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A THEORY OF COUPLED TRANSPORT IN CELLS

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SUMMARY

1. Simple physical descriptions of living cells involving only binding enzymes and semipermeable membranes lead to a diffusion and confined reaction description of coupled transport in cells. Diffusive fluxes, such as those of Na^+ and glycine or alanine, are strongly coupled when the diffusion occurs through regions of confined chemical reactions.

2. A general theory of the diffusion of chemical species through a region of confined chemical reactions containing a multiple binding enzyme is developed. An equilibrium assumption is made such that an analytical function can be obtained for the flux of transferring species through the region of confined reaction. This function depends upon the concentrations of transferring chemical species at the boundaries of the confined reaction region.

3. The theory is applied to experimental data from the literature. In particular, it is applied to the coupling of Na^+ and alanine in rabbit ileum and to the coupling of Na^+ and glycine in pigeon red cells. The agreement of the theory to these data, as well as other reported data, indicates that confined reactions are possibly the cause of flux coupling in living systems.

INTRODUCTION

Many types of cells are capable of accumulating metabolites, *i.e.* they are able to maintain a concentration difference of amino acids or sugars between the interior and exterior of the cell. At first, the ability to maintain these concentration differences of metabolites and also to transport these metabolites against the concentration differences was called active transport because no physical processes were known at that time which could explain this phenomenon. Recently, it has been found that the transport of sugar and amino acids in various cells appear to depend upon the simultaneous transport of Na^+. This discovery simplified the problem of the transport of these metabolites against concentration gradients by transferring it from the realm of active transport into coupled transport. However, the sodium transport remains active.

With experiments performed in 1952, CHRISTENSEN and co-workers[1,2] showed that the replacement of sodium by potassium in the extracellular medium of Ehrlich ascites cells caused a decrease in the transfer of amino acids into the cells. At first,

they attributed this loss of the ability to accumulate amino acids to the presence of K^+ rather than the absence of Na^+. They also understood that the energy for the accumulation of the amino acids could come from the potential energy inherent in the electrochemical gradients of Na^+ or K^+. By replacing sodium with potassium, magnesium, or choline in the mocosal medium, CSAKY AND THALE[3] presented evidence that the Na^+ gradient was required for the transport of sugar across the intestinal wall of the toad. Besides these studies, others have shown a linkage between the transfer of the Na^+ and amino acids or sugars for Ehrlich ascites tumor cells[4], red blood cells[5–9], leukocytes[10], skeletal muscle[11], kidney[12], and Ehrlich ascites cells[13–15]. From these experiments, it appears that this coupling phenomenon is found in a wide variety of living cells.

These experiments show that there are several general properties associated with these coupled transport systems. Experiments performed by changing the extracellular metabolite concentration, while holding all other concentrations constant, appear to indicate that the transfer of the metabolite is occurring by two different processes; one being diffusional and the other facilitated. The diffusion process appears to be linear in the extracellular metabolite concentration. The rate of transport by the facilitated mechanism appears to follow a Michaelis–Menten type curve, *i.e.* the rate of transport is constant at large values of the metabolite concentration and linear during the low range. Also, if the ratio of intracellular metabolite to extracellular metabolite is measured during steady state, it appears that this ratio is inversely proportional to the extracellular metabolite concentration. Facilitated transfer appears to be in the same direction as that of the sodium gradient. Since the sodium gradient usually is such that the extracellular concentration is higher, the usual facilitated transport of metabolite is from the extracellular medium into the cell. However, if the direction of the Na^+ gradient is reversed, experimental evidence seems to indicate that the direction of facilitated metabolite transfer also changes. Thus, it appears that both intracellular and extracellular sodium concentrations (and not just the sodium gradient) are important in the facilitated transport mechanism.

Other questions about the type of transport have been experimentally investigated. For example the energy source for transport has been tested to determine if all the energy for transport of the amino acid or sugar comes from the Na^+ gradient, or if some of this energy comes from cellular metabolism. The question of a bound species of amino acid or Na^+ in the cell has been investigated. Other experimentors have been interested in the stoichiometric relationship between the metabolite transferred and the sodium transferred. All of these investigations have been rather general (based upon conservation of energy and mass) because no suitable theory has been proposed that would suggest more detailed experimentation.

Previous theories which have been developed to explain the phenomena described above are summarized in a book by STEIN[16], and elsewhere by LAUGER[17]. STEIN[16] suggests the division of these theories into four categories, mobile carrier models, pore models, superficial enzymes, and transport enzymes as part of the cell membrane. All of these models view the transport as occurring solely in the membrane. The most extensively used model assumes a carrier to exist in the membrane. This carrier reacts on one side with the metabolite, translocates across the membrane by some process and releases a metabolite on the other side. The carrier then returns to the opposite side of the membrane to repeat the process. Various carrier systems

have been proposed; some of these models envision a complicated cycling of the carriers while others suppose a complicated series of reactions on one side or the other of the membrane followed by the simple translocation reaction.

The purpose of this paper is to present a molecular theory of coupled transport which explains many of the phenomena described above. This theory is based on a simplified view of the cell postulating only diffusion, reaction, and a semipermeable membrane. The reactions involved are assumed to be of a particular enzymatic type. The equations describing the concentration profiles and fluxes of the transferring species as well as the enzyme and its complexes are derived. Since these equations cannot be solved, equilibrium assumptions are used to obtain analytical expressions for the flux of the transferring species. Predictions from these flux equations are then compared with experimental observations. Two of these comparisons are given in detail in this paper, others are discussed.

Physical description of the cell

Probably there are many physical descriptions of cells which will give rise to the diffusion and confined reaction scheme proposed in this paper. Thus the same basic mechanism may be working in cells of quite different construction. Two different generally known simplified descriptions of cells will be presented, both of which lead to the same diffusion and confined reaction theory for the explanation of the coupled transport.

The first description of the cell assumes that the cell contents are separated from the external media by the cell membrane, which is permeable to ions and metabolites but impermeable to complex biological molecules contained therein. This semipermeability is the only property required of the membrane; in contrast, previous theories have postulated very complex properties (*e.g.* carriers) for the membrane. The interior of the cell may be either compartmentalized or homogeneous. Near the inside of the cell membrane, there is assumed to be a layer (or film) of liquid across which the concentration gradients of sodium and metabolites are assumed to exist. This film could be well structured if the cell is compartmentalized, or it could be a rather arbitrary distance (*i.e.* film theory mass transfer) into the cell in the case of a homogeneous cell. An enzyme capable of forming complexes with the transferring chemical species by a series of reversible reactions is postulated to exist in this liquid film as well as throughout the cell.

The second description of the cell does not involve the cell membrane, rather it involves enzymes permanently attached to the cell wall. In this case, the enzymes are assumed to be long chain molecules attached at one end with the enzymatic activity at the other. Thus the enzymatic portion of the molecule is able to diffuse and react in a restricted region near the cell wall. This description once again gives rise to a region of a confined reaction through which Na^+ and metabolites diffuse. The confined region of reaction is maintained in this case by enzyme attachment to the cell wall.

These are just two of many possible physical situations which could give rise to regions of confined reactions through which chemical species diffuse. Fig. 1 shows the general phenomena to be considered in this paper. Phase I would correspond to the outside of the cell; Phase II to the reaction zone, and Phase III to the inside of the cell.

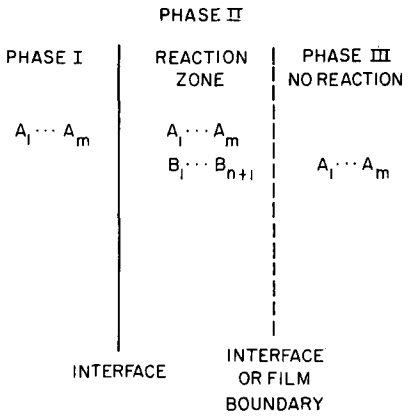

Fig. 1. Region of confined reaction. The distance variable X is equal to zero at the interface and equal to L at the film boundary. Phase I represents the exterior of the cell and Phase III represents the interior of the cell.

Theory of confined reactions

From Fig. 1 it can be seen that the problem is to analyze the diffusion of various species through the region of reaction, Phase II. The formulation of this problem depends upon the type of reaction occurring in Phase II. For biological reactions the following series of reaction types appears to be of the proper form. Also, rarely are more than two molecules involved in a reaction.

$$\begin{aligned} B_1 + A_1 &\rightleftharpoons B_2 \\ B_2 + A_2 &\rightleftharpoons B_3 \\ &\vdots \\ B_n + A_n &\rightleftharpoons B_{n+1} \end{aligned} \tag{1}$$

where n = reactions; n + 1 = different nontransferring species B_i; and m = different transferring species A_i.

In these equations the B_i's are the biological enzymes and complexes which are unable to diffuse out of the region of reaction. The A_i's are the Na^+, metabolites, or other species which transfer through the region of reaction. All the A_i's do not have to be different, *i.e.* $A_i = A_j$, $i \neq j$; thus there are m ($m \neq n$) distinct transferring species. For the purposes of further development we will define the matrix $\bar{\bar{P}}$ such that

$$P_{ij} = \begin{cases} 1 \text{ if the } i^{th} \text{ transferring species occurrs in the } j^{th} \text{ reaction} \\ 0 \text{ otherwise} \end{cases} \tag{2}$$

Also note that

$$\sum_{i=1}^{m} P_{ij} = 1 \qquad j = 1, \ldots n$$

This says that only one transferring species appears in each reaction. In these equations $P_{11} = 1$ which specifies A_1 in the first reaction.

Mass balances performed on the m transferring species A's in Phase II, then gives the following m equations.

$$D_i^t \frac{d^2C_i^t}{dx^2} = \sum_{j=1}^{n} P_{ij}r_j \qquad i = 1,\ldots m \tag{3}$$

where D_i^t = diffusivity of i^{th} transferring species; C_i^t = concentration of i^{th} transferring species; r_j = net forward reaction rate for the j^{th} reaction; x = distance parameter in Phase II (left boundary of Phase II, $x = 0$).

Similar mass balances can be performed on the n+1 nontransferring species, B's, in Phase II leading to the following equations.

$$D_j^{nt} \frac{d^2C_j^{nt}}{dx^2} = r_j - r_{j-1} \qquad j = 1\ldots n+1 \tag{4}$$

where D_j^{nt} = diffusivity of j^{th} nontransferring species; C_j^{nt} = concentration of j^{th} nontransferring species.

These equations assume constant diffusivity with no diffusive coupling and the electrical effects of transfer are neglected. In these equations the following is true.

$$r_0 = r_{n+1} = 0 \tag{5}$$

Associated with these (n + m +1) second order differential equations are the 2(n + m + 1) boundary conditions. The concentrations of the transferring chemical species are known at the boundaries, and the fluxes of the nontransferring chemical species are known to be zero at the boundary. These conditions are given by the following equations.

at $x = 0$		and at $x = L$	
$C_i^t = C_{i0}$	$i = 1\ldots m$	$C_i = C_{iL}^t$	$i = 1\ldots m$ (6)
$\frac{dC_j^{nt}}{dx} = 0$	$j = 1\ldots n+1$	$\frac{dC_j^{nt}}{dx} = 0$	$j = 1\ldots n+1$ (7)

If all of the above boundary conditions were independent, the desired 2(n + m + 1) conditions would be completed. However, it can be shown that one more boundary condition is required; this is the condition that the total concentration of all complexing molecules in the reacting zone, Phase II, is constant, *i.e.*

$$\int_0^L \left(\sum_{j=1}^{n+1} C_j^{nt} \right) dx = LC_T \tag{8}$$

Thus given the reaction rate functionalities, r_i, the description of the concentration profiles in Phase II and the flux of transferring chemical species across Phase II are found from the solutions of Eqns. 3 and 4 with boundary conditions, Eqns. 6–8. These equations cannot be solved easily analytically, but approximations to the solution of these equations can be obtained by using the equilibrium assumption.

Equilibrium solution

In order to obtain analytical solutions to these equations, each reversible reaction is assumed to be at (or near) equilibrium. Qualitatively, this says that the

reaction processes considered are much more rapid than the diffusion process across distance L. The equilibrium assumption allows the removal of the reaction rate functionalities, r_j's. The equilibrium conditions for the n reactions are given by the following equations where K_i is the equilibrium constant for the i^{th} reaction.

$$K_1 = \frac{C_2^{nt}}{C_2^{nt} C_1^t} \tag{9}$$

$$K_2 = \frac{C_3^{nt}}{C_2^{nt}\left(\sum_{i=1}^{m} P_{i2} C_i^t\right)}$$

$$K_j = \frac{D_{j+1}^{nt}}{C_j^{nt}\left(\sum_{i=1}^{m} P_{ij} C_i^t\right)}$$

$$K_n = \frac{C_{n+1}^{nt}}{C_n^{nt}\left(\sum_{i=1}^{m} P_{in} C_i^t\right)}$$

Also in order to obtain an analytical solution, the diffusivities of the nontransferring complexes are assumed to be the same. For large biological molecules this is probably a very good approximation. With this assumption from Eqn. 4 the following equation is found.

$$\sum_{j=1}^{n+1} D_0^{nt} \frac{d^2 C_j^{nt}}{dx^2} = 0 \text{ or } \sum_{j=1}^{n+1} \frac{d^2 C_j^{nt}}{dx^2} = 0 \tag{10}$$

$$D_0^{nt} = D_j^{nt} \qquad j = 1 \ldots n$$

Integration of Eqn. 10 once gives

$$\sum_{j=1}^{n+1} \frac{dC_j^{nt}}{dx} = 0 \tag{11}$$

The constant of integration is zero because no net flux of complexes can occur across the boundaries. The fluxes of each of the n + 1 nontransferring species need not be zero at the boundaries because under the assumption of equilibrium reaction, the equivalent of a surface reaction can occur at the boundaries. However, the sum must be such that no net transfer of nontransferring species occurs across the boundaries (see GODDARD *et al.*[19] and BASSETT AND SCHULTZ[20] for discussion of this point).

Application of Eqns. 8–10 then gives

$$\sum_{j=1}^{n+1} C_j^{nt} = C_T \tag{12}$$

Also from Eqn. 4 it can be seen that

$$r_j = \sum_{l=1}^{j} D_l^{nt} \frac{d^2 C_l^{nt}}{dx^2} = D_0^{nt} \sum_{l=1}^{j} \frac{d^2 C_l^{nt}}{dx^2} \tag{13}$$

Substituting Eqn. 13 into Eqn. 3 gives

$$D_i^t \frac{d^2 C_i^t}{dx^2} = \sum_{j=1}^{n} P_{ij} \left(D_0^{nt} \sum_{l=1}^{j} \frac{d^2 C_l^{nt}}{dx^2} \right) \qquad i = 1 \ldots m \tag{14}$$

Thus with the equilibrium assumption, the m second order differential equations, Eqn. 14, must be solved with 2m boundary conditions, Eqn. 6. The extra n + 1 conditions which determine the concentration of the n + 1 nontransferring species are given by Eqn. 9 and Eqn. 12.

Solution of equilibrium problem

Integration of Eqn. 14 gives

$$D_i^t C_i^t - D_0^n \sum_{j=1}^{n} P_{ij} \left(\sum_{l=1}^{j} C_l^{nt} \right) = a_i x + b_i \qquad i = 1 \ldots m \tag{15}$$

where a_i and b_i are constants of integration.

By defining C_{j0}^{nt} and C_{jL}^{nt} to be the concentration of the j^{th} nontransferring chemical species at the x = 0 boundary and at the x = L boundary respectively, the flux (see BASSETT AND SCHULTZ[20]) of the i^{th} diffusing species is given by

$$a_i = -J_i = \frac{D_i^t}{L} (C_{iL}^t - C_{i0}^t) - \frac{D_0^{nt}}{L} \sum_{j=1}^{n} P_{ij} \left[\sum_{l=1}^{j} (C_{lL}^{nt} - C_{l0}^{nt}) \right] \tag{16}$$

Now from the equilibrium conditions, Eqn. 9, the following is obtained.

$$C_j^{nt} = C_1^{nt} \prod_{k=1}^{j-1} \left(\sum_{i=1}^{m} P_{ik} K_k C_i^t \right) \tag{17}$$

Note for

$$j = 1 \qquad \prod_{k=1}^{0} \left(\sum_{i=1}^{m} P_{ik} K_k C_i \right) \equiv 1.$$

From the conservation of complexes, Eqn. 12, with the substitution of Eqn. 17, it is found that

$$\sum_{j=1}^{n+1} C_j^{nt} = C_T = C_1^{nt} \sum_{j=1}^{n+1} \left(\prod_{k=1}^{j-1} \left(\sum_{i=1}^{m} P_{ik} K_k C_i^t \right) \right)$$

or

$$C_1^{nt} = \frac{C_T}{\sum_{j=1}^{n+1} \left(\prod_{k=1}^{j-1} \left(\sum_{i=1}^{m} P_{ik} K_k C_i \right) \right)} \tag{18}$$

Evaluation of Eqns. 17 and 18 at both boundaries and substituting into Eqn. 16 gives

$$-\bar{J}_i = \frac{D_i^t}{L}(C_{iL}^t - C_{i0}^t) - \frac{D_0^{nt}C_T}{L}\sum_{j=1}^{n} P_{ij}\left[\sum_{l=1}^{j}\left(\frac{\prod_{k=1}^{l-1}\left(\sum_{s=1}^{m} P_{sk}K_kC_{sL}^t\right)}{\sum_{g=1}^{n+1}\left(\prod_{k=1}^{g-1}\left(\sum_{s=1}^{m} P_{sk}K_kC_{sL}^t\right)\right)}\right.\right.$$
$$\left.\left. - \frac{\prod_{k=1}^{l-1}\left(\sum_{s=1}^{m} P_{sk}K_kC_{s0}^t\right)}{\sum_{g=1}^{n+1}\left(\prod_{k=1}^{s-1}\left(\sum_{s=1}^{m} P_{sk}K_kC_{s0}^t\right)\right)}\right)\right] \qquad (19)$$

Thus for any group of rapid reactions of stoichiometry of the type shown in Eqn. 1, the mass flux for the i^{th} transferring chemical species is given by Eqn. 19 in terms of the concentration of the transferring species at the boundaries. Various properties of confined reaction systems with a single reaction have been investigated by the authors[21].

EXAMPLES

The applicability of the theory will be demonstrated by a detailed comparison with the experimental results of two investigations. Many other experimental comparisons were made but the two given here should be sufficient to illustrate the theory. The first experimental data used for comparison were generated by SCHULTZ *et al.*[22] and CURRAN *et al.*[23] who measured the transport of alanine and sodium across the mucosal border of rabbit ileum. In the second example, the theory was applied to the experimental results of VIDAVER[5–8] who determined the effect of sodium concentration upon the transport of glycine in pigeon red cells. Radio isotope measurement techniques were employed in both of these investigations.

Alanine transport in rabbit ileum

The experimental technique of SCHULTZ *et al.*[22] for the determination of alanine and sodium flux in the rabbit ileum involved removing a portion of the terminal ileum after the animal had been sacrificed. The segment of intestine was then placed in a special apparatus with the mucosal side up facing a oxygenated solution and the serosal side down against a moistened filter paper. The intestinal segment was preincubated for 30 min with Ringer solutions of various Na^+ compositions and containing no alanine or glucose in most cases. After preincubation, the test solution containing isotope labeled alanine and Na^+ was exchanged for the preincubation solutions. After 10–60 sec the test solution was removed and the volume occupied by the solutions was washed with cold mannitol solution which suddenly terminated the exposure of the tissue. A piece of the intestinal segment was then analyzed for $^{22}Na^+$ and isotope alanine.

The data are analyzed by CURRAN *et al.*[23] and these authors suggest a scheme of reactions to be occurring at the boundaries in a carrier type model. This same reaction sequence appears to be very reasonable for our model. The reactions assumed

to be occurring in the confined reaction zone (Phase II of Fig. 1) are given by the following equations.

$$\begin{aligned} B_1 + A_1 &\rightleftharpoons B_2 \\ B_2 + A_2 &\rightleftharpoons B_3 \end{aligned} \tag{20}$$

where B_1 is a binding enzyme; B_2 is a complex formed of B_1 and A_1; B_3 is a complex formed of B_1, A_1 and A_2; A_1 is alanine; and A_2 is Na^+.

From this proposed reaction scheme and the other assumptions used in the general development, the expression for alanine flux can be obtained directly from Eqn. 19.

$$J_1 = \frac{D_1^t(C_{10}^t - C_{1L}^t)}{L} - \frac{D_o^{nt}C_T}{L}\left[\frac{1}{1 + K_1C_{10}^t(1 + K_2C_{20}^t)} - \frac{1}{1 + K_1C_{1L}(1 + K_2C_{2L}^t)}\right] \tag{21}$$

Since the alanine flux being measured is in fact the isotope tagged alanine and since the preincubation solutions never contained any isotope tagged alanine, the initial concentration of isotope tagged alanine in the cells is zero. However, the internal alanine concentration would affect the transport of labeled alanine by exchange diffusion. This effect is not considered in the theoretical development and is presumed to be small.

$$C_{iL}^t = 0$$

This simplifies the flux relationship considerably to the following form.

$$J_1 = \frac{D_1C_{10}^t}{L} + \frac{D_o^{nt}C_T}{L}\left[1 - \frac{1}{1 + K_1C_{10}^t(1 + K_2C_{20}^t)}\right] \tag{22}$$

It should be noted that this equation (Eqn. 22) predicts that the flux of isotope tagged alanine should be independent of the concentration of alanine or sodium inside the cell (C_{1L}^t and C_{2L}^t), respectively). This is what in fact was found experimentally by SCHULTZ[22]. However, by referring to Eqn. 21, it can be seen that the natural alanine flux should be a function of intracellular alanine and sodium concentrations. The magnitude of this dependency may be such that experimental determination of the effect would be difficult.

The first term of the right hand side of Eqn. 22 represents the Ficks law flux of alanine and the second term represents the flux of alanine caused by the sodium gradient as coupled through the confined reaction. For the particular experimental conditions of SCHULTZ[22], it appears that the first term (Ficks law flux) is negligible; this may be caused by the fact that most of the alanine is in a bound form for low concentrations of alanine, *i.e.*

$$D_o^{nt}C_T \gg D_1C_{10}^t$$

Eliminating the Ficks law term, the relationship for alanine flux reduces to the following equation (Eqn. 23). Note that Eqn. 23 represents the flux greater than Ficks law flux.

$$J_1 = \frac{D_o^{nt} C_T}{L}\left[1 - \frac{1}{1 + K_1 C_{10}^t (1 + K_2 C_{20}^t)}\right] \tag{23}$$

The maximum value of the flux above Ficks law is given by

$$J_1^m = \frac{D_o^{nt} C_T}{L} \tag{24}$$

By rearrangement Eqn. 23 can be put in the following form for comparison with the results of CURRAN *et al.*[23].

$$J_1 = \frac{J_1^m C_{10}^t}{\frac{1}{K_1(1 + K_2 C_{20}^t)} + C_{10}^t} \tag{25}$$

CURRAN *et al.*[23] represent the measured alanine flux with the following relationship.

$$J_1 = \frac{J_m C_{10}^t}{K_T + C_{10}^t} \tag{26}$$

where J_m is the maximal influx and K_T is the apparent Michaelis constant.

By comparison, it can be seen that the following is true.

$$J_m = J_1^m = \frac{D_o^{nt} C_T}{L} \tag{27}$$

and

$$K_T = \frac{1}{K_1(1 + K_2 C_{20}^t)} \tag{28}$$

The experimental evidence indicates that J_m is constant and that $1/K_T$ is linear in sodium concentration, *i.e.* the K_T dependency follows Eqn. 28. These comparisons demonstrate that the theory presented here qualitatively predicts the experimental results. Thus there is a theoretical basis for choosing equations similar to Eqn. 26 and Eqn. 28 for representing the dependency flux of isotope tagged alanine on mucosal bathing solution concentration of alanine and sodium.

The pertinent equation (Eqn. 25) can be arranged such that the constants J_1^m, K_1, and K_2 can be estimated from straight line graphical procedures. By taking the reciprocal of both sides of this equation the following equation is obtained.

$$\frac{1}{J_1} = \frac{1}{J_1^m} + \frac{1}{J_1^m} \frac{1}{K_1(1 + K_2 C_{20}^t)} \frac{1}{C_{10}^t} \tag{29}$$

A plot of $1/J_1$ *versus* $1/C_{10}^t$ as taken from CURRAN *et al.*[23] (Fig. 2 in their paper) is given in Fig. 2. It can be seen that this plot does give straight lines for constant values of sodium concentration. The intercept is the same and independent of the sodium concentration as predicted by the theory.

From the intercept of the lines drawn in Fig. 2 the value of J_1^m is found. $1/J^m = 0.185$ h·cm²·μmoles^{-1} or $J_1^m = 5.4$ μmoles·h^{-1}·cm^{-2}.

The slope of the line marked [Na^+] = 0 (*i.e.* $C^t_{20} = 0$) is calculated and by using Eqn. 29 the value of K_1 is found.

$$\frac{1}{J^m_1 K_1} = 7.51 \text{ or } K_1 = 0.0246 \text{ mM}^{-1}$$

Using the slope of the line marked [Na] = 140 in Fig. 2, the value of K_2 can be calculated by the following sequence of steps.

$$\frac{1}{J^m_1 K_1(1 + 140K_2)} = 1.82$$

$$J^m_1 = 5.4 \qquad K_1 = 0.0246$$

$$K_2 = 0.0224 \text{ mM}^{-1}$$

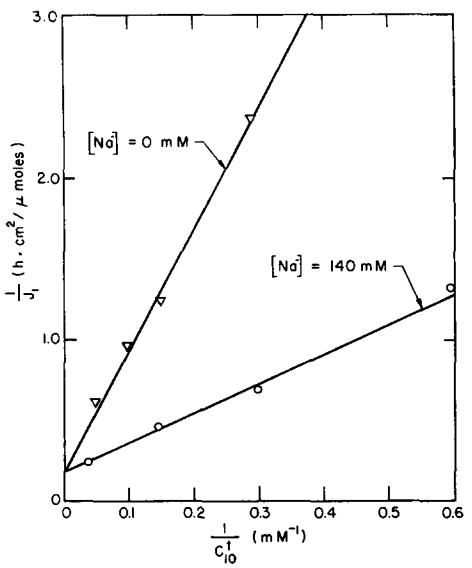

Fig. 2. Data of CURRAN[23]. This plot indicates the linear variation of inverse flux (ordinate) and inverse alanine concentration (abscissa) for the transport of alanine into the rabbit ileum.

These values then can be used to predict the shape of the $1/K_T$ *versus* sodium concentration (C^t_{20}) curve. Inverting Eqn. 28 and substituting in values for K_1 and K_2 gives the following equation.

$$\frac{1}{K_T} = 0.0246 + 0.00055\, C^t_{20} \tag{30}$$

Eqn. 30 is plotted in Fig. 3 along with the data of CURRAN *et al.*[23]. It can be seen that the straight line predicted by the theory does not compare very well with the data. This probably indicates that the data in Fig. 3 were not obtained from the same set of experiments as those in Fig. 2. The data in Fig. 2 are apparently not typical of the numbers obtained in most experiments since the value of J^m_1 calculated from the data

in Fig. 2 is 5.4, yet the average value for this quantity at zero sodium concentration is 6.8 $\pm$ 0.7.

This example illustrates the application of the theory to a specific case. In general, the theory predicts the phenomena qualitatively and quantitavely very well. Other predictions concerning the relative fluxes of sodium and alanine could be attempted, but such comparisons are complicated by the influence of the normal alanine upon the isotope tagged sodium flux and other such influences. The theory also suggests that the independence of the alanine flux on intracellular concentration of sodium and alanine could possibly be an artifact of the experimental technique. The calculations indicate the ease of the application of the theory and its predictive capabilities.

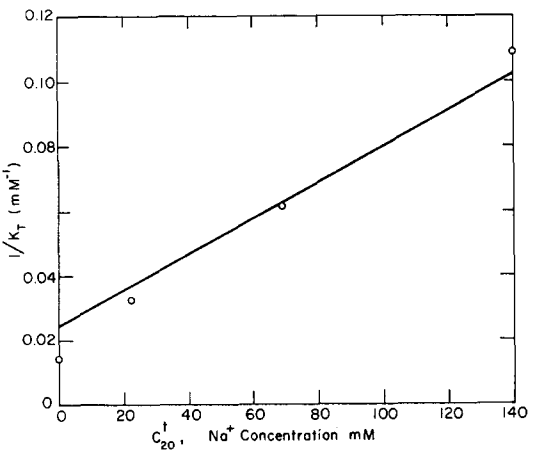

Fig. 3. Data of CURRAN[23]. The line represents the theoretical prediction of the variation of inverse Michaelis constant (ordinate) *versus* sodium concentration in the mucosal solution. The dots are experimental measurements.

Glycine transport in pigeon red cells

VIDAVER[5–8] in a remarkable series of experiments measured the effect of sodium on the transport of glycine into pigeon red cells. Blood was collected from adult commercial pigeons, filtered through cheese cloth, centrifuged and held in cold storage until needed. The blood cells were centrifuged into a pellet prior to being placed in the incubation solution containing isotope tagged glycine for 15–25 min. The amount of tagged glycine in the cells was then measured to obtain the flux of glycine. The concentrations of sodium ion and glycine were varied in the incubation solution.

In the discussion of the experimental results VIDAVER[5] presents a series of reversible reactions which appear appropriate in this case with a change in the order of the reactions. Actually the order of the reactions is probably immaterial as long as the equilibrium constants are such that the dominant complex is the one containing the two sodium ions and the glycine molecule. The series of reactions used in this derivation are listed below.

$$\begin{aligned} B_1 + A_1 &\rightleftharpoons B_2 \\ B_2 + A_2 &\rightleftharpoons B_3 \\ B_3 + A_2 &\rightleftharpoons B_4 \end{aligned} \qquad (31)$$

where B_1 = binding enzyme; B_2 = complex containing glycine; B_3 = complex containing glycine and one Na^+; B_4 = complex containing glycine and two Na^+; A_1 = glycine; and A_2 = Na^+.

For this particular example we have the following values for the parameter P_{ij}.

$$P_{11} = 1 \qquad P_{12} = 0$$
$$P_{21} = 0 \qquad P_{22} = 1$$
$$P_{31} = 0 \qquad P_{32} = 1$$

Using these values the glycine flux into the cell can be obtained from Eqn. 19.

$$J_1 = \frac{D_1^t}{L}(C_{10}^t - C_{1L}^t) + \frac{D_0^{nt}C_T}{L}\left[\frac{1}{1 + K_1C_{1L}^t(1 + K_2C_{2L}^t(1 + K_3C_{2L}^t))} - \frac{1}{1 + K_1C_{10}^t(1 + K_2C_{20}^t(1 + K_3C_{20}^t))}\right] \tag{32}$$

For the measurement of glycine entry into the pigeon red cells, the initial intracellular isotope labeled glycine concentrations was zero ($C_{1L}^t = 0$). Thus the formula for entry flux of glycine is simplified. This formula also applies to the case of labeled glycine transport into an unlabeled medium; in this case $C_{10}^t = 0$ and the flux is in the opposite direction. The previous discussion concerning the effects of unlabeled metabolite again applies.

$$J_1 = \frac{D_1^t}{L}C_{10} + \frac{D_0^{nt}C_T}{L}\left[1 - \frac{1}{1 + K_1C_{10}^t(1 + K_2C_{20}^t(1 + K_3C_{20}^t))}\right]$$

or (33)

$$J_1 = \frac{D_1^t}{L}C_{10} + \frac{D_0^{nt}C_T}{L}\left[\frac{C_{10}^t}{\frac{1}{K_1 + K_1K_2C_{20}^t + K_1K_2K_3C_{20}^{t2}} + C_{10}^t}\right]$$

This formula indicates that the flux of glycine is composed of two components; one obeying Michaelis–Menten kinetics (the second term), and one resembling simple diffusion as found experimentally by VIDAVER[5]. From the experimental data of VIDAVER[5], it appears that the following inequalities are true for all measured values of C_{20}^t.

$$K_3C_{20}^t \gg 1 \tag{34}$$
$$K_2K_3C_{20}^{t2} \gg 1$$

Actual calculations on the data indicate this to be true except in the range of low C_{20}^t concentration. The formula for flux, Eqn. 33 can be simplified to

$$J_1 = \frac{D_1^t}{L}D_{10} + \frac{D_0^{nt}C_T}{L}\left[\frac{C_{10}^t}{\frac{1}{K_1K_2K_3C_{20}^{t2}} + C_{10}^t}\right]. \tag{35}$$

If the simple diffusion term is subtracted from the total flux, the Michaelis-Menten flux, J_1^*, can be put in the following form.

$$\frac{1}{J_1^*} = \frac{1}{J_2^m}\left[1 + \frac{1}{K_1K_2K_3C_{20}^{t2}}\frac{1}{C_{10}^{i}}\right] \tag{36}$$

This equation is in qualitative agreement with the experimental data of VIDAVER[5] *i.e.* the plots of the inverse of the labeled glycine entry rate above simple diffusion *versus* the inverse of the labeled glycine concentration in solution and *versus* the inverse of the solution sodium concentration squared give straight lines. Figs. 4 and 5 as taken from Figs. 1 and 2 in VIDAVER[5] indicate the straight line functionalities.

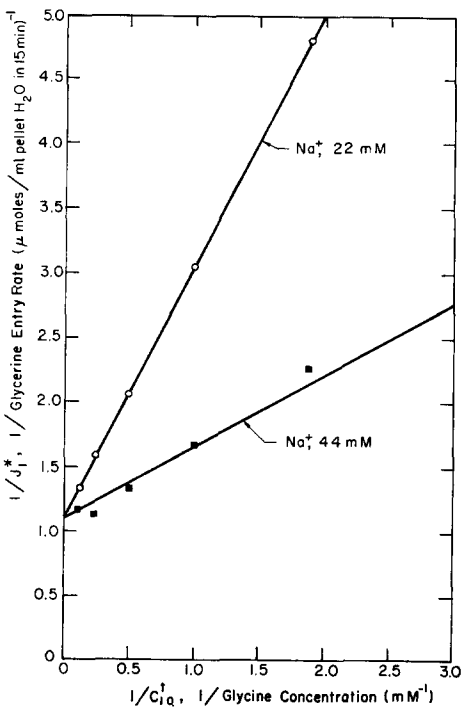

Fig. 4. Data of VIDAVER[5]. This plot of inverse Michaelis glycine entry rate *versus* inverse of glycine concentration in solution is linear as predicted by theory.

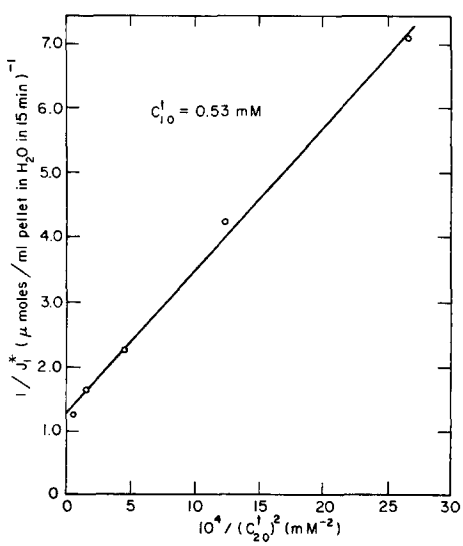

Fig. 5. Data of VIDAVER[5]. The theory predicts the linearity between the inverse of the Michaelis glycine entry rate and the inverse of the sodium concentration in solution squared.

Similar calculations as were performed on the data of SCHULTZ *et al.*[22] and CURRAN *et al.*[23] could be presented here to yield values for J_1^m, and $K_1K_2K_3$ and give a quantitative agreement.

Eqn. 35 is not quite the same as that which VIDAVER[5] suggests as applying to the data. However, other variations of Eqn. 33 can be considered, *e.g.* only the linear term in C_{20}^t might be eliminated. This would give a curved line in Fig. 3 of VIDAVER[5] and a curved line in Fig. 5 of his paper. The curvature would improve the fit of both, but debate of this minor point appears unwarranted.

VIDAVER[5] also measured the labeled glycine exit rate from intact pigeon red cells and found that this exit rate was essentially independent of the unlabeled glycine or sodium concentration in the medium. This would be expected since, as was stated before, Eqn. 33 is applicable to the transport of labeled glycine into a medium containing no labeled glycine with the changes of $C^t_{1_0}$ to C^t_{1L} and $C^t_{2_0}$ to C^t_{2L}. Thus we would not expect an effect of sodium concentration in the solution $C^t_{2_0}$ or glycine concentration in the solution on the exit rate except for the phenomena of exchange diffusion. This was found by VIDAVER[5]. He did, however, find an effect of intracellular sodium concentration C^t_{2L} on exit rate, as would be anticipated from the theory.

In further experiments, VIDAVER[6] was able to alter the intracellular concentration of the cells by lysing and restoring the cells. He found that if the sodium concentration outside the cell was higher than that inside the cell, the cell tended to establish a higher intracellular glycine concentration than extracellular concentration, and if the sodium concentration in the medium was less than that in the cell, the cell depleted its intracellular glycine concentration. If no sodium or glycine gradient existed across the cell, the inlet and exit rates of glycine were equal and there was no tendency to establish a glycine gradient. All of these results are in qualitative agreement with Eqn. 32. If the inlet glycine rate equals the exit rate, then J_1 equals zero, and it is possible to calculate the expected glycine accumulation in the cell if the sodium and glycine concentration outside the cell are known and the sodium concentration in the cell is known.

In experiments in another paper, VIDAVER[7] replaced Cl^- with mucate ion and found a significant effect upon the transport rates of glycine into the cell. In particular he found that the mucate ion lowered the value of J_2^m in Eqn. 36. If it is postulated that the anion, either chloride or mucate, diffuses with the complexes B_2,B_3,B_4, then the diffusivity of these complexes should be much less with the mucate ion than with the chloride ion. Since J_2^m is proportional to the diffusivities of the complexes the lowered value of this constant with mucate is expected. Also changes in the slopes of the Lineweaver-Burke plots are expected since the equilibrium constants for sodium mucate are likely to be different from those of NaCl. Also there are the Nernst–Plank electrical charge effects.

Overall, the theory when applied to the data of VIDAVER[5–8] gives excellent qualitative and quantitative agreement. Other sequences of reactions than those given in Eqn. 31 were developed but all reduce to the same form if B_1 and B_4 are the predominant complexes in the solution. In this case, the system could be treated as a single reaction[21]. Finally, it should be pointed out that this application is an example of a case where the number of transferring species is less than the number of reactions.

Also comparisons were performed with the data of HELMREICH AND KIPNIS[24]. In this case, theoretical qualitative comparisons were made with the ratio of α-amino isobutyric acid as a function of extracellular α-amino isobutyric acid concentration. Similar comparisons, as well as some transport comparisons, were made with the data of HEINZ[25] and some of the data given by VIDAVER AND SHEPHERD[9]. The data of THIER *et al.*[26] were also included in the comparisons. All of the above comparisons gave good agreement with the theory.

Some other data (*e.g.* refs. 4 and 13) were considered for comparison but were not included in the comparison because the experimental technique involved direct

measurement of intracellular sodium or metabolyte concentration. In such cases, the method probably determined the total sodium or metabolyte in the cell, *i.e.* the free sodium and that bound by the equilibrium complexes.

DISCUSSION AND CONCLUSIONS

This paper presents two particular physical descriptions of cells which could give rise to confined chemical reactions. Many other descriptions are possible; for example, each entire cell of a membrane may act as a confined reaction involving binding enzymes for transport across the membrane. Such may be the case for many living membranes. Since the theory developed involves only known physical concepts, such as binding enzyme reactions, simple diffusion, and cell wall attachments or semi-permeable membranes, it is thought that this theory is probably closer to the actual phenomena found in cells than theories postulating unusual transport steps such as "translocation."

The theory is developed from these simple physical concepts and has much generality since most reactions are of the form given in Eqn. 8. The assumption of equal diffusivity of the enzyme on complexes probably does not cause serious error as found by BASSETT AND SCHULTZ[20]. The assumption of equilibrium can cause serious error or it can be a very good approximation (ref. 20 and F. H. VERHOFF AND K. R. SUNDARESAN, unpublished results); in the cases presented it appears, surprisingly, to be a good approximation. Theory development is continuing using the present confined reaction scheme but including more than one enzyme and also allowing for competition among several chemical species (*e.g.* alanine and leucine) for the same site on a binding enzyme.

This expanded theory will also be necessary to explain the "Trans" effects of the experiments of VIDAVER AND SHEPHERD[9]. The effect of unlabeled "trans" glycine on labeled glycine transport is similar to single component facilitated diffusion as discussed by BASSETT AND SCHULTZ[20] and KUTCHAI *et al.*[27]. Also it should be noted that the net reaction in the region of chemical reaction is zero in the theory presented in this paper; thus all the energy for chemical species transfer against the gradient is obtained from the gradient of another chemical species. Work is now progressing on a theory which will allow the chemical reaction to proceed; in this case, chemical energy can be coupled *via* the confined reaction to the transport of substances against the chemical gradient.

In this paper two examples of the detailed application of the theory were presented. The theory was also applied to the data of other authors, and the qualitative and quantitative comparisons indicate that theory predicts the experiments. From all this evidence, it is strongly suspected that confined binding enzyme reactions are responsible for the coupled transport found in cells.

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