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Simulation of Differential Effects on Rates in Membrane Transport†

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Solute uptake by a mobile carrier mechanism has been simulated with an analog computer for the case where the movement of the carrier across the membrane is rate-limiting. The ratio of the rate constants for the translocation of the loaded carrier to those for the translocation of the empty carrier was varied to simulate three important conditions of transport: a ratio greater than unity, which led as previously predicted to *trans* stimulation; a ratio less than unity, which led as predicted to *trans* inhibition; and a ratio equal to unity, which eliminated any effect of solute concentration on the *trans* flux. In addition, transients related to the relative amount of the mediator species are recorded. When previous loading of the cells with the solute was simulated, we obtained overshoots in the uptake of labeled solute corresponding to those often seen experimentally. The inhibitory action of 2,4-dinitrofluorobenzene on a well characterized laboratory example of *trans* stimulation was analyzed in terms of the model. Whereas the simulation shows that a change in the ratio of the rate constants for translocation can modify the intensity of the *trans* phenomenon, the action of dinitrofluorobenzene proved instead to arise from decreases in the two carrier fluxes that cause the transport in question to be uphill. The distribution of the influence between these two fluxes may assist identification of the mode of energization of the process.

1. Introduction

Many transport systems have been shown to concentrate a substrate by a *trans* membrane exchange for another substrate of the same system. In a few instances the question can even be raised whether the transport system accomplishes anything but exchange (Brock & Moo-Penn, 1962; Thomas & Christensen, 1971). For the majority of the cases, however, the exchange

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phenomenon is recognized to be closely associated with net transport by the same system. Exchange between solutes proves the physical separation of the entry and the exit events, and provides evidence for the existence of a transport site sequentially but not simultaneously accessible to the substrate from the two sides of the membrane. We will continue here the conventional use of the word *carrier* to imply the mobility thus indicated for the mediating structure, without implications as to how large a part of the structure actually moves.

Heinz & Walsh (1958) showed that it was the *trans* flux (i.e., the flux towards the perturbing mass of solute) which is stimulated in an accelerative exchange (*trans* stimulation), and Heinz & Durbin (1957) provided a theoretical explanation for this behavior. They showed that under the usual transport models a translocation of the solute–carrier complex more rapid than that of the empty carrier could lead to *trans* stimulation.

It is sometimes overlooked that Heinz & Durbin developed in the same paper the prediction that *trans* inhibition would result instead, given the reverse situation that the empty carrier is translocated more rapidly than the solute–carrier complex. The term *trans* inhibition implies that a perturbing mass of transport substrate decreases the flux of the same substrate or its analogs across the membrane toward the perturbing mass. We subsequently showed in this laboratory that *trans* inhibition could be caused to replace *trans* stimulation in a given transport system by chemical modification of the substrate structure (Christensen, 1972).

Oxender & Whitmore (1966) showed that the treatment of Ehrlich ascites tumor cells with 2,4-dinitrofluorobenzene could largely eliminate the vigorous *trans* stimulation normally shown by Na^+-independent system *L*, with only minor effects on net transport by this system. This result could have implied that the exchange phenomenon is really not an inherent part of the transport process, even though inhibition analysis has suggested that it is (Christensen & Handlogten, 1968). To us, it has seemed more likely, however, that this reagent might produce its effect by causing a slowing of the translocation of the loaded carrier, a slowing not shared proportionately by the empty carrier; thus, the reagent may modify the flux differential presumably responsible for *trans* stimulation. To examine the theoretical behavior of such systems, we have tested the effects of changing each of the translocation rate constants under analog computer simulation.

The simulation results substantiate the prediction that a differential slowing of the translocation of the two carrier species can decrease or increase *trans* stimulation. The laboratory test of the effect of dinitrofluorobenzene on amino acid transport showed, however, that the reagent appears instead to decrease the asymmetry between the rate constants that account for the uphill character

of the transport. Accordingly, an interference with energization appears more likely.

The results of the computer simulation are in agreement with the mathematical treatments of carrier transport provided by Stein (1967), Geck (1971), Britton (1965), and Kotyk (1973), among others, and involve no new assumptions. The simulation has the advantage of allowing us an easy manipulation of the mathematical model not only to obtain solutions under complex conditions but also to test for good fit between theoretical and experimentally determined values.

2. The Mobile Carrier Model

Figure 1 illustrates a conventional carrier transport mechanism which can apply equally well to active or passive transport. The model involves the binding of the solute to a carrier at the membrane surface (side 1), the move-

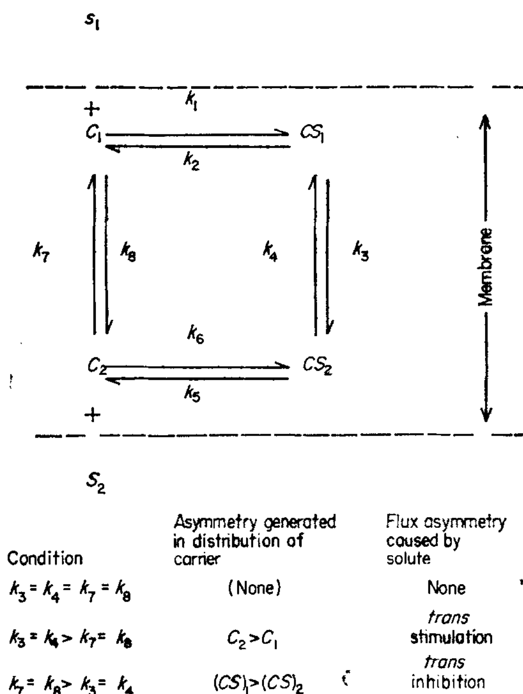

FIG. 1. Proposed model of mediated transport. S_1 and S_2 are the solute concentrations on the outside and on the inside of the membrane respectively, and C is a mobile carrier. The model can apply either to an active transport if an energy input is provided, or to a facilitated diffusion.

ment of the carrier–solute complex across the membrane, and the release of the solute on the other side (side 2). Distribution of the solute and the carrier species can be described in terms of the model by differential equations (1) through (5):

$$\frac{d[C]_1}{dt} = k_2[CS]_1 + k_7[C]_2 - (k_1[S]_1 + k_8)[C]_1 \quad (1)$$

$$\frac{d[CS]_1}{dt} = k_1[S]_1[C]_1 + k_4[CS]_2 - (k_2 + k_3)[CS]_1 \quad (2)$$

$$\frac{d[CS]_2}{dt} = k_3[CS]_1 + k_6[S]_2[C]_2 - (k_4 + k_5)[CS]_2 \quad (3)$$

$$\frac{d[C]_2}{dt} = k_5[CS]_2 + k_8[C]_1 - (k_6[S]_2 + k_7)[C]_2 \quad (4)$$

$$\frac{d[S]_2}{dt} = k_5[CS]_2 - k_6[S]_2[C]_2 \quad (5)$$

and the mass-balance equation

$$C_{total} = [C]_1 + [CS]_1 + [CS]_2 + [C]_2. \quad (6)$$

Solution of these equations can be simplified by the following conventional restriction: Reaction of the solute with the carrier at either membrane surface will be assumed to proceed at a much faster rate than the movement of either the loaded or the empty carrier across the membrane. In terms of the model, the magnitudes of the rate constants k_1, k_2, k_5 and k_6 are assumed much greater than those of k_3, k_4, k_7 and k_8.

We will call a passive transport in which the loaded and the unloaded carrier show the same translocation rate constant, i.e., in which $k_3 = k_4 = k_7 = k_8$, a *balanced* transport. We might have proposed the term *symmetrical* transport, except that the latter term has already been pre-empted. Stein (1967) defines a symmetrical transport as one having $k_1 = k_6$, $k_3 = k_4$, $k_2 = k_5$ and $k_7 = k_8$, i.e., a facilitated diffusion for which K_m and V_{max} are the same in both directions. The three conditions of transport which we will consider, namely *trans* stimulation, *balance* and *trans* inhibition, are thus symmetrical transports under the definition of Stein.

On removing the restriction of *balance*, conditions of *trans* stimulation and of *trans* inhibition are possible. For a system showing a *trans* stimulation, the rate constants for the translocation of the loaded carrier are assumed to be larger than those of the unloaded carrier, that is $k_3 = k_4 > k_7 = k_8$. Conversely, for a system showing a *trans* inhibition, the rate constants for the movement of the loaded carrier are assumed to be less than those of the empty carrier, $k_3 = k_4 < k_7 = k_8$.

A description of these *trans* effects has been simulated with an analog computer AD-64. The simulation solves equations (2) through (6) for the condition where the translocation steps are rate-limiting; other conditions are given in Table 1. Expessions for the influx and the efflux of the simulated uptakes were derived using the steady state approximation. The equations

TABLE 1

Conditions of the analog simulation

(1) The translocation reactions are rate limiting.
(2) The K_m and V_{max} of influx are equal to the K_m and V_{max} of efflux.
(3) k_1, k_2, k_5 and k_6 are equal to each other and to an arbitrary value of 10.
(4) We define the conditions as follows:
   (a) for *balance*, $k_3 = k_4 = k_7 = k_8$
   (b) for *trans* stimulation, $k_3 = k_4 = 5k_7 = 5k_8$
   (c) for *trans* inhibition, $5k_3 = 5k_4 = k_7 = k_8$.
(5) The simulation is solved when k_3 and k_4 are fixed at an arbitrary value of 0·2 and k_7 and k_8 varied, or when k_7 and k_8 are fixed at a value of 0·2 and k_3 and k_4 are varied.
(6) At zero time, no solute is present and all the carrier is in the empty form equally distributed between the two sides of the membrane.
(7) At the start of the program, the solute is introduced at saturating concentration in a medium of infinite volume.

were verified with the computer by individually changing the value of each rate constant and observing the resultant flux. The flux depends on the amount of the loaded carrier available at the respective membrane surfaces and the value of the rate constant for the rate-limiting step. For influx, velocity equals $k_3[CS]_1$ and for efflux, velocity equals $k_4[CS]_2$.

3. Analog Simulation of Net Transport

The approach toward equilibrium under the three *trans* conditions can be seen in Figs 2(a) through 4(a). The curves describe the net influx of the solute with time and the rate of the reverse process as the solute accumulates on the inside and begins to exit from the cell. For the curves obtained when k_7 and k_8 are fixed and k_3 and k_4 are varied, the approach toward equilibrium for the case of *trans* stimulation is at least twice as fast as for the other cases. The rates of transport, however, depend on the magnitude as well as on the ratio of the rate constants for the translocation process. Indeed by generating the curves when k_3 and k_4 are fixed and k_7 and k_8 are varied, we come to simulate a transport system showing a *trans* inhibition whose rates are greater than those of a system showing a *trans* stimulation. Therefore, to

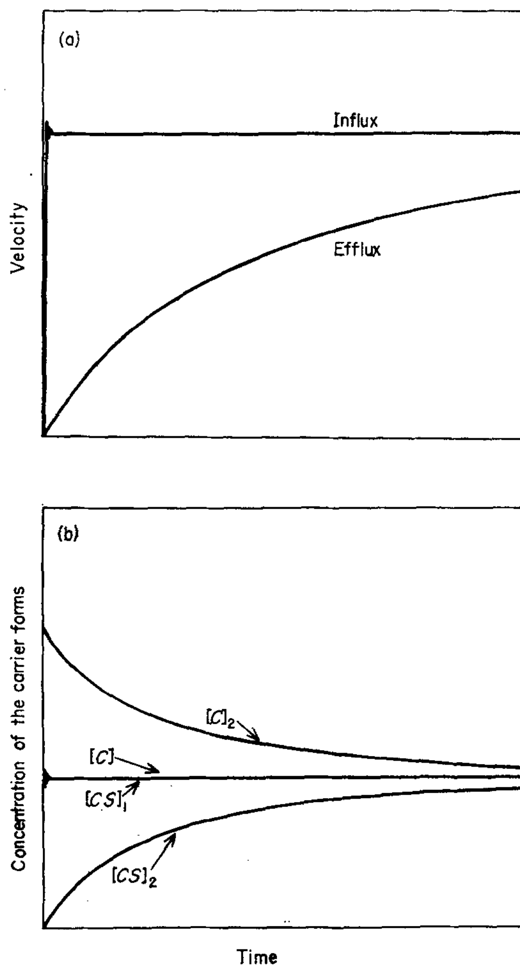

FIG. 2. Computer simulation: progress toward equilibrium under the condition of *balance*, (a) the fluxes and (b) the distribution of the carrier forms. The rate constants for the translocation of the empty and the loaded carrier are equal.

prevent any bias through the selection of the values for the rate constants, the velocities in Figs 2(a) through 4(a) have been normalized by dividing by the value of k_3 or k_4.

Figure 2(a) shows the simulated fluxes under the condition of a *balanced* transport. After the initial transient period, the influx remains constant because the condition of *balance* prevents any displacement of the distribution of the loaded carrier [Fig. 2(b)]. Efflux rises asymptotically as solute accumulates until equilibrium is reached and efflux equals influx.

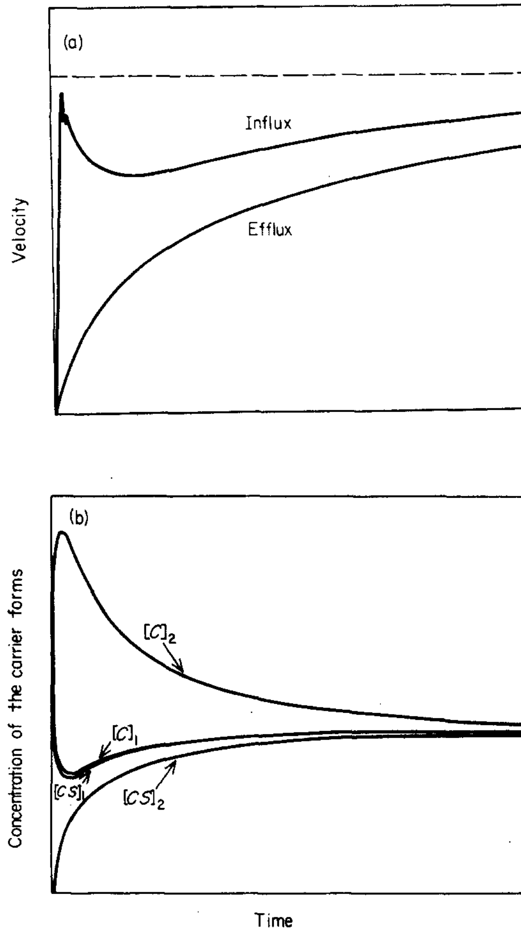

FIG. 3. Computer simulation: progress toward equilibrium under the condition of *trans* stimulation, (a) the fluxes and (b) the distribution of the carrier forms. The rate constants for the translocation of the loaded carrier are five times the magnitude of those of the empty carrier. The dashed straight line represents the equilibrium state.

The simulation of net uptake under the condition of *trans* stimulation is seen in Fig. 3. The rapid movement of the loaded carrier across the membrane results in almost an instantaneous maximization of the influx and forces most of the external carrier to the inside. This event depletes the concentration of the external carrier and since the rate of entry is given by the concentration of the loaded carrier, a quick decrease in the influx is also seen.

The decline in the influx is halted as the concentration of the loaded carrier rises on the inner side of the membrane. Since the equilibrium of

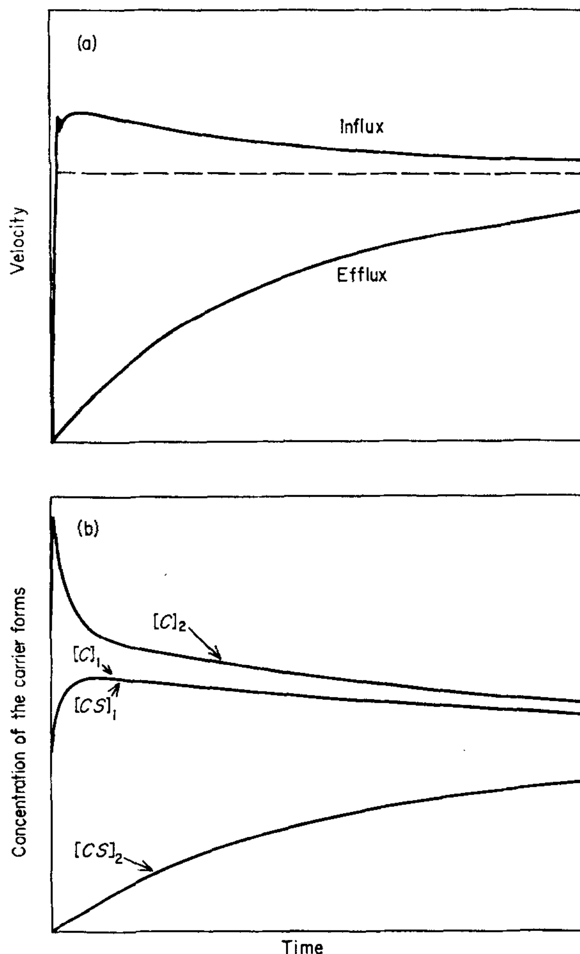

FIG. 4. Computer simulation: progress toward equilibrium under the condition of *trans* inhibition, (a) the fluxes and (b) the distribution of the carrier forms. The rate constants for the translocation of the loaded carrier are one-fifth the magnitude of those of the empty carrier. The dashed straight line represents the equilibrium state.

carrier species favors the formation of loaded carrier, as solute accumulates the state of the internal carrier rapidly shifts from the empty to the loaded form. The outward movement of the carrier accelerates and helps reduce the asymmetry in the carrier distribution.

Undoubtedly, the pre-steady-state character of the influx is exaggerated in the simulation since the concentration of the carrier was not greatly different from that of the substrate. In a physiological environment, the

concentration of the carrier may be less than one-millionth of that of the substrate, and the rate constants may accordingly be orders of magnitude greater than that represented on the computer. The approach to a steady state for the binding of solute to the carrier is probably instantaneous in the biological context.

The curves in Fig. 4(a) describe the influx and the efflux under the condition of *trans* inhibition. The rate-limiting step in the transport here is the slow translocation of the loaded carrier. The carrier combines with the solute and accumulates in the loaded form at the outer surface of the membrane [Fig. 4(b)]. As the uptake continues more of the carrier accumulates in this form, an effect that causes the influx to rise and become higher than the equilibrium influx.

At the inner surface of the membrane, the equilibrium among the forms of the carrier favors the formation of the empty carrier. Most of the loaded carrier will dissociate rather than cross the membrane, for our conditions in the ratio of 250:1. Therefore, a large amount of solute must be accumulated before an appreciable internal concentration of the loaded carrier is attained. As the concentration of the loaded carrier at the inner surface gradually increases and the carrier begins to redistribute itself, the rise in the influx is halted and the influx begins to decrease towards the equilibrium value.

The simulated fluxes of *trans* inhibition and *trans* stimulation may perhaps best be compared when the translocation of the empty carrier is held constant. In this extreme case, the appearance of solute inside the cell with time is over eight times faster under the condition of *trans* stimulation than under the condition of *trans* inhibition. We see how fast solute movement can be under the condition of *trans* stimulation, how slow uptake can be under the condition of *trans* inhibition. The rate of movement in a system showing a *trans* inhibition can be so slow as to give the appearance that the transport is inoperative.

4. Simulation of Exchange Transport

The analog computer simulation can be solved for the case where the solute is simultaneously introduced at both sides of the membrane, other conditions in Table 1 remaining the same, so that the phenomenon of exchange transport is simulated. The expected fluxes for an exchange uptake in a system showing a *trans* stimulation are seen in Fig. 5. Both fluxes are accelerated by the exchange in proportion to the concentration of the load. The initial fall in the influx seen in the absence of internal solute is lessened or eliminated depending on the load, since the exchange will be returning the carrier almost as fast as it enters.

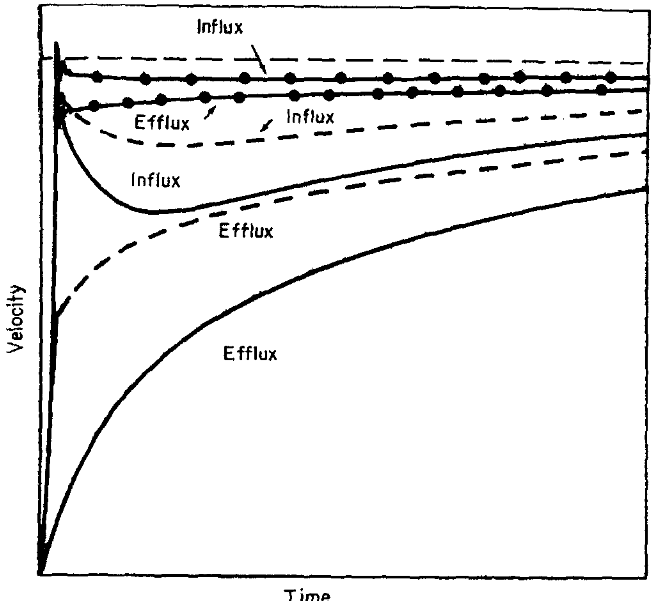

FIG. 5. Computer simulation: the effect of loading on the approach to equilibrium under the condition of *trans* stimulation. S_1 was 1 mM in all cases: S_2 was zero for the solid lines, 0·4 mM for the dashed lines and 1 mM for the dotted lines. The dashed straight line represents the equilibrium state.

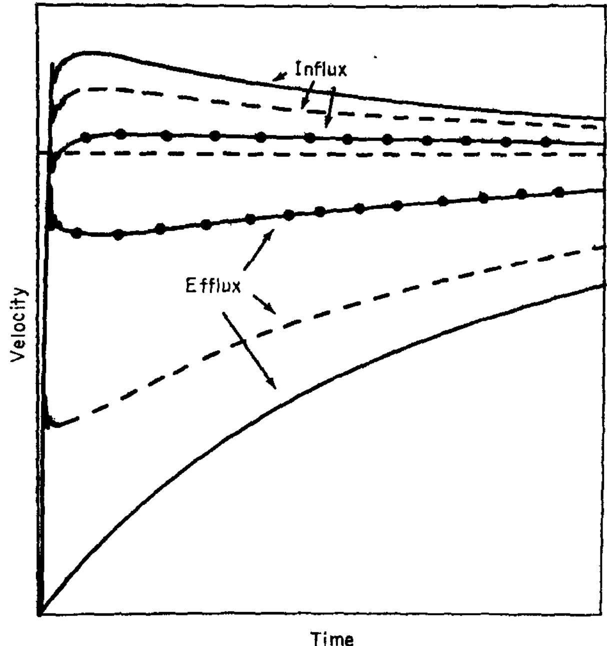

FIG. 6. Computer simulation: the effect of loading on the approach to equilibrium under the condition of *trans* inhibition. Conditions similar to those in Fig. 5.

Figure 6 shows the simulation of exchange uptake under the condition of *trans* inhibition. The exchange increases the efflux but decreases the influx. Because of the intracellular solute already present, carrier asymmetry is not as great as that observed without a load, and the initial rise in the influx above the equilibrium influx is less extreme.

We observe an interesting paradox in the nomenclature of *trans* inhibition and *trans* stimulation. Although the influx under the condition of *trans* inhibition will decrease with the extent of loading of the cell, the computer shows that the pre-equilibrium influx will always be greater than the influx at equilibrium (Fig. 6). Likewise, the influx under the condition of *trans* stimulation will increase with the extent of loading, yet the pre-equilibrium influx will always be less than the influx at equilibrium (Fig. 5).

5. Simulation of Tracer Exchange

To simulate tracer exchange with the analog computer requires that the model in Fig. 1 be modified to describe the transport of two species, R and S (Fig. 7). We assume that R and S are either analogs or different forms of

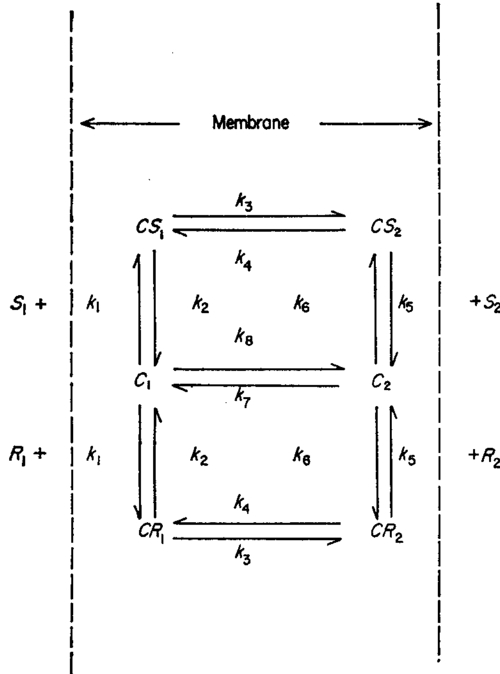

FIG. 7. Proposed model of carrier transport for the competition between two substrates and the carrier. R and S are either analogs or different forms of the same solute.

the same solute, and in this case specifically, that S is the nonradioactive species and that R is a radioactive tracer.

Since $S_1 \gg R_1$, R contributes negligibly to the distribution of the carrier forms, and equations (1) through (5) still describe the distribution of S and the carrier forms. At the start of the simulation, the unlabeled solute is simultaneously added to both sides of the membrane but the labeled solute is added only at the outer surface, other conditions in Table 1 remaining the same. The concentrations of S_1 and R_1 are again assumed to apply to a medium of infinite volume.

Figure 8 describes the appearance of labeled solute on the inside of the cell with time, with and without the simultaneous addition of a ten-fold higher concentration of solute on the opposite side of the membrane. A large overshoot above the equilibrium concentration of R is seen during exchange

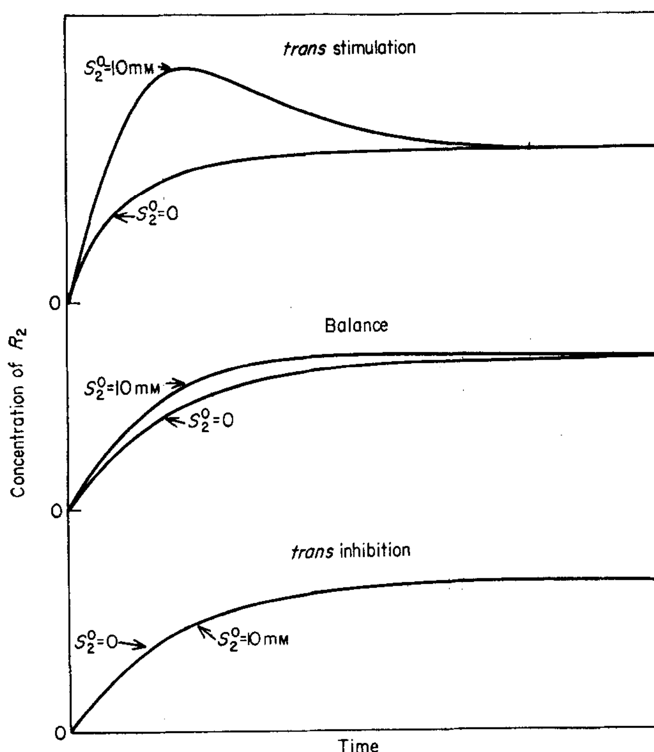

FIG. 8. Computer simulation: appearance of labeled solute inside the cell (R_2) with time under the three conditions. The equations of the model in Fig. 7 were solved when S_2 was equal to zero (net uptake) or to 10 mM (exchange uptake); S_1 was equal to 1 mM for both cases.

uptake under the condition of *trans* stimulation. The shape of the overshoot is consistent with experimental results obtained in this laboratory and elsewhere for *trans* stimulating systems. A more modest overshoot is seen during exchange uptake under the condition of *balanced* transport. These overshoots in the concentration of R disappear when the concentration of internal solute is equal to or less than the outside concentration.

The extreme slowness of the net movement of R under the condition of *trans* inhibition explains why only a slight additional decrease in the transport of R is seen during an exchange uptake. The experimental observation of *trans* inhibition is difficult and probably requires both a large concentration of the *trans* solute relative to the K_m, and a large difference in the translocation rate constants for the loaded and the empty carrier. Biological cases of *trans* inhibition may sometimes be overlooked or confused with a *balanced* transport through failure to use sufficient levels of substrate in radioactive tracer experiments.

Although we have limited our discussion to passive transports, the results of the analog computer simulation could apply equally well to uphill transport. Asymmetries such as have been suggested by Stein (1967) and by Geck (1971) will not alter the shape of the flux curves, but will change the steady states levels of substrate. Representative solutions for active transport under the condition of *trans* stimulation are presented in Figs 10 and 11. Similar solutions can be expected for the other conditions.

6. *Trans* Stimulation Phenomena in the Ehrlich Cell

A well characterized laboratory model of *trans* stimulation is that of system L of the Ehrlich ascites tumor cell (Oxender & Christensen, 1963). Reactivity with this system is high for the bulky neutral amino acids and for the synthetic amino acid 2-aminonorbornane-2-carboxylic acid (Tager & Christensen, 1971). It is not safe, however, to assume that system L is a passive system for any given substrate. As has been discussed elsewhere (Christensen, deCespedes, Handlogten & Ronquist, 1973), substrates with certain structures are strongly concentrated by this system by what may well be an exaggeration of a weak access to energy-coupling for the normal substrates. Nevertheless, *trans* stimulation by endogenously present amino acids plays a large role in the apparent uphill operation of system L.

Transport of the norbornane amino acid shows a vigorous *trans* stimulation so that net uptake can be demonstrated only with special care (Christensen, deCespedes, Handlogten & Ronquist, 1974). In order to slow down the exchange process and to help deduce which step in transport was rate-limiting, the Ehrlich cell was modified by treatment with 2, 4-dinitrofluoro-

benzene (DNFB). Treatment with this chemical at 1 mM prior to the uptake measurement reduced the initial rate of net and exchange uptake of the norbornane amino acid by approximately 50%, while the steady state level of the amino acid was reduced to only one-sixth the level observed in cells not so treated (Fig. 9).

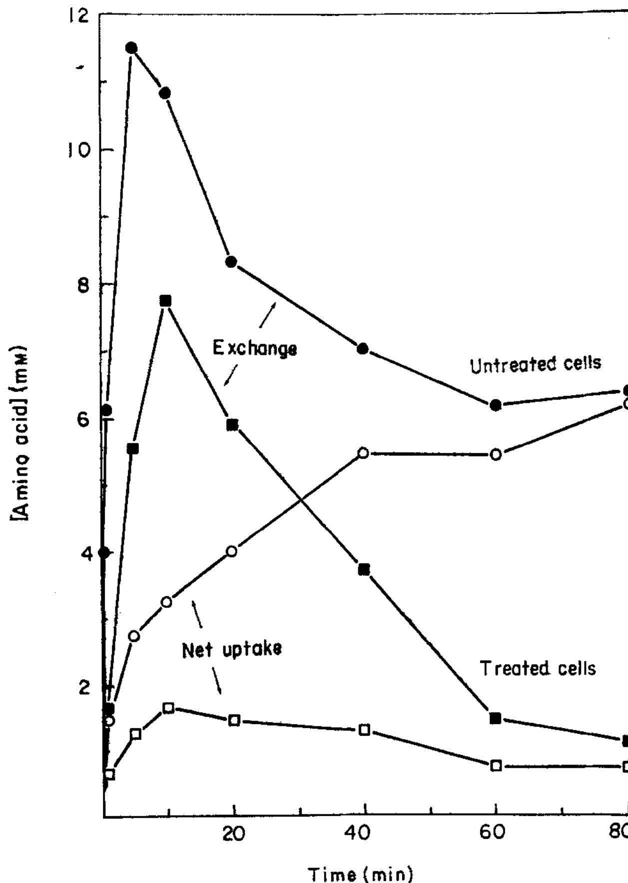

FIG. 9. Uptake of 0·4 mM norbornane amino acid in Ehrlich ascites tumor cells. For the exchange experiments, the cells were loaded for 20 min with 10 mM norbornane amino acid. The calculated internal concentration of the norbornane amino acid at the start of the experiment was 15 mM. For the net uptake experiments, the exchangeable amino acids in the intra-cellular pool were lowered by repeated incubation in Krebs–Ringer bicarbonate buffer containing 6 mM Na^+ and 0·1 mM α-aminoisobutyric acid. Each cell population was then divided in half. One half was incubated in 0·3% alcoholic Krebs–Ringer bicarbonate buffer containing 1 mM DNFB for 2 min; the other half served as the control and was incubated in the same buffer without DNFB. Uptake was measured at 37°C.

Assuming provisionally that a change in the steady state level reflects an asymmetric action of DNFB on the transport rate constants, we attempted to simulate this behavior on the computer, using the model of tracer exchange. An asymmetric action of DNFB on the rate constants for the translocation of the empty carrier would result in the uptake behavior seen in Fig. 10. Although the exchange overshoot was greatly reduced and a steady state level only one-fourth that of the control was reached in the treated cells, the simulated effect of DNFB on the initial rates of uptake did not correspond to the experimental behavior. Much better agreement with the experimental data was obtained when we assumed that the chemical acted asymmetrically on either k_1 and k_2 or k_3 and k_4 (Fig. 11). Additional experiments (not

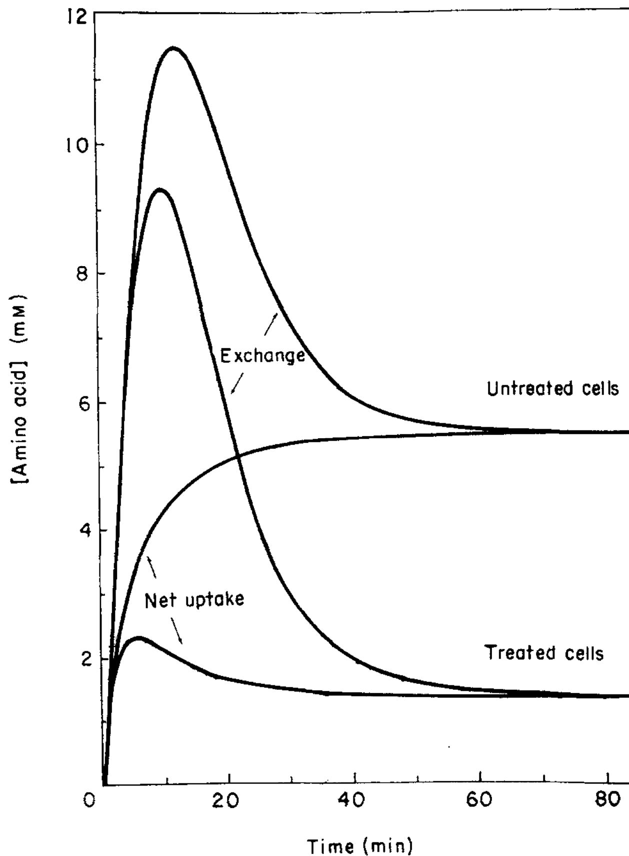

FIG. 10. Computer simulation: appearance of R_2 with time under the condition of *trans* stimulation where $k_7 = k_8$ (untreated cells) and where $k_7 = \frac{1}{4}k_8$ (treated cells). Conditions similar to those in Fig. 8.

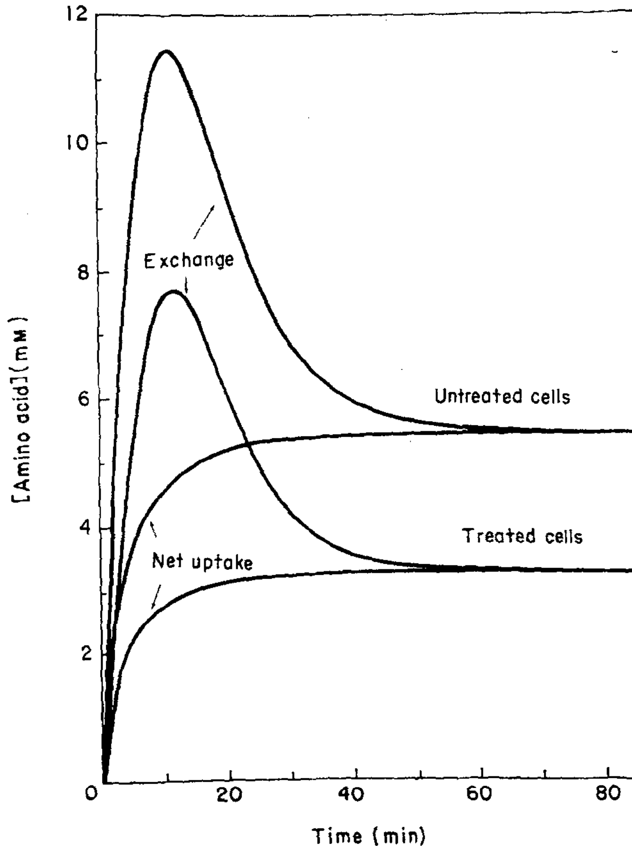

FIG. 11. Computer simulation: appearance of R_2 with time under the condition of *trans* stimulation where $k_3 = k_4$ (untreated cells) and where $k_3 = \frac{1}{2}k_4$ (treated cells). The same solution was obtained where $k_1 = k_2$ (untreated cells) and where $k_1 = \frac{1}{2}k_2$ (treated cells). Conditions similar to those in Fig. 8.

reported here) showed that *DNFB* treatment did not affect the K_m, but did reduce the V_{max} of uptake, a result which suggested that the major effect of the chemical was on the movement of the loaded carrier across the membrane.

Whereas the simulation confirms the prediction that a differential slowing of the movements of the loaded and the unloaded carrier can modify the intensity of the *trans* phenomenon, the sequence of changes produced by DNFB shows that this reagent does not act in that way. Instead, the fit of the simulation model with our experimental results was much better when we assumed that this reagent decreases both k_3 and k_7. Figure 12 shows the good fit obtained when k_7 is decreased twice as much as k_3.

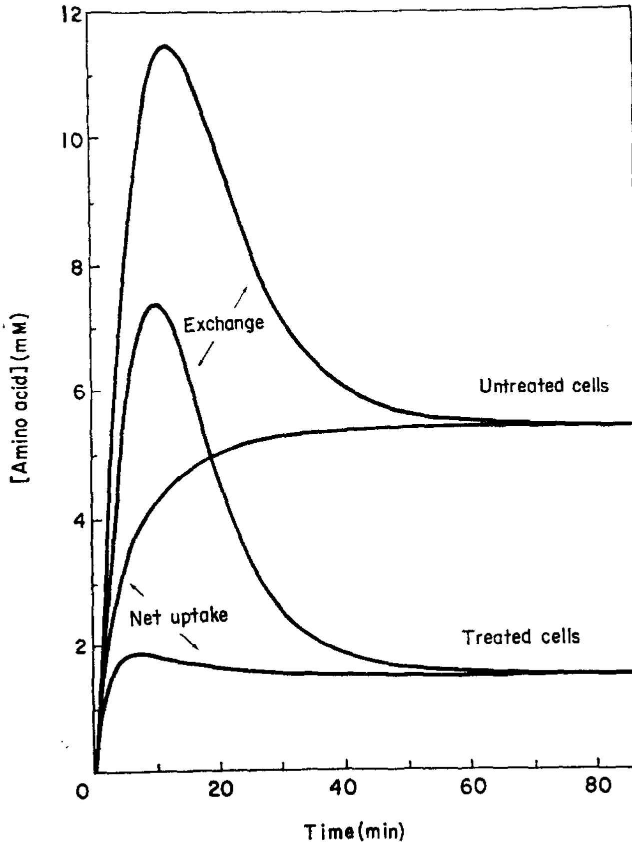

FIG. 12. Computer simulation: appearance of R_2 with time under the condition of *trans* stimulation where $k_3 = k_4$ and $k_7 = k_8$ for the untreated cells and where $k_3 = 0{\cdot}7\ k_4$ and $k_7 = 0{\cdot}4\ k_8$ for the treated cells. Conditions similar to those in Fig. 8.

The flaw in the earlier interpetation by Oxender & Whitmore (1966) was that too much attention was given to the rate changes in the first few minutes, too little attention to the lowered steady state toward which the poisoned process was tending (Fig. 9). Effects of DNFB on the steady state are also evident in the Oxender's unpublished results, so that Fig. 12 describes the action of DNFB on the transport of leucine as well as on that of the norbornane amino acid.

For an active transport process, decreasing either k_3 or k_7 will diminish the steepness of the uphill transport, effects that might arise through interference with an energy input (Figs 10 and 11). Our hypothesis that both rate constants are decreased by DNFB treatment appears to imply an energy

input for both of these steps, although we do not take the results of Fig. 12 to establish an unequal energization of the two. Most models of active transport postulate an energy requirement for the translocation of either the loaded or the empty carrier, but not both. Under one extreme type of transport model, the *pure vectorial-force* model, in which all the external energy serves to drive the empty or the loaded carrier across the membrane,

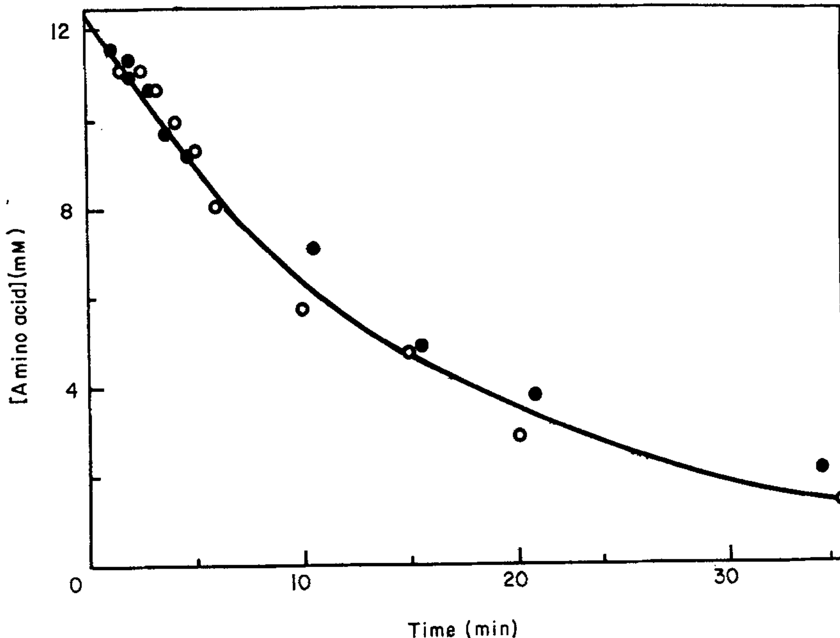

FIG. 13. Time course of the net exodus of the norbornane amino acid at 37°C from Ehrlich tumor cells. Cells were loaded for 20 min with 10 mM norbornane amino acid, then treated with or without 1 mM DNFB as described in Fig 9. The calculated internal concentration of the norbornane amino acid at the start of the experiment was 12·2 mM. Net exodus of the amino acid was measured into a large volume of medium to minimize recapture. The open circles represent the untreated cells; the filled circles, the cells treated with DNFB.

two sites of energy coupling might be necessary to explain our data. Although it would be biologically useful to drive the carrier in the loaded form in one direction and in the unloaded form in the other direction, a mechanistic basis for this duality is not obvious. Under another extreme type of transport model, the *pure affinity-change* model, in which all the external energy serves to modify the carrier to a form with a different affinity for the substrate (see Christensen, 1975), a single mode of interference with energy delivery might

well cause both of the translocation steps productive to uphill transport to appear to be slowed.

Furthermore, under intermediate models in which the external energy serves both to change the affinity of the carrier for the substrate and to provide a vectorial force across the membrane, one might also expect that an interference with energy delivery would decrease the apparent values of both k_3 and k_7. In that case these apparent values might show decreases because each may be a product of a true rate constant times an activity coefficient representing the proportion of the carrier in the form contributing to uphill transport. The action of DNFB might then be that of an uncoupling agent with the active form of the chemical serving to modify the asymmetry between the proportions of the carrier presented in its two forms at each surface of the membrane.

As a test of the hypothesis that both k_3 and k_7 are affected by DNFB treatment, two predictions can be made concerning exodus: first, if the

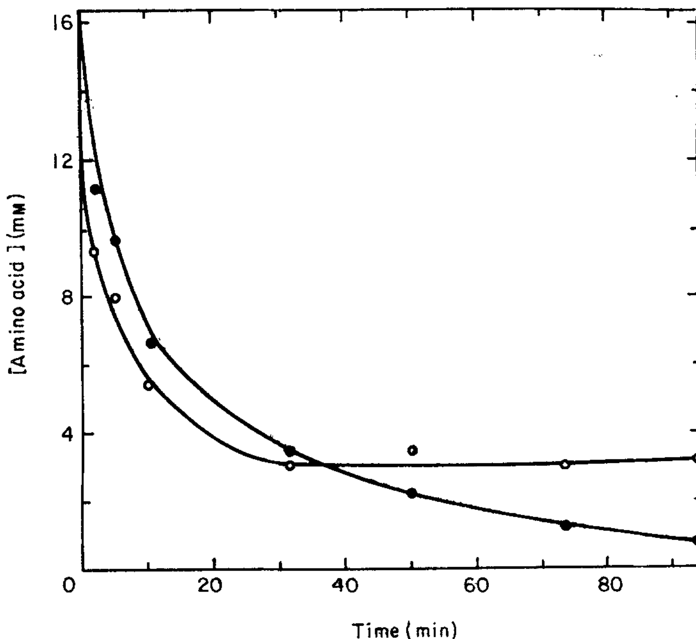

FIG. 14. Time course of the exchange exodus of the norbornane amino acid. The calculated internal concentration of the norbornane amino acid at the start of the experiment was 16·3 mM. Exchange exodus was measured into a small volume of medium containing 0·5 mM norbornane amino acid to maximize recapture of the lost amino acid. Other conditions similar to those in Fig. 13. The open circles represent the untreated cells; the filled circles, the cells treated with DNFB.

medium has an infinite volume that net exodus, which is independent of k_3 and k_7, should not be affected by the chemical treatment, and second, that exchange exodus, which depends on k_3 for the exchange stimulation, would be reduced by the treatment. Figures 13 and 14 show that both of these predictions have been confirmed for the exodus of the norbornane amino acid. Note that the rate of recapture of the escaped amino acid, which is directly proportional to k_3, is also reduced by the treatment (Fig. 14).

It thus seems probable to us that an asymmetric action of DNFB on the movements of the carrier into and out of the Ehrlich cell can account for our results and the preceding observations of Oxender & Whitmore (1966). Another asymmetric action for this chemical has been reported with sugar transport in the erythrocyte. In this system, which seems to be a *balanced* transport in our terminology, DNFB appears to bind only to the loaded carrier, and only at the outside surface of the membrane (Krupka, 1971, 1973; Edwards, 1973).

A different means of modifying the relation between the rates of translocation of the empty and the loaded carrier is to modify the structure of the substrate. Christensen (1972) was able to convert the *trans* stimulation shown by 2-aminoisobutyric acid into a *trans* inhibition by *N*-methylating the substrate. Since the transport system was otherwise unmodified in these studies and since a *trans* inhibition implies that the loaded carrier is moving slower than the empty carrier, we may presume that the effect of *N*-methylating the substrate falls on the magnitude of the rate constants for the translocation of the loaded carrier.

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