THE COUPLING OF AN ENZYMATIC REACTION TO TRANSMEMBRANE FLOW OF ELECTRIC CURRENT IN A SYNTHETIC "ACTIVE TRANSPORT" SYSTEM

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ABSTRACT If a chemical reaction is constrained to occur within an asymmetric structure, e.g. by the presence of bound or otherwise trapped enzyme, coupling of the reaction to the flow of one or more solutes, or to the flow of electric current, becomes possible. Such systems can serve as models in which transport is "driven" by chemical reaction. In this respect the processes involved are analogous to active transport, though the molecular mechanisms may be quite different from those in nature. A simple arrangement of this kind has been studied: a composite membrane consisting of two ion exchange membranes of opposite fixed charge, separated by an intermediate layer of solution containing papain. An uncharged substrate of low molecular weight acts as "fuel" for the system, N-acetyl-L-glutamic acid diamide. This material (not previously described) hydrolyzes in the presence of papain to ammonium N-acetyl-L-glutamine. The composite membrane gives rise to an electromotive force, ultimately reaching a stationary state, when clamped between two identical solutions in which the affinity of the reaction has been fixed. Onsager's reciprocity relation has not hitherto been tested in a case of coupling between chemical reaction and a vectorial flow (here electric current); its validity for this system, in which stationary-state coupling occurs, was established over the experimental range of affinities (up to 3 kcal/mole).

INTRODUCTION

We have devised a simple model in which stationary-state coupling between chemical reaction and flow of ionic electrical current can be demonstrated. It is essentially an enzymatic fuel cell, consisting of two large compartments I and II (regarded as infinite reservoirs) separated by an inner compartment i[1] in which the chemical reaction takes place. The composite structure of interest comprises two oppositely charged ion exchange membranes in series, α and β, sandwiching a solution of enzyme. The enzyme cannot permeate through the membranes.

[1] For symbols used in this paper see list preceding References at end of paper.

The fuel for this cell should be preferably an uncharged material of low molecular weight, able to pass freely through both membranes and to act as a substrate for the enzyme. It is required to yield a salt by hydrolysis. A suitable substrate has been found to be N-acetyl-L-glutamic acid diamide (AGDA), which in the presence of papain hydrolyzes to the ammonium salt of N-acetyl-L-glutamine (NAG).

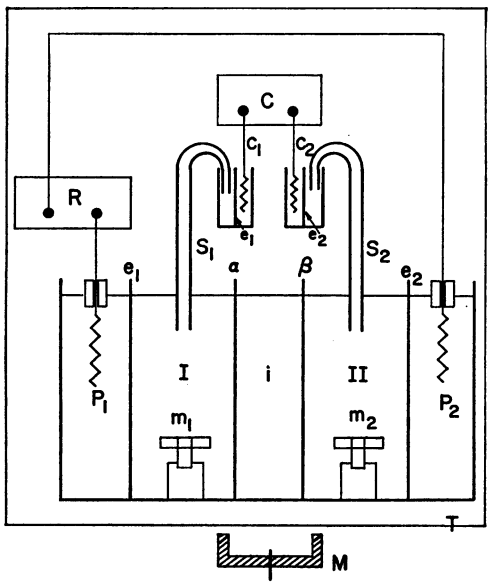
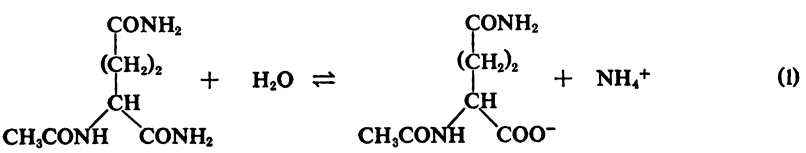


FIGURE 1 The cell. P_1 and P_2: potential-measuring half-cells; c_1 and c_2: current-carrying half-cells; e_1, e_2, and β: cation-exchange membranes; α: anion-exchange membrane; m_1 and m_2: Teflon-coated magnetic stirrers; S_1 and S_2: salt bridges; R: potential recorder; C: constant-current source; T: air thermostat; M: driving magnet; I and II: large (100 ml) outer compartments; i: inner compartment (10 ml). The concentration of papain in the inner compartment was always 1 mg/ml. Membrane area: 20 cm².

This salt gives a practically neutral reaction (pH = 7) in dilute solutions:



(I)

Upon introduction of electrodes reversible to the ammonium ion a well-defined potential difference can be measured while an electric current flows through the system. The electrodes form the outer compartments (Fig. 1). It is readily seen that the splitting of uncharged molecules in the sandwiched layer to produce cations and anions must give rise to an electric current—even though identical electrodes are introduced into identical solutions on either side. The following analysis shows that in the stationary state the process can be described by a set of two forces and two flows, and that the driving force is the affinity of the reaction in the outer solution.

Nonequilibrium Thermodynamics of the System

The starting point for a thermodynamic description of nonequilibrium systems is a calculation of the entropy production of the process. In isothermal processes it is convenient to use the dissipation function defined by:

$$\Phi = T\frac{d_i S}{dt} \tag{2}$$

For a discontinuous system the dissipation function per unit area is obtained by applying the Gibbs equation to all its parts (1):

$$\Phi = \sum_k \Delta\tilde{\mu}_k J_k + AJ_r \tag{3}$$

where J_k is the flux of the kth species from one compartment to another, and $\Delta\tilde{\mu}_k$ is its conjugate force. The reaction flow—the number of moles substrate consumed per square centimeter of cell cross-section—is J_r. The affinity of the reaction, A, is defined by:

$$A = -\sum_i \nu_i \mu_i \tag{4}$$

In this analysis it will be assumed that water is at equilibrium across each membrane and the influence of water flow on other species is negligible. Identical concentrations, both in substrate and in product (salt), are maintained in solutions I and II. We may write for the dissipation function of the total system:

$$\Phi = \Delta\mu_a{}^{\alpha}J_a{}^{\alpha} + \Delta\tilde{\mu}_+{}^{\alpha}J_+{}^{\alpha} + \Delta\tilde{\mu}_-{}^{\alpha}J_-^{\alpha} + \Delta\mu_a{}^{\beta}J_a{}^{\beta} + \Delta\tilde{\mu}_+{}^{\beta}J_+{}^{\beta} + \Delta\tilde{\mu}_-^{\beta}J_-^{\beta} + A^iJ_r \tag{5}$$

where

$$\begin{aligned}\Delta\tilde{\mu}_k{}^{\alpha} &= \tilde{\mu}_k{}^{\mathrm{I}} - \tilde{\mu}_k{}^{i}\\ \Delta\tilde{\mu}_k{}^{\beta} &= \tilde{\mu}_k{}^{i} - \tilde{\mu}_k{}^{\mathrm{II}}\end{aligned} \tag{6}$$

Substrate, cation, and anion are denoted respectively by the subscripts a, $+$, and $-$; the flows and forces over the separate membrane elements are denoted by the superscripts α and β.

Designating the affinity in the inner compartment by A^i and that in compartments I and II by A^o, we write for the affinity of the reaction in each compartment (regarding the reaction as pseudounimolecular, since the hydrolysis takes place in very dilute solution):

$$\begin{aligned}A^i &= \mu_a{}^i - \mu_s{}^i = \mu_a{}^i - (\tilde{\mu}_+{}^i + \tilde{\mu}_-{}^i)\\ A^o &= \mu_a{}^{\mathrm{I}} - \mu_s{}^{\mathrm{I}} = \mu_a{}^{\mathrm{I}} - (\tilde{\mu}_+{}^{\mathrm{I}} + \tilde{\mu}_-{}^{\mathrm{I}})\end{aligned} \tag{7}$$

Combining equations (6) and (7) we find:

$$A^i = A^o - \Delta\mu_a{}^\alpha + \Delta\mu_s{}^\alpha = A^o - \Delta\mu_a{}^\alpha + \Delta\tilde{\mu}_+{}^\alpha + \Delta\tilde{\mu}_-{}^\alpha \tag{8}$$

From the condition of equality of concentrations in compartments I and II we obtain:

$$\Delta\mu_a = \Delta\mu_a{}^\alpha + \Delta\mu_a{}^\beta = 0$$

and

$$\Delta\mu_s = \Delta\mu_s{}^\alpha + \Delta\mu_s{}^\beta = \Delta\tilde{\mu}_+{}^\alpha + \Delta\tilde{\mu}_-{}^\alpha + \Delta\tilde{\mu}_+{}^\beta + \Delta\tilde{\mu}_-{}^\beta = 0 \tag{9}$$

so that

$$\begin{aligned} \Delta\mu_a{}^\alpha &= -\Delta\mu_a{}^\beta \\ (\Delta\tilde{\mu}_+{}^\alpha + \Delta\tilde{\mu}_+{}^\beta) &= \Delta\tilde{\mu}_+ = -\Delta\tilde{\mu}_- = -(\Delta\tilde{\mu}_-{}^\alpha + \Delta\tilde{\mu}_-{}^\beta) \\ \Delta\tilde{\mu}_+{}^\beta &= \Delta\tilde{\mu}_+ - \Delta\tilde{\mu}_+{}^\alpha \\ \Delta\tilde{\mu}_-{}^\beta &= -\Delta\tilde{\mu}_+ - \Delta\tilde{\mu}_-{}^\alpha \end{aligned} \tag{10}$$

Substitution of the expressions for A^i, $\Delta\mu_a{}^\beta$, $\Delta\tilde{\mu}_+{}^\beta$, and $\Delta\tilde{\mu}_-{}^\beta$ from equations (8) and (10) into equation (5), and rearranging, gives:

$$\begin{aligned} \Phi = {} & \Delta\mu_a{}^\alpha(J_a{}^\alpha - J_a{}^\beta - J_r) + \Delta\tilde{\mu}_+{}^\alpha(J_+{}^\alpha - J_+{}^\beta + J_r) \\ & + \Delta\tilde{\mu}_-{}^\alpha(J_-{}^\alpha - J_-{}^\beta + J_r) + \Delta\tilde{\mu}_+(J_+{}^\beta - J_-{}^\beta) + A^oJ_r \end{aligned} \tag{11}$$

By definition,

$$\Delta\tilde{\mu}_+ = FE \tag{12}$$

where E is the overall potential difference between the reversible electrodes in compartments I and II, and

$$(J_+{}^\beta - J_-{}^\beta)F = I \tag{13}$$

where I is the electric current density in the system.

In a stationary state the concentrations in the inner compartment are constant and hence the flows are not independent:

$$\begin{aligned} -J_r + J_a{}^\alpha - J_a{}^\beta &= 0 \\ J_r + J_+{}^\alpha - J_+{}^\beta &= 0 \\ J_r + J_-{}^\alpha - J_-{}^\beta &= 0 \end{aligned} \tag{14}$$

Applying these conditions, the first three terms in equation (11) disappear and the dissipation function reduces to:

$$\Phi = EI + A^{o}J_{r} \tag{15}$$

It is seen that during the approach to the stationary state the flows conjugate to the unfixed forces, depending on concentrations in the inside compartment, vanish. This is a general property of stationary states (1).

In the stationary-state dissipation function, the membranes α and β and the inner solution act as a single complex membrane in which a chemical reaction takes place. The phenomenological equations are:

$$\begin{aligned} E &= R_{11}I + R_{12}J_{r} \\ A^{o} &= R_{21}I + R_{22}J_{r} \end{aligned} \tag{16}$$

Alternatively we can arrive at equation (16) by writing the phenomenological equations corresponding to the full dissipation function [equation (5)] and introducing the boundary and stationary-state conditions into these equations (cf. Appendix). The test of Onsager's theorem has been given for many coupled phenomena (2), but hitherto not for an example of coupling between chemical reaction and flux of material. In our case the reaction is coupled to a flow of ions, which may be measured as an electric current according to equation (13).[2] In the absence of ideally permselective membranes, however, the system is *incompletely* coupled even though no side reactions take place. In other words a stationary state is reached at open circuit in which the reaction continues to advance. This is in contrast to the complete coupling between chemical reaction and electron flow which occurs in a reversible electrochemical cell. A quantitative description of the degree of coupling is discussed below.

A convenient set of measurements to test Onsager's theorem is given by the following relationship between the derivatives:

$$(\partial E/\partial A^{o})_{I} = -(\partial J_{r}/\partial I)_{A^{o}} \tag{17}$$

From equation (16)

$$(\partial E/\partial A^{o})_{I} = R_{12}/R_{22} \tag{18}$$

$$-(\partial J_{r}/\partial I)_{A^{o}} = R_{21}/R_{22} \tag{19}$$

[2] The dependence of I on J_r is a case of "stationary-state coupling" as long as the space between the two membranes is considered as a separate compartment. If, however, all measurements are carried out on the outside only, one finds direct coupling: transport takes place between identical solutions at $E = 0$. In this sense our system is not analogous to that described by Prigogine (1), although the basic phenomenon in both cases is the modification of local μ_i's by a chemical reaction. It may well be that active transport across a phase membrane will eventually be considered as stationary-state coupling on a molecular scale.

For reasons of experimental convenience we measure the differential coefficients; confirmation of equation (17) implies symmetry of the matrix of coefficients. The two independent sets of experiments which subject equation (17) to a test are: (i) The electrical potential difference between the two outer solutions as a function of the affinity of the reaction at constant current, i.e., equation (18). (ii) The flow of salt as a function of electric current at constant affinity, i.e., equation (19).

While the chemical reaction and electric current in our case are interdependent, the ratio between them is not constant. In this sense the system cannot be treated as an ordinary electrochemical cell. The tightness of coupling between two processes may be expressed quantitatively by the degree of coupling q, defined by reference 3.

$$q = -\frac{R_{12}}{\sqrt{R_{11}R_{22}}} \tag{20}$$

in the case where Onsager symmetry holds. (In the absence of symmetry, equation (20) defines q_{21}.) Then $q^2 \leqslant 1$, where the equality sign refers to fully coupled systems. In an asymmetrical system $q_{12}q_{21} \leqslant 1$, but in the limit of complete coupling $q_{12}^2 = q_{21}^2 = 1$ and the cross-coefficients must be equal (4).

From equation (16) it is readily seen that, for a given affinity,

$$\frac{(J_r)_{I=0}}{(J_r)_{E=0}} = 1 - q^2 \tag{21}$$

Thus, a determination of the rate of reaction at open circuit and at short circuit measures the degree of coupling directly.

Another method of determining the degree of coupling directly is based on the relation:

$$\left(\frac{\partial I}{\partial J_r}\right)_E \left(\frac{\partial J_r}{\partial I}\right)_{A^\circ} = q^2 \tag{22}$$

In this case it is only necessary to determine short circuit reaction rate and current to obtain the first term above, since

$$\left(\frac{\partial I}{\partial J_r}\right)_E = \left(\frac{I}{J_r}\right)_{E=0} \tag{23}$$

The second term may be obtained by varying the potential at constant affinity in the outside baths.

EXPERIMENTAL

A Lucite cell was constructed which consists of five parts, two 50 ml electrode compartments P_1 and P_2 (for measuring potentials), two 100 ml compartments I and II in which Teflon-coated magnetic stirrers are inserted, and one 10 ml inner com-

partment *i*. The stirrers are driven by an arrangement constructed to fit the air thermostat used. The latter consists of a wooden box with a glass window, fitted with a heating coil and a bimetallic thermoregulator (American Instrument Company, Silver Spring, Maryland); the air is circulated by two muffin fans (Rotron Mfg. Co., Woodstock, N. Y.). The experiments were done at a constant temperature of 40.0 ± 0.5°C. The electromotive force was monitored by a recorder (Varian G-11a) (Varian Associates, Palo Alto, Calif.) and accurate measurements were carried out by a voltmeter [both Keithley Electrometer 620 (Keithley Instruments, Inc., Cleveland, Ohio) and Philips microvoltmeter GM 6020 (Philips Co., Eindhoven, Holland) were used]. Current was passed by means of a constant current supply (Regatron C612, Electronic Measurements, Co., Inc., Eatontown, N. J.). The electrodes reversible to the ammonium ion are the following half-cells P_1 and P_2 :

$$\text{Ag/AgCl, } NH_4Cl(0.01 \text{ N}) \mid M \mid$$

The current-carrying electrodes c_1 and c_2 consist of the half-cells:

$$\text{Ag/AgCl, } NH_4Cl(0.01 \text{ N}) \mid M \mid \text{ } NH_4NAG \text{ } 0.01 \text{ N} \mid \text{salt bridge} \mid$$

In this system M is a permselective cation-exchange membrane (AMF ion C-60, American Machine & Foundry Co., New York) equilibrated with the ammonium ion. The salt bridges are saturated NH_4NAG-agar bridges made of polythene tubing. The current-carrying half-cells are made in such a way that no chloride can enter the system. Care must be taken that only the uncharged substrate (AGDA) and the neutral salt (NH_4NAG) are present in the system. Reversible silver–silver chloride electrodes were made by electrolytic deposition of silver chloride on silver wire according to a procedure described by Janz (5).

For the composite inner structure two sets of membranes were used with different properties. Both sets contain the same membrane β: a cation-exchange membrane (TNO C60) equilibrated with ammonium ion. However, they differ in membrane α. In the first set an anion-exchange membrane α_I (AMF ion A-60, American Machine & Foundry Co.) is used, in the second set, an anion exchange membrane α_{II} (TNO A60) which was treated with strong alkali (~1 N NaOH) for 24 hr, and accordingly lost most of its charged groups. Both membranes α_I and α_{II} are equilibrated with the N-acetyl glutamine ion as negative counterion. The membrane area is 20 cm^2.

A good deal of work was invested in the search for a suitable substrate. The resulting material, N-acetyl-L-glutamic acid diamide (AGDA) mp 194°C, $[\alpha]_D^{30°} = 5.4$ (in 1 % H_2O) has not previously been reported and was synthesized by a straightforward procedure. The N-acetyl-L-glutamine (NAG), mp 199–200°C $[\alpha]_D^{30°} = -12.2$ (in 5% H_2O) was synthesized according to a procedure for synthesis of

acetylglutamine by Karrer et al (6). In later experiments, both substrate and product (salt) were obtained commercially (Yeda Research & Development Co. Ltd., Rehovoth, Israel). The enzyme papain (2 × crystallized, Worthington Biochemical Corp., Freehold, N. J.) was assayed pH-statically by a titrator in conjunction with a Titrigraph (TTT1/SBR2 Radiometer Co., Copenhagen, Denmark) on the substrate benzoyl-L-arginine ethyl ester before use. As a chelating and reducing agent BAL (1,2-dimercaptopropanol) was added to the medium. BAL is virtually an uncharged material, in contrast to the usual reagent cysteine-EDTA.

The progress of the reaction was evaluated by measuring the ammonia production by the Conway microdiffusion technique (7). The AGDA was analyzed by hydrolysis in a Kjeldahl microdistillation apparatus and subsequent titration.

The first series of experiments (i) was carried out under conditions of zero current. For each run, the affinity in the outside compartments was varied by adjusting the concentrations of substrate and product. A stationary value for the potential was reached after a maximal time of 24 hr. Some experiments were started off at zero time with a potential above the stationary-state value, by adding a more concentrated salt solution in the central compartment. Each experiment was repeated at least twice, and the following blank experiments were also done: (*a*) without papain but with substrate—to check whether hydrolysis takes place without papain; (*b*) with papain but without substrate—to check whether a potential is built up through the products of self-digestion of papain. In both cases the potential remained zero in the course of an experiment (24 hr). To show that the activity of the papain does not decrease during this period, the papain was assayed on benzoyl-L-arginine ethyl ester before and after each experiment. In order to obtain the value for R_{12}/R_{22} the stationary-state potential was determined for each value of the affinity.

The second series of experiments (ii) was carried out under conditions of constant affinity. We measured the flow of products, by analysis of ammonia, for a series of values of constant current. The variations in concentration of the product which were measured did not exceed 20% during an experiment, while the variations in substrate concentration were very small, less than 1%. The variations in the substrate and product concentrations alter the external affinity no more than 7%. A large number of experiments were carried out in order to find the right conditions for measuring reaction flow as function of electrical current, since a current of 1 ma causes electrolysis of water in the central compartment. Apparently, the rate of flow of salt out of the inner compartment at this current is much too high in comparison with the rate of reaction, and this compartment is consequently desalinated. An applied potential of 9 v is enough to split water. Experiments done at currents up to 300 μa do not show any of these side effects. After about 12 hr, a stationary state was reached (observed by recording the potential, which is time-independent in this state) and samples were taken for chemical analysis at intervals of 8 hr approximately. A typical run lasted 48 hr.

The properties of the separate elements of the composite "membrane" were measured independently as follows: (*a*) Transport number τ_1, by measurement of membrane potential for a given concentration difference (8); (*b*) Permeability coefficient ω, by measurement of flow for a given concentration difference; (*c*) Electrical conductance κ, by passing a given current and measuring the potential at the surface of the membrane by two probe electrodes (9); (*d*) The kinetic constants of the reaction, by carrying out a series of experiments in solution for different concentrations of substrate and product, and measuring the rate of reaction by the Conway microdiffusion method (7) on samples taken at different time intervals.

These kinetic measurements were carried out using the same enzyme concentration (1 mg/ml) as used in the active transport system.

The degree of coupling was determined in the following way: the system was allowed to approach the stationary state at zero current, and in the stationary-state

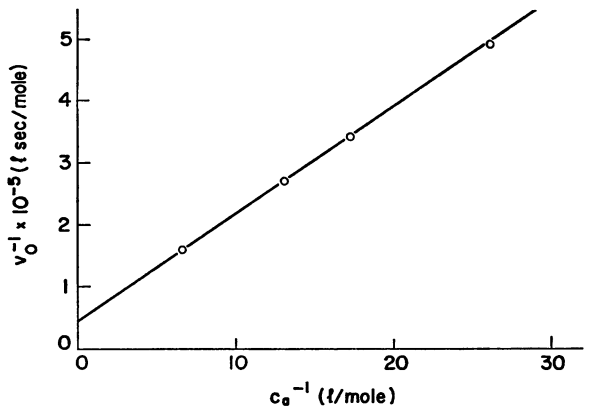


FIGURE 2 Lineweaver-Burke plot: V_0 is the initial velocity, i.e. in the absence of product, c_a is the concentration of substrate. The measurements were carried out at 1 mg/ml enzyme concentration. $V = 2.5 \times 10^{-5}$ mole $l^{-1}sec^{-1}$; $K_m = 0.432$ mole l^{-1}.

samples were taken at 8-hr intervals. Subsequently, current was passed and adjusted manually until the potential became zero. When the short circuit current reached stationarity (after approximately 8 hr) samples were taken and analyzed.

RESULTS AND DISCUSSION

The purpose of the present investigation is the clarification of the cross-relations between chemical reaction and electric current in a synthetic system. Since the substrate had not previously been described, it was necessary to determine first the equilibrium constant and kinetic coefficients of the reaction itself.

The kinetic experiments were conducted in the nonsaturated region, and a Lineweaver-Burke plot was drawn to obtain values for the Michaelis-Menten constant K_m and the maximum velocity V.

TABLE I

THE VALUES OF THE KINETIC (k_a, k_s) AND EQUILIBRIUM (K) CONSTANTS OF THE REACTION

$$a \underset{k_s}{\overset{k_a}{\rightleftharpoons}} s^2$$

FROM MEASUREMENTS OF THE REACTION VELOCITY v AT TWO DIFFERENT SUBSTRATE CONCENTRATIONS (c_a) AND A SERIES OF DIFFERENT PRODUCT CONCENTRATIONS (c_s).

The reaction velocity v is denoted v_o when the product concentration c_s is zero. The measurements were carried out at 1 mg/ml enzyme concentration.

c_a	c_s	v $\left(\frac{\text{mole}}{\text{cm}^3\text{sec}}\right)$ $\times 10^9$	$v_o - v$ $\left(\frac{\text{(mole)}}{\text{cm}^3\text{sec}}\right)$ $\times 10^9$	$k_a = \frac{v_o}{c_a}$ $(\text{sec}^{-1}) \times 10^5$	$k_s = \frac{v_o - v}{c_s^2}$ $(\text{sec}^{-1}\text{M}^{-1}) \times 10^4$	$K = k_a/k_s$
M	M					M
0.0382	—	2.035	—	5.42	—	—
0.0382	0.0139	1.865	0.170	5.42	8.80	0.0613
0.0382	0.0173	1.865	0.170	5.42	5.68	0.0952
0.0382	0.0208	1.750	0.285	5.42	6.60	0.0820
0.0382	0.0242	1.677	0.358	5.42	6.12	0.0883
0.0574	—	2.93	—	5.10	—	—
0.0574	0.0173	2.65	0.28	5.10	9.36	0.0545
0.0574	0.0208	2.55	0.38	5.10	8.78	0.0575
0.0574	0.0242	2.53	0.40	5.10	6.83	0.0745
		Average		5.26	7.18	0.0733

Table I shows that the velocity of the reaction decreases with increasing salt concentration. The equilibrium constant was determined from these measurements in the following way. Assume the usual steady-state reaction scheme

$$E + S \underset{k_{-1}}{\overset{k_{+1}}{\rightleftharpoons}} ES \underset{k_{-2}}{\overset{k_{+2}}{\rightleftharpoons}} EP \underset{k_{-3}}{\overset{k_{+3}}{\rightleftharpoons}} E + P \tag{24}$$

where E, S, and P represent enzyme, substrate, and product, respectively. It is readily shown that the net rate of reaction from left to right, per unit total concentration of enzyme, is given in reference 10:

$$v = \frac{k_{+1}\,k_{+2}\,k_{+3}[S] - k_{-1}\,k_{-2}\,k_{-3}[P]}{k_{-1}k_{-2} + k_{-1}k_{+3} + k_{+2}k_{+3} + k_{+1}(k_{+2} + k_{-2} + k_{+3})[S] + k_{-3}(k_{-1} + k_{+2} + k_{-2})[P} \tag{25}$$

In our case the substrate is an amide, while the product is a salt which we may consider fully dissociated at the low concentrations used. If the amide and salt concentrations are given by c_a and c_s respectively, we have

$$[S] \equiv c_a$$
$$[P] \equiv c_s^2 \tag{26}$$

Introducing equations (26) we can write the following useful transformation of equation (25):

$$v = \frac{V[1 - (c_s^2/Kc_a)]}{1 + (K_m/c_a) + (Bc_s^2/c_a)} \tag{27}$$

where

$$V = \frac{k_{+2}\, k_{+3}}{k_{+2} + k_{-2} + k_{+3}}$$

$$K = \frac{k_{+1}\, k_{+2}\, k_{+3}}{k_{-1}\, k_{-2}\, k_{-3}}$$

$$K_m = \frac{k_{-1}\, k_{-2} + k_{-1}\, k_{+3} + k_{+2}\, k_{+3}}{k_{+1}(k_{+2} + k_{-2} + k_{+3})}$$

$$B = \frac{k_{-3}(k_{-1} + k_{+2} + k_{-2})}{k_{+1}(k_{+2} + k_{-2} + k_{+3})}$$

When $c_s \simeq 0$, equation (27) reduces to the Michaelis-Menten equation. We denote the value of v measured under these conditions by v_o. The quantity K is seen to be the over-all equilibrium constant for the reaction. Now if the following conditions hold,

$$c_a \ll K_m$$

$$c_s^2 \ll K_m/B \tag{28}$$

equation (27) takes the form

$$v = k_a c_a - k_s c_s^2 \tag{29}$$

where

$$k_a = \frac{V}{K_m} \qquad k_s = \frac{V}{KK_m} \tag{30}$$

Consequently,

$$K = k_a/k_s. \tag{31}$$

In the linear region, where both substrate and product concentrations are low, the conditions (28) are almost certainly satisfied: at zero product concentration K_m may be regarded as the substrate concentration required for half-maximal velocity. An expression of the same form as equation (29) would be obtained directly on

the basis of the simple reaction scheme

$$S \underset{k_s}{\overset{k_a}{\rightleftharpoons}} P \tag{32}$$

The quantities k_a and k_s are most readily determined by measuring the reaction velocity, at a given substrate concentration, in the absence and presence of product. In Table I the velocities at two amide concentrations and several different salt concentrations, and the corresponding values of k_a, k_s, and K, are given. The average value for K from a series of nine experiments is 0.073 M.

The affinity, A^o, may be written as follows, since we are dealing with very dilute

TABLE II

STATIONARY-STATE ELECTROMOTIVE FORCE OBTAINED AT DIFFERENT VALUES OF THE OUTER AFFINITIES FOR TWO DIFFERENT ACTIVE TRANSPORT SYSTEMS I AND II

System	External affinity A^o	Electromotive force ε (volt) $\times 10^3$
	kcal/mole	
I	1.31	28.2
	1.53	31.0
	1.60	35.1
	1.96	42.0
	2.17	47.5
	2.34	48.0
	2.53	52.2
	2.63	53.4
II	1.31	15.5
	1.55	19.1
	1.91	25.0
	2.10	27.5
	2.39	33.2
	3.03	39.8

solutions:

$$A^o = RT \ln (K c_a^o/(c_s^o)^2) \tag{33}$$

If reaction and electric current are indeed coupled an electric potential will develop across the composite membrane at open circuit when $A^o \neq 0$.

In Table II the open circuit potential is given at different external affinities for the two sets of membranes. Fig. 3 shows the electromotive force multiplied by the Faraday (in kilocalories/mole) versus A^o. Following convention the sign of the electro-

motive force of the cell, $\mathcal{E}$, is opposite to that of E as defined by equation (12): $\mathcal{E} = -E_{I=o}$ (3). It is remarkable that the response of the electromotive force to the change of the affinity is linear, even at high affinities.

In Fig. 4 stationary-state reaction flow is plotted against applied current for the two sets of membranes. The experiments were carried out at an external affinity of $A^o = 2.6$ kcal/mole. The reaction flow is measured as the flow of the ammonium ion and is expressed as NH_4^+ current.

All the data on the measurement of the Onsager coefficients were subjected to a regression analysis as reported in Table III. Although the agreement between observed values of the ratios $R_{12}F/R_{22}$ and $R_{21}F/R_{22}$ is satisfactory in both membrane sets, a slight but possibly significant deviation from symmetry was found at the lower degree of coupling.

As shown by the value of K the equilibrium is at nearly complete hydrolysis

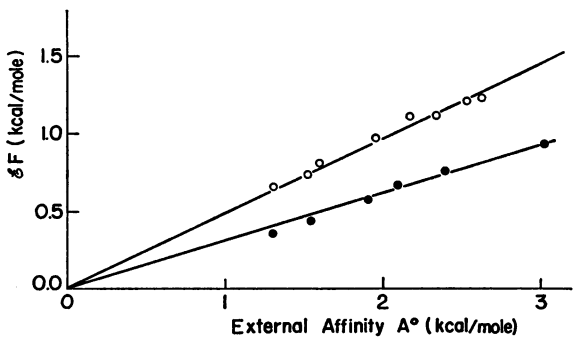


FIGURE 3 Stationary-state electromotive force ($\mathcal{E}$): a plot of $\mathcal{E}F$ in kilocalories/mole (volts × 23.1) versus the external affinity of the reaction (A^o) for the two active transport systems. ○, system I; ●, system II; the experiments were carried out at zero current.

under the condition (28). The back reaction is not accessible by chemical analysis. However, in our setup the reverse reaction in the presence of an excess of salt can be detected. Production of AGDA and depletion of NH_4NAG in the central compartment should give rise to a negative potential. The same concentration of papain was used as previously, and to all compartments a solution of 0.1 N NH_4NAG was added. With set I membranes, a stationary-state potential of -2 mv was obtained.

The finite value of R_{12} in this system does not stem from a direct coupling of I and J_r at any point, but from the localization of the reaction and the permselectivity of the membranes (stationary-state coupling). All coefficients can thus be expressed in terms of the reaction kinetics and the transport coefficients through the membrane. A starting point for this analysis is given by the equations for each separate membrane element at zero volume flow (11):

$$I/\kappa_\alpha = E_\alpha - \tau_1^\alpha \frac{\Delta\pi_s^\alpha}{Fc_s^{*\alpha}}$$

$$I/\kappa_\beta = E_\beta - \tau_1^\beta \frac{\Delta\pi_s^\beta}{Fc_s^{*\beta}} \tag{34}$$

$$J_s^\alpha = \omega_s^\alpha \Delta\pi_s^\alpha - (\tau_1^\alpha/F)I$$

$$J_s^\beta = \omega_s^\beta \Delta\pi_s^\beta - (\tau_1^\beta/F)I \tag{35}$$

in which $\Delta\pi_s$ is the difference in salt osmotic pressure, $J_s = J_-$ is the flow of the NAG ion, and c_s^* is the average concentration, as defined previously (11), between the internal membrane compartment and the external compartments. The coefficients are: the permeability ω, the electrical conductance κ, and the transport number of

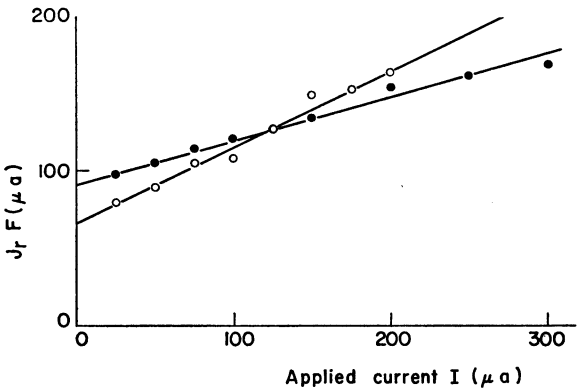


FIGURE 4 Stationary-state reaction flow (J_r) versus the applied current (I). The reaction flow is measured as the flow of ammonium ion in the stationary state and is expressed as current flow in μa. The experiments were carried out at an external affinity A^o = 2.6 kcal/mole on the two active transport systems. ○, system I; ● system II. (The measured total fluxes are given instead of flux densities since only the slopes of the lines are of interest.)

the NAG ion τ_1. It is assumed here that there is negligible coupling between the flow of AGDA and the flows of the NAG or ammonium ions.

From identity of concentrations in compartments I and II:

$$\Delta\pi_\alpha = -\Delta\pi_\beta = \Delta\pi \quad \text{and} \quad c_s^{*\alpha} = c_s^{*\beta} = c_s^* \tag{36}$$

Combining equations (34) and making use of equations (36):

$$I(1/\kappa_\alpha + 1/\kappa_\beta) = E - \frac{\tau_1^\alpha - \tau_1^\beta}{F} \frac{\Delta\pi_s}{c_s^*} \tag{37}$$

in which $E = E^\alpha + E^\beta$. The flow of salt obeys the stationarity conditions, equations (14).

From equations (14) and (35) and making use of equations (36):

$$J_r + (\omega_s^{\alpha} + \omega_s^{\beta})\Delta\pi_s - \frac{\tau_1^{\alpha} - \tau_1^{\beta}}{F} I = 0 \tag{38}$$

Introducing $\Delta\pi_s$ from equation (38) into (37) and rearranging:

$$E = \left[- \frac{\tau_1^{\alpha} - \tau_1^{\beta}}{c_s^*(\omega_s^{\alpha} + \omega_s^{\beta})F} \right] J_r + \left[1/\kappa_{\alpha} + 1/\kappa_{\beta} + \frac{(\tau_1^{\alpha} - \tau_1^{\beta})^2}{c_s^*(\omega_s^{\alpha} + \omega_s^{\beta})F^2} \right] I \tag{39}$$

In order to evaluate J_r we assume a linear relationship between the rate of chemical reaction and the affinity of the reaction in the inner compartment, an assumption

TABLE III

THE RATIOS OF PHENOMENOLOGICAL COEFFICIENTS (R_{12}/R_{22} AND R_{21}/R_{22}) OBTAINED BY REGRESSION ANALYSES OF TWO INDEPENDENT SETS OF MEASUREMENTS

From measurements of the stationary electromotive force ($\mathcal{E}$) as a function of the outer affinity (A^o) at zero electric current the ratio R_{12}/R_{22} is obtained, while from the measurements of stationary reaction flow (J_r) as a function of the electric current (I) at constant outer affinity the ratio R_{21}/R_{22} is obtained. The measurements were carried out on two active transport systems (I and II), which differ in their degree of coupling (q). The values given for q are taken from Table VI. F is the Faraday constant.

System	Degree of coupling q	Cross-coefficient functions		
		Measurement	Value from regression analysis	Standard error
I	0.80	$\mathcal{E}F$ vs. A^o: $-R_{12}F/R_{22}$	0.47	0.02
		J_rF vs. I: $-R_{21}F/R_{22}$	0.50	0.03
II	0.53	$\mathcal{E}F$ vs. A^o: $-R_{12}F/R_{22}$	0.31	0.01
		J_rF vs. I: $-R_{21}F/R_{22}$	0.27	0.02

which will hold close to equilibrium:

$$J_r = L_r A^i \tag{40}$$

In view of equation (8),

$$A^0 = A^i + \frac{\Delta\pi_a}{c_a^*} - \frac{\Delta\pi_s}{c_s^*} \tag{41}$$

since

$$\Delta\mu_s^{\alpha} = \Delta\pi_s^{\alpha}/c_s^{*\alpha} = \Delta\pi_s/c_s^* \tag{42}$$

and

$$\Delta\mu_a{}^{\alpha} = \Delta\pi_a{}^{\alpha}/c_a{}^{*\alpha} = \Delta\pi_a/c_a{}^{*} \tag{43}$$

where $\Delta\pi_a$ and $c_a{}^{*}$ are the osmotic pressure and average concentrations of AGDA between inner and outer compartments. (Any terms arising from a small pressure difference between the internal and external solutions will essentially cancel in A^o and are neglected.)

The rate of disappearance of the amide is balanced by the influx through both membranes (cf. equations (14)):

$$J_r = (\omega_a{}^{\alpha} + \omega_a{}^{\beta})\Delta\pi_a \tag{44}$$

Substitution of the expressions for A^i, $\Delta\pi_a$, and $\Delta\pi_s$ from equations (40), (44), and (38) into equation (41) gives

$$A^o = \left[\frac{1}{L_r} + \frac{1}{c_a{}^{*}(\omega_a{}^{\alpha} + \omega_a{}^{\beta})} + \frac{1}{c_s{}^{*}(\omega_s{}^{\alpha} + \omega_s{}^{\beta})}\right] J_r - \left(\frac{\tau_1{}^{\alpha} - \tau_1{}^{\beta}}{c_s{}^{*}(\omega_s{}^{\alpha} + \omega_s{}^{\beta})F}\right) I \tag{45}$$

If we compare equations (39) and (45) with equations (16) we see that we can identify the phenomenological coefficients with the single membrane and kinetic coefficients in our derivation:

$$R_{11} = \left[1/\kappa_{\alpha} + 1/\kappa_{\beta} + \frac{(\tau_1{}^{\alpha} - \tau_1{}^{\beta})^2}{c_s{}^{*}(\omega_s{}^{\alpha} + \omega_s{}^{\beta})F^2}\right] \tag{46}$$

$$R_{12} = R_{21} = -\frac{\tau_1{}^{\alpha} - \tau_1{}^{\beta}}{c_s{}^{*}(\omega_s{}^{\alpha} + \omega_s{}^{\beta})F} \tag{47}$$

$$R_{22} = [1/L_r + 1/c_a{}^{*}(\omega_a{}^{\alpha} + \omega_a{}^{\beta}) + 1/c_s{}^{*}(\omega_s{}^{\alpha} + \omega_s{}^{\beta})] \tag{48}$$

Since the reaction in equations (16) is a scalar process, while the current is a one-dimensional flow, R_{12} is the corresponding component of a vectorial cross-coefficient. The directionality of the composite membrane shows up in R_{12} in the difference of the transport numbers. By assuming symmetrical cross-coefficients in the transport equations for the individual elements, this treatment predicts the validity of the Onsager reciprocal relations for the system as a whole *when the internal reaction is close to equilibrium* ($A^i \ll RT$).

Membrane conductance, permeability coefficients, and transport numbers were determined for each of the single membranes. The values of these transport coefficients are given in Table IV.

The values of R_{ij} can be predicted from equations (46)–(48) only if the entire system is close to equilibrium, otherwise the average concentrations are unknown.

Even then, since we cannot carry out kinetic measurements close to equilibrium we have no way of estimating the reaction coefficient L_r.

In the treatment above we assume linear relations between *all* forces and flows. While permeabilities and transport numbers in charged membranes may in fact depend only weakly on the electrolyte concentration, this approximation is certainly not justified for the chemical reaction coefficient. A better approach is to use the correct kinetic equations for the reaction velocity together with linear transport equations for the membranes; the inner concentrations of amide and salt at the stationary state can then be obtained in the following way.

From equation (29)

$$J_r = \bar{k}_a c_a{}^i - \bar{k}_s (c_s{}^i)^2 = J_r{}^o - \bar{k}_a \Delta c_a + \bar{k}_s \Delta c_s \tag{49}$$

TABLE IV

TRANSPORT COEFFICIENTS, MEASURED FOR EACH MEMBRANE SEPARATELY

α_I and α_{II} are anion exchange membranes used in the two different active transport systems, while β is the cation exchange membrane used in both systems.

Coefficient		Membrane α_I	Membrane α_{II}	Membrane β
Transport No. τ_1		0.86	0.63	0.12
$\tau_1{}^\alpha - \tau_1{}^\beta$		0.74	0.51	
Permeability ω_s	(mole/dyne sec)	1.36×10^{-17}	4.21×10^{-17}	1.26×10^{-17}
$\omega_s{}^\alpha + \omega_s{}^\beta$	(" " ")	2.62×10^{-17}	5.47×10^{-17}	
ω_a	(mole/dyne sec)	6.4×10^{-16}	7.40×10^{-16}	6.40×10^{-16}
$\omega_a{}^\alpha + \omega_a{}^\beta$	(" " ")	1.28×10^{-15}	1.38×10^{-15}	
Resistance $1/\kappa$	$\left(\frac{\text{dyne sec cm}^3}{(\text{e.s.u.})^2}\right)$	2.6×10^{-9}	1.7×10^{-8}	3.3×10^{-11}
$1/\kappa^\alpha + 1/\kappa^\beta$	(" ")	2.6×10^{-9}	1.7×10^{-8}	

where

$$\Delta c_a = c_a{}^o - c_a{}^i \qquad \Delta c_s = c_s{}^o - c_s{}^i \tag{50}$$

and

$$J_r{}^o = \bar{k}_a c_a{}^o - \bar{k}_s (c_s{}^o)^2 \tag{51}$$

which with equation (33) can be transformed into

$$J_r{}^o = (c_s{}^o)^2 \bar{k}_s (e^{A^o/RT} - 1) \tag{52}$$

Since we have defined J_r per cm^2 of cell cross-section, we use in these equations $\bar{k}_{a,\,s} = \frac{V_i}{\alpha} k_{a,\,s}$, where V_i is the volume of the inner compartment and α the

area of the cell cross-section. The quantity $\bar{k}_s'$ is defined by

$$\bar{k}_s' = 2\bar{k}_s c_s^o \left(1 - \frac{\Delta c_s}{2c_s^o}\right) \tag{53}$$

We assume $\Delta c_s \ll 2c_s^o$ so that $\bar{k}'$ will remain approximately constant.

The stationary-state conditions, equations (38) and (44) together with equation (49) give the two linear equations for Δc_s and Δc_a

$$J_r^o - \bar{k}_a \Delta c_a + (p_s + \bar{k}_s')\Delta c_s - \frac{\tau_1^\alpha - \tau_1^\beta}{F} I = 0 \tag{54}$$

$$-J_r^o + (p_a + \bar{k}_a)\Delta c_a - \bar{k}_s' \Delta c_s = 0 \tag{55}$$

where

$$\begin{aligned} p_s &= 2RT(\omega_s^\alpha + \omega_s^\beta) \\ p_a &= RT(\omega_a^\alpha + \omega_a^\beta) \end{aligned} \tag{56}$$

The solution of these equations is

$$\Delta c_s = -\frac{J_r^o - (1 + \bar{k}_a/p_a)(\tau_1^\alpha - \tau_1^\beta)I/F}{p_s(1 + \bar{k}_s'/p_s + \bar{k}_a/p_a)} \tag{57}$$

$$\Delta c_a = \frac{J_r^o + (\bar{k}_s'/p_s)(\tau_1^\alpha - \tau_1^\beta)I/F}{p_a(1 + \bar{k}_s'/p_s + \bar{k}_a/p_a)} \tag{58}$$

By inserting these expressions for Δc_s and Δc_a back into equation (49) and substituting equation (52) for J_r^o we obtain

$$J_r = \frac{(c_s^o)^2 \bar{k}_s (e^{A^o/RT} - 1)}{1 + \bar{k}_a/p_a + \bar{k}_s'/p_s} + \frac{(\tau_1^\alpha - \tau_1^\beta)I}{F(1 + p_s/\bar{k}_s' + p_s \bar{k}_a/p_a \bar{k}_s')} \tag{59}$$

and thus

$$(J_r/I)_{A^o=0} = (\partial J_r/\partial I)_{A^o} = \frac{(\tau_1^\alpha - \tau_1^\beta)}{F(1 + p_s/\bar{k}_s' + p_s \bar{k}_a/p_a \bar{k}_s')} \tag{60}$$

This value for $-R_{21}/R_{22}$ can be calculated from the separately measured values of p and $\bar{k}$. The values for the transport numbers and permeabilities are taken from Table IV and those for the reaction rate constants from Table I. As seen in Table V the result is in good agreement with the directly measured slope $(\partial J_r/\partial I)_{A^o}$.

Equation (59) shows that, in the absence of an electric current, J_r is not a linear function of A^o. Expanding $e^{A^o/RT}$ and writing equation (59) as $J_r = (1/R_{22})A^o - (R_{21}/R_{22})I$, it is seen that $1/R_{22}$ depends on A^o (cf. equations 16).

The degree of coupling, q, is defined by a ratio of the R_{ij}'s and hence may be calculated from their measured values. Alternatively, the relation given in equation (21) may be used for a direct determination of q: For tight coupling the rate of reaction at short circuit must be much higher than at open circuit. The results for q from both methods are given in Table VI. These were obtained in a series of experi-

TABLE V

CALCULATED AND DIRECTLY MEASURED VALUES OF THE RATIOS OF PHENOMENOLOGICAL COEFFICIENTS R_{21}/R_{22} FOR THE TWO DIFFERENT ACTIVE TRANSPORT SYSTEMS (I AND II)

The values are calculated according to equation (60) from the values of individual transport parameters taken from Table IV, and those of the reaction rate coefficients taken from Table I. The measured values are taken from Table III.

System	$-R_{21}F/R_{22}$	
	Calculated from equation (60)	Measured
I	0.51	0.50
II	0.26	0.27

TABLE VI

THE DEGREE OF COUPLING (q) FOR TWO DIFFERENT ACTIVE TRANSPORT SYSTEMS (I AND II)

For a series of values of the outer affinity (A^o) measurements were made (in the stationary state) of the short circuit current (I^{sc}), the reaction rate at short circuit (J_r^{sc}), and the reaction rate at open circuit (J_r^{oc}). The values of q are then calculated from these measurements according to equations (21) and (22). The values of $(\partial J_r/\partial I)_{A^o}$ are taken from Table III. (The measured total fluxes are given instead of flux densities since only ratios are involved.)

System:	I			II	
A^o, *kcal*	1.3	2.0	2.6	1.8	2.6
I^{sc}, *μa*	145	175	220	100	110
J_r^{sc}, *μmoles/hr*	4.5	5.2	7.0	3.5	4.1
J_r^{oc}, *μmoles/hr*	1.7	1.9	2.5	2.5	3.0
$q = \sqrt{1 - J_r^{oc}/J_r^{sc}}$	0.79	0.80	0.79	0.54	0.53
$q = \sqrt{(\partial I/\partial J_r)_E(\partial J_r/\partial I)_{A^o}}$	0.77	0.79	0.77	0.53	0.52

ments using the two sets of membranes and a series of different affinities A^o. The fifth and sixth rows in this Table give the degree of coupling determined according to equations (21) and (22).

CONCLUSIONS

In the description of this "active transport" system according to the formalism of irreversible thermodynamics we assumed linear phenomenological equations, re-

lating the chemical reaction and the electric current to both driving forces. The experimental results show that the assumption of a linear relation between chemical flow and affinity is not justified. It is well known that the linear approximation for chemical reactions holds only close to equilibrium. In most membranes Ohm's law holds in a wide range, but in the bipolar arrangement used here, voltage-current curves are nonlinear (12). Nevertheless, measurements on the system as a whole, as well as calculations from the separately examined properties of the elements show that the reaction rate at constant affinity is a linear function of the electric current. Also, the open circuit potential is a linear function of the affinity, A^o. Moreover, the slopes are equal as expected from the Onsager relation:

$$\left(\frac{\partial J_r}{\partial I}\right)_{A^o} = -\left(\frac{E}{A^o}\right)_{I=0}$$

In other words, if without prior knowledge of the system we would have attempted to derive the affinity of the driving reaction from measurements of $(\partial J_r/\partial I)_{A^o}$ and the open circuit potential, assuming reciprocal relations, we would have obtained the correct result. Investigation of other experimental and mathematical models will show whether this depends on some specific property of our model system or has more general validity.

The linear relation between J_r and I might be regarded as the apparent "stoichiometry" of the system. Clearly this does not reflect either tight coupling between transport and reaction or linear kinetics.

It is of interest to consider the "fuel cell" aspect of our system using an analysis of the efficiency of energy conversion by coupled processes (3). The efficiency of energy conversion η may be defined for isothermal processes as the fraction of energy expenditure of spontaneous processes which undergoes conversion. This has been shown to depend essentially on the degree of coupling and ratio of forces. It has a maximum value given by $\eta_{max} = q^2/(1 + \sqrt{1 - q^2})^2$. The values for η_{max} calculated from measured values of q for the two sets of membranes are 25% for set I and 7% for set II. A very high degree of coupling—in our case highly permselective membranes—is necessary for energy conversion with reasonable efficiency.

The authors are greatly indebted to Prof. A. Berger for his help in choosing and synthesizing the substrate. We gratefully acknowledge a Weizmann Fellowship which made possible Dr. Caplan's stay in Rehovoth.

The investigation was supported by PHS research grants GM-09432-02 to GM-09432-04 from the National Institute of General Medical Sciences.

Received for publication 15 March 1967.

APPENDIX

When we write out all the flows which appear in the dissipation function of equation (5)

as a function of the forces we obtain the following equation:

$$\begin{bmatrix} J_r \\ J_a^{\alpha} \\ J_a^{\beta} \\ J_+^{\alpha} \\ J_-^{\alpha} \\ J_+^{\beta} \\ J_-^{\beta} \end{bmatrix} = \begin{bmatrix} L_r & 0 & 0 & 0 & 0 & 0 & 0 \\ 0 & L_a^{\alpha} & 0 & 0 & 0 & 0 & 0 \\ 0 & 0 & L_a^{\beta} & 0 & 0 & 0 & 0 \\ 0 & 0 & 0 & L_+^{\alpha} & L_{\pm}^{\alpha} & 0 & 0 \\ 0 & 0 & 0 & L_{\pm}^{\alpha} & L_-^{\alpha} & 0 & 0 \\ 0 & 0 & 0 & 0 & 0 & L_+^{\beta} & L_{\pm}^{\beta} \\ 0 & 0 & 0 & 0 & 0 & L_{\pm}^{\beta} & L_-^{\beta} \end{bmatrix} \begin{bmatrix} A^i \\ \Delta\mu_a^{\alpha} \\ \Delta\mu_a^{\beta} \\ \Delta\tilde{\mu}_+^{\alpha} \\ \Delta\tilde{\mu}_-^{\alpha} \\ \Delta\tilde{\mu}_+^{\beta} \\ \Delta\tilde{\mu}_-^{\beta} \end{bmatrix} \tag{A-1}$$

We assume that only the flows of the positive and negative ions are intrinsically coupled: $L_{\pm} = L_{\mp} \neq 0$. We can reduce this 7 by 7 set of coefficients to a 5 by 5 set by imposing the boundary conditions on the electrochemical potential differences according to equations (9) and (10). We thereby transform into a new set of flows and forces:

$$\begin{bmatrix} J_r \\ J_a^{\alpha} - J_a^{\beta} \\ J_+^{\alpha} - J_+^{\beta} \\ J_-^{\alpha} - J_-^{\beta} \\ J_+^{\beta} - J_-^{\beta} \end{bmatrix} = \begin{bmatrix} L_r & 0 & 0 & 0 & 0 \\ 0 & L_a^{\alpha} + L_a^{\beta} & 0 & 0 & 0 \\ 0 & 0 & L_+^{\alpha} + L_+^{\beta} & L_{\pm}^{\alpha} + L_{\pm}^{\beta} & L_{\pm}^{\beta} - L_+^{\beta} \\ 0 & 0 & L_{\pm}^{\alpha} + L_{\pm}^{\beta} & L_-^{\alpha} + L_-^{\beta} & L_-^{\beta} - L_{\pm}^{\beta} \\ 0 & 0 & L_{\pm}^{\beta} - L_+^{\beta} & L_-^{\beta} - L_{\pm}^{\beta} & L_+^{\beta} - 2L_{\pm}^{\beta} + L_-^{\beta} \end{bmatrix} \begin{bmatrix} A^i \\ \Delta\mu_a^{\alpha} \\ \Delta\tilde{\mu}_+^{\alpha} \\ \Delta\tilde{\mu}_-^{\alpha} \\ \Delta\tilde{\mu}_+ \end{bmatrix} \tag{A-2}$$

Inverting equation (A-2) to a set of forces as a function of flows we obtain:

$$\begin{bmatrix} A^i \\ \Delta\mu_a^{\alpha} \\ \Delta\tilde{\mu}_+^{\alpha} \\ \Delta\tilde{\mu}_-^{\alpha} \\ \Delta\tilde{\mu}_+ \end{bmatrix} = \begin{bmatrix} R_r & 0 & 0 & 0 & 0 \\ 0 & R_a & 0 & 0 & 0 \\ 0 & 0 & R_+ & R_{\pm} & R_{+c} \\ 0 & 0 & R_{\pm} & R_- & R_{-c} \\ 0 & 0 & R_{+c} & R_{-c} & R_c \end{bmatrix} \begin{bmatrix} J_r \\ J_a^{\alpha} - J_a^{\beta} \\ J_+^{\alpha} - J_+^{\beta} \\ J_-^{\alpha} - J_-^{\beta} \\ J_+^{\beta} - J_-^{\beta} \end{bmatrix}. \tag{A-3}$$

When we invoke the stationary-state conditions equations (14), substitute the outer affinity according to equation (8), and substitute for $\Delta\tilde{\mu}_+$ and $J_+^{\alpha} - J_-^{\beta}$ according to equations

(12) and (13), we obtain the 2 by 2 matrix:

$$\begin{bmatrix} A^o \\ E \end{bmatrix} = \begin{bmatrix} (R_r + R_a + R_+ + 2R_\pm + R_-) & -\dfrac{(R_{+c} + R_{-c})}{F} \\ -\dfrac{(R_{+c} + R_{-c})}{F} & R_c/F^2 \end{bmatrix} \begin{bmatrix} J_r \\ I \end{bmatrix}. \quad \text{(A-4)}$$

SYMBOLS

A	affinity of the reaction: $A \equiv -\sum \nu_i \mu_i$ (the ν_i are stoichiometric coefficients)
α	cell cross-section
B	$\equiv \dfrac{k_{-3}(k_{-1} + k_{+2} + k_{-2})}{k_{+1}(k_{+2} + k_{-2} + k_{+3})}$
$c_{a,s}$	concentration of amide and salt
$c^*_{a,s}$	average concentrations of amide and salt between inner and outer compartments defined by $c^* \equiv \Delta c/\Delta \ln c$
E	electrical potential difference between reversible electrodes in compartments I and II
$\mathcal{E}$	electromotive force of the cell
F	Faraday constant
I	electrical current density
$J_k^{\alpha,\beta}$	flow of species k through membranes α, β
J_r	flow of chemical reaction: number of moles converted per unit cell cross-section
J_r^o	$\equiv \bar{k}_a c_a^o - \bar{k}_s (c_s^o)^2$
K	equilibrium constant of the reaction
K_m	Michaelis-Menten constant
k_l	rate constant for every intermediate step in the enzyme reaction $l = \pm 1, \pm 2, \pm 3$
$k_{a,s}$	rate constants for the over-all chemical reaction
$\bar{k}_{a,s}$	rate constants for J_r; $\bar{k}_{a,s} \equiv k_{a,s} V_i \alpha /$, $\bar{k}'_s \equiv 2\bar{k}_s c_s^o (1 - \Delta c_s/2c_s^o)$
L_r	reaction coefficient
$p_{a,s}$	total permeability constant of amide and salt for both membranes $p_a \equiv RT(\omega_a^\alpha + \omega_a^\beta) \qquad p_s \equiv 2RT(\omega_s^\alpha + \omega_s^\beta)$
q	degree of coupling
R_{ij}	phenomenological coefficients for the complex membrane system
V	maximum velocity of the chemical reaction
v	velocity of the chemical reaction
v_o	initial value of v (measured when $c_s = 0$)
V_i	volume of the inner compartment
$\Delta\tilde{\mu}_k^{\alpha,\beta}$	electrochemical potential difference of species k between inner and outer compartments $\Delta\tilde{\mu}_k^\alpha = \tilde{\mu}_k^{\mathrm{I}} - \tilde{\mu}_k^i \qquad \Delta\tilde{\mu}_k^\beta = \tilde{\mu}_k^i - \tilde{\mu}_k^{\mathrm{II}}$
$\Delta\pi_{a,s}^{\alpha,\beta}$	osmotic pressure difference of amide and salt between inner and outer compartments $\Delta\pi_{a,s}^\alpha = \pi_{a,s}^{\mathrm{I}} - \pi_{a,s}^i = \Delta\pi_{a,s}$ $\Delta\pi_{a,s}^\beta = \pi_{a,s}^i - \pi_{a,s}^{\mathrm{II}} = -\Delta\pi_{a,s}$
$\omega_{a,s}^{\alpha,\beta}$	permeability of amide and salt through membranes α and β
$\kappa^{\alpha,\beta}$	electrical conductivity of membranes α and β
$\tau_1^{\alpha,\beta}$	transport number of membranes α and β for the anion

Superscripts

α, β anion-exchange and cation-exchange membranes
i, o inner and outer compartments
I, II outer compartments I and II

Subscripts

a amide
s salt
$+, -$ cation and anion

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