DETERMINATION OF THE DISTRIBUTION OF CATALYST ACTIVITY ACROSS A PERMEABLE MEMBRANE CONTAINING AN IMMOBILIZED ENZYME

Indeterminacy of a Functional Approach to a Structural Problem

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ABSTRACT Porous membranes were fabricated from collodion and impregnated with papain, inhomogeneously through the thickness of the membrane. These membranes were placed between reservoirs containing *N*-α-benzoyl arginineamide, a substrate for the enzyme papain. The progress of the reaction was monitored by sampling the reservoirs on each side for ammonia, a reaction product. From these data the diffusion coefficient, enzyme activity, and distribution of enzyme activity of the membrane were estimated. The limitations of this approach are discussed in the context of the analysis of biological transport systems.

INTRODUCTION

It is generally believed that many biological transport processes involve transport proteins incorporated into cellular membranes, which are functionally analogous to enzymes. In attempting to elucidate the mechanism of the transport process, biophysicists use measurements of transport rates that depend on the concentrations of the transported substance or other agents on both sides of the membrane, for example, see Sachs (1977). This approach was motivated by standard biochemical methods of analysis, in which the dependence upon substrate and product concentrations of enzyme reaction rates in solution is used to infer their mechanisms. Since the biochemical research has been remarkably productive, we anticipated comparable success for transport systems. Such success has not been achieved in even a single case. Shamoo (1975) presents a fair indication of the current understanding of a number of transport systems. The contrast between the indirectness of interpretation and the heavy use of analogy in speaking of transport systems, and the precise molecular detail with which many enzyme-catalyzed reactions have been described, e.g., Steitz (1981), is striking.

One reason for the primitive state of our understanding of biological transport processes is that an enzyme compartmentalized in a membrane is a much more complicated system than an enzyme in free solution. This difference arises because the membrane is macroscopic. The apparent kinetics of an enzyme within a membrane depends upon where the enzyme is located relative to the substrate supply. As a trivial example, suppose that the membrane were impermeable, and that the enzyme were located on one surface. Place this membrane between two reservoirs, one containing a substrate for the enzyme while in the other the substrate is absent. In the first instance, the active surface is placed in contact with the reservoir containing the substrate; reaction will occur. In the second instance the membrane is turned around; now, of course, there will be no reaction at all. More generally, the apparent kinetics of an enzyme within a membrane is influenced by the distribution of the enzyme within that membrane. The reason for this dependence is that the apparent kinetics of an enzyme within a membrane can be modulated by mass transfer processes governing substrate access to the active site of the enzyme.

Recent structural studies have provided considerable qualitative data about the distribution of enzymes within membranes, e.g., whether the protein lies predominantly or entirely on one side or the other of the membrane (Carraway, 1975). For some proteins, more detailed geometric placement can be determined by x-ray diffraction or electron microscopic methods (Caspar et al., 1977; Henderson and Unwin, 1975). Despite this structural detail, we have only qualitative knowledge of the accessibility of substrates to the active sites of enzymes in membranes.

This state of ignorance, combined with the predicted

dependence of enzyme reaction rates in membranes on their position, suggests that many attempts to deduce the molecular mechanism of biological transport processes from externally measured transport rates have likely been inconclusive because quite distinct mechanisms associated with different distributions of the transport proteins within the membrane can produce the same observed rate of transport. Nevertheless, some kinds of statements, the most important of which relate to functional symmetry of placement of transport proteins within the membrane, may be determined conclusively, as we shall illustrate.

Mitz (1967) and others have suggested medical and technological applications of immobilized enzyme in membranes involving inhomogeneous distribution of enzymatic activity as well as transport properties across the membrane. To exploit these applications, we require means to evaluate the distribution of catalyst activity within the membrane. Because not all of the enzyme in such membranes is active, measures of protein content do not suffice.

In this manuscript we pursue the question, What can be learned about the distribution of enzyme across a membrane from observation of the flow of reaction products across its surfaces, as that flow depends upon the composition of the reservoirs bathing the two surfaces? Our approach was to fabricate macroscopic, enzymatically active model membranes that are catalytically inhomogeneous. From the apparent kinetics of the membrane-bound enzyme we attempted to infer the true distribution of the enzyme. Estimates of inhomogeneity obtained from kinetic analysis were compared with those obtained directly by examination of the internal structure of the membrane.

MATERIALS AND METHODS

Membranes

Cellulose nitrate from (collodion, DuPont Co., Wilmington, DE) was dissolved in alcohol-ether and cast into membranes of ~400 μm thickness, following the method of Sollner (Carr and Sollner, 1945). Papain (E.C.2.1.1.1.1.7) was obtained as the Hg-dimer (Sigma Chemical Co., St. Louis, MO). Disks of collodion were exposed to a suspension of the enzyme (10 mg/ml) in Tris buffer, as described by Goldman (1965, 1968), except that only one surface of the membrane was exposed. The membranes remained in this gradient of enzyme for 150–200 h, at 4%C. Following adsorption of the enzyme, it was irreversibly trapped within the membrane matrix by cross-linking with a bifunctional diazonium reagent, bis(diazobenzidinesulfonic acid), prepared after Kabat and Meyer (1952) from 4,4′, *p*-aminobiphenyl,3,3′-disulfonic acid (Eastman Kodak Co., Rochester, NY, model T6303). The completion of the reaction was signaled by the appearance of a honey-colored zone in the membrane.

Measurement of Papain Activity

To avoid the complexities arising from pH effects, *N*-α-benzoylargininamide (BAA) (Miles Laboratories, Inc., Elkhardt, IN) was selected as a substrate for papain. Hydrolysis of BAA by papain liberates NH_4^+, conveniently analyzed by microdiffusion analysis following Conway (1944). Calibration experiments established that the assay was adequately linear over the range 1–4 mM, with reproducibility of ±0.05 mM.

All of the kinetic studies on the membranes were conducted using a buffer (0.05 M Tris-phosphate, 0.01 M EDTA, 0.025 M cysteine [free base], at pH 6.15). When the membranes were assayed, 100-μl samples were taken at 5, 15, 30, 45, 60, and 90 min after filling the chambers. No correction was made for change in chamber volume resulting from the removal of each successive aliquot, since the change in the physical parameters was less than their uncertainty.

Chambers for Transport Measurement

The membranes were mounted in a lucite cell with two compartments of 8.7 ml volume and 1.77 cm^2 exposed membrane area. Each compartment housed a magnetic stirring bar, and a resealable port from which samples could be removed with a microsyringe (Hamilton Co., Reno, NY). The experimental arrangement is illustrated in Fig. 1.

Temperature Control

The chambers were placed in a thermostat-controlled air bath, consisting of a 3/4 in. plywood box, 450 cf/m circulating fans (Rotron Inc., Woodstock, NY, model FIC2), a pair of 2,500 W heater coils energized by a 10 A variac (General Radio, Concord, MA). A sensing thermocouple (American Instrument Co., Inc., Silver Spring, MD, model 4-235F) and relay (American Instrument Co., Inc., model 5-6500) constituted the feedback loop. Continuous cooling was provided by a constant flow at the rate of 5 liters/min of 4°C water through a small heat exchanger. An additional 150 cf/min fan removed heat from the magnetic stirrer base used to agitate the chambers. A 4 mm styrofoam pad insulated the chamber from the magnetic stirrer base. This apparatus sufficed to maintain temperature within the chambers at 37°C ±0.25°C for the 2–4 h duration of an experiment. This temperature was chosen because it is near the optimum for the enzyme.

MATHEMATICAL MODEL FOR THE KINETICS OF AN IMMOBILIZED PAPAIN MEMBRANE

The experimental results that are to be modeled are relatively uncomplicated: after the membrane is placed in contact with the reservoirs, the product concentration rises on both sides of the membrane. Any model for this phenomenon should be simple because complex models are certain to be under-determined. The following model will be demonstrated to be sufficient. In deriving this model, we have made the following simplifying assumptions: (*a*) Substrate and product have the same diffusion coeffi-

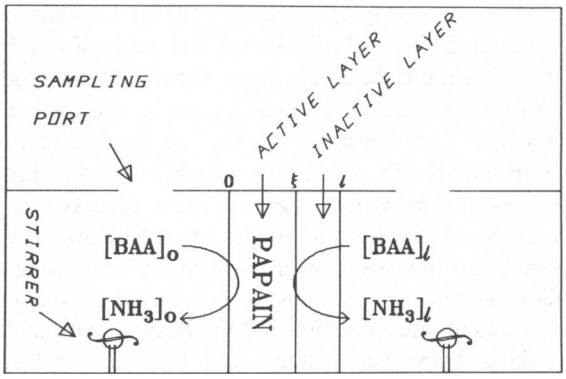

FIGURE 1 Diagram of the experimental arrangement. The two-layer membrane is placed between stirred chambers. The substrate, BAA, is more easily accessible to the active layer from the *o* bath than from the *l* bath. The product, NH_3, is assayed by sampling on both chambers through the sampling port. The entire apparatus is housed in a constant-temperature bath. In some experiments, either the *o* or the *l* bath is perfused, to maintain zero concentration of both substrate and product.

cient in both zones of the membrane; (*b*) electro-chemical effects on transport and reaction may be ignored; (*c*) the kinetics of the enzyme in the membrane microenvironment are irreversible and first order in substrate concentration; (*d*) the enzyme activity is constant in behavior. There are two zones, one with zero activity; (*e*) the concentration profile within the membrane is always in a stationary state with respect to the current bath compositions.

The assumptions are justified as far as possible in the Discussion. On the basis of these assumptions, the equations of continuity for substrate A, and product B within the active and inactive layers of the membrane are

$$\begin{aligned}
&DA_{xx} = \kappa A \quad 0 < x < \xi \quad A(0) = A^{\circ}(t) \quad A(\xi) = A^{\xi}(t)\\
&DB_{xx} = -\kappa A \quad 0 < x < \xi \quad B(0) = B^{\circ}(t) \quad B(\xi) = B^{\xi}(t)\\
&DA_{xx} = 0 \quad \xi < x < l \quad A(\xi) = A^{\xi}(t) \quad A(l) = A^{l}(t)\\
&DB_{xx} = 0 \quad \xi < x < l \quad B(\xi) = B^{\xi}(t) \quad B(l) = B^{l}(t)
\end{aligned}$$

where D is the diffusion coefficient of substrate or product, and the subscript xx denotes the second spatial derivative. The functions $A^z(t)$ $z = 0, \xi, l$, are different for the three experimental designs. The appropriate forms appear in Appendix A. The flows of A and B are continuous across $x = \xi$, so that

$$A_x(\xi^-) = A_x(\xi^+)$$
$$B_x(\xi^-) = B_x(\xi^+)$$

There are three parameters in the model: the diffusion coefficient D, the pseudo-first-order rate constant, $\kappa = V_{max}/K_m$, and ξ, the position of the boundary between the active and inactive regions of the membrane. How these equations are solved and related to the experimentally measured concentrations in this problem will be discussed in Appendix A.

EXPERIMENTAL RESULTS

Three different kinds of experiments were performed: (*a*) equal initial substrate concentration on both sides of the membrane; (*b*) substrate initially present on the active side and absent on the other side; (*c*) substrate initially present on the inactive side and absent on the other side. Typical results of measuring NH_4^+ concentrations in the chambers on the two sides of the membrane in the three types of experiments are shown in Figs. 2–4. Each type of experiment was repeated twice with the same membrane. Using the model described above, we obtained least squares estimates of D, κ, and ξ for the membrane. The experimental results are summarized in Table I. The standard errors shown are asymptotic estimates. We took the average of each parameter over all of the experiments to obtain the following characterization of the membrane in terms of the model where $D = 7 \times 10^{-6}$ cm^2/s; $\kappa = 0.1$ h^{-1}; and $\xi = 140$ μm. D and κ are probably accurate to $\pm 50\%$, while ξ is $\pm 25\%$. The missing standard error estimates presumably resulted from ill-conditioning of the variance-covariance matrix. With sparse and noisy data, as in the experimental data described here, such ill conditioning is not uncommon. The implication of such a finding is that there is an extended domain in the parameters (D, κ, ξ) where any choice of values fits about equally well.

Evidently, comparing the curves in Figs. 2–4 to the data points in the same curves, the model gives an adequate fit. Can we go further to argue that the model is correct in some sense? The diffusion coefficient of BAA was measured by DeSimone (1970) and DeSimone and Caplan (1973) in membranes prepared in the same way, except that the enzyme was uniformly dispersed through the entire membrane. The diffusion coefficient was determined with no reaction, and 7×10^{-6} cm^2/s was a typical value. DeSimone also measured κ in the homogeneous membranes. Because κ is a locally defined quantity, we can compare values between membranes. κ-values of ~ 0.1 h^{-1} were obtained. Finally, as mentioned in the section on membrane preparation, the cross-linking process introduced a characteristic color into the membrane. Optical microscopic inspection of a membrane in cross-section shows a frontier between the colored and clear regions of the membrane that was quite sharp. For the case of the membrane used for the experiments in Figs. 2–4, this frontier was located $\sim 150 \pm 10$ μm from the active surface of the membrane. Thus the two-region model is not only descriptive, but its parameters are even in rather good

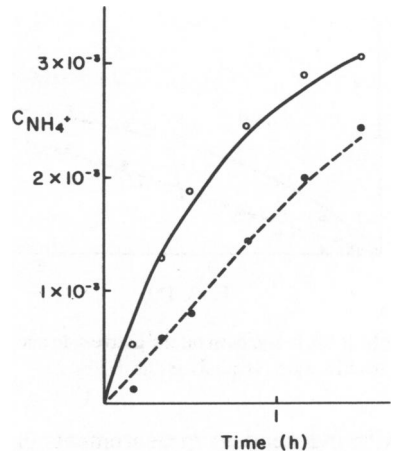

FIGURE 2 NH_4^+ concentration in baths starting with 4 mM BAA in both baths. The open circles (o) are the measurd data for the bath facing the active surface of the membrane. The closed circles (●) are the data for the bath facing the inactive membrane surface. The solid (—) and dashed (---) lines are obtained by using the model with the least squares optimum parameters.

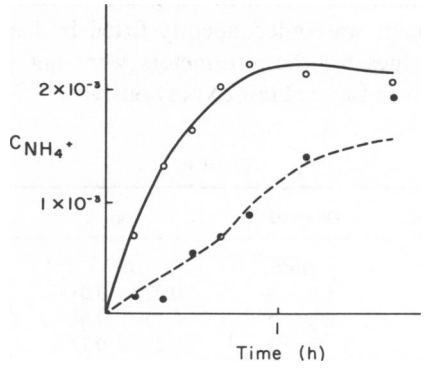

FIGURE 3 4 mM BAA in the bath on the active side and 0 mM BAA the bath on the inactive side. Symbols are as in Fig. 2.

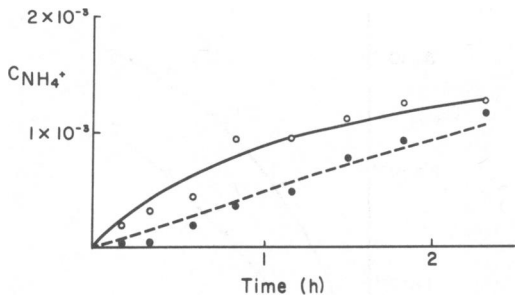

FIGURE 4 0 mM BAA in the bath on the active side and 4 mM BAA in the bath on the inactive side. Symbols are as in Fig. 2.

agreement with independent measurements of the specific membrane parameters obtained in independent measurements.

The ultimate test of a model is its ability to predict the out-come of new experiments. To explore this capacity, we converted one of the chambers in the lucite cell to a continuous flow, and washed out the chamber with a buffer in which no substrate was present. The effect of washing, was to maintain the surface of the membrane at zero concentration, both for the substrate and the product. The mathematical analysis of this experimental design differs slightly from that for the original experiment in that the concentrations in the bath that is cleared are replaced by constant zero values. This case is analyzed in Appendix B. Under these conditions, the progress of the reaction is slower, so eight samples were taken, spread over 2.5 h. This experiment was performed with the membrane in each of its two distinguishable orientations: (*a*) the active side washed, while the inactive side was bathed with buffer initially containing 4 mM substrate, and (*b*) the inactive side washed, while the active side was exposed to substrate initially at 4 mM.

Figs. 5 and 6 show the outcomes of these experiments. The dots are the measured data, and the solid curves are the predicted outcomes for this membrane using the average membrane parameters determined from previous experiments. The agreement is satisfactory, given the uncertainty in the measurements. A somewhat smaller sum of squares was obtained when the model for this type of experiment was independently fitted by least squares, but the values for the parameters were not statistically different from those obtained previously.

TABLE I

Experiment	$D(\times 10^{-6})$	κ	ξ
	cm^2/s	h^{-1}	μm
4 = 4	13 ± 4	0.07 ± 0.015	140 ± 40
4 = 4	20 ± 5	0.15 ± 0.06	90 ± 40
4 = 0	9 ± 1	0.2 ± 0.13	140 ± —
4 = 0	7 ± 1	0.13 ± —	110 ± 40
0 = 4	6 ± 2	0.14 ± 0.03	140 ± —
0 = 4	3 ± 0.6	0.3 ± —	190 ± —

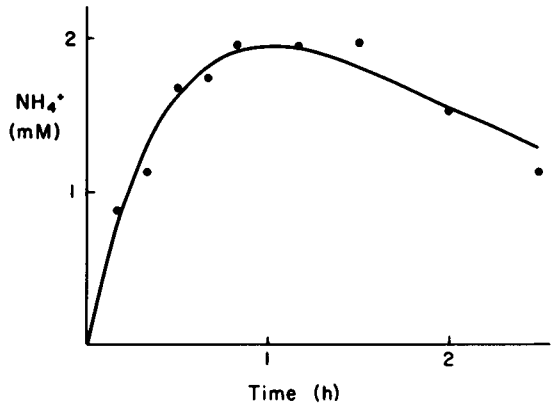

FIGURE 5 NH_4^+ concentration in bath starting with 4 mM BAA in the bath facing the active side, while the other side is washed with 0 mM BAA. The closed circles (●) are the measured data; the solid line is the prediction made using the best-fitting parameters for the same membrane in the previous set of experiments.

DISCUSSION

What conclusions can we draw from the satisfactory agreement between the model and the experiments? Have we shown that the model is correct? Because the describing model is linear, the mathematical form of the time variation of product concentration we have derived is independent of any choice for spatial distribution of enzyme activity or diffusion coefficients. The only effect of choosing a different distribution is to alter the values for the best-fitting parameters in the new model. To illustrate this effect, we consider an alternative model in which all of the enzyme activity is concentrated in a thin layer at $x = \xi$ within an otherwise homogeneous membrane. Fitting the data of Figs. 2–4 with this model, we obtained the following values for the parameters: $D = 18 \times 10^{-6}$ cm^2/s, $\kappa = 0.008$ h^{-1}, and $\xi = 85$ μm. The value for D is outside the physically reasonable range for membranes of this type. We can probably reject this model.

On the other hand, any number of models having appropriately chosen inhomogeneous distributions of D and κ can be constructed that do not lead to physically

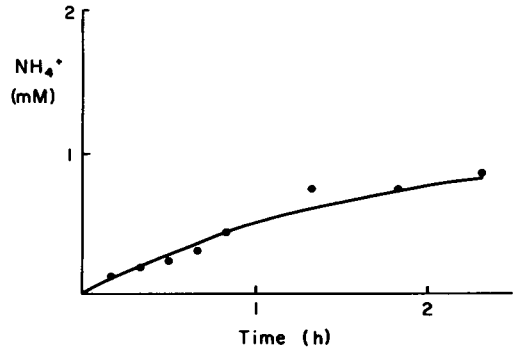

FIGURE 6 NH_4^+ concentration in bath starting with 4 mM BAA in the bath facing the inactive side, while the other side is washed with 0 mM BAA. Symbols are as in Fig. 5.

unrealistic values of the parameters that match data from the experiments described here. On the basis of the present experiments, there would be no way of distinguishing among them. For example, we suppose that the enzyme activity varied linearly across the entire membrane. Analysis of this model is complicated by the fact that the mathematical statement of the concentration profile involves modified Bessel functions of imaginary argument and orders 0 and 1. We omit the details; see Bunow (1970). This model was also fitted to the data of Figs. 2–4. The best estimates of D and and the average value of κ turn out to be within 50%, i.e., within the experimental uncertainty, of those obtained using the piecewise constant activity model. This model, then, cannot be excluded on physical grounds. We can, however, eliminate all symmetrical enzyme distributions on the basis of the outcomes of the experiments reported in Figs. 2–4, since a symmetrical environment (equal substrate concentrations in the baths on both sides) developed unsymmetrical product concentrations between the two sides.

The assumptions made in developing the model did not prevent it from performing satisfactorily. Several of the assumptions are subject to independent, albeit partial, verification. We comment here on these verifiable assumptions.

Equal Diffusion Coefficients

The papain-collodion membranes used in these experiments are highly hydrated, >90% water by weight. At this porosity, the Stokes-Einstein relation is probably appropriate for estimating diffusion coefficients, up to a tortuosity factor. On this basis, the diffusion coefficients of the substrate and product differ by a factor of <25%, i.e., within our experimental uncertainty, so the assumption of equal diffusion coefficients is acceptable.

No Electrochemical Effects

The buffer electrolyte used in all of the experiments was 0.1 M Tris-phosphate. This high ionic strength, combined with the buffering capacity of the medium, almost certainly overcame any local pH or electrochemical effects. The concentration of the electrolytes associated with the reaction, NH_4^+ and N-α-benzoyl-arginine, nowhere exceeded 0.01 M.

Irreversible, First-Order Reaction Kinetics

The equilibrium constant for hydrolysis of BAA is sufficiently large (DeSimone, 1970; DeSimone and Caplan, 1973) that no reverse reaction could have occurred with any concentration of substrates and products obtainable from the initial substrate concentration. This research also showed that the K_m of papain with BAA is ~10 mM. Hence, the substrate concentration, never >4 mM in the external reservoirs, was <50% of the K_m. Inside the membrane, substrate concentrations were even lower, due to the reaction. Hence, the assumption of first-order kinetics is adequately justified.

Constant Enzyme Activity Within the Active Layer, Zero Activity in the Inactive Layer

The first part of this assumption is not verifiable within the confines of the model, as we have already shown. The second part of the assumption is likely to be correct, given the lack of color resulting from the cross-linking reaction in that region.

Steady State of Reactant and Product Concentration Profiles

Concentration profile within the membrane during the experiment is really determined by partial differential equations, which express material balance for each of the species. We solved these equations by numerical methods, with the parameter values determined using the steady state assumption used in the text. The numerical solution showed that, after the first 5–10 min, i.e., before our experimental data were taken, there was no significant difference between the correct profile and that obtained using the steady state assumption. This time to relax to a pseudo–steady state was independent of the initial conditions, assuming them to lie within the range of concentrations: 0 and 4 mM. Hence, this assumption does not affect our outcome.

SIGNIFICANCE OF RESULTS

The membranes described in this manuscript certainly did not perform active transport. Phenomenologically, i.e., in the sense of nonequilibrium thermodynamics applied to biological transport processes (Katchalsky and Curran, 1963), however, there is coupling between the driving force for reaction and the mean transport rates of substrate and product across the membrane (Bunow, 1970, 1978*a*, *b*). It can be seen from examination of Figs. 2–4 that, when our membrane is placed between reservoirs initially at equal concentrations, a difference in concentration between the two reservoirs develops as time goes on. Furthermore, if the membrane is placed between reservoirs at different concentrations of substrate or product, the difference can be maintained for a time much longer than would be required to relax it by diffusion alone in the absence of reaction. In principle, although we have not demonstrated it here, the system of membrane and reservoirs could perform external work by way of the electrochemical gradients produced between the reservoirs in these experiments. The free energy of the reaction supplies the energy that is transiently stored in these gradients. Our membranes convert chemical energy into concentration differences, as do actively transporting biological membranes.

The measurements we have made on our membranes are also comparable with observations made in studying membrane transport: rate of flow of transported species across the membrane surfaces and their dependence and influence on the concentrations on the two sides of the membrane. The results presented here suggest that it would be relatively easy to build any number of models that are compatible with data of this sort, and quite difficult to justify the selection of one or another among them. On the basis of these observations, we argue that many published papers which propose mechanisms for membrane transport on the basis of succussfully fitting a model to flux data, studies with inhibitors, etc. are probably more speculative than deductive. Sachs (1977) illustrates the kind of paper we have in mind, although by no means do we intend to be specifically critical of that work, but see Bunow (1980).

The issue of nonuniqueness described here is distinct from the type of nonuniqueness that commonly arises in determining mechanism from observation of chemical kinetics. In that domain, one commonly makes use of Occam's razor to select the least complex of several competing mechanisms. Also, further analysis of mechanisms that are equivalent with respect to one set of experiments frequently suggests additional experiments that will discriminate among them. In the present case, it is not so obvious, for example, that a piecewise constant distribution of enzyme activity is less complex than a linear near variation. Furthermore, we have been unable to think of any new variations on flux-type measurements that provide any better discrimination among the several models for distribution of enzyme activity.

The problem, as we see it, is that there is a disparity in scale between the questions asked in studies of biological membrane transport and the kinds of measurements performed in attempting to answer those questions. Such questions as, Is the pump located at the serosal or the mucosal surface of the epithelium? cannot be answered by experiments that really report on the entire thickness of the membrane.

APPENDIX A

Derivation of the Model Equations

The continuity equations of the text are linear, and may be solved in closed form to obtain the concentration profiles for substrate and product across the membrane. Instead, the time dependence of concentration of product in the reservoirs on the two sides of the membrane was observed. Here we obtain the differential equations obeyed by the reservoir concentrations:

$$\begin{aligned}
A &= [A^\circ \sinh \beta(\xi - x) + A^\xi \sinh \beta x]/S && 0 < x < \xi \\
A &= A^l - (A^l - A^\xi)(l - x)\nu && \xi < x < l \\
B &= A^\circ + B^\circ + (A\xi - A^\circ + B^\xi - B^\circ)x/\epsilon \\
&\quad + [A^\circ \sinh \beta(\xi - x) + A^\xi \sinh \beta x]/S && 0 < x < \xi \\
B &= B^l - (B^l - B^\xi)(l - x)\nu && \xi < x < l
\end{aligned}$$

where the concentrations of A and B at $x = \xi$ are

$$\begin{aligned}
A^\xi &= (\beta A^\circ + A^l \nu S)/(\beta C + \nu S) \\
B^\xi &= B^\circ(1 - \xi/l) + B^l \xi/l \\
&\quad + (A^\circ - A^\xi)(1 - \xi/l) \\
&\quad + \beta\xi(1 - \xi/l)(A^\circ - A^\xi C)/S
\end{aligned}$$

and $\nu = 1/l - \xi)$, $\beta^2 = \kappa/D$, $C = \cosh \beta\xi$, and $S = \sinh \beta\xi$.

Equations for the Case of Both Baths Permitted to Relax

To compare this model with data measured as concentrations at the boundaries $x = 0$ and $x = l$, we need to obtain the equations of conservation of mass for the reservoirs. It follows from the cylindrical shape of the chambers with ratio of area to length L, that

$$\begin{aligned}
dA^\circ/dt &= D\beta/L(A^\xi - A^\circ C)/S \\
dA^l/dt &= D(A^\xi - A^l)\nu/L \\
dB^\circ/dt &= D/L[(B^\xi - B^\circ - A^\xi)/\xi + \beta(A^\xi - A^\circ C)/S] \\
dB^l/dt &= D/L(B^\xi - B^l)\nu
\end{aligned}$$

where the expressions derived for A^ξ and B^ξ are used.

Values of D, κ, and ξ for the experimental membranes were determined by use of the method of least squares, applied to the differential equations. The resulting nonlinear normal equations were solved by Newton-Kantorovich methods (Himmelblau, 1972).

APPENDIX B

Equations for the Case of One Bath Held at Zero Concentration

The concentration profiles within the membrane, as well as the interfacial concentrations have the same algebraic form as in the previous case, but here they are specialized for the relevant zero concentrations in one bath or the other. We proceed in the same way to obtain the differential equations for the concentrations of substrate and product in the bath which is not maintained at zero concentration.

There are two cases. For the case in which the active surface of the membrane is held at zero concentration, the interfacial concentrations are

$$\begin{aligned}
A^\xi &= A^l \nu S/(\beta C + \nu S) \\
B^\xi &= (B^l \nu\xi + A^\xi)/\nu l
\end{aligned}$$

and the conservation equations for the compartment on the unwashed side are

$$\begin{aligned}
dA^l/dt &= -D\nu/L(A^\xi - A^l) && A^l(0) = A_l \\
dB^l/dt &= -D\nu/L(B^\xi - B^l) && B^l(0) = 0.
\end{aligned}$$

For the case in which the inactive surface is held at zero concentration, the interfacial concentrations are

$$\begin{aligned}
A^\xi &= \beta A^\circ/(\beta C + \nu S) \\
B^\xi &= (B^\circ + A^\xi)/\nu l
\end{aligned}$$

and the material balance equations for the unwashed compartment are

$$\begin{aligned}
dA^\circ/dt &= -D\beta/L(A^\circ - A^\xi C)/S && A^\circ(0) = A^\circ \\
dB^\circ/dt &= -D/L(B^\xi - B^\circ - \beta\xi A^\xi)/\xi && B^\circ(0) = 0.
\end{aligned}$$

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