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The Kinetics of Transport Inhibition by Noncompetitive Inhibitors

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Summary. A new analysis of the conventional carrier model shows that noncompetitive inhibitors can give rise to either competitive, noncompetitive or uncompetitive kinetics; the true mechanism and also the relative affinity of the inhibitor on each surface of the membrane can be decided from the patterns of inhibition observed in different transport experiments. The principles governing the kinetics of inhibition apply to both reversible and irreversible inhibitors, for in either case the substrate may increase or decrease inhibition or be without effect. Ambiguity arises if the noncompetitive inhibitor acts on only one side of the membrane and if the substrate, in the course of being transported, alters the steady-state distribution of the carrier between inner and outer forms. In facilitated transport systems only equilibrium exchange should give rise to noncompetitive kinetics, whatever the location of the inhibitor. In active systems even the interpretation of exchange in the final steadystate is complicated if the energy-coupling mechanism produces a large displacement in the distribution of the free carrier or the substrate complex: the inhibition could be competitive or uncompetitive, depending on the location of the inhibitor. The actual mechanism is revealed in the uncoupled system.

Key Words kinetics · transport inhibition · noncompetitive · competitive · inhibition mechanism · carrier model

Introduction

In studying the inhibition of biological transport, it is well to keep in mind that the observed kinetic behavior is sometimes misleading. For example, competitive inhibitors can produce noncompetitive kinetics if the substrate and inhibitor are on opposite sides of the membrane [1–3]. It is now clear that similar problems are encountered with noncompetitive inhibitors. The analysis given here shows when ambiguity should arise, and how the underlying mechanism can be determined.

Rate equations for substrate transport in the presense of noncompetitive inhibitors are derived below. The treatment is based on the conventional carrier model shown in Fig. 1, in which the carrier alternates between inward-facing and outward-facing conformations. The inhibitor is assumed to act directly on the carrier, and the complex formed with the carrier is assumed to be immobile, i.e. not to undergo reorientation in the membrane.

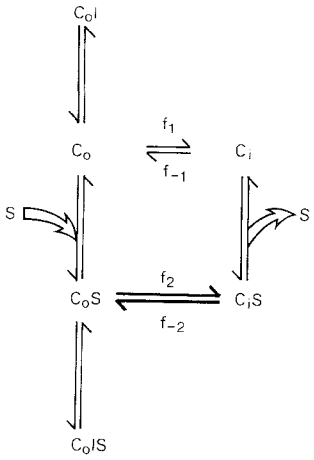

Fig. 1. The conventional cyclic carrier model: a substrate site is alternately exposed on the outer and inner surfaces of the cell membrane. C_o and C_i are the outward-facing and inward-facing forms of the free carrier, respectively, and C_oS and C_iS are the corresponding forms of the carrier-substrate complex. It is often convenient to refer to the interconversion of the inner and outer forms as "movement," though this could involve a conformational change in a carrier molecule spanning the membrane, rather than diffusion of the carrier from one surface of the membrane to the other. In the example shown here, a noncompetitive inhibitor I is present in the suspending medium but not inside the cell, and a substrate S in the external solution is represented as moving inward faster than the free carrier returns, causing the carrier to accumulate in the inner form, away from the inhibitor. The inhibition would therefore be partly or wholly competitive

Table. Inhibition behavior expected of a noncompetitive inhibitor in different transport experiments[a]

Experiment	Inhibitor location	Substrate *trans* effect	Inhibition type
Zero *trans*	*cis*	+	C to M
		−	U
	trans	+	U
		−	C to M
Equilibrium exchange	*cis*	+	N
		−	N
	trans	+	N
		−	N
Infinite *trans*	*cis*	+	M
		−	U
	trans	+	U
		−	M

[a] The observed inhibition may be competitive (C), noncompetitive (N), mixed competitive and noncompetitive (M) or mixed noncompetitive and uncompetitive (U), as illustrated in Fig. 2. The inhibitor is assumed to be confined to one side of the membrane, either in the same compartment as the transported substrate (*cis*) or in the opposite compartment (*trans*). The substrate may exhibit either *trans* acceleration, indicated by a plus sign (where exchange is faster than zero *trans* flux), or *trans* inhibition, indicated by a minus sign (where exchange is slower than zero *trans* flux).

In two cases the inhibition should be noncompetitive in all experiments: (i) when the inhibitor adds to the carrier on both sides of the membrane, or when addition is independent of carrier orientation; and (ii) when equilibrium exchange and zero *trans* rates are equal.

Before examining the rate equations, it may be an advantage to consider the principles which emerge from the analysis, as well as the inhibition kinetics in various experiments resulting from the operation of these principles. In the interest of simplicity, the inhibitor is first assumed to add with equal affinity to the free carrier and the carrier-substrate complex; that is, to be purely noncompetitive in mechanism, though the rate equations are more general and allow for the possibility of unequal affinities. A summary of the conclusions is given in the Table, which may serve as a convenient reference in interpreting or designing experiments.

The kinetics of inhibition can be observed in several different types of experiment. The three most common are as follows: (i) In zero *trans* experiments, the substrate concentration in the *trans* compartment is initially equal to zero. By convention, the *trans* compartment is on the opposite side of the membrane to the substrate whose movement is followed; the *cis* compartment is the one occupied by this substrate. (ii) In infinite *trans* experiments the substrate concentration in the *trans* compartment is saturating. (iii) In equilibrium exchange experiments the substrate concentrations in the two compartments are equal.

General Principles

Facilitated Transport Systems. By altering the steady-state distribution of the carrier between the two membrane faces, a substrate can either increase or decrease the inhibition produced by a noncompetitive inhibitor restricted to one side of the membrane. For example, with an inhibitor outside the cell but not inside, a substrate in the external solution reduces the inhibition if the carrier-substrate complex moves inward through the membrane faster than the free carrier returns. In the steady state the substrate causes the carrier to accumulate in the inward-facing form out of reach of the inhibitor, as illustrated in Fig. 1, and because of this the inhibition is partly or wholly competitive.

It is a simple matter to estimate the relative rates at which the carrier-substrate complex and the free carrier undergo reorientation in the membrane. If the movement of the complex is faster, the maximum rate of equilibrium exchange should be faster than zero *trans* flux, for the former depends on movement of the complex only, and the latter on movement of both the complex and the free carrier (*see* Fig. 1). Such behavior is called accelerated exchange and has been observed, for example, in the glucose [11, 16, 17], choline [10, 14], and leucine [9] transport systems of human erythrocytes.

If instead of zero *trans* entry, zero *trans* exit of the same substrate were followed (with the inhibitor confined to the external solution as before), the inhibition would grow stronger as the substrate concentration rose, for the substrate, in being transported out of the cell, would tend to drive the carrier into the external face of the membrane, in contact with the inhibitor. This behavior resembles uncompetitive inhibition (Fig. 2).

The opposite is true if the complex moves more slowly than the free carrier. The substrate then exhibits *trans* inhibition rather than accelerated exchange; i.e. zero *trans* flux is faster than exchange. In zero *trans* entry experiments the substrate, which is outside the cell, causes the carrier to accumulate on the outer surface in contact with the inhibitor, making the inhibition stronger and in this sense uncompetitive. In exit experiments the substrate, now inside

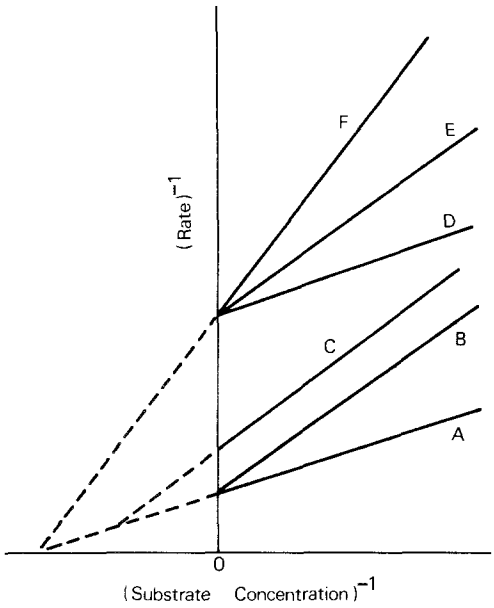

Fig. 2. A diagrammatic representation of different types of inhibition, as seen in reciprocal plots. The pattern of inhibition is determined by the relative values of the substrate-dependent and substrate-independent inhibition constants. The former is calculated from the ratio of slopes of reciprocal plots in the presence and absence of the inhibitor, and the latter from the ratio of intercepts. *A*, control rates in the absence of the inhibitor; *B*, competitive inhibition; *C*, mixed competitive and noncompetitive inhibition; *D*, pure uncompetitive inhibition; *E*, mixed uncompetitive and noncompetitive inhibition; *F*, pure noncompetitive inhibition

the cell, holds the carrier on the inner surface, protecting it from an external inhibitor; the inhibition therefore has a competitive component.

In contrast to zero *trans* entry and exit experiments, equilibrium exchange is usually unambiguous. This is because the substrate is present at equal concentrations on both sides of the membrane, leaving the carrier distribution independent of the relative mobility of the free carrier and the complex. Hence, the substrate should neither protect the carrier nor expose it to the inhibitor, and the observed behavior, like the actual mechanism, should be noncompetitive. An exception to this rule is met in extremely unsymmetrical transport systems, as is apparent from the rate equations for the inhibition of exchange (Eqs. 7 and 8, below). Extreme asymmetry is unlikely in facilitated systems, but if it occurs it would be revealed by differing inward and outward transport rates and could be taken into account. Asymmetry is implicit in active transport, which is considered later.

There should be no ambiguity whatever if a noncompetitive inhibitor is bound to the carrier on both sides of the membrane, or what is equivalent, if it is restricted to one membrane surface but reacts to impair transport, whatever the orientation of the carrier. Either way, the inhibition is independent of the carrier distribution. Also, there is no ambiguity if equilibrium exchange and zero *trans* rates for the substrate are equal: here the free carrier and the complex move at the same speed, so that the carrier distribution is independent of the substrate. In such cases, the substrate has no effect on the inhibition, which is therefore noncompetitive.

Reversible and Irreversible Inhibitors. The kinetic analysis described in a later section applies specifically to reversible inhibitors, but the same principles apply to irreversible inhibitors as well. With either, the inhibition may be competitive, noncompetitive, or uncompetitive; that is, the substrate may protect the system against inactivation, fail to protect, or increase the inhibition, respectively. The kinetic treatment of irreversible inhibition involves rates of inactivation rather than rates of substrate transport in the presence of the inhibitor. Lieb and Stein [13] and Devés and Krupka [5] have treated the general case, where substrates and irreversible inhibitors are present on either side of the membrane, where the inhibitor may react specifically with any of the carrier forms, and where the reaction occurs either within, or outside, the substrate site (competitive or noncompetitive, respectively).

The Sidedness of Inhibition. It is possible for an inhibitor which passively equilibrates across the cell membrane to add specifically to either the inner or outer carrier form. For example cytochalasin B, when added to the external solution, binds with high affinity, and in competition with internal substrate, to the inward-facing glucose carrier in red cells, but does not bind to the outward-facing form [1, 3]. It was previously shown how the relative affinity of a reversible competitive inhibitor for inner and outer carrier forms (the sidedness of binding) can be determined from the kinetics of substrate transport [2, 3], and by applying the rules outlined above it is clear that a similar strategy could be used to determined the sidedness of a noncompetitive inhibitor as well. From the summary in the Table it appears that

equilibrium exchange and zero *trans* experiments should be capable of deciding this question.

Active Transport Systems. Although the rules outlined above are generally applicable to both equilibrative and active systems, the latter can present a special problem. In theory this occurs when the energy coupling mechanism, which is responsible for building up a concentration gradient of the substrate, shifts the carrier distribution between inner and outer forms. For example, where energy is harnessed to accelerate the inward movement of the carrier-substrate complex, thus concentrating the substrate inside the cell, the carrier is driven into the inner face of the membrane in the presence of the substrate. This would occur not only in zero *trans* uptake but in exchange in the final steady state as well. As a result, the inhibition by a noncompetitive inhibitor restricted to the external medium should be competitive in both experiments. On the other hand, an inhibitor attacking only the inner carrier form would produce partially uncompetitive inhibition, which becomes stronger at increasing substrate concentrations. The actual mechanism should be apparent in the uncoupled system, where energy is not available to drive the complex inward, and where exchange should therefore be inhibited noncompetitively.

Correspondingly, the rates of inactivation of the coupled system by an irreversible inhibitor could either decrease or increase in the presence of the substrate, depending on whether the inhibitor attacks the outer or inner carrier form. In the uncoupled system, the substrate should have little effect. Such observations would provide evidence on the coupling mechanism, otherwise difficult to determine.

An Example of Competitive Inhibition by a Noncompetitive Inhibitor. The irreversible inhibition of choline transport in human red cells by N-ethylmaleimide, first described by Martin [15], is in complete accord with the theoretical predictions for a facilitated transport system. It has been shown that N-ethylmaleimide, which rapidly equilibrates across the cell membrane by simple diffusion, reacts only with the inward-facing form of the carrier. Nontransported substrate analogs bound in competition with internal substrate fail to protect the carrier against the inhibitor; the reaction is therefore outside the substrate site and the inhibition mechanism is noncompetitive [5–7, 15]. Considering this, it might seem surprising that high concentrations of choline inside the cell reduce the inactivation rate to almost zero. The explanation is that the carrier-substrate complex moves outward far more rapidly than the free carrier returns, as accelerated exchange of choline proves [10, 14]; the carrier therefore accumulates in the outer form, which does not react with N-ethylmaleimide. The behavior corresponds to the competitive inhibition predicted in zero *trans* experiments with a substrate exhibiting accelerated exchange and occupying the same compartment as the inhibitor.

Given this effect of internal choline, external choline is expected to increase rather than decrease the inhibition by N-ethylmaleimide, and this is that is found. The carrier is now driven into the inner face of the membrane as the substrate is transported inward; the behavior corresponds to the partially uncompetitive inhibition predicted when the substrate and inhibitor are in opposite compartments.

The behavior of slowly transported substrates, which exhibit *trans* inhibition instead of accelerated exchange, as well as the behavior of nontransported substrate analogs, reverses the relations found with choline [5], just as we would predict. Such substrate analogs, when inside the cell, draw the carrier into the inward-facing form and therefore accelerate inhibition by N-ethylmaleimide; but analogs outside draw the carrier to the outer surface of the membrane and protect.

The predictions of theory have been expressed here qualitatively, but in fact quantitative agreement between rates of inactivation of the choline system and the rates of substrate transport were demonstrated [5]. Hence, the findings substantiate an analysis based on the conventional carrier model.

Theory

In order to work out the kinetics of reversible inhibition in a variety of experiments, it will be useful to write a general equation for the transport of two substrates, S and T, in the presence of a noncompetitive inhibitor I. The equation can then be simplified for individual cases; for example unidirectional rates of exchange can be found by letting one substrate represent the labeled, and the other the unlabeled, species. This equation was derived before [2], based on the cyclic carrier model in Figs. 1 and 3, and on the

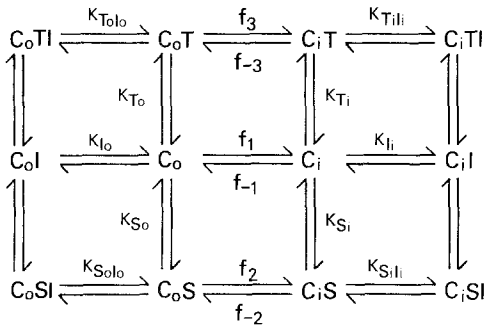

Fig. 3. Transport scheme for two substrates S and T in the presence of a noncompetitive inhibitor I. Subscripts o and i refer to carrier forms on the outer and inner surfaces of the membrane, respectively, and $f_{\pm i}$ are rate constants for reorientation of carrier in the membrane. K_{So}, K_{To}, K_{Io}, etc. are dissociation constants

assumption that the substrate and inhibitor are in rapid equilibrium with the carrier.[1] When written in terms of experimental parameters the rate equation is as follows:

$$v=\frac{\frac{\bar{V}_{Si}}{\bar{K}_{Si}}([S_i]-\alpha[S_o])+\frac{\tilde{V}^T_{Si}}{\tilde{K}^S_{To}\bar{K}_{Si}}\left([S_i][T_o]-\frac{\alpha}{\beta}[S_o][T_i]\right)}{1+\frac{[I_o]}{\bar{K}_{Io}}+\frac{[I_i]}{\bar{K}_{Ii}}+\frac{[S_o]}{\bar{K}_{So}}\left(1+\frac{[I_i]}{\tilde{K}^S_{Ii}}+\frac{[I_o]}{\bar{K}_{SoIo}}\right)+\frac{[S_i]}{\bar{K}_{Si}}\left(1+\frac{[I_o]}{\tilde{K}^S_{Io}}+\frac{[I_i]}{\bar{K}_{SiIi}}\right)}$$
$$+\frac{[T_o]}{\bar{K}_{To}}\left(1+\frac{[I_i]}{\tilde{K}^T_{Ii}}+\frac{[I_o]}{\bar{K}_{ToIo}}\right)+\frac{[T_i]}{\bar{K}_{Ti}}\left(1+\frac{[I_o]}{\tilde{K}^T_{Io}}+\frac{[I_i]}{\bar{K}_{TiIi}}\right)+\frac{[S_o][S_i]}{\bar{K}_{So}\tilde{K}^S_{Si}}\left(1+\frac{[I_o]}{\tilde{K}^S_{SoIo}}+\frac{[I_i]}{\tilde{K}^S_{SiIi}}\right)$$
$$+\frac{[T_o][T_i]}{\bar{K}_{To}\tilde{K}^T_{Ti}}\left(1+\frac{[I_o]}{\tilde{K}^T_{ToIo}}+\frac{[I_i]}{\tilde{K}^T_{TiIi}}\right)+\frac{[S_o][T_i]}{\bar{K}_{So}\tilde{K}^S_{Ti}}\left(1+\frac{[I_o]}{\tilde{K}^T_{SoIo}}+\frac{[I_i]}{\tilde{K}^S_{TiIi}}\right)+\frac{[S_i][T_o]}{\bar{K}_{Si}\tilde{K}^S_{To}}\left(1+\frac{[I_o]}{\tilde{K}^S_{ToIo}}+\frac{[I_i]}{\tilde{K}^T_{SiIi}}\right). \quad (1)$$

The definitions of most of these constants, as well as the relationships existing between them, and equivalent expressions in terms of individual rate constants in the transport scheme in Fig. 3, were given earlier [4].[2] Only the inhibition constants need concern us here, and those for addition to the free carrier are as follows:

$$\bar{K}_{Io}=K_{Io}(1+f_1/f_{-1})$$
$$\bar{K}_{Ii}=K_{Ii}(1+f_{-1}/f_1)$$
$$\tilde{K}^T_{Io}=K_{Io}(1+f_1/f_{-3})$$
$$\tilde{K}^T_{Ii}=K_{Ii}(1+f_{-1}/f_3).$$

In the analysis, S and T represent the same compound, either labeled or unlabeled; hence the constants for S and T are equivalent:

$$\tilde{K}^T_{Io}=\tilde{K}^S_{Io}=K_{Io}(1+f_1/f_{-2})$$
$$\tilde{K}^T_{Ii}=\tilde{K}^S_{Ii}=K_{Ii}(1+f_{-1}/f_2).$$

The constants for addition to the carrier-substrate complex are

$$\bar{K}_{SoIo}=K_{SoIo}(1+f_2/f_{-1})$$
$$\tilde{K}^S_{SoIo}=K_{SoIo}(1+f_2/f_{-2})$$
$$\bar{K}_{SiIi}=K_{SiIi}(1+f_{-2}/f_1)$$
$$=$$
$$\tilde{K}^S_{SiIi}=K_{SiIi}(1+f_{-2}/f_2)$$
$$\tilde{K}^T_{SoIo}=\tilde{K}^S_{ToIo}=\tilde{K}^S_{SoIo}$$
$$\tilde{K}^T_{SiIi}=\tilde{K}^S_{TiIi}=\tilde{K}^S_{SiIi}.$$

α and β are the equilibrium ratios of substrate concentrations: $\alpha=([S_i]/[S_0])_{\text{final}}$ and $\beta=([T_i]/[T_0])_{\text{final}}$. In equilibrating systems α and β are equal to unity, but in active systems they may be very large.

To explore the predictions of the conventional carrier model, four different experiments may be considered: zero *trans* entry, equilibrium exchange, infinite *trans* entry, and Sen-Widdas net exit.

[1] This assumption is probably justified; in the case of the choline transport system of human erythrocytes evidence was presented [5] to show that dissociation of the carrier-substrate complex is a rapid step with all substrate analogs tested, including (i) choline, which exhibits accelerated exchange, (ii) analogs with a low maximum velocity, which are subject to *trans* inhibition, and (iii) analogs which are bound at the substrate site but not transported.

[2] The convention followed in naming the experimental constants, which is intended to suggest the significance of each constant directly, may be summarized as follows. The subscripts S_o, S_i, T_o and T_i denote the substrate transported and its location, outside or inside the cell respectively. The superscripts denote the nature of the experiment in which the constant is determined: "$-$" refers to a zero *trans* experiment, "$\sim$" to an infinite *trans*, and "$=$" to equilibrium exchange; the superscripts S and T designate the saturating *trans* substrate. For example, $\bar{K}_{Si}$ is the substrate half-saturation constant inside, and $\bar{V}_{Si}$ is the maximum rate of exit, both in zero *trans* experiments.

Zero Trans Entry

An expression for the rate of zero *trans* entry may be written by substituting $[S_i]=[T_0]=[T_i]=0$ into Eq. (1):

$$v=\frac{\bar{V}_{So}}{1+\frac{[I_o]}{\bar{K}_{SoIo}}+\frac{[I_i]}{\tilde{K}^S_{Ii}}+\frac{\bar{K}_{So}}{[S_o]}\left(1+\frac{[I_o]}{\bar{K}_{Io}}+\frac{[I_i]}{\bar{K}_{Ii}}\right)}. \quad (2)$$

If the inhibitor is restricted to the external solution ($[I_i]=0$) the Equation, written in reciprocal form, becomes

$$\frac{1}{v}=\frac{1}{\bar{V}_{So}}\left(1+\frac{[I_o]}{\bar{K}_{SoIo}}+\frac{\bar{K}_{So}}{[S_o]}\left(1+\frac{[I_o]}{\bar{K}_{Io}}\right)\right). \quad (3)$$

From Eq. (3), the inhibition is competitive if $\bar{K}_{SoIo}\gg\bar{K}_{Io}$. The ratio of the constants is

$$\frac{\bar{K}_{SoIo}}{\bar{K}_{Io}}=\frac{K_{SoIo}(1+f_2/f_{-1})}{K_{Io}(1+f_1/f_{-1})}. \quad (4)$$

Assuming that $K_{SoIo}=K_{Io}$, as in a purely noncompetitive mechanism, the ratio can be large, and the inhibition purely competitive, if $f_2\gg f_{-1}$ (with $f_1\simeq f_{-1}$). Mixed competitive and noncompetitive inhibition would result from somewhat lower ratios of f_2/f_{-1}. Pure noncompetitive inhibition would be seen with a substrate for which $f_2=f_{-1}$ (making $\bar{K}_{SoIo}=\bar{K}_{Io}$).

With a slowly transported substrate, showing *trans* inhibition instead of accelerated exchange ($f_2<f_{-1}$), the predictions are different.[3] The ratio of inhibition constants in Eq. (4) is now less than unity, and in consequence the inhibition becomes stronger as the substrate concentration rises (still assuming that the inhibitor is confined to the external solution). In reciprocal plots, the intercept rises more than the slope (Eq. 3). The plot is intermediate between pure uncompetitive and pure noncompetitive inhibition, as illustrated in Fig. 2, and may be termed partially uncompetitive.

When the substrate and inhibitor are in opposite compartments, the inhibition patterns are reversed. Equation (2) may be rewritten in reciprocal form for the case where $[I_o]=0$:

$$\frac{1}{v}=\frac{1}{\bar{V}_{So}}\left(1+\frac{[I_i]}{\tilde{K}^S_{Ii}}+\frac{\bar{K}_{So}}{[S_o]}\left(1+\frac{[I_i]}{\bar{K}_{Ii}}\right)\right). \quad (5)$$

The inhibition pattern now depends on the ratio of the internal constants:

$$\frac{\tilde{K}^S_{Ii}}{\bar{K}_{Ii}}=\frac{1+f_{-1}/f_2}{1+f_{-1}/f_1}. \quad (6)$$

If the substrate exhibits *trans* acceleration ($f_2>f_{-1}$), the ratio is less than unity, and the inhibition is partially uncompetitive. With a slowly transported substrate ($f_2<f_{-1}$) the ratio of inhibition constants is greater than unity, and the inhibition is partly competitive.

Equilibrium Exchange

The rate equation for equilibrium exchange is found by substituting $[S_i]=\alpha[T_o]$ and $[S_o]=[T_i]=0$ into Eq. (1). The symbol T is then replaced by S, since S and T represent the same substrate, one being radioactively labeled. After taking account of certain obligatory relationships among the experimental parameters, as demonstrated earlier [4], the equation reduces to

$$v=\frac{\bar{\bar{V}}_S}{1+\frac{[I_o]}{\tilde{K}^S_{SoIo}}+\frac{[I_i]}{\tilde{K}^S_{SiIi}}+\frac{\bar{\bar{K}}_{Si}}{[S_i]}\left(1+\frac{[I_o]}{\bar{K}_{Io}}+\frac{[I_i]}{\bar{K}_{Ii}}\right)}. \quad (7)$$

The type of inhibition now depends on the ratio

$$\frac{\tilde{K}^S_{SoIo}}{\bar{K}_{Io}}=\frac{K_{SoIo}(1+f_2/f_{-2})}{K_{Io}(1+f_1/f_{-1})}. \quad (8)$$

Provided $f_2\simeq f_{-2}$ and $f_1\simeq f_{-1}$, these ratios will not be seriously distorted, and a noncompetitive inhibitor will give noncompetitive kinetics.

Infinite Trans Entry

In this experiment, cells are loaded with a saturating concentration of unlabeled substrate, and the rate of uptake of labeled substrate at various concentrations is measured. The rate equation is found by setting $[S_i]=[T_o]=0$, and $[T_i]\to\infty$ (where T is the nonradioactive form of substrate S present inside the cells), and by taking into account the previously demonstrat-

[3] The relative sizes of the rate constants f_2 and f_{-2} for the carrier-substrate complex, compared with f_1 and f_{-1} for the free carrier (Fig. 1), may be estimated from the relative rates of exchange and zero *trans* entry or exit, as discussed in references [8–10, 12, 18 and 19].

ed [4] relationship $\bar{K}_{To}/\bar{K}_{Si}=\tilde{K}^S_{To}/\tilde{K}^T_{Si}$, as well as the identity of constants involving S and T:

$$v=\frac{\bar{\bar{V}}_S}{\left\{1+\frac{[I_o]}{\tilde{K}^S_{SoIo}}+\frac{[I_i]}{\tilde{K}^S_{SiIi}}+\frac{\tilde{K}^S_{So}}{[S_o]}\left(1+\frac{[I_o]}{\tilde{K}^S_{Io}}+\frac{[I_i]}{\bar{K}_{SiIi}}\right)\right\}}. \tag{9}$$

With an inhibitor restricted to the external solution, the inhibition pattern depends on the ratio $\tilde{K}^S_{SoIo}/\tilde{K}^S_{Io}$; and with an inhibitor acting on the internal surface alone, it depends on $\tilde{K}^S_{Si}/\bar{K}_{SiIi}$. These ratios, found from the constants listed under Eq. (1), do not become large whatever the relative mobilities of the substrate complex and the free carrier (f_2 and f_{-2} compared with f_1 and f_{-1}), and therefore competitive kinetics should not be seen.

Sen-Widdas Exit (Infinite cis net exit)

Here the cells are loaded with a saturating concentration of substrate, and the net rate of exit is determined in the presence of varying concentrations of the same substrate in the external medium ($[S_i]\to\infty$; $[T_o]=[T_i]=0$).

$$v=\frac{\bar{V}_{Si}}{1+\frac{[I_o]}{\tilde{K}^S_{Io}}+\frac{[I_i]}{\bar{K}_{SiIi}}+\frac{[S_o]}{\tilde{K}^S_{So}}\left(1+\frac{[I_o]}{\tilde{K}^S_{SoIo}}+\frac{[I_i]}{\tilde{K}^S_{SiIi}}\right)}. \tag{10}$$

The observed inhibition parallels that in infinite *trans* entry. The substrate distribution in the two experiments is the same, and for this reason the pattern of inhibition depends on the same ratios of constants: $\tilde{K}^S_{SoIo}/\tilde{K}^S_{Io}$ with an external inhibitor, and $\tilde{K}^S_{SiIi}/K_{SiIi}$ with an internal inhibitor. Competitive kinetics would therefore not be seen if the mechanism is noncompetitive.

Active Transport Systems

The interpretation of the inhibition of active transport is more complicated. This is because, in the final steady state, a substrate gradient is established at the expense of metabolic energy: in terms of Eq. (1), an equivalent statement is that α has a value far larger than unity:

$$\alpha=\left(\frac{[S_i]}{[S_o]}\right)_{\text{final}}=\frac{f_{-1}f_2K_{Si}}{f_1f_{-2}K_{So}}. \tag{11}$$

In facilitated transport $\alpha=1$ and the relationship among the constants is dictated by the principle of microscopic reversibility. In active transport, any of the constants could in theory be changed from its value in the equilibrium system to give $\alpha\gg1$. If energy coupling disturbs either the free carrier distribution ($f_{-1}\gg f_1$), or the distribution of the carrier-substrate complex, ($f_2\gg f_{-2}$), the inhibition kinetics will be different in the coupled and uncoupled systems. The characteristic inhibition is predictable from the effects of these inequalities on the substrate-dependent and substrate-independent inhibition constants in various experiments (Eqs. 4, 6 and 8).

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