could be used to alter the equilibrium of a reversible ATPase within the mitochondrial membrane, thereby generating ATP. Mitchell later amended this hypothesis to propose that when ATP hydrolysis is strictly coupled to the translocation of 2 protons from phase R to L per ATP hydrolysed then:

$$\frac{[\mathrm{ADP}]\cdot[\mathrm{PO_4}]}{[\mathrm{ATP}]} = K_{\mathrm{H_2O}} \frac{[\mathrm{H}]_{\mathrm{R}}^2}{[\mathrm{H}]_{\mathrm{L}}^2}$$

Since the energy required to maintain the observed distribution of ATP to ADP and $PO_4^{3-}$ is 40 kJ/mol (7 pH units), and the total amount of electrochemical potential from the proton gradient is only 21 kJ/mol, (1 pH unit + 150 mV), the polyvalent stoichiometry of the ATPase II provides a necessary buttress to the chemiosmotic hypothesis.

The above systems have been described by the following thermodynamic relations.

*For sugar cotransport:*

$$RT \ln\left(S^2/S^1\right) = n\left(RT \ln\left(\mathrm{Na}^1/\mathrm{Na}^2\right) + zF\Delta\psi\right) \qquad (1.1)$$

*For proton-coupled cotransport of electroneutral solute*

$$RT \ln\left(S^2/S^1\right) = n\left((2.3RT/F)\,\Delta\mathrm{pH} + \Delta\psi\right) \qquad (1.2)$$

*For $Ca^{2+}$-$Na^+$ exchange:*

$$RT \ln\left(\mathrm{Ca}^2/\mathrm{Ca}^1\right) = nRT \ln\left(\mathrm{Na}^2/\mathrm{Na}^1\right) + (n - z\mathrm{Ca})F\Delta\psi \qquad (1.3)$$

*For chemiosmotic synthesis of ATP:*

$$\log\frac{\mathrm{ATP}}{\mathrm{ADP}} = \log(\mathrm{PO_4}) - n\left(\Delta\mathrm{pH} + \frac{\Delta\psi}{60}\mathrm{mV} + 5\right) \qquad (1.4)$$

Where $R$ is the gas constant; $T$ is temperature (Kelvin); $F$ is the Faraday constant, $\Delta\psi$ the membrane potential; $S^1$ and $S^2$, are the internal and external concentrations of sugar S, or any electroneutral cotransported solute S; $n$ is the stoichiometric coefficient relating either $Na^+$, or proton cotransport to solute flux; $z$ is the charge on $Ca^{2+}$ and 5 in Eqn. 1.4 refers to the hydrolysis constant of ATP at 300 K.

The energy coupling of cotransport has been reviewed by Mitchell and more recently by West [18].

In all the cases reviewed above the potential energy required to drive the cotransported solute accumulation is in excess of the energy available from the electrochemical potential difference of the driving solute across the transporting membrane; so a second hypothesis is required to account for the coupled flow. Polyvalent binding of the driving molecule to the cotransport system within the membrane is claimed to increase the energy available for cotransport. The basis for this widely held belief is the analysis by Mitchell [2–5]; Vidaver [12]; Blaustein and Hodgkin [6]; Rottenberg [16]; Weber [19]; Stein and Honig [20], and Aronson [21].

As there is no limit to the stoichiometry of solute interaction with carriers, neither is there an upper limit to the amount of force obtainable from ion gradients linked to cotransport, (Aronson [21]). If the stoichiometric coefficient for Na-dependent sugar accumulation were 10 : 1, the theoretical maximum sugar accumulation ratio in Kimmich's system [8] would be $9 \times 10 = 90$ kJ/mol, which is equivalent to a static head accumulation of $10^{16}$-fold.

## Antiport

Antiport is a special form of membrane cotransport process which facilitates the antiparallel flow of ligands [2,3]. Two examples of this form of cotransport have been already mentioned $Na^+$-$Ca^{2+}$ exchange [6] and proton-catecholamine exchange [17], which employ multivalent stoichiometry.

The antiport process is assumed to involve binding of two ligands, A and B to the same carrier. The ligands are assumed to traverse the

system via non-interfering routes. Passive downhill movement of one ligand caused by a maintained concentration difference is considered to induce a counterflow of the other ligand. This counterflow is driven by the transference of energy from one ligand to the other through the agency of the antiporter.

This model of antiport requires that the system should reach a state where the energy flow of the driving ligand will be balanced by energy flow in the opposite direction of the driven ligand. At this point there will be zero net energy flow, assuming that there are no leaks via shunt pathways and the system is at static-head equilibrium.

## Analysis of the thermodynamics of cotransport

*1. Current view*

Cotransport is the process whereby a flow of one solute down its electrochemical potential gradient, the driving solute, can induce a coupled flow of another solute, the driven solute, either in the same direction, symport, or in the opposite direction, antiport. The symport process is considered to lead directly to solute accumulation.

'Static-head equilibrium' across a cotransporter is assumed to occur when the electrochemical potential difference of the driven solute is sufficient to prevent the flow of the driving solute, in the case of a symport system this requires that the electrochemical potential of the driven solute has opposite polarity to that of the driving solute and for an antiport system both the driven and driving solutes have the same polarity. Since net flux of both the driving and driven solutes is zero, assuming no leaks via a shunt pathways, this steady state can be considered as an equilibrium state. It is different from 'true equilibrium' where the solutes are uniformly distributed across a membrane with equal temperature and pressure on either side.

Static-head equilibrium can also be differentiated from a 'static-head stationary-state', which implies here a state where there is zero net flow of driven solute across the membrane, but there is a steady-state flow of driving solute.

*2. Symport*

A simplified model of a multivalent cotransport system, similar to that described by Ramos and Kaback [15] for proton-dependent cotransport and by Kimmich [8] for $Na^+$-dependent sugar cotransport illustrates how Eqns 1.1–1.3 are derived (Fig. 1a).

*Electrical short-circuit condition*

It is assumed that at static-head equilibrium unidirectional flows of c′ and c″, the unliganded carrier and of ($c'Na_nS$) and ($c''Na_nS$), the carrier-ligand complex, across the membrane are equal and symmetrical i.e. $k = k$ ($k$ is equal to both the forward and backward rate of movement of both free carrier and carrier-ligand complex).

For simplicity, it is assumed that the overall carrier-substrate dissociation constants for each side of the membrane are symmetrical: i.e. $K' = K''$, where $K = K_{Na}{}^n \cdot K_S$ ($K_{Na}$ and $K_S$ are the dissociation constants of $Na^+$ and $S^+$ for the carrier.)

The following reactions are assumed to take place at each side of the membrane.

$$\begin{aligned} n\mathrm{Na}' + \mathrm{S}' + \mathrm{c}' &\rightleftharpoons (\mathrm{c}'\mathrm{Na}_n\mathrm{S}) \\ n\mathrm{Na}'' + \mathrm{S}'' + \mathrm{c}'' &\rightleftharpoons (\mathrm{c}''\mathrm{Na}_n\mathrm{S}) \end{aligned} \tag{2.1}$$

Hence

$$K' = \frac{(\mathrm{Na}')^n \cdot S' \cdot c'}{(\mathrm{c}'\mathrm{Na}_n\mathrm{S})}\ ;\ K'' = \frac{(\mathrm{Na}'')^n \cdot S'' \cdot c''}{(\mathrm{c}''\mathrm{Na}_n\mathrm{S})} \tag{2.2}$$

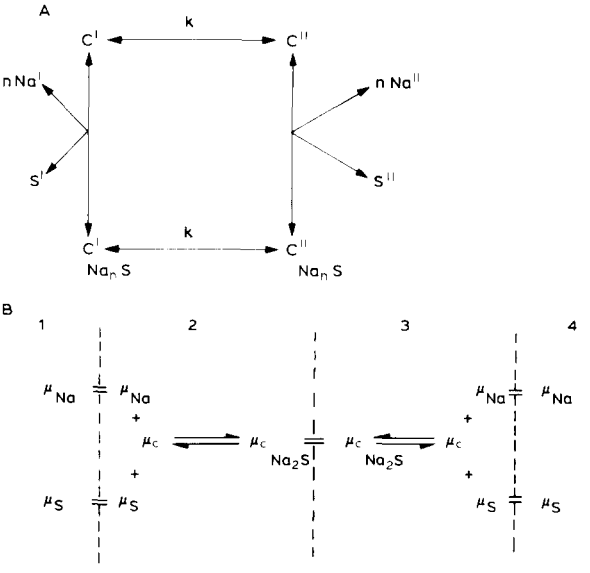



Fig. 1. (A) Simple kinetic model of a symporter. (B) Phase equilibrium model of a symporter.

At static-head equilibrium, when the electrical potential difference across the membrane $\Delta\psi = 0$, the carrier-substrate complex is equally distributed between the two sides of the membrane.Thus:

$$(c'Na_nS)\cdot k = (c''Na_nS)\cdot k \tag{2.3}$$

Since $K' = K''$; and $k$ (forward) $= k$ (reverse), it follows from Eqns. 2.2 and 2.3 that:

$(Na')^n \cdot S' \cdot c' = (Na'')^n \cdot S'' \cdot c''$ and hence,

$$[Na']^n \cdot [S'] = [Na'']^n \cdot [S''] \tag{2.4}$$

*Open-circuit cotransport*

It is assumed that the electrostatic potential difference across the membrane acts directly only on the charged substrate-carrier complex (Geck and Heinz [22]).

If $\Delta\psi \neq 0$; then at static-head equilibrium, the effect of the electric field on the equilibrium distribution of carrier-substrate complex is:

$$(c''Na_nS)/(c'Na_nS) = X; \text{ where } X = \exp(n^c F\Delta\psi/RT) \tag{2.5}$$

i.e. $\bar{\mu}'_{(cNa_nS)} = \bar{\mu}''_{(cNa_nS)}$

$n^c$ is the net charge number on the transported ligand-carrier intermediate.

Hence in Eqn. 2.5 $n^c = n \cdot z_{Na} - z_S$, where $z_{Na}$ is the charge number and sign of Na and $z_S$ is the charge number and sign of the sugar charge. Here $z_S$ is zero and it is assumed that the carrier is uncharged, thus $n^c = n$.

At static-head equilibrium, the forward and reverse rates of carrier movement between all nodes are equal; hence the distribution of the uncharged carrier c within the membrane is:

$$c' = c'' \tag{2.6}$$

From Eqns. 2.2, 2.5 and 2.6

$$\frac{c'\cdot(Na')^n\cdot S'\cdot X}{c''\cdot(Na'')^n\cdot S''} = \frac{(Na')^n\cdot S'\cdot X}{(Na'')^n\cdot S''}$$

or,

$$(Na'/Na'')^n\cdot\exp(nF\Delta\psi/RT) = (S''/S') \tag{2.7}$$

*3. Antiport*

It will be assumed that A the driving solute and B, the driven solute are nonpolar species which take part in an antiport system. It is assumed that two ligands, A and B, bind to the same carrier and that each ligand-carrier complex, crosses the membrane separately (Fig. 2). The free carrier does not cross the membrane.

From these assumptions, it is deduced that static-head equilibrium will occur when:

$$Ac' = Ac'' \text{ and } Bc' = Bc'' \tag{3.1}$$

$K_a$ and $K_b$, are the dissociation constants of A and B for the free carrier, $c$ on both sides of the antiporter.

Thus:

$$Ac' = \frac{c'\cdot A'}{K_a};\ Ac'' = \frac{c''\cdot A''}{K_a}$$

$$Bc' = \frac{c'\cdot B'}{K_b};\ Bc'' = \frac{c''\cdot B''}{K_b} \tag{3.2}$$

Hence

$$c' = \frac{Ac'\cdot K_a}{A'} = \frac{Bc'\cdot K_b}{B'}$$

and

$$c'' = \frac{Ac''\cdot K_a}{A''} = \frac{Bc''\cdot K_b}{B''} \tag{3.3}$$

From Eqns. 3.1, 3.2 and 3.3, it follows that:

$$\frac{Ac'\cdot K_a}{Bc'\cdot K_b} = \frac{[A']}{[B']} = \frac{[A'']}{[B'']} \tag{3.4}$$

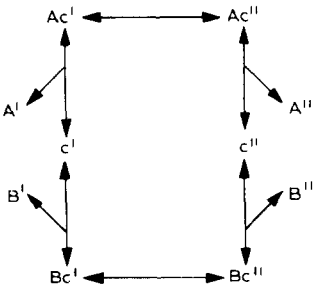



Fig. 2. A simple kinetic model of an antiporter.

Since

$$\Delta\mu_A^{1-2} = RT\ln(A_1/A_2) \text{ and } \Delta\mu_B^{1-2} = RT\ln(B_1/B_2) \quad (3.5)$$

where $\Delta\mu_i^{1-2}$ is the chemical potential difference of component $i$ between solutions 1 and 2; $R$, the gas constant and $T$, temperature (Kelvin).

It follows that the energy flow via the antiport transport system is zero when:

$$\Delta\mu_A^{1-2} = \Delta\mu_B^{1-2} \quad (3.6)$$

*4. The Gibbs free energy function G and cotransport [23–25]*

The Gibbs free energy function $G$ of an isothermal, isobaric reaction in a single phase is:

$$G = \sum_i n_i\mu_i \quad (4.1)$$

At equilibrium, the Gibbs free energy of the system is at a minimum, hence the change in free energy $dG/dn_i = 0$

$dG$ is the change in free energy for a change in $n_i$ assuming that $\mu$ for all components remains unchanged.In an open system, such as occurs with flow of solutes across a membrane between two reservoirs, where $\mu$ of all components is controlled,

$$dG_{tr} = \sum_i dn_i(\Delta\mu_i^{1-2}) \quad (4.2)$$

(subscript $i$ refers to transfer of $n$ moles of component $i$, $n$ is positive for flow from 2-1 and negative for flow from 1-2. $\Delta\mu$ is the difference in electrochemical potential between 1 and 2 of component, $i$; $dG_{tr}$ refers to the free energy change due to transport across the membrane of $n$ moles of components $i$.

$G$ is an extensive state variable defining the amount of work performed (Joules) by the system on moving $n$ moles.

When a system is at equilibrium it performs no work.

$$\text{Hence } dG = 0 \quad (4.3)$$

From the relationships shown in Eqns. 4.2 and 4.3 Mitchell [2] deduced that the transport ratio of symported solutes could affect static-head equilibrium.

Symport of A and B reaches static-head equilibrium when:

$$dG = dn_A\Delta\mu_A^{1-2} + dn_B\Delta\mu_B^{1-2} = 0 \quad (4.4)$$

hence:

$$(n_A/n_B)\Delta\mu_A^{1-2} = -\Delta\mu_B^{1-2} \quad (4.5)$$

or

$$\left(\frac{A^1}{A^2}\right)^{n_A/n_B} = \left(\frac{B^2}{B^1}\right) \quad (4.6)$$

*5. Conventional thermodynamics of antiport [2,21]*

If the system contains two mobile components A and B whose transport is coupled so that movement of 1 mole of A induces a counterflow of 1 mole of B then

$$dG = dn_A\Delta\mu_A^{1-2} - dn_B\Delta\mu_B^{2-1}$$

or

$$dG = dn_A\Delta\mu_A^{1-2} + dn_B\Delta\mu_B^{1-2} \quad (5.1)$$

The sign of $dn$ is negative on flow from phase 1 to 2 and positive on flow from 2-1.

At equilibrium, the system can do not work, hence

$$dG/dn = 0 \quad (5.2)$$

Thus, Mitchell [2] deduced from Eqns. 5.1 and 5.2 that if

$$dn_A = -dn_B \quad (5.3)$$

Then at equilibrium

$$(n_A/n_B)\Delta\mu_A^{1-2} = \Delta\mu_B^{2-1} \quad (5.4)$$

or

$$\left(\frac{A^1}{A^2}\right) = \left(\frac{B^1}{B^2}\right) \quad (5.5)$$

*6. Cotransport in relation to the zeroth law of thermodynamics*

Acceptance of Eqns. 2.7, 4.6 and 5.5 requires

the following assumptions:

(1) A reversible chemical reaction between two ligands within the microscopic environment of the membrane can affect the equilibrium between the ligands in the macroscopic external environment in which the ligands do not react.

(2) The ratio of moles of ligands transported by the carrier affects the size of the driving force, (affinity) of the cotransport reaction.

(3) The mode (symport or antiport) of membrane cotransport controls the direction of the net driving force, (affinity) across the membrane.

The following hypothetical examples illustrate the difficulty in accepting the proposition that the stoichiometry of the coupling reaction can affect the static-head equilibrium.

Assume that between aqueous phases 1 and 2, there is a cotransporter which couples 2 moles of A to the flow of a mole of B.

Thus, according to the theory of multivalent cotransport, static-head equilibrium will be obtained when:

$$[A^1]^2/[A^2]^2 = [B^2]/[B^1]$$

If, between aqueous phases 2 and 3, 1 mole of A is coupled to the flow of 1 mole of B, via a cotransporter, then static-head equilibrium between 2 and 3 will be obtained when:

$$[A^2]/[A^3] = [B^3]/[B^2]$$

thus: if $A^1 = 100$ mM; $A^2 = 10$ mM; $B^1 = 10$ mM and $B^3 = 10$ mM, then static-head 'equilibrium' between 1 and 2 occurs when $B^2 = 1000$ mM and between 2 and 3 when $A^3 = 1000$ mM (Table I)

TABLE I

| | Concentration (mM) | | |
|---|---|---|---|
| | 1 | 2 | 3 |
| A | 100 | 10 | 1000 |
| B | 10 | 1000 | 10 |

Hence, if phase 2 is in equilibrium with phase 3, as well as with phase 1, according to the Zeroth Law of Thermodynamics, phase 1 should also be in equilibrium with phase 3. This is a statement of the generalized form of a Zeroth Law (Buchdahl [26]).

However, since the concentrations of B in phases 1 and 3 are equal, whereas A in 1 is not equal to A in 3, there is no possibility for any form of static-head equilibrium state existing between 1 and 3.

A similar problem arises with antiporters: assume that between compartments 1 and 2, there is an antiporter embedded within the membrane which can transport ligands A and B and between compartments 2 and 3, there is a symporter, which also transports A and B (Table II).

TABLE II

| | Concentration (mM) | | |
|---|---|---|---|
| | 1 | 2 | 3 |
| A | 100 | 10 | 100 |
| B | 100 | 10 | 1 |

As static-head equilibrium across an antiporter occurs when $(A^1/A^2) = (B^1/B^2)$ and across a univalent symporter when $(A^2/A^3) = (B^3/B^2)$; it follows that if the concentrations (mM) of A and B in compartments 1 and 2 are; $A^1 = 100$, $B^1 = 100$ and $A^2 = 10$, then the static-head stationary state across membrane 1-2 will occur when $B^2 = 10$. Similarly, if the concentration of $A^3$ is fixed at 100, static-head across the symporter between 2 and 3 occurs when $B^3 = 1$. Although compartments 1 and 3 are apparently in equilibrium with compartment 2, they cannot be in equilibrium with each other. There is no concentration difference of A between compartment 1 and 3, whereas a larger concentration difference of B exists between 1 and 3 than between 1 and 2, or 2 and 3.

The fact that a prediction of the antiport hypothesis is untenable, means that the assumptions, on which this hypothesis is based, are false.

It could be argued that the Zeroth Law of Thermodynamics pertains only to thermal equilibrium states. Tisza [27] has shown that if a thermodynamic wall is non-restrictive of at least some chemical species, i it is also non-restrictive of entropy and energy. The criterion for equilibrium

across such a wall consists of the simultaneous conditions

$\mu_i' = \mu_i''$

$T' = T''$

The statistical form of the zeroth law then applies: "Two ensembles that have been in statistical equilibrium with a reservoir are in statistical equilibrium with each other, regardless of the presence or absence of the reservoir." (Tisza and Quay [28]).

It could also be argued that in both examples, the thermodynamic walls between compartments 1 and 2 and 2 and 3 differ, so that the equilibrium between compartments 1 and 2 is dependent on a different process from that between 2 and 3 and hence, there is no necessity for an equilibrium state to exist between compartments 1 and 3. This argument is only correct at the microscopic level in considering the forms of mobile species which cross the membrane. However, at the macroscopic level, to which the thermodynamic laws all pertain, there is no distinction between any of the thermodynamic walls separating the compartments. They all permit the flow of components A and B. The relative rate at which A and B flow cannot affect the equilibrium state, which is time-independent.

*7. Refutation of current views on cotransport thermostatics*

*(a) Macroscopic thermodynamics*

In sections 2–5 it is shown that Eqns. 1.1–1.3 are derived on the basis of the Law of Mass Action. However, these equations apply to closed macroscopic systems. The cotransport chemical reactions within the membrane do not take place within a closed system. The membrane is an open system, i.e. exchange of transported ligands takes place between the membrane and the external reservoirs at both surfaces. The membrane is closed only to the species involved in chemical transformations and translocations within the membrane phase. The reservoirs are also open to the external environment.

In Eqns. 4.4 and 5.1 it is assumed that only internal change occurs.

This is true only for a closed environment [24,25,29].

Internal change is defined as follows:

$$d_i n_k = d_r n_k + d_{tr} n_k \tag{7.1}$$

Subscripts i, r, tr and $k$ refer to the changes due to total internal change, change due to chemical reaction and change due to transport of any component, $k$ from one internal phase to another internal phase, respectively.

Thus if the membrane phases were extended into the external reservoirs and these reservoirs were closed to the external environment, i.e. the membrane were a closed system, which only permits coupled transport across the membrane then $d_i n_k$ would be the only change observed.

However, in an open system such as actually exists, the infinitesimal change of state

$$dn_k = d_i n_k + d_e n_k \tag{7.2}$$

where $dn$ refers to the total change and $d_e$ to the external change.

It should be noted that $dn_k$ is the only exact differential in Eqn. 7.2 (Haase [24]).

Since any of the components, $k$ can be added independently to the open reservoirs, the total change occurring in an open system is the sum of all the separate changes of the components $k$. This means that the coupled reactions which occur only within the confines of the membrane are not the exclusive agents of change within the total open system. The transported ligands can be added to, or subtracted independently from, the external reservoirs and do not react chemically with each other within the reservoirs.

Hence, use of stoichiometric coefficients of the ligand interactions within the intermediate reactions to define the equilibrium of the complete open system is inappropriate.

*(b) Stoichiometry of cotransport*

*Definitions (Prigogine and Defay [25]).* The Law of Definite Proportions is: the increase in mass, $M_k$ of a component, $k$, which is being formed in a reaction r, is proportional to its mass $M_k$ and to its stoichiometric coefficient $\nu_k$. The sign of $\nu_k$ is negative for a component (reactant) consumed and positive for a component produced (product) by the reaction.

Where a series of reactions r, occur in several phases $\alpha$, the Law of Conservation of Mass applied to the resultant reaction gives:

$$0 = \sum_{\alpha} \sum_{k} \nu_{k,r} \cdot M_k \tag{7.3}$$

The term $\nu_r$ the resultant stoichiometric coefficient, may be used to define the stoichiometry of a series of multiphase reactions.

Hence, Eqn. 7.3. may be rewritten as follows

$$0 = \sum_{k} \nu_{k,r} M_k \tag{7.4}$$

In the case of the transference of component $k$ between phases, as $k$ is conserved on moving from one phase to another, the resultant stoichiometric coefficient $\nu_r$ of the phase transfer reaction is zero.

$$\begin{array}{cccc} & A^1 & = & A^2 \\ \nu & -1 & & +1 \\ \nu_r & & 0 & \end{array} \tag{7.5}$$

Hence, the stoichiometric coefficient of component $k$ of one-way transfer between phases is always 1 (Prigogine and Defay [25]).

*The affinity, A of a chemical reaction.* The unambiguous way to define the equilibrium state of a chemical system is to use the affinity function, $A$. This is an intensive state variable (Prigogine and Defay [25] and Sanfeld [23]).

$$A = -\sum_{k} \nu_k \cdot \mu_k \tag{7.6}$$

where, $\mu_k$ is the chemical potential of $k$. As $\nu_k$ is a dimensionless coefficient and $\mu_k$ is an intensive variable, $A$ is also an intensive variable;
When $A > 0$ the reaction velocity $v \geqslant 0$
When $A < 0$, $v < 0$
When $A = 0$, $v = 0$
This last statement defines true equilibrium.

However when $A \neq 0$ and $v = 0$ this is false equilibrium [23,25] False equilibrium occurs when a reaction does not proceed despite the presence of a chemical driving force. In a real system this might occur as a result of a metastable state. It could also be generated by inappropriate use of Eqns. 4.4 and 5.1. where no account is taken of the fact that cotransport exists within an open system.

*The affinity of cotransport.* In a multiphase system the resultant affinity of a sequence of reactions is

$$A_r = -\sum_{\alpha} \sum_{k} \nu_k \cdot \mu_k \tag{7.7}$$

i.e., the resultant affinity of a linked series of reactions $j$, between several phases is:

$$A_r = \sum_{j} A_j \tag{7.8}$$

At equilibrium the resultant affinity and the affinities of all the intermediate reactions of the sequence = 0

$$A_r = \sum_{j} A_j = 0 \tag{7.9}$$

The cotransport system should be regarded as a linked sequence of reactions occurring between three phases. This involves (see Fig. 1b):

(1) Movement of the 'reactant' solutes from the open phase 1 to phase 2, the membrane phase.

(2) A series of chemical transformations and translocations within the membrane phases and which may involve multivalent interactions, which result in, translocation of the mobile ligand-carrier complex or complexes, in the case of an antiporter, across the membrane from phase 2 to phase 3.

(3) Movement of the 'product' solutes from the membrane, phase 3 to the second aqueous phase, 4.

At equilibrium

$$A_r = \sum_{i} \nu_i \cdot \mu_i \text{ (reactants)} - \sum_{i} \nu_i \cdot \mu_i \text{ (products)} = 0 \tag{7.10}$$

The affinity of all the intermediate reactions in the sequence is zero when the overall reaction is at equilibrium (Eqn. 7.9).

Since the transported ligands do not react with each other in the external reservoirs, the stoichiometric coefficient for their reaction in phases 1 and 4 is zero.

When Eqn. 7.10 is applied to the cotransport reaction between $Na^+$ and sugar as defined in Eqn. 1.1, the static-head relationship is

$$A_r = \begin{array}{ll} \mu^1_{Na} - & \mu^4_{Na} = 0 \\ & = 0 \\ \mu^1_S - & \mu^4_S = 0 \end{array} \tag{7.11}$$

Eqn. 7.11 shows that in an open system, the distribution of reactants and products in the reservoirs on either side of the membrane at static-head equilibrium is independent of any reaction which may take place in the intermediate phase. This indicates that static-head equilibrium does not exist in open systems, like membrane cotransport and the only equilibrium state for an open system is true phase equilibrium, where

$$\mu_k^1 = \mu_k^4 \tag{7.12}$$

(Prigogine and Defay [25] chapter 6)

*8. Cotransport stoichiometry*

As the principles involved in the previous section lead to a radical alteration in the conventional interpretation of the thermostatics of cotransport, c.f. Eqns. 1.1–1.3 and 7.12, it is worth giving worked examples of the stoichiometric equations of cotransport.

*Symport*

When A and B are both cotransported across a membrane according to the following reaction scheme: (Fig. 1b)

*(1) Phase transfer reaction 1-2* (superscripts refer to phase number, subscripts to the stoichiometric coefficients of the individual reaction, bracketed numbers are the resultant stoichiometric coefficients).

$$\begin{array}{lccc} & nA^1 & = & nA^2 \\ \nu & -n & & +n \\ \nu_r & & (0) & \end{array}$$

$$\begin{array}{lccc} & B^1 & = & B^2 \\ \nu & -1 & & +1 \\ \nu_r & & (0) & \end{array} \tag{8.1}$$

*(2) Cyclic reactions within the membrane of carrier c with n*A *and* B

$$\begin{array}{llllll} (a) & nA^2 + & B^2 + & c^2 = & A_nBc^2 & \\ \nu & -n & -1 & -1 & +1 & \end{array} \tag{8.2a}$$

$$\begin{array}{llll} (b) & A_nBc^2 & = & A_nBc^3 \\ \nu & -1 & & +1 \\ \nu_r & & (0) & \end{array} \tag{8.2b}$$

$$\begin{array}{llllll} (c) & A_nBc^3 & = & nA^3 & +B^3 + & c^3 \\ \nu & -1 & & +n & +1 & +1 \end{array} \tag{8.2c}$$

$$\begin{array}{llll} (d) & c^2 & = & c^3 \\ \nu & -1 & & +1 \\ \nu_r & & (0) & \end{array} \tag{8.2d}$$

*(3) Phase transfer reactions between 3 and 4*

$$\begin{array}{lccc} & nA^3 & = & nA^4 \\ \nu & -n & & +n \\ \nu_r & & (0) & \end{array}$$

$$\begin{array}{lccc} & B^3 & = & B^4 \\ \nu & -1 & & +1 \\ \nu_r & & (0) & \end{array} \tag{8.3a}$$

Since Reactions 8.2 (a–d) are cyclic, addition of 8.2 (a, b, c, d) leads to reduction of the resultant stoichiometric coefficients of the individual components in the cycle to zero.
i.e, the sum of Eqns. 8.2a + 8.2b + 8.2c + 8.2d = 0

$$\begin{array}{llllll} & nA^2 & +B^2 & = & nA^3 & +B^3 \\ \nu & -n & -1 & & +n & +1 \\ \nu_r & & & (0,0) & & \end{array} \tag{8.2e}$$

Eqn. 8.2e is the resultant equation for transfer of the cotransported solutes across the membrane from phase 2 to phase 3.

As there is no chemical reaction between A and B in the external phases 1 and 4 the absence of reaction is represented as follows

$$\begin{array}{llllllll} & A^1 & + & B^1 & = & A^1 & + & B^1 \\ \nu & 0 & & 0 & & 0 & & 0 \\ & A^4 & + & B^4 & = & A^4 & + & B^4 \\ \nu & 0 & & 0 & & 0 & & 0 \end{array} \tag{8.3b}$$

The phase transfer reaction is dependent on the phase transfer reactions across the membrane boundaries with the external solution. Hence addition of Eqns. 8.1, 8.2e and 8.3a,b gives

$$\begin{array}{lccc} & nA^1 & = & nA^4 \\ \nu & -n & & +n \\ \nu_r & & (0) & \end{array}$$

$$\begin{array}{lccc} & B^1 & = & B^4 \\ \nu & -1 & & +1 \\ \nu_r & & (0) & \end{array} \tag{8.4}$$

Eqn. 8.4 shows that the stoichiometric coeffi-

cient for the phase transfer reaction across a membrane for all transported components is always 1. This result can be generalized to the simple formula that equilibrium distribution of solutes across a membrane is unaffected by the stoichiometry of ligand interaction with microscopic components existing within the membrane compartment.

*Antiport*

The same arguments can be applied to antiporters as has been used above for symporters: e.g.,

Transport from reservoir 1 to the membrane phase 2 can be described as follows

$$\begin{array}{lccc} & A^1 & = & A^2 \\ \nu & -1 & & +1 \\ \nu_r & & 0 & \end{array} \qquad (8.5)$$

$$\begin{array}{lccc} & B^1 & = & B^2 \\ \nu & +1 & & -1 \\ \nu_r & & 0 & \end{array}$$

The antiport reactions are

$$\begin{array}{lccc} & A^2 + c^2 & = & Ac^2 \\ \nu & -1 -1 & & +1 \end{array} \qquad (8.6a)$$

$$\begin{array}{lccc} & B^2 + c^2 & = & Bc^2 \\ \nu & +1 +1 & & -1 \end{array} \qquad (8.6b)$$

Transport of transport intermediates between membrane phases 2-3

$$\begin{array}{lccc} & Ac^2 & = & Ac^3 \\ \nu & -1 & & +1 \\ \nu_r & & 0 & \end{array} \qquad (8.6c)$$

$$\begin{array}{lccc} & Bc^2 & = & Bc^3 \\ \nu & +1 & & -1 \\ \nu_r & & 0 & \end{array}$$

Reaction of components in phase 3

$$\begin{array}{lcccc} & Ac^3 & = & A^3 & c^3 \\ \nu & -1 & = & +1 & +1 \\ & Bc^3 & = & B^3 & +c^3 \\ \nu & +1 & & -1 & -1 \end{array} \qquad (8.6d)$$

Transport of components between reservoir 4 and membrane phase 3

$$\begin{array}{lccc} & A^3 & = & A^4 \\ \nu & -1 & & +1 \\ \nu_r & & 0 & \end{array}$$

$$\begin{array}{lccc} & B^3 & = & B^4 \\ \nu & +1 & & -1 \\ \nu_r & & 0 & \end{array} \qquad (8.7)$$

Addition of Eqns. 8.6a, 8.6b, 8.6c, 8.6d shows that the resultant stoichiometric coefficients for all components taking part in the cyclic reaction are zero, i.e.,

$$8.6a + 8.6b + 8.6c + 8.6d = 0 \qquad (8.6e)$$

The resultant stoichiometric coefficient, $\nu_r$ reaction of the antiport system is

$$8.5 + 8.6e + 8.7 = 0 \qquad (8.8)$$

There is no chemical reaction between A and B in phases 1 and 4

$$\begin{array}{lcccc} & A^1 + & B^1 & = & A^1 + & B^1 \\ \nu & 0 & 0 & = & 0 & 0 \end{array}$$

$$\begin{array}{lcccc} & A^4 + & B^4 & = & A^4 + & B^4 \\ \nu & 0 & 0 & & 0 & 0 \end{array} \qquad (8.9)$$

Hence, the distribution of the components A and B between reservoirs 1 and 4 at static-head equilibrium requires that

$$\mu_A^1 = \mu_A^4$$

$$\mu_B^1 = \mu_B^4 \qquad (8.10)$$

The equations above indicate that the only equilibrium which exists across a cotransporter is true equilibrium, i.e., the solutions on either side of the transporter have uniform composition.

*9a. Microscopic thermostatics*

Whilst the above thermodynamic arguments show that static-head equilibrium does not exist in open macroscopic thermodynamic systems, the kinetic arguments of Weber [19] and Stein and Honig [20] remain apparently unchallenged. There is a mismatch between the predictions of the Law of Mass Action, when applied to carrier cotrans-

port and the thermodynamic propositions that:
(a) phase equilibrium occurs only when:

$$\mu_k^1 = \mu_k^2 \tag{9.1}$$

(Prigogine and Defay [25]).
(b) reversible cyclic processes, such as those within the membrane phase during cotransport, make no net contribution to the forces determining equilibrium of the external system (Katchalsky and Spangler [30]).

Except for boundary conditions, thermostatic relationships do not take account of the mechanism, by which equilibrium is attained. However, cotransport theory makes several large assumptions about the mechanism of solute equilibration across the systems.

In particular
(a) that the transported ligands bind to a finite number of binding sites within the membrane.
(b) The nature of the reaction at the ligand sites determines the size and direction of static-head equilibrium, e.g., static-head equilibrium depends on whether the reaction is to a monovalent, or multivalent symporter, or antiporter. Additionally, the net charge of the mobile carrier-ligand complex is assumed to affect the static-head distribution in an electric field (Geck and Heinz [22]).
(c) The Law of Mass Action, as applied to macroscopic systems, can be applied unaltered to macroscopic and microscopic systems. Hence the electrochemical potential of the ligand-carrier complex is assumed to be the same as if it were a product of a macroscopic reaction.

Assumption (c) is incompatible with (a), because with a finite number of binding sites, the number of ligand-carrier complexes is not linearly related to the chemical potential of ligands in the macroscopic reservoirs, as saturation of the sites occurs. Assumption (c) is incompatible with (b) because when two or more ligand-carrier forms coexist, there is competition for occupancy of sites.

Hill [31,32], has shown that the Law of Mass Action cannot be simply transferred from macroscopic to microscopic levels. At the microscopic level the Gibbs free energy function

$$G = \hat{\mu}_i \cdot N_i \tag{9.2}$$

where (mu hat) $\hat{\mu}_i = G/N_i$ is the integral chemical potential. In microscopic systems, $\hat{\mu}_i$ differs from $\mu_i = \mathrm{d}G/\mathrm{d}n_i$ the differential chemical potential. In a finite system the integral chemical potential cannot be considered as a pure intensive state variable as it is also a function of $N$, the number of systems available. The Law of Mass Action cannot be applied without considerable modification to the relationship between the integral chemical potential $\hat{\mu}$ of a higher order form, e.g., ABc, which is the product of two or more unimolecular reactions and the chemical potentials of the reactant components A, B and c.

However, by using the lattice-gas approach applied to membrane transport systems introduced by Hill and Kedem [33], Hill [34] it is possible to calculate the relative number of any particular form of ligand-complex occupying the cotransporter matrix. At equilibrium, as there is no net flow of ligands, the need to consider any of the possible transport cycles is eliminated; furthermore, the individual forward or reverse rates of the ligand binding to sites need not be considered, only the dissociation constant. The only relationships requiring definition are those between the ligand concentrations in the external reservoir and the relative number of particular ligand forms on the adjacent lattice layer (see Fig. 3). This involves a simple computation of competitive Langmuir binding for multiple forms of ligand complex.

### *9b. Analysis of microscopic forms*

#### *(1) Symport lattice membrane*

In the symport cotransport lattice (Fig. 3a), it is assumed that ligand A binds first to the lattice and B binds to the bound form of A to form AB.

It will be assumed that $A$ and $B$ are the reduced concentrations of [A] and [B], equivalent to $[A]/K_a$ and $[B]/K_b$, respectively.

Where $K_a$ and $K_b$ are the dissociation constants of A and B for the lattice sites, $c$ the vacant sites
Hence

$$x_A = (1 - x_A \cdot B)\frac{A}{1+A} \tag{9.3}$$

where $x_A$ = fractional number of sites occupied by

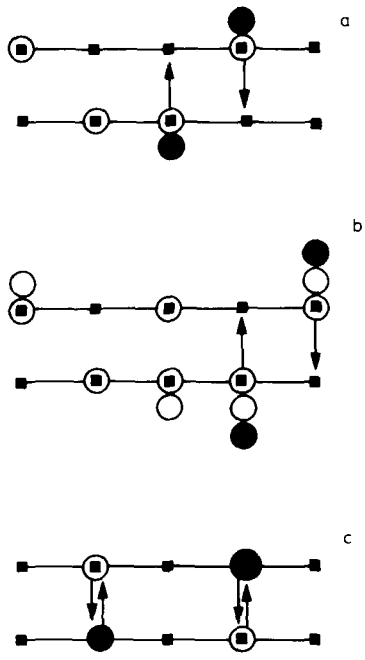



Fig. 3. Lattice model of static head stationary states of (a) a simple symport, (b) a multivalent symport, (c) an antiport.

A on the adjacent lattice row [35]

$$c = 1 - x_A - x_A \cdot B \tag{9.4}$$

Hence

$$x_A = \frac{A}{1 + A + A \cdot B} \tag{9.5}$$

$$x_{AB} = \frac{A \cdot B}{1 + A + A \cdot B} \tag{9.6}$$

$$x_c = \frac{1}{1 + A + A \cdot B} \tag{9.7}$$

At equilibrium

$$x'_{AB} = x''_{AB} \tag{9.8}$$

and

$$c' = c'' \tag{9.9}$$

Hence from Eqns. 9.9 and 9.7

$$1 + A' + A' \cdot B' = 1 + A'' + A'' \cdot B'' \tag{9.10}$$

and from Eqns. 9.6, 9.8 and 9.10

$$1 + A' = 1 + A'' \tag{9.11}$$

Hence

$$A' = A'' \tag{9.12}$$

and from Eqns. 9.12 and 9.8

$$B' = B'' \tag{9.13}$$

The unique solution for ligand distribution at static-head across a simple cotransport system is:

$$\mu'_A = \mu''_A \text{ and } \mu'_B = \mu''_B$$

The same arguments can be applied to a cotransport system where ligand B is bound to the lattice first, in this case

$$x_{AB} = \frac{A \cdot B}{1 + B + A \cdot B} \tag{9.14}$$

and

$$x_c = \frac{1}{1 + B + A \cdot B} \tag{9.15}$$

*(2) Multivalent symporter*

It is assumed that ligand A binds to the lattice, that another A bind to Ac and B binds to $A_2c$ (Fig. 3b)

$$x_A = \frac{A}{1 + A}(1 - x_A \cdot A - x_A \cdot A \cdot B) \tag{9.16}$$

Thus,

$$x_A = \frac{A}{1 + A + A^2 + A^2 \cdot B} \tag{9.17}$$

$$x_{A_2} = \frac{A^2}{1 + A + A^2 + A^2 \cdot B} \tag{9.18}$$

$$x_{A_2B} = \frac{A^2 \cdot B}{1 + A + A^2 + A^2 \cdot B} \tag{9.19}$$

$$x_c = \frac{1}{1 + A + A^2 + A^2 \cdot B} \tag{9.20}$$

At equilibrium

$$x'_{A_2B} = x''_{A_2B} \tag{9.21}$$

and

$$c' = c'' \tag{9.22}$$

from Eqns. 9.20 and 9.22

$$\frac{1 + A' + A'^2}{1 + A'' + A''^2} = 1 \tag{9.23}$$

Hence from Eqns. 9.21 and 9.23, it follows that

$A' = A''$ and hence $B' = B''$

Thus for a multivalent symporter there is a unique solution at which the lattice forms are in equilibrium when:

$\mu'_A = \mu''_A$ and $\mu'_B = \mu''_B$

*(3) Antiport lattice*

Static-head across an antiporter occurs when (Fig. 3c)

$$x'_A = x''_A \text{ and } x'_B = x''_B \tag{9.24}$$

$$x_A = \frac{A}{1 + A} \cdot (1 - x_B); \; x_B = \frac{B}{1 + B} \cdot (1 - x_A)$$

Thus

$$x_A = \frac{A}{1 + A + B}; \; x_B = \frac{B}{1 + A + B} \tag{9.25}$$

$$x_c = 1 - x_A - x_B = 1/(1 + A + B) \tag{9.26}$$

Hence from Eqns. 9.24, 9.25 and 9.26

$$\frac{A'}{A''} = \frac{1 + A' + B'}{1 + A'' + B''} = \frac{B'}{B'} = \frac{A' + B'}{A'' + B''} \tag{9.27}$$

Thus

$$\frac{A'}{A''} = \frac{B'}{B''} = 1 \tag{9.28}$$

Hence at static-head equilibrium

$B' = B''$ and $A' = A''$

Thus for an antiporter the only equilibrium state occurs when

$\mu'_A = \mu''_A$ and $\mu'_B = \mu''_B$

The following general points can be made concerning equilibrium at the microscopic level involving second or higher order ligand interactions which occur exclusively in a microscopic environment. As all third, or higher order forms on the lattice are produced by a series of unimolecular reactions between ligands and lower order lattice forms, it follows that production of the third-order form ABc′, requires the coexistence of at least one second-order lattice form, either Ac′, or Bc′.

Similarly, if a fourth-order form is required, say $A_2Bc'$, then the minimal number of other forms required as precursors are one second order form, either Ac′ or Bc′ and one third order form, either, ABc′ or, $A_2c'$. However, all the second- and third-order forms may coexist on the lattice, if formation of the intermediate is random and indeed, a myriad of fifth-, six- and higher order forms may be present. These higher forms could produce, $A_2Bc'$ by degeneration, or could simply occupy lattice sites.

The necessity for a plurality of lattice forms implies that the only way that an equal number of any particular form of complex formed from two or more ligands can be obtained on both sides of the membrane-lattice say, ABc′ = ABc″, is when there is a uniform distribution of both ligands on either side of the lattice.

i.e., $\mu'_A = \mu''_A$; $\mu'_B = \mu''_B$

The same argument also applies to antiporters and multivalent cotransporters. This analysis merely extends that of Hill [34] and Hill and Kedem [33] who showed in with their cotransport model (model 10), that equilibrium requires a uniform distribution of ligands between both baths. However, this finding has not been previously cited as a reason for rejecting the concept of a static-head equilibrium state.

Thus, it can now be seen that there is no divergence between the predictions of microscopic and macroscopic thermodynamic interpretations of cotransport. Both approaches lead to the conclusion that static-head equilibrium cannot exist in an open system.

*9c. Multivalent binding does not affect the chemical potential difference of the bound ligand complex across a membrane*

Tanford [36] has shown that the chemical

potential $\mu_{Lb}$ of a ligand L bound to a protein surface which contain $N_L$ sites, No unoccupied sites and $N$ sites binding ligand L is

$$\mu_{Lb} = \mu^0_{Lb} + RT \ln\left(\frac{\theta}{1-\theta}\right) \tag{9.29}$$

Where $\theta = N_L/N_0$ and $\mu^0_{Lb}$ is the standard free energy of the bound ligand L.

$\theta$ is related to the concentration, $C$ of ligand in the aqueous phase by the Langmuir binding relation, $K$ is the equilibrium constant.

$$\frac{\theta}{1-\theta} = KC \tag{9.30}$$

In the case where the ligand binds cooperatively to the sites, the relationship between bound ligand and aqueous concentration is

$$\frac{\theta}{1-\theta} = KC^n \tag{9.31}$$

It is assumed that $n$ moles occupy each binding site and the affinity $K$ of the ligand for the site changes rapidly on binding successive ligands so that each site is either fully occupied with $n$ ligands, or fully vacant [36].

The number of sites occupied is now $N_L/(n \cdot N_0)$. $N_L$ remains the number of individual ligands bound.

The chemical potential of the bound ligand complex $\mu_{bc}$ is now

$$\mu_{bc} = \mu^0_{bc} + \frac{RT}{n} \ln\left(\frac{\theta}{1-\theta}\right) \tag{9.32}$$

where $\mu^0_{bc}$ is the standard free energy of the bound complex.

Substitution of Eqn. 9.31 in 9.32 gives

$$\mu_{bc} = \mu^0_{bc} + \frac{RT}{n} \ln(KC^n) \tag{9.33}$$

or

$$\mu_{bc} = \mu^0_{bc} + \frac{RT}{n} \ln K + RT \ln C \tag{9.34}$$

Eqn. 9.34 shows that the component of chemical potential of the bound ligand complex related to the concentration, $C$ of ligand in the aqueous phase is unrelated to $n$, the number of molecules bound per site. The component of chemical potential related to $n$ is entirely incorporated into the concentration independent factors relating to the standard free energy of bound ligand.

Since the chemical potential difference of bound ligand complex is considered to be the driving force for a number of cotransport, chemiosmotic and active transport reactions, Eqn. 9.34 indicates that

$$\mu'_{bc} - \mu''_{bc} = RT \ln C' - RT \ln C''$$

$$= RT \ln(C'/C'') \tag{9.35}$$

Eqn. 9.35 shows that the chemical potential difference of a multivalently bound ligand across a membrane is unrelated to the number of ligands bound per site even in non-equilibrium situations. This application of Tanford's [36] treatment of the chemical potential of bound ligands, shows that the thermodynamic affinity of transport systems is entirely independent of the 'stoichiometry' of the process.

*10a. The requirement for entropy driven cotransport systems*

The failure of equilibrium thermodynamics to account adequately for static-head accumulation or depletion of driven solute indicates that models of cotransport where the driving force is solely determined by the thermodynamic affinities of the solutes taking part in the transport process are inappropriate.

An alternative mode of cotransport involving flow interactions will now be considered. In this mode cotransport is entropy driven. This process can produce concentration differences of the driven solute, quite sufficient to account for the highest observed solute accumulations in, or out of any living cell.

Prigogine [37] has described such a model of cotransport and this model has been adapted for use here, with only minor simplifications.

The rate of entropy production, $dS/dt$ due to an irreversible process is always greater than zero.

$$\frac{dS}{dt} = \frac{1}{T} A_k v_k > 0 \tag{10.1}$$

Where $A$ is the affinity of any reaction $k$ and $v$ is its rate. $T$ is absolute temperature.

When two simultaneous reactions are coupled, providing

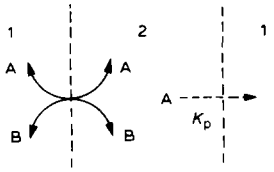
$$A_1 v_1 + A_2 v_2 > 0 \tag{10.2}$$

then it is possible for $A_1 v_1 < 0$ if $A_2 v_2 \gg 0$

Hence, one 'natural' irreversible reaction can drive a coupled reaction uphill against its conjugate affinity.

Since this coupled flow is dependent on entropy production, the coupled reaction will only proceed in the non-equilibrium condition.

Consider the following set of reactions (Scheme 1).




Scheme 1.

A coupled symport or antiport reaction takes place between A and B within the membrane separating phases 1 and 2. Within phase 2 there is a chemically activated pump such as the $Na^+$-pump or $Ca^{2+}$-pump which couples a chemical reaction to the flow of A, thereby maintaining the affinity of the transfer reaction of A between phases 1 and 2. No coupled interaction between A and B, or the pump and B, occurs at the pump site.

The appropriate phenomenological equations describing coupled flow of A and B, $J_A$ and $J_B$, respectively, and pump flux, $J_p$ are:

$$\begin{aligned} J_A &= L_{11}\Delta\mu_A^{1-2}/T + L_{12}\Delta\mu_B^{1-2}/T \\ J_B &= L_{21}\Delta\mu_A^{1-2}/T + L_{22}\Delta\mu_B^{1-2}/T \\ J_p &= L_{31}\left(A_p - \Delta\mu_A^{1-2}\right)/T \end{aligned} \tag{10.3}$$

$A_p = RT\ln(K_p \cdot \mathrm{ATP}/(\mathrm{ADP} \cdot \mathrm{PO_4}))$ is the affinity of the scalar ('chemical') pump reaction. $K_p$ is the equilibrium constant of the ATPase reaction (the affinity of the ATPase estimated on the basis of stoichiometric relations of the $Na^+$-pump is 40–50 kJ/mol, Chapman and Johnson [38]). $\Delta\mu_A^{1-2}$ and $\Delta\mu_B^{1-2}$ are the electrochemical potential differences of A and B between phases 1 and 2. $L_{11}$ and $L_{22}$ are the straight conductance coefficients which relate the flows, $J_A$ and $J_B$ to the affinities of A and B between 1 and 2, respectively. The coefficients $L_{12}$ and $L_{21}$ are the coupling coefficients relating the affinity of B to the flow of A and the affinity of A to the flow of B, respectively. $L_{31}$ is the coefficient relating the affinity of the pump reaction, $(A_p)$ to the flow of A from 2 to 1, $T$ is the absolute temperature, and $K_p$ is the equilibrium constant of the pump reaction. Onsager has shown [37] that near equilibrium

$$L_{12} = L_{21} \tag{10.4}$$

At stationary-state the concentrations of A and B in phases 1 and 2 are constant. This means that the rate of entry of A into the system is the same as the rate of exit via the pump. As there is no exit route for B except via the cotransport system, the stationary state implies that $J_{net}$ for B is zero as inflow and outflow are equal.

Hence

$$J_p = -J_A \text{ and } J_B = 0 \tag{10.5}$$

Thus

$$L_{21} \cdot \ln\left(\frac{A^1}{A^2}\right) = L_{22} \cdot \ln\left(\frac{B^2}{B^1}\right) \tag{10.6}$$

Also

$$L_{31}\left(\ln\left(\frac{A^2}{A^1}\right) + A_p\right) = L_{11} \cdot \ln\left(\frac{A^2}{A^1}\right) + L_{12} \cdot \ln\left(\frac{B^2}{B^1}\right) \tag{10.7}$$

The following relationships can be deduced from Eqns. 10.3–10.7

At stationary state

$$\ln\left(\frac{A^1}{A^2}\right) = \frac{L_{31} \cdot A_p}{L_{31} - \dfrac{(L_{12})^2}{L_{22}} + L_{11}} \tag{10.8}$$

and

$$\frac{[B^2]}{[B^1]} = \left(\frac{[A^1]}{[A^2]}\right)^{L_{12}/L_{22}} \tag{10.9}$$

Eqn. 10.8 shows that the distribution ratio of A, the driving solute is related to the affinity of the pump and inversely related to coupled flow and the conductance of A, via the pump and cotransporting membrane (the leak pathways).

Eqn. 10.9 is of considerable interest, it shows that the non-equilibrium stationary state distribution of the driven solute, B between phases 1 and 2 in dependent on

(a) the affinity of the driving solute A and

(b) is a power function of the ratio $L_{12}/L_{22}$.

*10b. Phenomenological stoichiometry of stationary state cotransport*

To maintain the stationary state, entropy production from the irreversible processes within the cotransport reaction must always exceed zero.

Thus application of Eqn. 10.2 to the cotransport reaction gives:

$$\frac{dS}{dt} = \frac{J_A \cdot A_A}{T} + \frac{J_B \cdot A_B}{T} > 0 \qquad (10.11)$$

By combining Eqns. 10.3–10.4 and 10.11, it can be shown [37,39] that the requirement for positive entropy production to maintain the stationary-state transport process implies that:

$$L_{12}^2 > L_{11} \cdot L_{22} \qquad (10.12)$$

Kedem and Caplan [39] have shown that the degree of coupling, $q$, which is a measure of the tightness of coupling may be estimated from the following relationship:

$$q = \frac{L_{12}}{\sqrt{L_{11} \cdot L_{22}}} \qquad (10.13)$$

Since $L_{12}$, $L_{21}$ can have either a positive, or negative values, it follows from Eqns. 10.12 and 10.13 that $q$, the degree of coupling must lie between $-1$ and $+1$. Since the only constraints on the relationship between $L_{12}$ and $L_{22}$ are those imposed by Eqns. 10.4 and 10.13, it can be deduced that

$$\frac{L_{12}}{L_{22}} = \frac{q}{\sqrt{L_{22}/L_{11}}} \qquad (10.14)$$

Eqn. 10.9 can now be rewritten, for symport, where $1 > q > 0$ and for antiport, where $-1 < q < 0$.

$$\left(\frac{B^2}{B^1}\right) = \left(\frac{A^1}{A^2}\right)^{q/\sqrt{L_{22}/L_{11}}} \qquad (10.15)$$

Fig. 4A shows the effect of variation of $L_{22}/L_{11}$ on the stationary state distribution ratio of driven solute, $B^2/B^1$, in the presence of a constant affinity ($A^1/A^2 = 10$) of the driving solute at several degrees of coupling. If the sign of $q$ is negative, then the same graph could be used to plot $B^1/B^2$ against the same values of $L_{22}/L_{11}$

At a constant $q$, $0.5 < q < 1.0$, reduction of $L_{22}$ increases the ratio $L_{12}/L_{22}$. The effect of reducing the straight conductance coefficient of the driven solute, $L_{22}$ to very low values relative to $L_{11}$, is to increase accumulation of the driven solute by very large amounts.

The distribution ratio of the driven solute can rise very considerably above that of the driving solute if the ratio $L_{12}/L_{22}$ is greater than 1. The constraints of Eqns. 10.12 and 10.4 require that the ratio $L_{12}/L_{22}$ can only rise to high values when $L_{22}$, the straight conductance coefficient of the driven solute, is much less than the straight con-

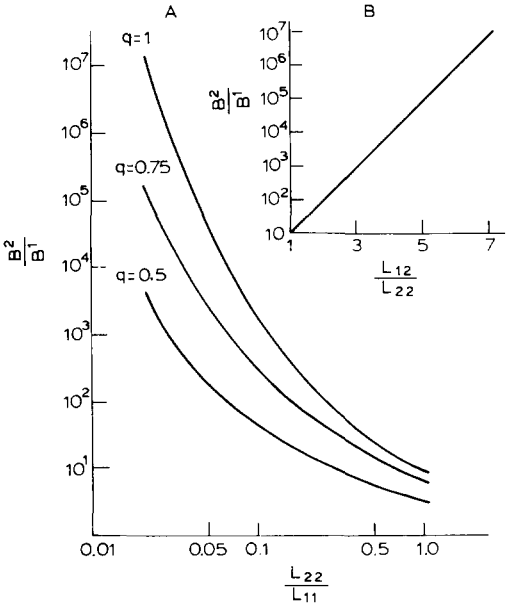



Fig. 4. Calculations of the distribution ratio $B^2/B^1$ of the driven solute for a fixed ratio of driving solute $A^1/A^2 = 10$. (A) The effect of changing the ratio of conductance $L_{22}/L_{11}$ at different degrees of coupling, $q$. (B) The effect of changing the ratio of conductances $L_{12}/L_{22}$ on accumulation.

ductance coefficient of the driving solute, $L_{11}$ within the cotransport system. The coupling coefficient $L_{12}$ must also be less than $L_{11}$, but sufficient to give a high degree of coupling, $q$.

It cannot be assumed that chemical coupling will automatically ensure a high degree of coupling. Hill [31,34] has shown that as the lattice sites of the membrane become saturated, movement of bound ligand complex within the membrane in 'wasteful cycles' comes to exceed the rate of ligand movement between the membrane and the external reservoirs and this leads to a reduced degree of coupling. Lagarde [40] has made a similar point.

**Phenomenological stoichiometry**

There is a close resemblance between Eqns. 10.9, 10.15 and Eqn. 4.6.

If $L_{12}/L_{22}$, or $q/\sqrt{(L_{22}/L_{11})}$ were substituted for the stoichiometric ratio $n_A/n_B$ in Eqn. 4.6 the conductance ratios could be considered as 'phenomenological stoichiometric coefficients'.

Kedem and Caplan [39] previously suggested that the term

$$Z = (L_{11}/L_{22})^{1/2}$$

resembles a stoichiometry of coupling. However, this is only a resemblance of form. The ratio $n_A/n_B$ in Eqn. 4.6 is a flow ratio and is independent of any of the membrane parameters which control flow. This fact has not always been fully appreciated. The terms $Z$ and $L_{12}/L_{22}$ or $q/\sqrt{(L_{22}/L_{11})}$ are conductance ratios which are locally determined within the membrane. As the phenomenological stoichiometric coefficients are conductance ratios they cannot completely determine the coupled flux ratio. The coupled flux ratio is completely determined only by application of the phenomenological equation. The coupled flux ratio is a function of the separate affinities both of the cotransported ligands across the cotransporter and the membrane conductances. Since wasteful cycles are unavoidable, even far from equilibrium [33], the degree of coupling, $|q|$ will always be less than unity.

Rottenberg [41] has made the point that when $q$ approaches 1, $Z$ the phenomenological stoichiometry is equivalent to the mechanical stoichiometry. Stucki [42] has noted that this view is mistaken.

Heinz [43] has adopted a 'quasi-chemical' approach to the treatment of coupled flow processes. He incorporates a fixed stoichiometry between the driving and driven flows into the coupled flow equations. This ad hoc assumption requires that the degree of coupling $q$ is always 1. When $q$ is 1, no entropy dissipation can occur, hence no coupled flow can take place. To avoid this pitfall, additional shunt pathways are postulated. These shunts reduce $q$ and hence this system has 'quasi variability' of flow stoichiometry which is operationally identical to other coupled systems.

**Practical considerations**

*(1) High distribution ratios require a low $L_{22}/L_{11}$ ratio*

In systems where the distribution ratio of the driven solute is very much higher than that of the driving solute ($>$ 1000-fold), e.g., the $Na^+/Ca^{2+}$ antiporter in cardiac muscle and squid axon [44–46] and with the proton/catecholamine antiporter in chromaffin granules [17], the ratio $L_{12}/L_{22}$ must exceed 3. If the degree of coupling $q$ of these antiport systems is $\leqslant -0.7$, then the ratio $L_{22}/L_{11} \ll 0.010$ (Figs. 4A and 4B). This means that high distribution ratios of driven solute can only be obtained when the straight permeability coefficient of the driven solute is much lower than that of the driving solute.

*(2) The necessity for a second barrier to achieve concentrative uptake*

The model for stationary-state coupling of flows has an important feature which is not normally considered necessary in thermostatic models of cotransport. This is the second barrier. Whilst it is assumed that the driving solute can be removed from the internal compartment via a pump flux $J_p$, it is also assumed that the driven solute remains behind within the compartment. The only exit for the driven solute B is via the cotransporter. If the driven solute is free to leave the internal compartment at a rate comparable to that of the driving solute, there will be no concentrative accumulation of driven solute, because the rate of inflow $J_A > J_B$ via the cotransporter.

Thus there are two requirements for concentrative accumulation of driven solute;
(1) that $L_{11} > L_{22}$ (see above) and
(2) the presence of a second barrier which permits outflow of the driving solute via the pump flux but retards outflow of the driven solute. This results in concentration polarization of the driven solute within the enclosed compartment. Attention has previously been drawn to the requirement for concentration polarization to attain concentrative uptake [4,47] and it is recognized that reduction of the leak permeabilities to driven solutes will increase accumulation [8,9]. It can now be seen that concentration polarization is necessary to attain any form of concentrative accumulation by a symport process. It should be noted that the pump and cotransport systems may coexist in the same membrane of a non-polarized cell, so that the second barrier can be considered as the absence of shunt pathways for driven solute.

*(3a) High sensitivity of cotransported solute distribution to pump flux*

The affinity of the driving solute, A within the cotransport system is controlled by the pump flux, $J_p$ (Eqn. 10.8). The stationary state distribution ratio of the driven solute, B is a power function of the non-equilibrium stationary state distribution ratio of the driving solute, A (Eqn. 10.9). Consequently, if the driven solute accumulation, or depletion, attains high distribution ratios, this distribution will be exquisitely sensitive to changes in pump flux and affinity of the driving solute.

*(3b) Application to small systems (vesicles, granules)*

It is a requirement to high distribution ratio transport systems, that the straight conductance coefficient of the driving solute, $L_{11}$ should greatly exceed that of the driven solute $L_{22}$ (Eqn. 10.15). In such a system, following a step increase in the affinity of the driving solute, (e.g., raising the external Na concentration in a vesicular cotransport system), the affinity of driving solute will decrease faster than the affinity of the driven solute can increase. In small volume systems, without a pump to maintain the affinity of the driving solute (e.g., membrane vesicles), high accumulation of driven solute may never occur as the driving solute gradient will be dissipated before the maximal possible distribution ratio of the driven solute can be established.

*(4) Explanation of high distribution ratios in the absence of high stoichiometric ratios*

A problem with the thermostatic view of cotransport is that it does not account for the numerous cases where the observed stoichiometric ratios of cotransport are insufficiently large to support the distribution of the driven solute. For example, it has recently been observed that a stoichiometric ratio of 3:1 for $Na^+$-$Ca^{2+}$ exchange in ferret cardiac muscle is absent, despite the presence of a highly active $Na^+/Ca^{2+}$ exchanger in this tissue [48]. There is no problem in reconciling such data with a thermodynamic, in contrast to a thermostatic, stationary-state, as this requires that the flux ratio of driving: driven solutes to vary as the affinities of the transported ligands alter (Eqn. 10.3)

*(5) Estimates of stoichiometry*

The stoichiometry of cotransport systems have been obtained by plotting the log (distribution ratio of driven solute) against the affinity of the driving solute (units in mV). The slope of this line according to conventional theory, predicts the stoichiometric ratio of cotransport either $n_A/n_B$ for a symporter or $-n_A/n_B$ for an antiporter [15,17,44–46]. The present analysis shows that these plots predict that the slope is equivalent to $q/\sqrt{L_{22}/L_{11}}$. Non-linearity of this line could arise if the conductance coefficients $L_{12}$ and $L_{22}$ decrease as the ligand concentration in the external solution is raised [40].

*(6) Electrical charge effects*

The sensitivity of the distribution ratio of driven solute to changes in electrical potential has suggested that there is multivalent binding of transporter ligands to the carrier complex, e.g, $3Na^+ : 1Ca^{2+}$ (net antiporter charge = 1 + ) [17,44–46]. The present analysis indicates that an antiporter for solutes having identical charge, e.g. $1Na^+ : 1H^+$, could also respond to changes in electrical potential.

In any antiport system where $L_{12}/L_{22} > -1$, the stationary-state for the driven solute is attained before that of the driving ligand, i.e., net

flow of the driving solute will continue, when the driven ligand distribution ratio has reached at stationary state. A change in electrical potential across this system will affect the affinities of both the driving and driven ligands, to an equal and opposite extent. However, as $-L_{12} > L_{22}$ and $L_{11} \gg L_{22}$, the effects of equal and opposite changes in affinity of the antiporting ligands affect the rate of flow of the driving ligand A more than that of the the driven ligand B. Hence, a change in potential difference will affect the stationary-state distribution of driven solute and the antiporter will appear to be electrogenic, although the true stoichiometric coefficients of the transport reaction are fixed at 1.

*(7) Applications to active transport processes*

Active transport can be considered as a form of cotransport system, where the driving force for coupled flow of the actively transported ligands is generated by the flow of the terminal phosphate group from ATP via the transport process (Hill and Eisenberg [49]). The implications of this view of active transport with respect to the current analysis of cotransport are that active transport systems cannot be considered to have a fixed stoichiometry and the reversal potential of the $Na^+$-pump (static-head equilibrium) should not be calculated on the basis of the equivalence of the flux ratios of $Na^+$, $K^+$ and ATP hydrolysis at non-equilibrium steady-states to mechanical stoichiometric coefficients [38].

A non-equilibrium thermodynamic view of active transport has the advantage that concentrative systems need not be constrained by the limits imposed by the free energy of hydrolysis of ATP and the mechanical stoichiometry of the transport system (see Fig. 4). In the case of the gastric proton pump protons are pumped against a pH gradient of 7 units, equivalent to 42 kJ/mol. Since the affinity of the phosphate group transfer reaction is approx. 50 kJ/mol, the mechanical stoichiometry is limited to 1 mole H transported per mole of ATP hydrolyzed. However Sachs et al. [50] find that 2 protons per ATP hydrolysed are transported at pH 6.1. These experimental findings are not inconsistent with active transport system in which the conductance coefficient for protons ($L_{22}$) is lower than for the phosphate group transfer system ($L_{11}$).

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