Biochimica et Biophysica Acta, 426 (1976) 198–217

BBA 77211

A NON-EQUILIBRIUM THERMODYNAMICS ANALYSIS OF ACTIVE TRANSPORT WITHIN THE FRAMEWORK OF THE CHEMIOSMOTIC THEORY

ALAIN E. LAGARDE

Laboratoire de Microbiologie (406), Institut National des Sciences Appliquées de Lyon, 69621 Villeurbanne (France)

(Received October 3rd, 1975)

SUMMARY

The proton circuit devised by Mitchell in the chemiosmotic theory was subjected to analysis using the formalism of irreversible thermodynamics. The phenomenological coefficients and the degree of coupling relating co-permeant flows were derived for anion/H^+, substrate/H^+, cation/H^+ and anion/anion biporter models. Linearity and equality of the cross-coefficients in Onsager relations were always satisfied. Macroscopic flows leading to charges splitting, such as oxido-reduction, hydro-dehydratation and transhydrogenase, are driven by a composite thermodynamic force which includes the proton-motive component. Multiple coupling occurs in the circuit when it is assumed that the net inward flux of protons becomes zero, i.e. when the circulation of protons reaches a stationary state. Under these conditions, oxidative phosphorylation, ATPase- or respiration-linked transhydrogenase and uptake of anion or cation against their electrochemical gradient may be predicted, in agreement with known experimental evidence.

INTRODUCTION

The chemiosmotic theory formulated by Mitchell [1] has attracted many workers in the field of energy-conserving and energy-transducing mechanisms taking place within biological membranes. Originally devoted to elucidation of an example of coupled processes in mitochondria (oxidative phosphorylation), concepts were extended to interpret transport phenomena [2–6]. Increasing evidence has been accumulated for the translocation of numerous substrates and ions to be connected in some way to the electrochemical potential difference of protons ($\Delta\tilde{\mu}_H$) across mitochondrial, chloroplast and bacterial membranes [7–10]. The vectorial functioning of the membrane-bound redox chain and of the ATPase complex was shown to be responsible for the generation of such a proton-motive force. Specific carrier molecules (symporters and antiporters) were further postulated so that the flow of protons and the flows of neutral molecules, cations and anions may be coupled [2]. The overall proton circuit may then be visualized as a multiple-flow system fuelled by a reversible proton pile and is analogous to electrical networks.

The present paper attempts to analyze such a circuit using the methods and notations of non-equilibrium thermodynamics [11–14]. The main problem to be dealt with is how the diffusion flux of a solute can be connected to all the driving forces operating in the complex system under consideration so that large concentration gradients can be obtained (i.e. active transport). In order to obviate a too generalized thermodynamic treatment, as already undertaken by others [14, 15, 25], a number of simple carrier models were chosen to illustrate the complexity of resulting equations. We have restricted our purpose to the following. (1) Derivation of carrier-mediated flux equations in terms of linear Onsager relations and the evaluation of the corresponding phenomenological coefficients. Calculation of the degree of coupling between the flows of two co-permeant species, as defined by Kedem and Caplan [16] was easily deduced. (2) The "quasi-chemical" notation used by Heinz [15] served to describe the charge-splitting reactions catalyzed by the redox chain, the ATPase complex and the transhydrogenase. (3) The total circuit was reconstituted and thermodynamic conditions leading to coupling between unrelated fluxes were sought.

ANALYSIS OF CARRIER – MEDIATED FLOWS

The systems to be studied consist in a membrane separating two aqueous phases of infinite size. Carrier molecules are specific components incorporated within the membrane, involved in the recognition of substrates and in their transfer from one side to the other. We adopted the thermodynamic approach given by Katchalsky and Spangler [13] for the treatment of carrier-mediated flows. We will examine in detail only the symport model, since the general procedure remains valid for any other model. Common assumptions are the following.

(a) We assumed ideal behaviour for all components present either in the solutions or within the membrane, so that activities were replaced by concentrations. The expression for the electrochemical potential of a component i, at constant pressure, takes the usual form:

$$\tilde{\mu}_i = \mu_i^0 + RT\ln C_i + z_i F \psi \tag{1}$$

Symbols used are listed at the end of the paper.

(b) The empty carrier and the membrane as a whole are electrically neutral.

(c) Suffix o stands for external or facing outwards and suffix *i* for internal or facing inwards. By convention: $\Delta\tilde{\mu}_i = (\tilde{\mu}_i)_o - (\tilde{\mu}_i)_i$ and $\Delta\psi = \psi_o - \psi_i$.

(d) Positive diffusional net fluxes (per unit membrane area) are directed from outside to inside, perpendicular to the membrane interfaces. They were computed according to the Nernst-Planck equation:

$$J_i = \omega_i C_i (-\mathrm{grad}\,\tilde{\mu}_i) \tag{2}$$

the thermodynamic driving force being:

$$\mathrm{grad}\,\tilde{\mu}_i = \frac{\mathrm{d}\tilde{\mu}_i}{\mathrm{d}x} = \frac{RT}{C_i}\frac{\mathrm{d}C_i}{\mathrm{d}x} + z_i F\frac{\mathrm{d}\psi_i}{\mathrm{d}x} \tag{3}$$

(e) The microscopic fluxes J_i are not coupled to other driving force than $(-\text{grad}\ \tilde{\mu}_i)$.

(f) We used the Goldman constant field assumption [17] to describe the flux of electrically charged species:

$$\frac{\mathrm{d}\psi i}{\mathrm{d}x} = -\frac{\Delta\psi}{\Delta x} \tag{4}$$

so that the fluxes of a neutral component ($z = 0$), a monovalent cation ($z = +1$), a monovalent anion ($z = -1$) are described by Eqns 5, 6 and 7, respectively:

$$J = P(C_0 - C_i) \tag{5}$$

$$J = \frac{F}{RT}\Delta\psi P \frac{C_i - C_0 e^{F\Delta\psi/RT}}{1 - e^{F\Delta\psi/RT}} \tag{6}$$

$$J = -\frac{F}{RT}\Delta\psi P \frac{C_i - C_0 e^{-F\Delta\psi/RT}}{1 - e^{-F\Delta\psi/RT}} \tag{7}$$

The anion/H^+ symport

Since the exact mechanisms of a symport system is unknown at present, we were led to include in the model depicted in Fig. 1 both fragmental experimental evidence and additive postulates. However, it looks basically similar to the Na^+-dependent amino acid transport reviewed by Schultz and Curran [18] and further examined by Heinz and colleagues [26].

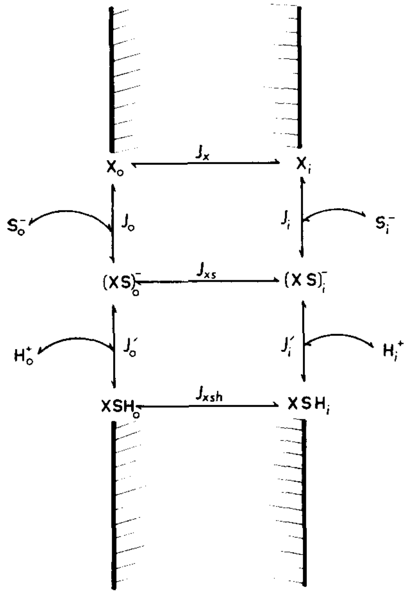


Fig. 1. The anion/H^+ symport model. X is the symbol for the symporter, S^- for the anion, H^+ for the proton. o stands for external or facing outwards, i for internal or facing inwards. J_0 J_i J'_0 J'_i are chemical flows occurring at the interfaces and J_x J_{xs} J_{xsh} are diffusion flows of the carrier complexes.

(1) The substrate molecule is supposed to exist as a fully dissociated anion. This may correspond to the situation of a monocarboxylic acid being translocated at a physiological pH well above its pK_a. The consideration of the partially dissociated substrate leads to more complicated equations.

(2) The carrier molecule takes three different forms exclusively: neutral and free (X), negatively charged binary complex (XS^-), and neutral ternary complex (XSH). In view of the low intrinsic conductance of biological membranes to protons, we exclude the possibility of the complex (XH^+) being formed [1, 7].

(3) There is an ordered sequence for binding and dissociating steps of the two ions. The carrier molecule bears two non-interacting sites with dissociation constants K_s and K_h, respectively. No asymmetry is introduced into the model, such that these constants have equal values on both sides of the membrane.

(4) The co-permeant species are not allowed to penetrate the membrane by free diffusion, so that the reactions with the carrier take place at the interfaces only (see Blumenthal and Katchalsky [19] for continuous models).

(5) For the sake of convenience we will assume in first approximation that all three carrier forms diffuse across the membrane with the same permeability coefficient.

The starting point is to define explicitely the dissipation function for the system (see Fig. 1), which under isothermal condition takes the form:

$$\Phi = J_o A_o + J'_o A'_o + J_i A_i + J'_i A'_i + J_x \Delta\mu_x + J_{xs} \Delta\tilde{\mu}_{xs} + J_{xsh} \Delta\mu_{xsh} \tag{8}$$

with the set of driving forces:

$$A_o = \mu_{x_o} + \tilde{\mu}_{s_o} - \tilde{\mu}_{x_o s_o} \tag{9}$$

$$A_i = \mu_{x_i} + \tilde{\mu}_{s_i} - \tilde{\mu}_{x_i s_i} \tag{10}$$

$$A'_o = \tilde{\mu}_{x_o s_o} + \tilde{\mu}_{h_o} - \mu_{x_o s_o h_o} \tag{11}$$

$$A'_i = \tilde{\mu}_{x_i s_i} + \tilde{\mu}_{h_i} - \mu_{x_i s_i h_i} \tag{12}$$

$$\Delta\mu_x = \mu_{x_o} - \mu_{x_i} \tag{13}$$

$$\Delta\tilde{\mu}_{xs} = \tilde{\mu}_{x_o s_o} - \tilde{\mu}_{x_i s_i} \tag{14}$$

$$\Delta\mu_{xsh} = \mu_{x_o s_o h_o} - \mu_{x_i s_i h_i} \tag{15}$$

When imposing the steady-state conditions for the membrane:

$$\begin{aligned}
\frac{d[X_o]}{dt} &= 0 = -J_o - J_x & \qquad \frac{d[X_i]}{dt} &= 0 = J_x - J_i \\
\frac{d[X_o S_o]}{dt} &= 0 = J_o - J'_o - J_{xs} & \qquad \frac{d[X_i S_i]}{dt} &= 0 = J_{xs} + J_i - J'_i \\
\frac{d[X_o S_o H_o]}{dt} &= 0 = J'_o - J_{xsh} & \qquad \frac{d[X_i S_i H_i]}{dt} &= 0 = J'_i + J_{xsh}
\end{aligned} \tag{16}$$

the following relations result:

$$\begin{aligned} J_x &= J_i = -J_o \\ J_{xsh} &= J'_o = -J'_i \\ J_{xs} &= J_o - J'_o = J'_i - J_i \end{aligned} \tag{17}$$

From Eqn. 17 we deduce the equation for the circulation of the carrier:

$$J_x + J_{xs} + J_{xsh} = 0 \tag{18}$$

The dissipation function becomes:

$$\Phi = J_x(A_i - A_o + \Delta\mu_x) + J_{xs}\Delta\tilde{\mu}_{xs} + J_{xsh}(\Delta\mu_{xsh} + A'_o - A'_i) \tag{19}$$

Taking into account the expressions of the affinities and electrochemical gradients Eqns 9–15 and rearranging terms we get:

$$\Phi = (J_x + J_{xs} + J_{xsh})\Delta\tilde{\mu}_{xs} + J_{xsh}(\tilde{\mu}_{h_o} - \tilde{\mu}_{h_i}) - J_x(\tilde{\mu}_{s_o} - \tilde{\mu}_{s_i}) \tag{20}$$

In view of Eqn. 18 the first term on the right in Eqn. 20 vanishes and when we further introduce the relations:

$$J_s = J_{xs} + J_{xsh} = -J_x \tag{21}$$

$$J_h = J_{xsh} \tag{22}$$

$$\Delta\tilde{\mu}_s = \tilde{\mu}_{s_o} - \tilde{\mu}_{s_i} \tag{23}$$

$$\Delta\tilde{\mu}_h = \tilde{\mu}_{h_o} - \tilde{\mu}_{h_i} \tag{24}$$

the steady-state dissipation function takes the final form:

$$\Phi = J_s\Delta\tilde{\mu}_s + J_h\Delta\tilde{\mu}_h \tag{25}$$

Two macroscopic flows contribute only to the dissipation function.

The problem we are concerned with now is to try and formulate the fluxes J_s and J_h according to the Onsager linearized relations:

$$\begin{aligned} J_s &= L_{11}\Delta\tilde{\mu}_s + L_{12}\Delta\tilde{\mu}_h \\ J_h &= L_{21}\Delta\tilde{\mu}_s + L_{22}\Delta\tilde{\mu}_h \end{aligned} \tag{26}$$

We are led to impose another restriction on the system by supposing that chemical reactions taking place at the membrane interfaces are in local equilibrium. The reason is that carrier diffusion flows are expected to be rate-limiting steps. Consequently we have the relations:

$$\begin{aligned} K_s &= \frac{[X_o][S_o]}{[X_oS_o]} = \frac{[X_i][S_i]}{[X_iS_i]} \\ K_h &= \frac{[X_oS_o][H_o]}{[X_oS_oH_o]} = \frac{[X_iS_i][H_i]}{[X_iS_iH_i]} \end{aligned} \tag{27}$$

According to Eqns. 5 and 7 we can write:

$$J_x = P_x([X_o] - [X_i]) \tag{28}$$

$$J_{xsh} = P_{xsh}([X_oS_oH_o]-[X_iS_iH_i]) \tag{29}$$

$$J_{xs} = aP_{xs}([X_o S_o]e^{-F\Delta\psi/RT}-[X_i S_i]) \tag{30}$$

with

$$a = \frac{F\Delta\psi}{RT(1-e^{-F\Delta\psi/RT})}$$

Inserting Eqns. 28–30 into Eqn. 18 and assuming $P_x = P_{xs} = P_{xsh} = P$, we get

$$[X_o]+[X_o S_o H_o]+a[X_o S_o]e^{-F\Delta\psi/RT} = [X_i]+[X_i S_i H_i]+a[X_i S_i] = X \tag{31}$$

X appears to be proportional to the total concentration of the carrier within the membrane. Using Eqns. 27 and 31 we are now able to express the different carrier concentrations in terms of X:

$$\begin{aligned}
&[X_o] = \frac{XK_sK_h}{\Delta_o} && [X_i] = \frac{XK_sK_h}{\Delta_i}\\
&[X_oS_o] = \frac{XK_h[S_o]}{\Delta_o} && [X_iS_i] = \frac{XK_h[S_i]}{\Delta_i}\\
&[X_oS_oH_o] = \frac{X[H_o][S_o]}{\Delta_o} && [X_iS_iH_i] = \frac{X[H_i][S_i]}{\Delta_i}
\end{aligned} \tag{32}$$

with:

$$\Delta_o = a e^{-F\Delta\psi/RT}K_h[S_o]+K_sK_h+[H_o][S_o]$$

$$\Delta_i = a K_h[S_i]+K_sK_h+[H_i]\,[S_i]$$

Recalling Eqn. 22 and inserting Eqn. 32 into 29 we get:

$$J_h = PX\left(\frac{[S_o][H_o]}{\Delta_o} - \frac{[S_i][H_i]}{\Delta_i}\right)$$

Symplifying and gathering terms leads to:

$$J_h = \frac{PXK_h[H_o][S_i]}{\Delta_o\Delta_i}\left\{K_s\left(\frac{[S_o]}{[S_i]} - \frac{[H_i]}{[H_o]}\right) + a[S_o]\left(1-\frac{[H_i]}{[H_o]}e^{-F\Delta\psi/RT}\right)\right\} \tag{33}$$

Similarily, by inserting Eqn. 32 into 28 the equation:

$$J_s = PXK_sK_h\left(\frac{1}{\Delta_i} - \frac{1}{\Delta_o}\right)$$

leads after simplification to:

$$J_s = \frac{PXK_sK_h[S_i]}{\Delta_o\Delta_i}\left\{[H_o]\left(\frac{[S_o]}{[S_i]} - \frac{[H_i]}{[H_o]}\right) + aK_h\left(\frac{[S_o]}{[S_i]}e^{-F\Delta\psi/RT}-1\right)\right\} \tag{34}$$

Since:

$$\Delta\tilde{\mu}_s = RT \ln \frac{[S_o]}{[S_i]} - F\Delta\psi$$

$$\Delta\tilde{\mu}_h = RT \ln \frac{[H_o]}{[H_i]} + F\Delta\psi$$

we get the equivalences:

$$\frac{[S_o]}{[S_i]} = e^{\Delta\tilde{\mu}_s/RT} e^{F\Delta\psi/RT} \tag{35}$$

$$\frac{[H_o]}{[H_i]} = e^{\Delta\tilde{\mu}_h/RT} e^{-F\Delta\psi/RT} \tag{36}$$

Inserting Eqns. 35 and 36 into Eqns. 33 and 34 and gathering terms gives:

$$\begin{aligned} J_s &= L_{11}X_1 + L_{12}X_2 \\ J_h &= L_{21}X_1 + L_{22}X_2 \end{aligned} \tag{37}$$

with

$$X_1 = e^{\Delta\tilde{\mu}_s/RT} - 1 \tag{38}$$

$$X_2 = 1 - e^{-\Delta\tilde{\mu}_h/RT} \tag{39}$$

$$L_{11} = \frac{PXK_sK_h}{\Delta_o\Delta_i}[S_i](aK_h + [H_o]e^{F\Delta\psi/RT}) \tag{40}$$

$$L_{12} = L_{21} = \frac{PXK_sK_h}{\Delta_o\Delta_i}[H_o][S_i]e^{F\Delta\psi/RT} \tag{41}$$

$$L_{22} = \frac{PXK_h}{\Delta_o\Delta_i}[H_o][S_i](K_s e^{F\Delta\psi/RT} + a[S_o]) \tag{42}$$

We reach the conclusion that the fluxes J_s and J_h are not linearly related to the driving forces $\Delta\tilde{\mu}_s$ and $\Delta\tilde{\mu}_h$ but to exponential functions (X_1 and X_2) comprising $\Delta\tilde{\mu}_s$ and $\Delta\tilde{\mu}_h$. But close to equilibrium ($|\Delta\tilde{\mu}_s/RT| \ll 1$) and $|\Delta\tilde{\mu}_h/RT| \ll 1$), by expanding the exponentials in Eqns. 38 and 39 we get: $X_1 \simeq \Delta\tilde{\mu}_s$ and $X_2 \simeq \Delta\tilde{\mu}_h$, so that Onsager's relations (Eqn. 26) are born out when the restriction of proximity to equilibrium is admitted. However, the phenomenological coefficients remain unchanged whereas the system is close to equilibrium or not. At this step of our treatment several points deserve discussion:

(1) The requirement of equality of the cross-coefficients is fulfilled ($L_{12} = L_{21}$). The fact that L_{12} is positive indicates a mutual drag between J_s and J_h, characteristic of a symport system. The inequality $(L_{12})_{12}{}^2 \ll L_{11}L_{22}$ resulting from the condition $\Phi \geqslant 0$ is also satisfied.

(2) The phenomenological coefficients depend upon the characteristics of the carrier (through K_s, K_h and P), the concentration of the carrier (through X), on $\Delta\psi$ and upon the concentrations of the co-permeant species. They are not time independent. For $\Delta\psi < 26$ mV at 25 °C, the term $F\Delta\psi/RT$ tends to 1, so that simplifications obtain:

$$\begin{aligned} L_{11} &= \frac{PXK_sK_H}{\Delta_o\Delta_i}[S_i](K_h+[H_o]) \\ L_{12} = L_{21} &= \frac{PXK_sK_h}{\Delta_o\Delta_i}[S_i][H_o] \\ L_{22} &= \frac{PXK_h}{\Delta_o\Delta_i}[S_i][H_o](K_s+[S_o]) \end{aligned} \tag{43}$$

with

$$\Delta_o = K_sK_h+K_h[S_o]+[S_o]\,[H_o]$$

$$\Delta_i = K_sK_h+K_h[S_i]+[S_i]\,[H_i]$$

(3) The dimensionless coefficient expressing the coupling between J_s and J_h can be calculated according to Kedem and Caplan [16]:

$$q_{\mathrm{sym}} = \frac{L_{12}}{\sqrt{L_{11}L_{22}}} = \sqrt{\frac{K_s[H_o]}{(bK_h+[H_o])(K_s+b[S_o])}} \tag{44}$$

with

$$b = \frac{F\Delta\psi}{RT(e^{F\Delta\psi/RT}-1)}$$

For $\Delta\psi$ < 26 mV q_{sym} reduces to:

$$q_{\mathrm{sym}} = \sqrt{\frac{K_s[H_o]}{(K_h+[H_o])(K_s+[S_o])}} \tag{45}$$

The interesting conclusion is that q_{sym} depends only upon the affinities of the carrier molecule for H^+ and S^- and upon the external concentrations $[H_o]$ and $[S_o]$. If we assume that the external medium is of infinite size such that $[H_o]$ and $[S_o]$ can be considered as time-independent values, this means that the coupling between J_s and J_h is constant with time for a given set of $[H_o]$ and $[S_o]$. Now at fixed $[H_o]$, q_{sym} will tend to zero (complete uncoupling) when $[S_o]$ becomes very large, i.e. when the carrier is saturated. On the other hand q_{sym} will tend towards +1 (complete coupling) when both conditions $[H_o] \gg K_h$ and $[S_o] \ll K_s$ are fulfilled.

(4) Eqn. 26 may be written into the equivalent form:

$$\begin{aligned} \Delta\tilde{\mu}_s &= R_{11}J_s+R_{12}J_h \\ \Delta\tilde{\mu}_h &= R_{21}J_s+R_{22}J_h \end{aligned} \tag{46}$$

If the system is maintained at "static-head" [16] by allowing $\Delta\tilde{\mu}_h$ to remain constant, when $J_s = 0$, then:

$$\Delta\tilde{\mu}_{s(J_s=0,\,\Delta\tilde{\mu}_h)} = \frac{R_{12}}{R_{22}}\Delta\tilde{\mu}_h = -\frac{L_{12}}{L_{11}}\Delta\tilde{\mu}_h = -\frac{[H_o]}{bK_h+[H_o]}\Delta\tilde{\mu}_h \tag{47}$$

Since the driving force $\Delta\tilde{\mu}_h$ may be splitted into an osmotic component ($\Delta p\mathrm{H}$ =

$pH_o - pH_i$) and a purely electrical component ($\Delta\psi$) according to: $\Delta\tilde{\mu}_h = -2.3\,RT\,\Delta pH + F\Delta\psi$
The concentration ratio ($f = [S_i]/[S_o]$) that can be developped under these conditions can be derived from Eqn. 47:

$$\ln f = -\frac{L_{12}}{L_{11}} 2.3\Delta pH + \left(\frac{L_{12}}{L_{11}} - 1\right)\frac{F}{RT}\Delta\psi \tag{48}$$

The validity of such a relation can be experimentally tested in cases where it is possible to monitor independently ΔpH and $\Delta\psi$, as was attempted, for instance, by Kashket and Wilson [20].

(5) The dissipation function (Eqn. 25) may be written into another equivalent form by inserting new driving forces: $\Delta\mu_{sh} = \Delta\tilde{\mu}_s + \Delta\tilde{\mu}_h$ the gradient of neutral substrate and $\Delta p = \Delta\tilde{\mu}_h/F$ the proton-motive force as defined by Mitchell [1]. New flows are the substrate flux $J_{sh} = J_s$ and the total electrical current $I = F(J_h - J_s)$. The proton-motive force can be determined by introducing a pair of reversible electrodes to protons and by measuring the potential difference [21]. The dissipation function becomes:

$$\Phi = J_{sh}\Delta\mu_{sh} + I\Delta p \tag{49}$$

The Onsager relations become:

$$\begin{aligned} J_{sh} &= l_{11}\Delta\mu_{sh} + l_{12}\Delta p \\ I &= l_{21}\Delta\mu_{sh} + l_{22}\Delta p \end{aligned} \tag{50}$$

with the new phenomenological coefficients:

$$\begin{aligned} l_{11} &= L_{11} \\ l_{22} &= F^2(L_{11} + L_{22}) \\ l_{12} &= l_{21} = F(L_{12} - L_{11}) \end{aligned} \tag{51}$$

Since $L_{11} > L_{12}$ as predicted from Eqns. 40 and 41, then l_{12} is negative so that J_{sh} and I are driven in mutually opposite directions. The degree of coupling between J_{sh} and I is given by:

$$q = -\frac{bK_h}{\sqrt{\left([H_o] + \frac{b}{K_s}[H_o][S_o]\right)(bK_h + [H_o])}} \tag{52}$$

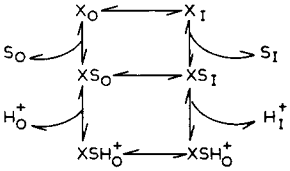
Other carrier - mediated flows

Following the general treatment detailed above for the anion/H$^+$ symporter, other carrier models have been analyzed in Table I, where the pertinent information is given. In any case the linearity and the symmetry of the Onsager relations are satisfied close to equilibrium. Contrary to symporter cases where the fluxes of the co-permeant species are dragged in the same direction ($q_{sym} > 0$), for antiporters the fluxes have obviously opposite direction ($q_{ant} < 0$) [15].

TABLE I

PHENOMENOLOGICAL COEFFICIENTS FOR CARRIER-MEDIATED FLOWS

A. Neutral substrate/H$^+$ symporter



$$q_{\mathrm{sym}} = +\sqrt{\frac{bK_s[\mathrm{H_o}]}{(K_h+b[\mathrm{H_o}])(K_s+[\mathrm{S_o}])}}$$

$$\begin{cases} J_S = L_{11}\Delta\mu_S + L_{12}\Delta\tilde{\mu}_H \\ J_H = L_{21}\Delta\mu_S + L_{22}\Delta\tilde{\mu}_H \end{cases}$$

$$L_{11} = \frac{P(\mathrm{SYM})}{RT\Delta_o\Delta_i} K_sK_h[\mathrm{S_i}](K_h+b[\mathrm{H_o}])$$

$$L_{22} = \frac{P(\mathrm{SYM})}{RT\Delta_o\Delta_i} bK_h[\mathrm{H_o}][\mathrm{S_i}](K_s+[\mathrm{S_o}])$$

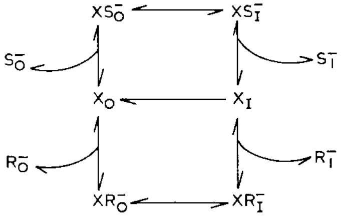
$$L_{12} = L_{21} = \frac{P(\mathrm{SYM})}{RT\Delta_o\Delta_i} K_sK_hb[\mathrm{H_o}][\mathrm{S_i}]$$

$$b = \frac{F\Delta\Psi}{RT(1-\exp -F\Delta\Psi/RT)}$$

$$\Delta_o = K_sK_h + K_h[\mathrm{S_o}] + b[\mathrm{H_o}][\mathrm{S_o}]$$

$$\Delta_i = K_sK_h + K_h[\mathrm{S_i}] + b\exp\frac{-F\Delta\Psi}{RT}[\mathrm{H_i}][\mathrm{S_i}]$$

B. Anion/anion antiporter



$$q_{\mathrm{ant}} = -b\sqrt{\frac{[\mathrm{R_o}][\mathrm{S_o}]}{(K_s+b[S_o])(K_r+b[\mathrm{R_o}])}}$$

$$\begin{cases} J_s = L_{11}\Delta\tilde{\mu}_s + L_{12}\Delta\tilde{\mu}_r \\ J_r = L_{21}\Delta\tilde{\mu}_s + L_{22}\Delta\tilde{\mu}_r \end{cases}$$

$$L_{11} = \frac{P(\mathrm{ANT})}{RT\Delta_o\Delta_i} K_sK_rb[\mathrm{S_o}](K_r+b[\mathrm{R_o}])$$

$$L_{22} = \frac{P(\mathrm{ANT})}{RT\Delta_o\Delta_i} K_sK_rb[\mathrm{R_o}](K_s+b[\mathrm{S_o}])$$

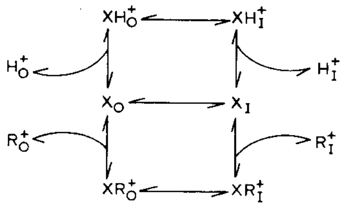
$$L_{12} = L_{21} = \frac{-R(\mathrm{ANT})}{RT\Delta_o\Delta_i} K_sK_rb^2[\mathrm{S_o}][\mathrm{R_o}]$$

$$b = \frac{F\Delta\Psi}{RT(\exp F\Delta\Psi/RT-1)}$$

$$\Delta_o = K_sK_r + bK_r[\mathrm{S_o}] + bK_s[\mathrm{R_o}]$$

$$\Delta_i = K_sK_r + bK_r\exp\frac{F\Delta\Psi}{RT}[\mathrm{S_i}] + bK_s\exp\frac{F\Delta\Psi}{RT}[\mathrm{R_i}]$$

C. Cation/H$^+$ antiporter



$$q_{\mathrm{ant}} = -b\sqrt{\frac{[\mathrm{R_o}][\mathrm{H_o}]}{(K_h+b[\mathrm{H_o}])(K_r+b[\mathrm{R_o}])}}$$

$$\begin{cases} J_r = L_{11}\Delta\tilde{\mu}_r + L_{12}\Delta\tilde{\mu}_h \\ J_h = L_{21}\Delta\tilde{\mu}_r + L_{22}\Delta\tilde{\mu}_h \end{cases}$$

$$L_{11} = \frac{P(\mathrm{ANT})}{RT\Delta_o\Delta_i} K_rK_hb[\mathrm{R_o}]\ (K_h+b[\mathrm{H_o}])$$

$$L_{22} = \frac{P(\mathrm{ANT})}{RT\Delta_o\Delta_i} K_rK_hb[\mathrm{H_o}](K_r+b[\mathrm{R_o}])$$

$$L_{12} = L_{21} = -\frac{P(\mathrm{ANT})}{RT\Delta_o\Delta_i} b^2K_rK_h[\mathrm{H_o}][\mathrm{R_o}]$$

$$b = \frac{F\Delta\Psi}{RT(1-\exp -F\Delta\Psi RT)}$$

$$\Delta_o = K_hK_r + bK_r[\mathrm{H_o}] + bK_h[\mathrm{R_o}]$$

$$\Delta_i = K_hK_r + bK_r[\mathrm{H_i}]\exp -\frac{F\Delta\Psi}{RT} + bK_h[\mathrm{R_i}]\exp -\frac{F\Delta\Psi}{RT}$$

CHARGE - SPLITTING REACTIONS

The biochemical reactions leading to oxygen consumption (respiration) and to the synthesis or hydrolysis of ATP (reversible ATPase) are responsible for the chemical conversion of particular reactants into products and for the simultaneous vectorial transfer of protons from one side of the membrane to the other. The asymmetric localization within the membrane of the components that catalyze such reactions is thought to allow such a dual function to occur (for details see refs. 1–3). Whatever the number, the nature and the sequence of the microscopic steps involved may be, a steady-state treatment similar to that used above for carrier-mediated flows leads to a dissipation function of the form: $\Phi = JA$, corresponding to a macroscopic reaction [15]:

$$\nu_s S + \nu_h H_i^+ \underset{k_2}{\overset{k_1}{\rightleftharpoons}} \nu_p P + \nu_h H_o^+ \tag{53}$$

where S and P stand for reactants and products, respectively, and where k_1 and k_2 are apparent rate constants. The expressions for the macroscopic flux and affinity are:

$$J = k_1[S]^{\nu_s}[H_i]^{\nu_h} - k_2[P]^{\nu_p}[H_o]^{\nu_h} \tag{54}$$

$$A = (\nu_s \mu_s - \nu_p \mu_p) - \nu_h \Delta\tilde{\mu}_h \tag{55}$$

Close to equilibrium the flux J is proportional to its chemical affinity ($J = LA$) and the phenomenological coefficient is simply:

$$L = \frac{k_1[S]^{\nu_s}[H_i]^{\nu_h}}{RT} = \frac{k_2[P]^{\nu_p}[H_o]^{\nu_h}}{RT} \tag{56}$$

As shown in Eqn. 55, the overall affinity can be splitted into a purely chemical component: $\nu_{ch} A_{ch} = \nu_s \mu_s - \nu_p \mu_p$ and an electro-osmotic component given by $-\nu_h \Delta\tilde{\mu}_h$. We can derive the equation which describes the proton translocation flux as:

$$J_h = -\nu_h J \tag{57}$$

and that of the associated purely chemical flux:

$$J_{ch} = \nu_{ch} J \tag{58}$$

In the absence of side reactions J_h and J_{ch} are completely coupled ($q = -1$):

$$\begin{aligned} J_{ch} &= L_{cc} A_{ch} + L_{ch} \Delta\tilde{\mu}_h \\ J_h &= L_{hc} A_{ch} + L_{hh} \Delta\tilde{\mu}_h \end{aligned} \tag{59}$$

with

$$L_{cc} = \nu_{ch}{}^2 L$$

$$L_{ch} = L_{hc} = -\nu_{ch} \nu_h L$$

$$L_{hh} = \nu_h{}^2 L$$

The negative sign in L_{ch} indicates that when the chemical flow J_{ch} proceeds in the direction given by A_{ch}, the proton flux J_h is directed outwardly.

TABLE II

PHENOMENOLOGICAL COEFFICIENTS IN CHARGE - SPLITTING REACTIONS*

Reaction	L	J_h	J_{ch}	A_{ch}	L_{cc}	$L_{ch} = L_{hc}$	L_{hh}
Redox chain**							
$RH_2+\frac{1}{2}O_2+2nH_i^+ \underset{k_{-ox}}{\overset{k_{ox}}{\rightleftharpoons}}$ $R+H_2O+2nH_o^+$	$L_{ox} = \frac{k^{ox}}{RT}[RH_2][O_2]^{\frac{1}{2}}[H_i]^{2n}$	J_h^{ox}	J^{ox}	A^{ox}	L^{ox}	$-2nL^{ox}$	$4n^2L^{ox}$
ATPase***							
$ATP+H_2O+2H_i^+ \underset{k_{-ATP}}{\overset{k_{ATP}}{\rightleftharpoons}}$ $ADP+P_i+2H_o^+$	$L^{ATP} = \frac{k_{ATP}}{RT}[ATP][H_2O][H_i]^2$	J_h^{ATP}	J^{ATP}	A^{ATP}	L^{ATP}	$-2L^{ATP}$	$4L^{ATP}$
Transhydrogenase							
$NADPH+NAD^++2H_i^+ \underset{k_{-NAD}}{\overset{k_{NAD}}{\rightleftharpoons}}$ $NADP^++NADH+2H_o^+$	$L^{NAD} = \frac{k_{NAD}}{RT}[NADPH][NAD^+][H_i^+]^2$	J_h^{NAD}	J^{NAD}	A^{NAD}	L^{NAD}	$-2L^{NAD}$	$4L^{NAD}$

* The explicit values of the coefficients in Eqn. 59 are given.

** n is the number of elementary oxido-reduction loops in the chain.

*** For simplicity, the un-ionized form of ATP, ADP and P_i are used.

Following the general scheme given above, the phenomenological coefficients were derived for the redox chain, the ATPase reaction and the transhydrogenase reaction by respecting the stoichiometry associated with each process (Table II).

COUPLING PHENOMENA IN THE PROTON CIRCUIT

We will consider a very general case in which several processes take place simultaneously as depicted in Fig. 2. Ten macroscopic flows contribute to the dissipation function of the system. Close to equilibrium, each flow is linearly dependent upon its conjugated driving force and upon $\Delta\tilde{\mu}_{\mathrm{h}}$, the electrochemical potential gradient of protons:

$$\begin{aligned}
J_{\mathrm{h}}^{\mathrm{ox}} &= -2nL^{\mathrm{ox}}A^{\mathrm{ox}}+4n^2L^{\mathrm{ox}}\Delta\tilde{\mu}_{\mathrm{h}}\\
J_{\mathrm{ox}} &= L^{\mathrm{ox}}A^{\mathrm{ox}}-2nL^{\mathrm{ox}}\Delta\tilde{\mu}_{\mathrm{h}}\\
J_{\mathrm{h}}^{\mathrm{ATP}} &= -2L^{\mathrm{ATP}}A^{\mathrm{ATP}}+4L^{\mathrm{ATP}}\Delta\tilde{\mu}_{\mathrm{h}}\\
J^{\mathrm{ATP}} &= L^{\mathrm{ATP}}A^{\mathrm{ATP}}-2L^{\mathrm{ATP}}\Delta\tilde{\mu}_{\mathrm{h}}\\
J_{\mathrm{h}}^{\mathrm{NAD}} &= -2L^{\mathrm{NAD}}A^{\mathrm{NAD}}+4L^{\mathrm{NAD}}\Delta\tilde{\mu}_{\mathrm{h}}\\
J^{\mathrm{NAD}} &= L^{\mathrm{NAD}}A^{\mathrm{NAD}}-2L^{\mathrm{NAD}}\Delta\tilde{\mu}_{\mathrm{h}}\\
J_{\mathrm{h}}^{\mathrm{sym}} &= L_{12}\Delta\tilde{\mu}_{\mathrm{s}}+L_{22}\Delta\tilde{\mu}_{\mathrm{h}}\\
J_{\mathrm{s}} &= L_{11}\Delta\tilde{\mu}_{\mathrm{s}}+L_{12}\Delta\tilde{\mu}_{\mathrm{h}}\\
J_{\mathrm{h}}^{\mathrm{ANT}} &= L'_{12}\Delta\tilde{\mu}_{\mathrm{r}}+L'_{22}\Delta\tilde{\mu}_{\mathrm{h}}\\
J_{\mathrm{r}} &= L'_{11}\Delta\tilde{\mu}_{\mathrm{r}}+L'_{12}\Delta\tilde{\mu}_{\mathrm{h}}
\end{aligned} \qquad (60)$$

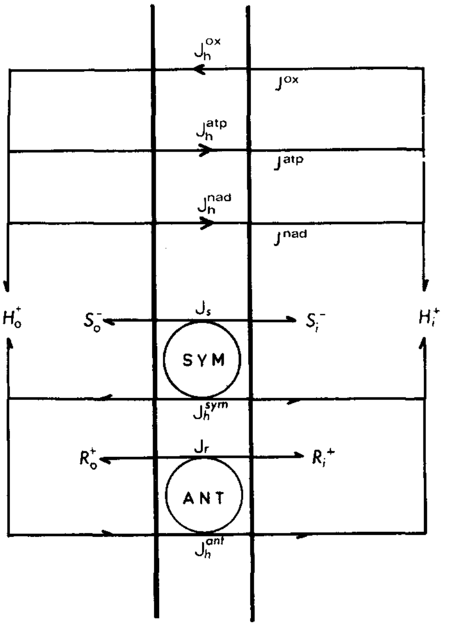


Fig. 2. A general proton circuit. The circuit is made of the redox chain (J^{ox}, $J_{\mathrm{h}}^{\mathrm{ox}}$), the ATPase complex ($J^{\mathrm{ATP}}$, J^{ATP}), the transhydrogenase (J^{NAD}, $J_{\mathrm{h}}^{\mathrm{NAD}}$), one symporter ($J_{\mathrm{s}}$, $J_{\mathrm{h}}^{\mathrm{sym}}$) and one antiporter ($J_{\mathrm{r}}$, $J_{\mathrm{h}}^{\mathrm{ant}}$).

The expressions for the coefficients L_{11}, L_{22} and L_{12} are given in Eqns. 40–42 and for L'_{11}, L'_{22} and L'_{12} in Table I. However, in such a system none of the individual proton flows can be measured independently and only the total proton flux is observable:

$$J_h^{tot} = J_h^{ox} + J_h^{ATP} + J_h^{NAD} + J_h^{sym} + J_h^{ant} \tag{61}$$

Consequently the 10-flow system is equivalent to a 6-flow system, one of them being the circulation of the protons:

$$\begin{aligned}
J^{ox} &= L^{ox}A^{ox} - 2nL^{ox}\Delta\tilde{\mu}_h \\
J^{ATP} &= L^{ATP}A^{ATP} - 2L^{ATP}\Delta\tilde{\mu}_h \\
J^{NAD} &= L^{NAD}A^{NAD} - 2L^{NAD}\Delta\tilde{\mu}_h \\
J_s &= L_{11}\Delta\tilde{\mu}_s + L_{12}\Delta\tilde{\mu}_h \\
J_r &= L'_{11}\Delta\tilde{\mu}_r + L'_{12}\Delta\tilde{\mu}_h \\
J_h^{tot} &= -2nL^{ox}A^{ox} - 2L^{ATP}A^{ATP} - 2L^{NAD}A^{NAD} + L_{12}\Delta\tilde{\mu}_s + L'_{12}\Delta\tilde{\mu}_r + \rho\Delta\tilde{\mu}_h
\end{aligned} \tag{62}$$

with

$$\rho = 4n^2L^{ox} + 4L^{ATP} + 4L^{NAD} + L_{22} + L'_{22}$$

It is seen that J_h^{tot} is the unique flow of the system which is dependent upon all the driving forces operating in it. Each flow will depend upon the other forces when a first-order stationary state is reached, given by a constant proton circulation:

$$J_h^{tot} = 0 \tag{63}$$

In that case:

$$\rho\Delta\tilde{\mu}_h = 2nL^{ox}A^{ox} + 2L^{ATP}A^{ATP} + 2L^{NAD}A^{NAD} - L_{12}\Delta\tilde{\mu}_s - L'_{12}\Delta\tilde{\mu}_r \tag{64}$$

By replacing Eqn. 64 in Eqn. 62 we get in matrix formulation:

$$\begin{pmatrix} J^{ox} \\ J^{ATP} \\ J^{NAD} \\ J_s \\ J_r \end{pmatrix} = (l_{ik})(A^{ox}A^{ATP}A^{NAD}\Delta\tilde{\mu}_s\Delta\tilde{\mu}_r) \tag{65}$$

Explicit l_{ik} phenomenological coefficients are listed in Table III. It can be seen that the symmetry of all cross-coefficients is respected. Recalling that L'_{12} is negative (see Table I), the signs of the coefficients in Table III allow trivial conclusions.

(a) $l_{12} = l_{21} < 0$ indicates that J^{ox} and J^{ATP} are driven in opposite directions so that respiration is coupled to ATP synthesis (oxidative phosphorylation) and ATP hydrolysis is reciprocally coupled to oxygen production from water (reversal of the electron chain). The partial coupling coefficient relating J^{ox} to J^{ATP} is given by:

$$\begin{aligned}
q_{ox/ATP} &= \frac{l_{12}}{\sqrt{l_{11}l_{22}}} \\
&= -\frac{4nL^{ox}L^{ATP}}{\sqrt{L^{ox}L^{ATP}(4L^{ATP}+4L^{NAD}+L_{22}+L'_{22})(4n^2L^{ox}+4L^{NAD}+L_{22}+L'_{22})}}
\end{aligned} \tag{66}$$

TABLE III

PHENOMENOLOGICAL COEFFICIENTS IN THE COUPLED PROTON CIRCUIT OF FIG. 4 IN THE STATIONARY STATE

$\rho \cdot l_{ik}$	ith flux	A^{ox}	A^{ATP}	A^{NAD}	$\Delta\tilde{\mu}_s$	$\Delta\tilde{\mu}_r$
	kth driving force	1	2	3	4	5
J^{ox}	1	$L^{ox}(4L^{ATP}+4L^{NAD}+L_{22}+L'_{22})$	$-4nL^{ox}L^{ATP}$	$-4nL^{ox}L^{NAD}$	$2nL^{ox}L_{12}$	$2nL^{ox}L'_{12}$
J^{ATP}	2	$-4nL^{ox}L^{ATP}$	$L^{ATP}(4n^2L^{ox}+4L^{NAD}+L_{22}+L'_{22})$	$-4L^{ATP}L^{NAD}$	$2L^{ATP}L_{12}$	$2L^{ATP}L'_{12}$
J^{NAD}	3	$-4nL^{NAD}L^{ox}$	$-4L^{NAD}L^{ATP}$	$L^{NAD}(4n^2L^{ox}+4L^{ATP}+L_{22}+L'_{22})$	$2L^{NAD}L_{12}$	$2L^{NAD}L'_{12}$
J_s	4	$2nL_{12}L^{ox}$	$2L_{12}L^{ATP}$	$2L_{12}L^{NAD}$	$L_{11}(4n^2L^{ox}+4L^{ATP}+4L^{NAD}+L_{22}+L'_{22})$	$-L_{12}L'_{12}$
J_r	5	$2nL'_{12}L^{ox}$	$2L'_{12}L^{ATP}$	$2L'_{12}L^{NAD}$	$-L'_{12}L_{12}$	$L'_{11}(4n^2L^{ox}+4L^{ATP}+4L^{NAD}+L_{22}+L'_{22})$

Complete coupling ($q_{\mathrm{ox/ATP}} = -1$) occurs only when either the transhydrogenase reaction and the carrier-mediated flows are absent from the system ($L^{\mathrm{NAD}} = L_{22} = L'_{22} = 0$) or the particular situation $4L^{\mathrm{NAD}}+L_{22}+L'_{22} = 0$ is realized. There is a competition between ATP synthesis and other processes in normal conditions for the energy available from the redox chain and the value of the coupling coefficient $q_{\mathrm{ox/ATP}}$ will depend upon the magnitude of the term $4L^{\mathrm{NAD}}+L_{22}+L'_{22}$ before $4L^{\mathrm{ATP}}$ and $4n^2L^{\mathrm{ox}}$.

(b) The reduction of NADP$^+$ by NADH, catalyzed by the energy-linked transhydrogenase, may be driven by respiration ($l_{31} < 0$), ATP hydrolysis ($l_{32} < 0$) and also by the net efflux of an anion ($l_{34} > 0$) or the net influx of a cation ($l_{35} < 0$).

(c) The symporter-mediated net inward flux of an anion may be driven by respiration ($l_{41} > 0$), ATP hydrolysis ($l_{42} > 0$), reduction of NAD$^+$ by NADPH ($l_{43} > 0$) or net influx of a cation through an antiporter ($l_{45} > 0$). It can also be predicted that, conversely, the net outward flux of the same anion should lead to the reversal of the electron chain, to ATP synthesis, to energizing of the transhydrogenase and to drag of the outflux of a cation. Experimental evidence for the occurrence of some of these coupled processes in vivo has been established (see the critical survey given by Greville [6]).

(d) We will consider a particular case of the proton circuit which received much experimental attention during the past few years. It concerns the transport of a substrate (say an anion) through a symporter in bacterial membranes. Closed membrane vesicles that conserved the original right-side-out orientation can be easily prepared. Respiration can be induced by supplying an electron donnor such as D-lactate and it was observed that a great number of molecules are accumulated inside against a concentration gradient [22]. The situation is described by the set of equations:

$$\begin{aligned} J^{\mathrm{ox}} &= l_{11}A^{\mathrm{ox}}+l_{14}\Delta\tilde{\mu}_{\mathrm{s}} \\ J_{\mathrm{s}} &= l_{41}A^{\mathrm{ox}}+l_{44}\Delta\tilde{\mu}_{\mathrm{s}} \end{aligned} \tag{67}$$

when the total proton flux reaches a stationary state. The coefficients are:

$$\begin{aligned} l_{11} &= \frac{L^{\mathrm{ox}}L_{22}}{\rho} \\ l_{44} &= \frac{L_{11}}{\rho} \\ l_{14} &= l_{41} = \frac{2nL^{\mathrm{ox}}L_{12}}{\rho} \end{aligned} \tag{68}$$

with $\rho = 4n^2L^{\mathrm{ox}}+L_{22}$.

The coefficient expressing the coupling between J^{ox} and J_{s} is:

$$q_{\mathrm{ox/sym}} = \frac{l_{14}}{\sqrt{l_{11}l_{44}}} = 2n\sqrt{L^{\mathrm{ox}}}\frac{L_{12}}{\sqrt{L_{11}L_{22}}} = 2n[\mathrm{H_i}]^n\sqrt{\frac{k_{\mathrm{ox}}[\mathrm{RH_2}][\mathrm{O_2}]^{\frac{1}{2}}[\mathrm{H_o}]K_{\mathrm{s}}}{RT[bK_{\mathrm{h}}+[\mathrm{H_o}]][K_{\mathrm{s}}+b[\mathrm{S_o}]]}} \tag{69}$$

Eqn. 69 shows the dependence of the coupling upon n, the number of oxido-reduction loops in the chain, and upon the concentrations of the feeding substrates RH_2 and O_2. The factor of concentration $f = [S_i]/[S_o]$ can be computed when a static-head situation is maintained:

$$\Delta\mu_{s(A^{ox}=\mathrm{const},\, j_s=0)} = -\frac{l_{14}}{l_{44}} A^{ox} = -2n \frac{L^{ox} L_{12}}{L_{11}} A^{ox} \tag{70}$$

The chemical affinity can be equated to:

$$A^{ox} = 2F\Delta E'$$

where $\Delta E'$ represents the oxido-reduction potential span between the first and the terminal electron acceptor in the chain, so that we get at last a linear relation similar to Eqn. 48:

$$\ln f = \frac{4nF}{RT} \frac{L^{ox} L_{12}}{L_{11}} \Delta E' - \frac{F}{RT} \Delta\psi \tag{71}$$

DISCUSSION

Concepts in irreversible thermodynamics have already served to describe puzzling problems such as oxidative phosphorylation [14], carrier-mediated flows [13, 19] or the coupling between a diffusion flux and a chemical reaction [15, 23]. Active transport phenomena involving a membranous carrier were interpreted rather unsatisfactorily until the chemiosmotic theory was proposed by Mitchell [1], because of the difficulty to attribute a physical meaning to the required energy. The description given here attempted to illustrate the fact that active transport can simply result from the thermodynamic coupling between unrelated driving forces operating in a given system under defined conditions. It was not used to help in proving or invalidating the theory, but rather to derive a number of consequences from admitted premises. The basal postulates of the chemiosmotic theory that we started from were the occurrence in the proton circuit of [1] (i) particular chemical reactions leading virtually to the transfer of protons from one side of the membrane to the other (electron-transfer chain, ATPase complex, transhydrogenase) and (ii) biporter molecules able to translocate H^+ simultaneously to another chemical species in the same or opposite direction. Our contribution, as compared to similar thermodynamic approaches in the field of transport [13, 15] was to avoid deliberately a too highly generalized description of the carrier-mediated processes, ignoring the parameters of the carrier molecules (affinity, mobility), and of the charge-splitting reactions abstracting their stoichiometry. Phenomenological coefficients appearing in the computed Onsager relations were then explicitly formulated so that their properties have been scrutinized.

The validity of the conclusions to be drawn is dependent upon a number of primordial conditions, restrictions and simplifying assumptions that we were led to introduce in the course of the mathematical treatment. (1) The contribution of the solvent (water) was not taken into account, so that further studies are open to include in the model osmo-osmotic and electro-osmotic phenomena. (2) In the Nernst-Planck equation used for computing the microscopic fluxes of charged carrier complexes, we assumed a constant electric field across an uncharged membrane [17]. Any other hypothesis concerning the distribution of fixed charges within the membrane should lead to different conclusions. (3) The treatment was applied at steady state so that only macroscopic flows appear in the dissipation function. We neglected the possibility for microscopic flows to be coupled to non-conjugated driving forces. (4) The permeability coefficients of the different carrier complexes were taken to be equal. It can easily be shown that the removal of such a restriction leads to more complicated equations without modifying the conclusions. (5) The reactions taking place at the membrane interfaces between the carriers and the ligands were assumed to be in local equilibrium. (6) Macroscopic chemical and diffusional fluxes are linearly connected to the driving forces only close to equilibrium. The extension of the treatment in the non-linear range [24] is open to further investigation. (7) The proton circuit has been idealized in the sense that we neglected all side reactions such as the free diffusion of protons and other ions across the membrane, as well as the translocation of P_i, ATP and ADP through specific transporters. It is expected that such processes participate in the modification of the efficiency of coupling between the remaining fluxes [15].

A conclusion already reached by several authors [11, 13] is that Onsager's phenomenological coefficient appearing in carrier-mediated flux equations depends upon state parameters such as internal and external concentrations of the co-permeant species. We found in addition that the degree of coupling between the fluxes mediated by the same carrier is completely governed by dissociation constants and by the external concentrations of the species. It was shown that the increasing saturation of the carrier with one of them leads to a corresponding reduction of q. On the other hand, q increases when the species are at very low external concentrations. Self-uncoupling is therefore an interesting property of biporter carriers. This kind of coupling modulation should give to the cell the ability to save energy when the concentration of a particular substrate is not limiting for growth or development.

In the multiple-flow system, the dependence of each flow upon all driving forces present emerges only when a first-order stationary state, given by a zero net inward proton flux, is reached. This is a well known case of "stationary state coupling" between diffusion processes and chemical reactions discussed by others [12, 23]. It remains to be tested experimentally whether a constant circulation of protons is rapidly established in vivo [6].

As shown, the translocation of a substrate against its electrochemical gradient (active transport) is made possible at the expense of a variety of other processes: examples of osmo-osmotic and chemico-osmotic couplings were illustrated in the last section. In agreement with the chemiosmotic model of Mitchell [2, 3], we predicted only "secondary active transport" [15], in the sense that we need not postulate two or more conformations for the carrier, one being the result of a specific chemical reaction between the carrier molecule and some energy-rich compound. The carrier function is restricted to the recognition and capture of a particular solute but the mediated fluxes are controlled by numerous forces operating within the system. If the carrier possesses interacting binding sites or subunits, new regulatory properties will emerge (see for instance the allosteric model of Blumenthal and Katchalsky [19]).

The treatment given in this paper may be easily extended to other circuits. Because of numerous analogies, the transposition is immediate for the Na^+ and K^+

circuit in eukaryotic cells where the reversible pile is the $(Na^+ + K^+)$-activated ATPase of the plasmatic membrane and where Na^+ plays the role of protons in symporters [18, 15].

LIST OF SYMBOLS

Vectorial flows are given per unit membrane area

$\mu_i\ \tilde{\mu}_i$	chemical, electrochemical potential of component i
μ_i^0	standard chemical potential of component i
z_i	valence of component i
F	Faraday constant
R	gas constant
$\psi, \Delta\psi = \psi_o - \psi_i$	electrical potential, electrical potential difference
L_{ii}, l_{ii}	straight coefficients relating the ith flux to the ith force
L_{ik}, l_{ik}	cross-coefficients relating the ith flux to the kth force
R_{ii}	resistance coefficient relating the ith force to the ith flux
R_{ik}	resistance coefficient relating the ith force to the kth flux
Φ	dissipation function, entropy production under isothermal conditions
Δp	proton - motive force
K	equilibrium constant
k	rate constant
A	chemical affinity
I	electrical current
q	degree of coupling between two fluxes or two forces
T	absolute temperature
ν_i	stoichiometric coefficient
P_i	carrier ith complex permeability coefficient
ω_i	molar mobility of component *i*
J_i	flux of component *i*
Δx	membrane width

ACKNOWLEDGMENTS

Financial support from the Délégation Générale à la Recherche Scientifique et Technique (Action Complementaire Coordonée "Interactions Moleculaires en Biologie") and from the Centre National de la Recherche Scientifique (ERA no. 177) is gratefully acknowledged. Thanks are due to the referees for helping me to clarify a number of points. This work will be part of the "Doctorat-es-Sciences" thesis to be held by the author at Université Claude Bernard de Lyon, under the guidance of Professor F. Stoeber.

REFERENCES

1 Mitchell, P. (1966) Biol. Rev. 41, 445–502

2 Mitchell, P. (1973) in Mechanisms in Bioenergetics (Azzone, G. F., Ernster L., Papa, S., Quagliariello, E. and Siliprandi, N., eds.), pp. 177–201, Academic Press, New York

3 Mitchell, P. (1969) in The Molecular Basis of Membrane Function (Tosteson, D. C., ed.), pp. 483–518, Prentice-Hall, New York
4 Skulachev, V. P. (1972) in Mitochondria and Biomembranes (FEBS meeting), Vol. 28, pp. 371–385
5 Mitchell, P. (1969) in Mitochondria and Biomembranes (FEBS meeting), Vol. 28, pp. 353–370
6 Greville, G. D. (1969) in Current Topics in Bioenergetics (Sanadi, D. R., ed.), Vol. 3, pp. 1–78, Academic Press, New York
7 Mitchell, P. and Moyle, J. (1969) Eur. J. Biochem. 7, 471–484
8 Scholes, P., Mitchell, P. and Moyle, J. (1969) Eur. J. Biochem. 8, 450–454
9 West, I. and Mitchell, P. (1973) Biochem. J. 132, 587–592
10 Griniuviene, B., Chielianskaiti, V. and Grinius, L. (1974) Biochem. Biophys. Res. Commun. 56, 206–213
11 Prigogine, I. (1961) in Introduction to Thermodynamics of Irreversible Processes, pp. 1–257, Interscience Publishers
12 Katchalsky, A. and Curran, P. F. (1967) in Non-Equilibrium Thermodynamics in Biophysics, pp. 1–244, Harvard University Press, Mass.
13 Katchalsky, A. and Spangler, R. (1968) Q. Rev. Biophys. 1, 127–175
14 Caplan, S. R. (1971) in Current Topics in Bioenergetics (Sanadi, D. R., ed.), pp. 1–79, Academic Press, New York
15 Heinz, E. (1974) in Current Topics in Membranes and Transport (Bronner F. and Kleinzeller, A., eds.), Vol. 5, pp. 137–159, Academic Press, New York
16 Kedem, O. and Caplan, S. R. (1965) Trans. Faraday Soc. 61, 1897–1911
17 Goldman, D. E. (1943) J. Gen. Physiol. 27, 37–60
18 Schultz, S. G. and Curran, P. F. (1970) Physiol. Rev. 50, 637–718
19 Blumenthal, R. and Katchalsky, A. (1969) Biochim. Biophys. Acta 173, 357–369
20 Kashket, E. R. and Wilson, T. H. (1973) Proc. Natl. Acad. Sci. U.S. 70, 2866–2869
21 Kedem, O. and Katchalsky, A. (1963) Trans. Faraday Soc. 59, 1918–1930
22 Kaback, R. H. (1972) Biochim. Biophys. Acta 265, 367–416
23 Katchalsky, A. and Oster, G. (1969) in The Molecular Basis of Membrane Function (Tosteson, D. C., ed.), pp. 1–44, Prentice Hall, New York
24 Glansdorff, P. and Prigogine, I. (1971) in Structure, Stabilité et Fluctuations, pp. 1–288, Masson, Paris
25 Delaage, M. A. (1975) Biochim. Biophys. Acta 394, 493–503
26 Heinz, E., Geck, P. and Wilbrandt, W. (1972) Biochim. Biophys. Acta 255, 442–461