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THE MEMBRANE VALVE: A CONSEQUENCE OF ASYMMETRICAL INHIBITION OF MEMBRANE CARRIERS

I. EQUILIBRATING TRANSPORT SYSTEMS

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Summary

Facilitated membrane transport systems act as valves, or rectifiers, when the substrate affinities on the two sides of the membrane differ substantially, i.e. when the system is strongly asymmetric. The asymmetry may be intrinsic or imposed by a reversible competitive inhibitor acting on only one side of the membrane. Under non-equilibrium conditions such systems allow net movements of substrate to proceed faster, sometimes much faster, in one direction than the other, though the final equilibrium is unaffected. Obligatory exchange systems may also function as valves when inhibited unsymmetrically, permitting exchange to occur more rapidly with one distribution of substrates than with the reversed distribution. Here, unequal flux rates do not depend on unequal concentrations of the substrate on either side of the membrane, but may also occur with equal concentrations, provided the affinities of the two substrates differ.

The kinetic theory leading to these conclusions is given here, and it is shown how individual parameters of a carrier system affect the efficiency, or tightness, of the valve. In addition, simple kinetic tests for the operation of a valve are outlined. Examples are cited of transport systems having inhibitor-binding sites on only one surface of the cell membrane, which could function normally as valves. Systems implicated are glucose transport in various cells, the ADP-ATP exchanger of mitochondria, the anion transporter of erythrocytes, and the Na^+-K^+ pump.

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Introduction

It may not be without physiological significance that an ordinary facilitated transport system in a biological membrane becomes a valve when it is acted upon by a reversible competitive inhibitor bound on only one surface of the cell membrane. In speaking of a valve, we mean that under non-equilibrium conditions the substrate may be transported in one direction at a very different rate than in the other, and this occurs without conflict with the principle of microscopic reversibility, and without affecting the final substrate concentrations, which in a non-active system are identical on either side of the membrane. Whether the system operates as a valve or not, as well as the direction of the effect (either inward or outward) could be established permanently in the cell or could be subject to metabolic regulation.

The physical basis of the behavior is as follows. When a carrier system is asymmetrically disturbed, for example by a competitive inhibitor acting on only one side of the membrane, the substrate concentrations required to saturate the carrier may differ on the two sides, and if so the maximum transport rates inward and outward must also differ.

Though the idea of the valve appears to be new, inhibition mechanisms have already been demonstrated experimentally which may now be seen to give rise to a valve action. Perhaps the most familiar example is the inhibition of the Na^+-K^+ pump by ouabain and strophanthidin. Briefly, inhibition is competitive with potassium, and the inhibitor-binding site exists only on the external surface of the cell membrane [1—3]. The full significance of this arrangement may become clear later when we explore the operation of valves in active transport systems. It will then be seen that the valve would automatically resist loss of accumulated potassium when the energy supply lapses or the external concentration falls without significantly reducing normal uptake.

Another example occurs in the facilitated transport system for glucose in erythrocytes. Cytochalasin B [4] and certain steroids (Devés, R. and Krupka, R.M., unpublished observations) have been found to inhibit the system by becoming bound to the carrier, in competition with the substrate, on the internal surface of the membrane only. Even in the absence of added inhibitor, the system exhibits a striking asymmetry in substrate affinities, which is of exactly the kind expected if an endogenous inhibitor binds at the cytochalasin site [5]. This system, therefore, would almost certainly act as a valve; as we shall see, it would retard sugar entry when the serum level rises, while allowing its release when the level in the serum falls.

The ADP-ATP exchanger of mitochondria furnishes a third example. A binding site for the reversible competitive inhibitor atractyloside was shown to be present on the external but not the internal surface of the inner membrane [6,7]; moreover, in the energized state of the mitochondrion, the affinities of ADP and ATP were shown to differ greatly [8]. These two circumstances lead to resistance to the exit but not entry of ADP, and conversely, to the entry but not exit of ATP.

Mechanism of the valve

The valve effect produced by a reversible competitive inhibitor depends on the following kinetic properties of carrier systems. When a competitive inhibitor adds to carrier on only one side of the membrane, a substrate on that side competes with the inhibitor and, if present at a sufficiently high concentration, overcomes the inhibition. Substrate on the other side, however, cannot compete, and inhibition is unabated whatever the substrate concentration. Substrate in the same compartment as the inhibitor may therefore be transported more rapidly than substrate at the same concentration in the opposite compartment (*trans* with respect to the inhibitor).

Net transport systems

The analysis described below leads to the following general conclusions.

(1) If the substrate concentration on one side of the membrane fluctuates, a valve tends to stabilize the concentration on the other side at a level either greater or less than that attained with a symmetrical transport system, depending on the direction of the valve.

(2) The valve tightens as the substrate and inhibitor concentrations rise.

(3) The effectiveness of the valve is heightened if the carrier-substrate complex undergoes a more rapid reorientation in the membrane than does the free carrier.

Kinetic analysis

(a) General rate equations. The rate of transport of a substrate S, in the presence of an inhibitor I on both sides of the membrane, is, on the basis of the scheme in Fig. 1, governed by the following equation [9]:

$$\frac{d[S_i]}{dt} = \frac{-d[S_o]}{dt} = \frac{f_1 f_{-2} C_t([S_o] - [S_i])/K_{S_i}}{\left(1 + \frac{[S_o]}{K_{S_o}} + \frac{[I_o]}{K_{I_o}}\right)\left(f_{-1} + f_{-2}\frac{[S_i]}{K_{S_i}}\right) + \left(1 + \frac{[S_i]}{K_{S_i}} + \frac{[I_i]}{K_{I_i}}\right)\left(f_1 + f_2\frac{[S_o]}{K_{S_o}}\right)} \quad (1)$$

where C_t represents the total amount of carrier in all forms, and the subscripts o and i designate the compartments outside and inside the cell, respectively.

When a valve is in operation the inhibitor binds on only one side; for the sake of illustration we shall assume that it binds on the outer side. The effect is most readily understood by considering zero *trans* rate measurements, that is, initial rates of substrate movement when the concentration of substrate on the far side of the membrane (*trans*) is zero. The initial rate of entry is found by setting $[S_i] = 0$:

$$\frac{-d[S_o]}{dt} = \frac{f_1 f_{-2} C_t [S_o]/K_{S_i}}{f_1 + f_{-1} + \frac{[S_o]}{K_{S_o}}(f_{-1} + f_2) + f_{-1}\frac{[I_o]}{K_{I_o}}} \quad (2)$$

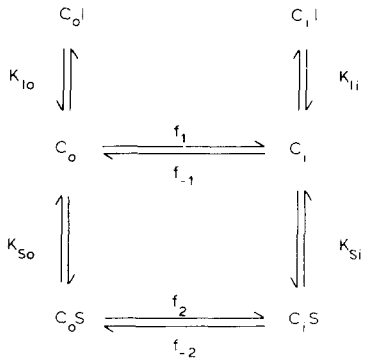

Fig. 1. Kinetic scheme for transport of substrate, S, in the presence of a competitive inhibitor, I, on both sides of the cell membrane. The subscripts o and i refer to forms of the carrier which combine with substrate or inhibitor in the external and internal solutions, respectively. K_{S_o}, K_{I_o} etc. are dissociation constants, and f_1, f_2 etc. rate constants for carrier reorientation steps in the membrane.

and the rate of exit by setting $[S_o] = 0$:

$$\frac{-d[S_i]}{dt} = \frac{f_1 f_{-2} C_t [S_i]/K_{S_i}}{f_1 + f_{-1} + \frac{[S_i]}{K_{S_i}}(f_1 + f_{-2}) + \frac{[I_o]}{K_{I_o}}\left(f_{-1} + f_{-2}\frac{[S_i]}{K_{S_i}}\right)} \tag{3}$$

The ratio of the rates of entry and exit, assuming that the same substrate concentrations are used ($[S_o] = [S_i] = [S]$), equals:

$$T = \frac{v_{in}}{v_{out}} = \frac{f_1 + f_{-1} + \frac{[S]}{K_{S_i}}(f_1 + f_{-2}) + \frac{[I_o]}{K_{I_o}}\left(f_{-1} + f_{-2}\frac{[S]}{K_{S_i}}\right)}{f_1 + f_{-1} + \frac{[S]}{K_{S_o}}(f_{-1} + f_2) + f_{-1}\frac{[I_o]}{K_{I_o}}} \tag{4}$$

T represents the 'tightness' of the valve and may be defined as the resistance to substrate flux in one direction compared to that in the other. When T is far from unity (either much larger or much smaller) the valve is tight; when $T = 1$ it offers no more resistance to passage one way than the other. In a symmetrical mechanism, in which $K_{S_o} = K_{S_i}$, $f_1 = f_{-1}$ and $f_2 = f_{-2}$, this ratio equals unity if $[I] = 0$, as may be shown by invoking the relationship,

$$\frac{f_1 f_{-2} K_{S_o}}{f_{-1} f_2 K_{S_i}} = 1 \tag{5}$$

Eqn. 5 is a necessary consequence of the principle of detailed equilibrium.

At sufficiently high substrate concentrations the ratio T approaches

$$T = \frac{K_{S_o}(f_1 + f_{-2} + f_{-2}[I_o]/K_{I_o})}{K_{S_i}(f_{-1} + f_2)} \tag{6}$$

while at vanishingly low substrate concentrations $T \to 1$. Understandably, rates can differ greatly at high but not at low substrate concentrations. Also, the effect is seen to be enhanced if the ratios f_{-2}/f_1 or $[I_o]/K_{I_o}$ are large.

(b) Dependence of valve tightness on transport parameters. A more general

equation for T may be written for the case where substrate at a concentration $[S_1]$, either inside or outside the cell, moves across the membrane into a solution of lower concentration, $[S_2]$. For simplicity we assume that $K_{S_i} = K_{S_o} = K_S$, $f_1 = f_{-1}$, and $f_2 = f_{-2}$:

$$T = \frac{v_{in}}{v_{out}} = \frac{A + \left(f_1 + f_2 \frac{[S_1]}{K_S}\right)\frac{[I_o]}{K_{I_o}}}{A + \left(f_1 + f_2 \frac{[S_2]}{K_S}\right)\frac{[I_o]}{K_{I_o}}} \tag{7}$$

where

$$A = 2f_1 + \frac{(f_1 + f_2)}{K_S}([S_1] + [S_2]) + \frac{2f_2}{K_S}[S_1][S_2]$$

In estimating the magnitude of the valve effect we shall first consider a gradient in which the upper substrate concentration exceeds K_S, while the lower one is less than K_S, in each case by a factor of 5: $[S_1]/K_S = 5$ and $[S_2]/K_S = 0.2$. If $[I_o]/K_{I_o} = 10$, T rises to a maximum as f_2/f_1 increases (Fig. 2A). With rising inhibitor concentrations, and f_2/f_1 set at 5, T again approaches a maximum (Fig. 2B). With $[I_o]/K_{I_o} = 20$, the entry rate is reduced by a factor of only 2, but exit drops by a factor of 14. Thus, a substantial asymmetry is rates is achieved under conditions where transport efficiency is not seriously impaired. Raising the upper substrate concentration (with $f_2/f_1 = 5$, $[I_o]/K_{I_o} = 10$, and $[S_2]/K_S = 0.2$) also elevates the value of T, and to a maximum as before (Fig. 2C).

The valve tightness, T, rises indefinitely if both the upper substrate concentration, $[S_1]$, and the inhibitor concentration are increased without limit. This is made evident upon substitution of the following values into Eqn. 7; $[S_1]/K_S = a[I_o]/K_{I_o}$; $[S_2] = 0$:

$$T = 1 + \frac{\frac{f_2}{af_1}\left(\frac{[S_1]}{K_S}\right)^2}{2 + \frac{[S_1]}{K_S}\left(1 + \frac{f_2}{f_1} + \frac{1}{a}\right)} \tag{8}$$

(c) Computer generation of concentration, time curves. The operation of a valve under circumstances where the external substrate concentration fluctuates in a regular way was followed by means of numerical integration of the general rate equation (1) by the Runge-Kutta method [11]. Fluctuating values for the external substrate concentration were generated from a sine curve which could be truncated at upper and lower limits. A computer program was written in which the quantity of substrate transported across the membrane was calculated at intervals of 0.016 times the length of one complete cycle. Integrations were carried out over several cycles, and values of internal and external substrate concentrations were printed out after 3 full cycles, when fluctuations in internal substrate concentration had become stabilized. The mean values of substrate concentrations inside and outside the cell were then calculated over one full cycle, as well as the maximum and minimum internal concentrations.

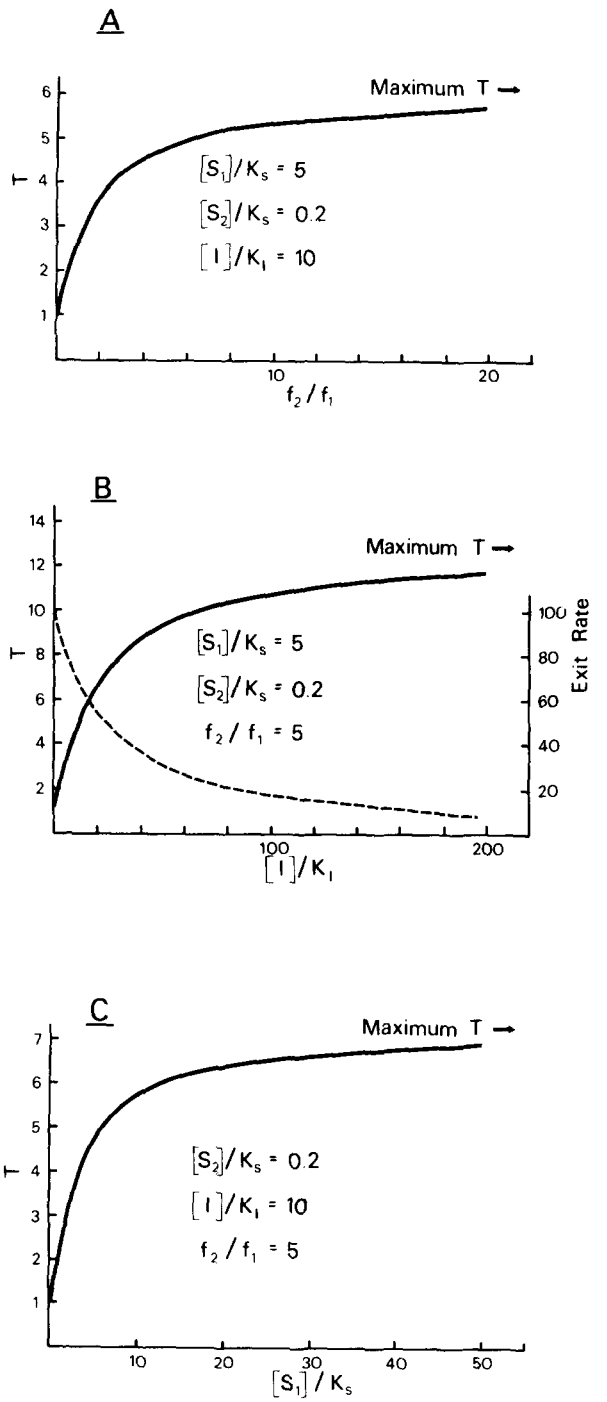

Fig. 2. The dependence of valve tightness, T (the relative resistance to substrate flux across the membrane in opposite directions), on several parameters of the transport system (see text). A. Dependence on the relative reorientation rates of carrier-substrate complex and free carrier. B. Dependence on the inhibitor concentration. (The dependence of transport rates in the favored direction on the inhibitor concentration is indicated by the dotted line and the scale on the right.) C. Dependence on the substrate concentration.

Calculations were carried out for four different cases: (1) The inhibitor binds only on the external surface of the membrane, as in the foregoing treatment. This valve may be referred to as 'inwardly directed', since inflow is allowed while outflow is impeded. (2) The inhibitor is only bound on the internal membrane face. The valve is now 'outwardly directed'. (3) The symmetry of the system is preserved by allowing the inhibitor to bind on both surfaces and in this case there is no valve. In order that the rate reduction be similar to that in cases (1) and (2), the inhibitor concentration is set at half the previous level (since the inhibitor binds on two surfaces instead of only one). (4) No inhibitor is present.

The plots in Figs. 3 and 4 show that a valve has the expected effect. Clearly an inwardly directed valve ($[I_i]/K_{I_i} = 0$) maintains a higher internal substrate concentration than the symmetrical control, while an outwardly directed valve $[I_o]/K_{I_o} = 0$) maintains a lower concentration. The possible advantage of an inwardly directed valve is easily seen under conditions where the external substrate is absent for a period of time before rising again (Fig. 4). Without a valve, or with an outwardly directed valve, $[S_i]$ falls to practically zero when the

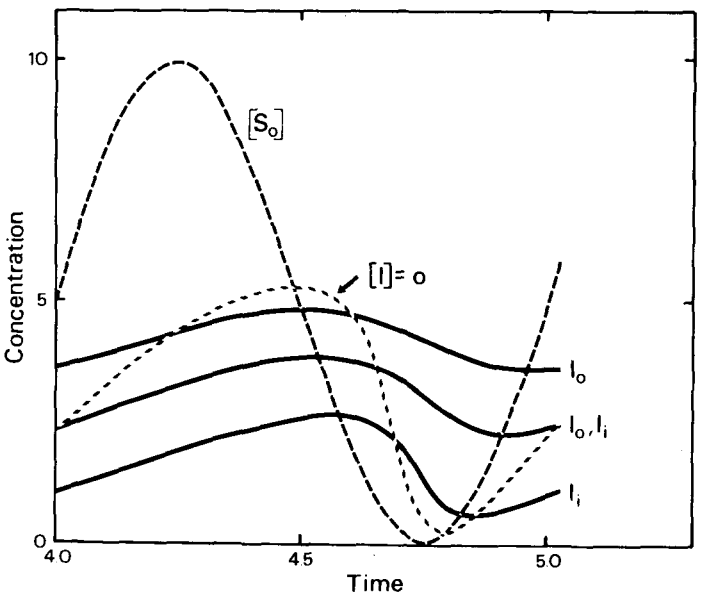

Fig. 3. The concentrations of substrate attained inside the cell in the presence of a regularly fluctuating substrate concentration outside the cell (broken line, $[S_o]$), with three different orientations of inhibiting sites (solid lines) or in the absence of inhibitor (broken line, $[I] = 0$). The internal substrate concentrations have reached stable levels after four full cycles of variation in the external concentration, and variations during the fifth cycle are shown. I_o, a non-symmetrical transport system in which a competitive inhibitor binds to the carrier at the outer surface of the cell membrane but not at the inner surface; this valve is described as 'inwardly directed'. I_i, a non-symmetrical system in which a competitive inhibitor binds to the carrier exposed at the inner membrane surface only; this valve is 'outwardly directed'. I_o, I_i, a symmetrical transport system, with inhibitor bound equally on both sides of the membrane, for comparison with inwardly and outwardly directed valves. A symmetrical system does not function as a valve. $[I] = 0$, a symmetrical system, in the absence of inhibitor. Transport parameters used in the calculation: $f_2/f_1 = 20$; $K_{S_o} = K_{S_i} = 5$; $f_1 = f_{-1}$; $f_2 = f_{-2}$; $[I]/K_I = 25$ (see text). Mean and minimum internal substrate concentrations relative to the mean external concentration over one full cycle are as follows. I_o: 0.85, 0.72; I_i: 0.35, 0.13; $I_o I_i$: 0.62, 0.46; $[I] = 0$: 0.66, 0.035.

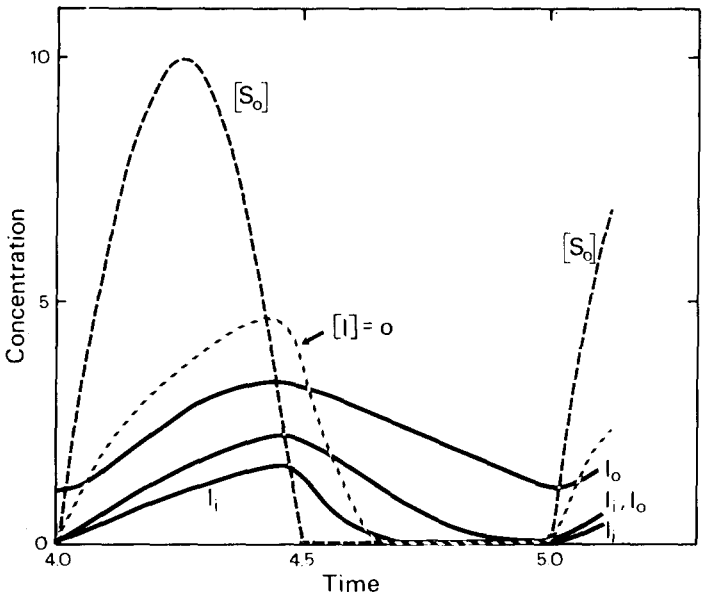

Fig. 4. Intracellular substrate concentrations (solid lines and broken line, $[I] = 0$) in the presence of regularly fluctuating external substrate concentrations (broken line, $[S_o]$) as in Fig. 3. Internal concentrations under the influence of inwardly and outwardly directed valves are labeled I_o and I_i, respectively, and the symmetrical system I_o, I_i, or $[I] = 0$ in the absence of inhibitor. Transport parameters are identical to those used for Fig. 3. Mean and minimum internal substrate concentrations relative to the mean external concentration over one full cycle are as follows. I_o: 0.74, 0.35; I_i: 0.18, $2 \cdot 10^{-4}$; $I_o I_i$: 0.34, $8 \cdot 10^{-3}$; $[I] = 0$: 0.53, $4 \cdot 10^{-14}$.

external substrate is removed, whereas it is sustained at a fairly high level when the valve is inwardly directed, with comparatively little fluctuation.

Carrier symmetry and asymmetric flux

The general rate equation (1) may be recast into a form which includes only experimental constants, as follows:

$$\frac{d[S_i]}{dt} = \frac{\left(\frac{V}{K_m}([S_o] - [S_i])\right)}{1 + \frac{[S_o]}{K_{m_o}} + \frac{[S_i]}{K_{m_i}} + \frac{[S_i][S_o]}{K_{m_i}K'_{m_o}}} \tag{9}$$

where

$$K_{m_o} = K_{S_o}\left\{\frac{1 + \frac{[I_o]}{K_{I_o}} + \frac{f_1}{f_{-1}}\left(1 + \frac{[I_i]}{K_{I_i}}\right)}{1 + \frac{f_2}{f_{-1}}\left(1 + \frac{[I_i]}{K_{I_i}}\right)}\right\}$$

$$K_{m_i} = K_{S_i}\left\{\frac{1 + \frac{[I_i]}{K_{I_i}} + \frac{f_{-1}}{f_1}\left(1 + \frac{[I_o]}{K_{I_o}}\right)}{1 + \frac{f_{-2}}{f_1}\left(1 + \frac{[I_o]}{K_{I_o}}\right)}\right\}$$

$$K'_{m_o} = K_{S_o}\left\{\frac{1 + \frac{f_1}{f_{-2}} + \frac{[I_o]}{K_{I_o}}}{1 + \frac{f_2}{f_{-2}}}\right\}$$

K_{m_o}, K_{m_i} and K'_{m_o} are the substrate affinity constants as determined in zero *trans* entry, zero *trans* exit, and infinite *cis* exit (Sen-Widdas) experiments, respectively, for the case where an endogenous inhibitor I may be present. V/K_m is the ratio of the maximum rate to the affinity constant as determined in zero *trans* entry, zero *trans* exit, or equilibrium exchange experiments. The same ratio is found in different experiments whether or not an unknown inhibitor disturbs the system, and this follows from the necessary relationship among the constants for any equilibrating transport system given in Eqn. 5.

The ratio of zero *trans* efflux and influx rates at saturating (and equal) substrate concentrations is given by K_{m_i}/K_{m_o}. Clearly, whenever these constants are unequal, whether because of uneven distribution of an inhibitor, or because of built-in asymmetry in the carrier and membrane structures, the ratio will deviate from unity and the system will behave as a valve, as described above.

Obligatory exchange systems

Certain facilitated diffusion systems, such as the ADP-ATP exchanger of the inner mitochondrial membrane, allow exchange but not net movement of substrate molecules across the membrane. This behavior may be explained if only the carrier-substrate complex, and not the free carrier, undergoes reorientation, or movement, associated with the process of transport; a condition represented by the transport scheme in Fig. 5. The rates of transport for two exchanging substrates S and P are then given by the following equation:

$$\frac{d[S_i]}{dt} = \frac{-d[P_i]}{dt} = \{f_{-2}f_3C_t([S_o][P_i] - [S_i][P_o])/K_{S_i}K_{P_o}\}$$
$$\times \left\{\left(1 + \frac{[S_o]}{K_{S_o}} + \frac{[P_o]}{K_{P_o}} + \frac{[I_o]}{K_{I_o}}\right)\left(f_{-2}\frac{[S_i]}{K_{S_i}} + f_{-3}\frac{[P_i]}{K_{P_i}}\right)\right.$$
$$\left. + \left(1 + \frac{[S_i]}{K_{S_i}} + \frac{[P_i]}{K_{P_i}} + \frac{[I_i]}{K_{I_i}}\right)\left(f_2\frac{[S_o]}{K_{S_o}} + f_3\frac{[P_o]}{K_{P_o}}\right)\right\}^{-1} \quad (10)$$

Expressions may be written for the tightness of the valve when the distributions of the substrates are reversed; thus, the flux of S, restricted to the outside of the cell, exchanging with P inside, may be compared to the flux of S inside exchanging with P outside. Expressions for valve tightness may then be written applying to two different conditions, either where the concentrations of S and P are assumed to be equal, or are assumed to be unequal.

In the case of unequal concentrations, we may consider S to be present on

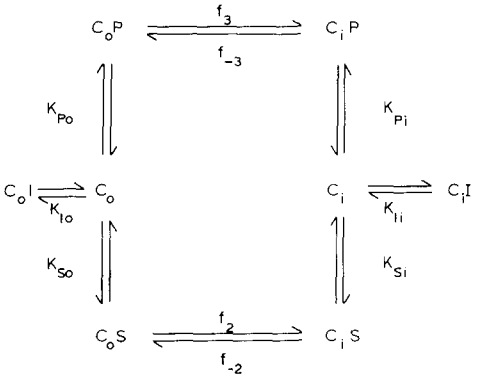

Fig. 5. Kinetic scheme for movement of substrates S and P in an obligatory exchange system, in the presence of a competitive inhibitor I on either side of the membrane. Subscripts and constants are defined as in Fig. 1.

one side of the membrane at a saturating concentration, and P to be present on the opposite side at a lower concentration equal to its affinity constant on the inner surface, K_{P_i}. Assuming that the inhibitor is only bound outside, and is present at a saturating concentration ($[I_o]/K_{I_o} >> 1$, and $[I_i]/K_{I_i} << 1$), we find the relative initial rates of movement of substrate S inward and outward to be:

$$T = \frac{v_{in}}{v_{out}} = \frac{K_{S_o}\{(f_{-2} + f_3)\, K_{P_i}/K_{P_o} + f_{-2}[I_o]/K_{I_o}\}}{K_{S_i}\left\{2f_2 + f_{-3} + f_{-3}\,\dfrac{[I_o]\; K_{S_o}}{K_{I_o}\;[S_o]}\right\}} \tag{11}$$

Obviously the efficiency of this valve depends both on the inhibitor concentration and on the affinity and flux constants for the two substrates, in a manner specified by the terms of Eqn. 11. The behavior resembles that of ordinary facilitated diffusion systems, as described above.

The corresponding equation may be written on the assumption that the substrate concentrations are equal, and also that $K_{S_o} = K_{S_i} = K_S$, $K_{P_o} = K_{P_i} = K_P$, $f_2 = f_{-2}$, and $f_3 = f_{-3}$:

$$T = \frac{1 + \dfrac{f_2[I_o]}{K_S K_{I_o} B}}{1 + \dfrac{f_3[I_o]}{K_P K_{I_o} B}} \tag{12}$$

where $B = \dfrac{f_2}{K_S} + \dfrac{f_3}{K_P} + [X]\,\dfrac{(f_2 + f_3)}{K_S K_P}$

and where $[X]$ is the concentration of substrate. If there is sufficient inhibitor to make the second terms dominant, it is seen that T approaches f_2K_P/f_3K_S in value. The valve will therefore be tight whenever the affinity and maximum exchange flux are greater for one substrate than for the other. It is of interest that here the valve is effective even at very low substrate concentrations, in contrast to our findings for net movement (Eqns. 1 and 4).

Relationships between exchange rates and concentrations of two substrates are plotted in Figs. 6 and 7, under conditions where the combined concentra-

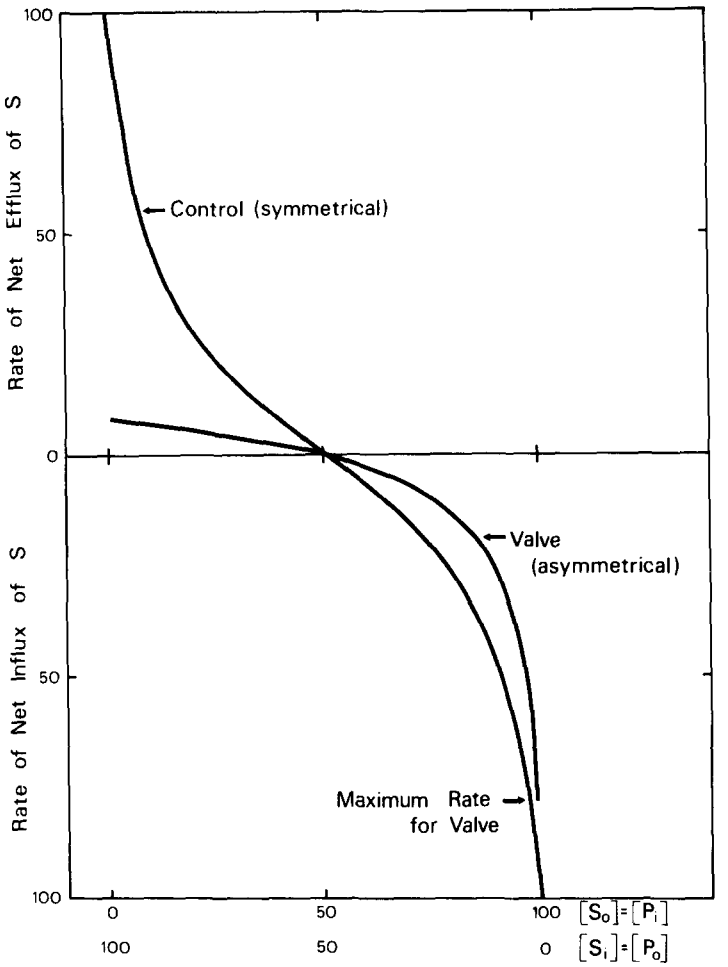

Fig. 6. Rates of exchange of two substrates, S and P, by an obligatory exchange transport system, with varying distributions of the substrates across the membrane, either in the absence of inhibitor (control) or in the presence of a competitive inhibitor acting on the external surface of the membrane only (valve). On the extreme left hand side of the figure, substrate S is present only inside the cell and substrate P only outside; on the right hand side the distribution is reversed. Transport parameters used in the calculations are, with reference to Fig. 5: $K_{S_o} = K_{S_i} = 10$; $K_{P_o} = K_{P_i} = 100$; $[I_o]/K_{I_o} = 25$; $f_2/f_3 = 4$; $f_2 = f_{-2}$; $f_3 = f_{-3}$. It is seen that under these conditions there is strong inhibition of the efflux but not influx of substrate S, and conversely, of the influx but not efflux of substrate P.

tions of both are constant, and equal, on each side of the membrane, but where their distribution is subject to alteration. In these calculations the affinities of the substrates have been assumed to differ by a factor of either 10 or 100, and their individual maximum flux rates by a factor of 4. Without a valve, the expected symmetry in rates with varying substrate distribution is seen; with a valve, striking asymmmetry is apparent.

Kinetic tests for a valve mechanism

The essential feature underlying the valve mechanism is asymmetry. As was noted above, asymmetry may be imposed upon a symmetrical transport system

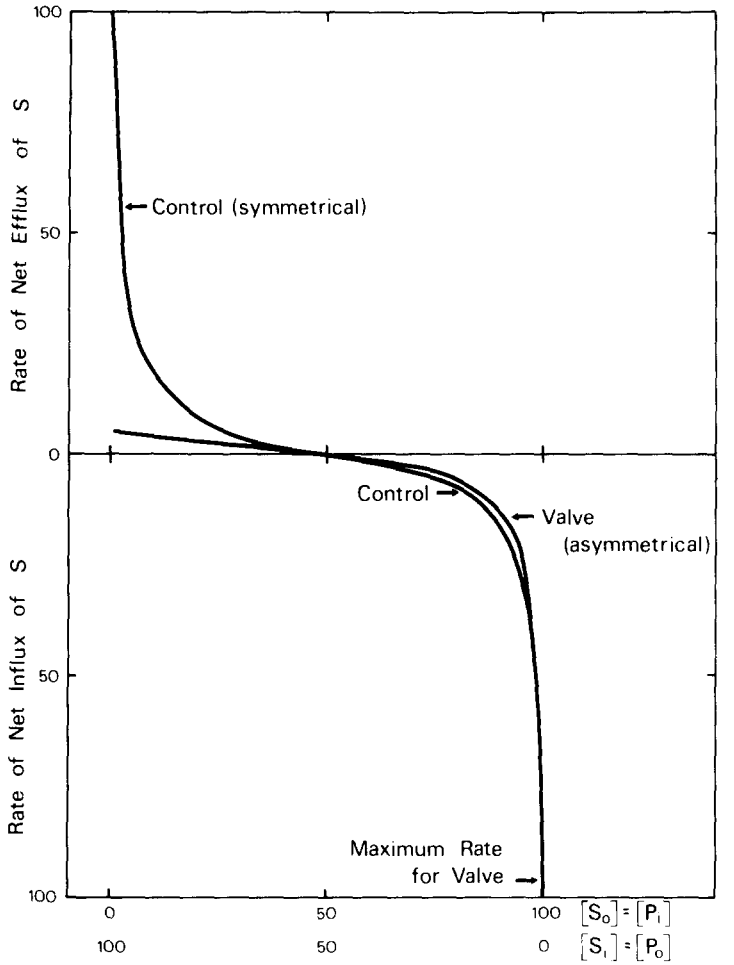

Fig. 7. The operation of a valve in an obligatory exchange system, as in Fig. 6. The assumed transport parameters are identical to those of Fig. 6, except for substrate affinites, which have the following values: $K_{S_o} = K_{S_i} = 3$; $K_{P_o} = K_{P_i} = 300$.

by an inhibitor acting on only on side of the membrane, or it may be intrinsic, resulting from asymmetries in the structures of the carrier and the cell membrane. In either case the experimental parameters for an equilibrating system, in zero *trans* flux measurements, must obey the following relationship:

$$\frac{V_{in}}{K_{m_o}} = \frac{V_{out}}{K_{m_i}} \tag{13}$$

where V_{in} and V_{out} are the maximum rates of influx and efflux, respectively, and K_{m_o} and K_{m_i} the half-saturation concentrations for external and internal substrate, respectively. If V_{in} and V_{out} are unequal, either entry or exit will be favored at substrate concentrations which approach a saturating level. It follows that the most direct evidence for a valve is strong asymmetry in zero

trans exit and entry experiments. In addition, asymmetric inhibition sites, which may be demonstrated by an experimental approach described before [9], are presumptive evidence.

Similar relationships among affinities and transport rates are found for obligatory exchange systems. If exchange of labeled and unlabeled pools of a substrate is measured with a constant and saturating substrate concentration on one side and a varying concentration on the other, then on the basis of Eqn. 10 the ratio of experimental half-saturation constants is given by,

$$\frac{[S_o]_{1/2}}{[S_i]_{1/2}} = \frac{f_{-2}K_{S_o}}{f_2K_{S_i}}(1 + [I_o]/K_{I_o}) \tag{14}$$

If transport rates are compared under similar conditions, with labeled substrate at a saturating level on one side exchanging with unlabeled substrate at a given, vanishingly low, concentration on the other side, the ratio of inward and outward rates is equal to

$$\frac{v_{in}}{v_{out}} = \frac{f_{-2}K_{S_o}}{f_2K_{S_i}}(1 + [I_o]/K_{I_o}) \tag{15}$$

Hence, a relationship identical in form to Eqn. 13 is observed in obligatory exchange. Again the system must function as a valve if asymmetry is pronounced, whatever its physical basis.

Discussion

The membrane valve is analogous to an ordinary mechanical valve located in a tube joining two liquid pools. The mechanical valve can affect the flow rate in one direction or the other when a pressure head is applied, but cannot alter the final levels of liquid, which are equal. Similarly, equilibrium substrate concentrations across the membrane are independent of the operation of a carrier valve. This is evident from Eqn. (1), which shows that net transport ceases when $[S_i] = [S_o]$, regardless of the presence or distribution of an inhibitor. A similar conclusion applies to obligatory exchange systems (Eqn. 10).

The possible advantage to the cell of a valve in an ordinary facilitated transport system is evident from plots of substrate concentration within the cell under conditions where the external concentration fluctuates (Figs. 3 and 4). Where accumulation of substrate and resistance of loss is desirable, an inwardly directed valve, with inhibitor binding restricted to the external surface of the membrane, is called for. Free living organisms such as bacteria and fungi would benefit, it would seem, from such an arrangement. Where there is reason to maintain the availability of the substrate in the medium outside the cell, an outwardly directed valve would serve.

In the glucose transport systems present in two types of blood cell, erythrocytes and leucocytes, there is evidence for an arrangement of this second kind, in that the site of attachment of the competitive inhibitor cytochalasin B is localized on the inner surface of the membrane [4]. As was noted earlier, asymmetries in substrate parameters have been demonstrated in the erythrocyte system [5], consistent with the natural occurrence of an inhibitor, as yet unidentified, which becomes bound to the cytochalasin site, producing an out-

wardly directed valve. In avian erythrocytes, asymmetry is abolished under conditions of anoxia [11], so that the valve would cease to function; this fact may suggest that the valve is subject to metabolic regulation. The outwardly directed valve which may function in these cells under aerobic conditions, as well as in human erythrocytes [5], would give rise to the kind of behavior illustrated in Figs. 3 and 4 (I_i), in response to changing glucose concentrations in the serum.

In view of the possible function of a valve in these cells, the reversal of orientation of the cytochalasin B site in other types of mammalian cells seems particularly significant: in chick embryo fibroblasts, Novikoff hepatoma cells and HeLa cells, the available evidence indicates that the cytochalasin B site is on the outer rather than the inner surface of the membrane [4]. Here, therefore, an inwardly directed valve may sustain the intracellular glucose concentration when the level in the surrounding medium declines. It is gratifying to notice that in these, as well as in blood cells, the orientation of the cytochalasin site, and hence the direction of the assumed valve, is reasonable in terms of known cellular function.

As we have already stated, the ADP-ATP exchanger of mitochondria possesses a site for attachment of the competitive inhibitor atractyloside on only the external surface of the inner membrane [6,7] and such a site obviously has the potential of forming a valve. We have seen, too, that a valve can act in an obligatory exchange system even when the total concentrations on either side are constant and equal, provided that the affinities or maximum transport rates for the two substrates differ. In the energized state of mitochondria the affinity of ADP as measured on the outer surface of the membrane is roughly 100 times that of ATP [8], and the above analysis shows that an externally acting competitive inhibitor would transform the system into a valve allowing ADP to enter the mitochondrion but impeding its exit, and conversely allowing ATP to leave but not enter (Eqn. 12; Figs. 6 and 7). This arrangement seems consistent with the expected cycling of ADP and ATP into and out of mitochondria in the energized state; and under conditions where the external ADP concentration falls sharply, it would prevent a reversal of the cycle.

The demands made in the de-energized state are different. Now ATP synthesis in the mitochondrion ceases, and in the presence of high external ADP the mitochondrion would become entirely depleted of ATP, were the valve to continue in operation. Actually the affinity for ATP is known to rise sharply in this state, becoming equal to that for ADP [8], with a consequent loss of valve function. The necessity for this, and for facilitating ATP entry during the de-energized condition, may be understood by considering that pyruvate carboxylase, a mitochondrial enzyme requiring ATP, is needed to funnel carbon into the citric acid cycle; it does so by forming oxaloacetate from pyruvate and carbon dioxide at the expense of phosphate-bond energy. Following a period in the deenergized state, therefore, resumption of activity in the citric acid cycle, which ultimately produces more ATP, depends on the prior availability of ATP to get the process started. Hence the requirement to override the valve in the de-energized state.

Recent work on another obligatory exchange system, that involved in anion movement in erythrocytes, has revealed a binding site for the competitive

inhibitor NAP-taurine (*N*-(4-azido-2-nitrophenyl)-2-aminoethyl sulfonate) which is present on only the external surface of the cell membrane [12—14]. By implication, this system too could function as a valve if an endogenous inhibitor is present in the serum.

Where an inhibitor and substrate can be shown to compete for the carrier, the restriction of inhibitor sites to one side of the membrane, in contrast to substrate sites which must obviously become exposed at both, could suggest the existence of separate sites linked by allosteric interaction. In this connection, recent experiments in our laboratory point to the conclusion that the sites for glucose and cytochalasin in the glucose system of erythrocytes are separate, even though glucose and cytochalasin compete for the carrier.

Finally, where asymmetric systems are found, the occurrence of endogenous inhibitors would obviously be of great interest. It is conceivable however that such substances could be covalently attached to the carrier or the membrane, in some way that still allows reversible adsorption at an inhibitor site. This would spatially limit the action of the inhibitor and could therefore be important in the cell or tissue economy. If so, their presence could be difficult to demonstrate, but should be manifest in the inherent asymmetry of the system, as well as in the restriction of inhibitor sites to one side of the membrane. Alternatively, membrane or wall structures could function to entrap transport inhibitors and so restrict their action, and in this case disruption of these structures could release the inhibitors.

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