F. Finally, the possibility that the heterozygote consists of two singly marked molecules fused together through partial pairing making a unit composed of four original strands cannot as yet be excluded.

Annealed samples were shaken with 80 per cent phenol, treated with chymotrypsin and shaken with chloroform-octanol to remove any protein which might have bound them together, but none of these treatments altered the ratio of doubles to singles.

With so many possible explanations for the low recovery of heterozygotes, some decisive experiments must precede further consideration of the mechanism. One additional result may be noted, however, in closing. Heated mixtures of markers which had been chilled rapidly (such as *d* in Table 1), and which consequently showed a low level of both markers, returned to 25–50 per cent of the initial transforming activity on reheating and annealing just as Marmur and Lane had found. More important for this discussion, heterozygotes which were absent from the chilled mixture formed during the annealing of the reheated DNAs. This shows that the rapid chilling which causes collapse of the separated strands,[3] does not, however, inflict irreparable damage on either marker or their capacity to form heterozygotes.

Summary.—Heat denaturation of a mixture of genetically different transforming DNAs from different stocks of *Hemophilus influenzae* followed by the annealing treatment recommended by Marmur and Lane led to the formation of heterozygotes, physical units carrying both genetic markers. A number of possible mechanisms for their formation have been considered.

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THE KINETICS OF CARRIER-MEDIATED ACTIVE TRANSPORT OF AMINO ACIDS*

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Amino acids are concentrated in many types of cells by an active transport process. However, it is only with the cells of Ehrlich ascites carcinoma that sufficient data have accumulated for us to consider setting up models of this process.

We proceed by first setting up some general models of active transport systems

and use the stationary state approximation to derive initial fluxes. These can then be compared with the experimental data on amino acid active transport.

Experimental Characteristics of the Amino–Active Transport System in Ehrlich Ascites Cells.—Let us first briefly review the major experimental features of amino acid transport in Ehrlich ascites cells.

Initial uptake velocity: The initial flux in a 1- to 2-min incubation increases rapidly as the extracellular concentration of amino acid rises and asymptotically approaches a linear function of the average gradient across the cell membrane. Thus, the initial flux may be written $F = k_p \cdot \langle c_e - c_i \rangle + F_T$, where k_p is the permeability constant for the cell membrane, $\langle c_e - c_i \rangle$ is the average gradient across the cell membrane during the period of incubation, and F_T is the net active transport. The latter shows saturation behavior as the extracellular concentration increases.

The steady state: After a two-hour incubation, the intracellular concentration of amino acid is fairly constant. A graph of the intracellular versus the extracellular concentration after two hours of incubation shows a high value for the ratio c_i/c_e at low c_e. This falls, approaching 1 as c_e increases. However, the data from steady-state studies at high extracellular concentrations must be used with caution, because there is evidence of some cell damage.

Competition among amino acids: Although many of the amino acids compete with one another for active transport, in some cases the initial flux of one amino acid is increased when another amino acid is present in roughly equimolar amounts. L-tryptophan and O-diazoacetyl-L-serine (azaserine) represent such a combination. When each is present at about 1 mM/L in the extracellular phase, the initial flux of L-tryptophan is increased and that of azaserine is decreased as compared to the fluxes when each is present alone at the same concentration. However, if the extracellular concentration of azaserine is increased to about 5 mM/L, the initial flux of L-tryptophan is decreased. Similar results are found if L-tryptophan is incubated with the Ehrlich ascites cells in the presence of L-histidine, L-2,4-diaminobutyric acid, and L-leucine.

Exchange diffusion: Heinz[1] reported that the initial uptake flux of C^{14}-labeled glycine was markedly increased when the cells had first been loaded with unlabeled glycine. Subsequently, Heinz and Walsh[2] reported a hetero-exchange diffusion in which the initial flux of alanine was increased by preloading the cells with glycine. We have shown that the initial flux of L-tryptophan is slightly decreased after preloading the cells with glycine. However, if the ascites cells are first loaded with L-tryptophan or azaserine, the initial flux of the other is markedly increased.

Effect of inhibitors: Many of the metabolic inhibitors markedly impair active transport of the amino acids.[3–5] Dinitrophenol and cyanide markedly inhibit the initial flux of L-tryptophan active transport but only slightly inhibit the much larger initial flux of L-tryptophan when the cells are preloaded with azaserine.[6] Thus, although active transport is directly dependent on cell metabolism, exchange diffusion is not.

Models of Active Transport.—Two major types of models have been suggested to explain transport across a cell membrane. These may be labeled adsorption-type and carrier-type models. The former is characterized by the presence of fixed sites which can combine with a substrate in the extracellular or intracellular

phase and can release the substrate into one or both phases. This type of model may be generalized to include a series of adsorption sites, a substrate being passed from one adsorption site to the next. Carrier models, on the other hand, are characterized by the presence of a mobile carrier which can combine with free substrate at one surface of the cell membrane to form a carrier-substrate complex capable of traversing the cell membrane by diffusion or by an active process and of releasing the substrate at the other surface of the cell membrane. In this case, the assumption is usually made that within the cell membrane the equilibrium constant for the reaction between substrate and carrier is such as to make dissociation of carrier substrate complex negligible.

Adsorption transport models: It is well to briefly consider the adsorption transport models first if for no other reason than to justify our dismissal of them as inadequate models. Detailed mathematical arguments will not be presented because the adsorption models are simpler than the carrier transport models and essentially the same type of treatment (but simpler) can be used with them as will be used with the carrier models.

Irreversible adsorption transport models: This model is illustrated in Figure 1. The area between the two vertical lines represents the cell membrane; e and i are the extracellular and intracellular phases respectively. All of the adsorption sites C are available to extracellular amino acid for formation of the CS complex. This is of interest for two reasons: The differential equations describing this model can be solved explicitly under the assumption of constant extracellular concentration and constant cell volume, conditions which can be approximated quite closely in amino acid uptake experiments up to extracellular concentrations of 10–15 mM/L. Secondly, it is surprising how closely one can fit the kinetic data on uptake of a single amino acid with this model. It is obviously unrealistic, for it cannot give any exchange diffusion because of its irreversibility.

Reversible adsorption transport: The previous model may be extended somewhat by assuming that all steps are reversible. This is essentially the model used byLeFevre[7] in discussing sugar transport in erythrocytes. This model can give exchange diffusion in the sense that the adsorption site

FIG. 1.—Schematic of model of irreversible adsorption transport.

can form a complex with an amino acid from one side of the membrane and release it on the other side in exchange for another amino acid. However, if two different amino acids are present, one on each side of the membrane, one can show that in this model they will compete for the adsorption site. Thus, it cannot give an increased net uptake flux of an amino acid after preloading the cell with another amino acid. This is obvious from the assumption that the adsorption sites are equally accessible to substrates in either phase.

The "bucket brigade" model: Danielli,[8] among others, has considered models in which the substrate is passed along a chain of non-mobile adsorption sites in the membrane or in a pore passing through the membrane. This too can give exchange diffusion in the same sense that the previous model can. But again
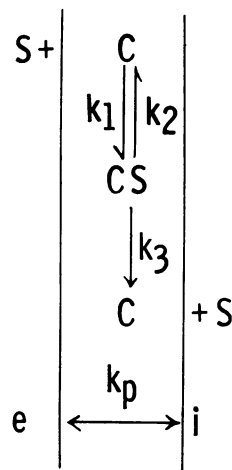
it cannot give an increased uptake flux of one amino acid after preloading the cell with the same or another amino acid. If we consider the ith adsorption site at the start of such a process, then amino acid (1) from site $(i - 1)$ must compete for site i with amino acid (2) from site $(i + 1)$.

Carrier transport models: The carrier transport models are generally more complicated than the adsorption transport models. Although one can consider extremely complicated carrier models involving various enzymatic reactions,[9] this seems needlessly complex at this stage of our knowledge. It is important to consider the implications of the various types of carrier models at the simplest possible level.

The carrier active transport models may be classified on the basis of the nature of the linkage between the transport system and cellular metabolism. We differentiate four main types:

    1. *Active transport of free carrier.* The linkage to cell metabolism is through
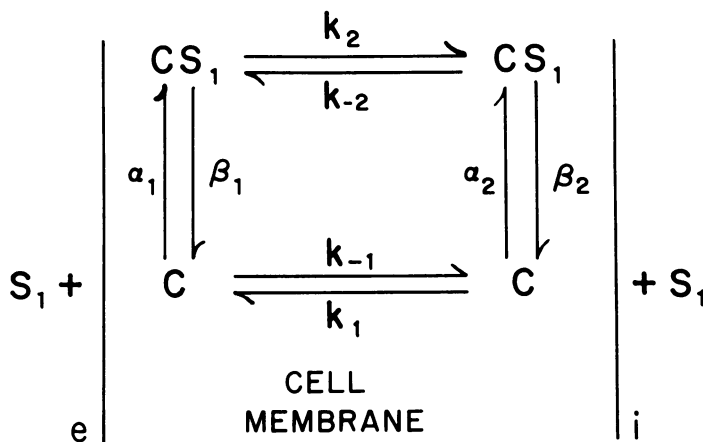

FIG. 2.—Schematic of generalized model of carrier active transport.
e = extracellular phase; i = intracellular phase; S_1 = substrate 1;
C = carrier; CS_1 = carrier-substrate complex.

one or more reactions which speed up the movement of free carrier from the inner towards the outer surface of the cell membrane.

    2. *Active transport of carrier-substrate complex.* Transfer of carrier-substrate complex from the outer to the inner surface of the cell membrane is speeded up by linkage to cellular metabolism.

    3. *Association reaction model.* The rate of formation of substrate-carrier complex at the outer surface of the cell membrane is increased by linkage to cell metabolism.

    4. *Dissociation reaction model.* The rate of dissociation of substrate-carrier complex at the inner surface of the cell membrane is increased by metabolically linked reactions.

One cannot of course rule out the possibility of combinations of these mechanisms.

Widdas[10] has used simplified versions of a carrier model in discussing sugar transport. Some of the carrier models proposed by Rosenberg and Wilbrandt[9] are similar to those above. However, they introduce the simplifying assumptions

(1) that the total concentration of carrier is equal on the two sides of the membrane, and (2) that the diffusion constants in the membrane are the same for free carrier and carrier-substrate complex. Neither of these is a physically reasonable assumption. Finally, it should be pointed out that the first two adsorption transport models can be viewed as special cases of the carrier models in which the diffusion rates in the membrane are much greater than the rates of reaction of substrate with carrier.
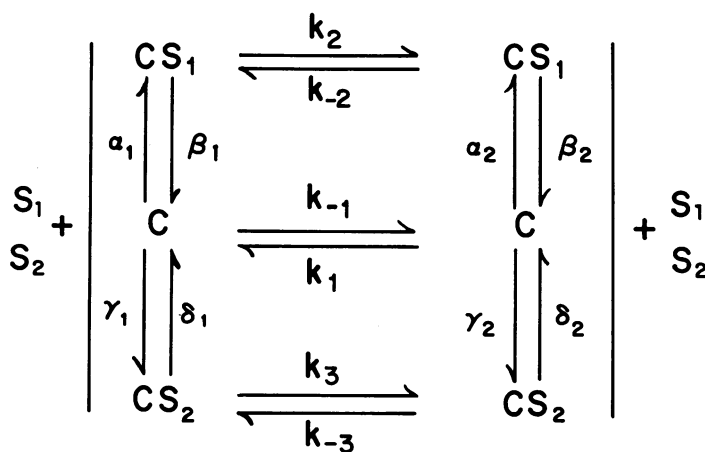

FIG. 3.—Schematic of model of carrier active transport with two substrates.

A simplified general model: We proceed by setting up a simplified generalize model which includes the above types as special cases. We neglect the amount of carrier or carrier-substrate complex which is in transit between the two surface phases of the cell membrane. Figure 2 is a schematic of the model when only one substrate is present. Figure 3 is the same model when two substrates are present.

Notation:

C_0 = total amount of carrier in mM/sq cm surface area.

x_e = concentration of free carrier at outer surface of cell membrane in units mM/sq cm.

x_i = concentration of free carrier at inner surface of cell membrane.

y_e = concentration of CS_1 at outer surface.

y_i = concentration of CS_1 at inner surface.

z_e = concentration of CS_2 at outer surface.

z_i = concentration of CS_2 at inner surface.

c_{e1}, c_{e2} = concentration of substrates 1 and 2 respectively in extracellular phase (mM/kg water).

c_{i1}, c_{i2} = concentration of substrates 1 and 2 respectively in intracellular phase.

k_{p1}, k_{p2} = permeability constant for free substrate 1 and 2 respectively.

A = surface area of cells.

V_e = volume of extracellular phase.

V = intracellular water, assumed constant.

The rate constants for the various reactions and transfers are indicated in Figures 2 and 3.

In order to keep the models as simple as possible, we assume symmetry in rate and diffusion constants between the·two sides of the cell membrane except for the portion of the transport system which is linked to cell metabolism. Although the linkage to cell metabolism may be quite complicated, we introduce it simply as an increase in the pertinent rate constant. Thus, the various types of models are characterized by the following conditions on the rate constants.

1. Active transport of free carrier:

$$\alpha_1 = \alpha_2, \quad \beta_1 = \beta_2, \quad k_2 = k_{-2};$$
$$\gamma_1 = \gamma_2, \quad \delta_1 = \delta_2, \quad k_3 = k_{-3};$$
$$k_1 > k_{-1}.$$

2. Active transport of carrier-substrate complex:

$$\alpha_1 = \alpha_2, \quad \beta_1 = \beta_2, \quad k_2 > k_{-2};$$
$$\gamma_1 = \gamma_2, \quad \delta_1 = \delta_2, \quad k_3 > k_{-3};$$
$$k_1 = k_{-1}.$$

3. Association-reaction model:

$$k_1 = k_{-1}, \quad k_2 = k_{-2}, \quad k_3 = k_{-3};$$
$$\beta_1 = \beta_2, \quad \alpha_1 > \alpha_2;$$
$$\delta_1 = \delta_2, \quad \gamma_1 > \gamma_2.$$

4. Dissociation-reaction model:

$$k_1 = k_{-1}, \quad k_2 = k_{-2}, \quad k_3 = k_{-3};$$
$$\alpha_1 = \alpha_2, \quad \gamma_1 = \gamma_2;$$
$$\beta_2 > \beta_1, \quad \delta_2 > \delta_1.$$

It should be emphasized that the above conditions do not violate the principle of detailed balancing because the rate processes shown in the model are not a closed system. It is assumed that the rate processes which are linked to cellular metabolism are linked via reactions which are not shown in the model but which provide the free energy to increase the rate of the process involved. The effect of this linkage is included at the simplest possible level as an increase in the rate constant of the process which is assumed linked to cellular metabolism. The effect of inhibitors of cellular metabolism would be to decrease this rate constant; and in complete inhibition of cellular metabolism, the inequality signs in the above cases would be replaced by equality signs, as would be required by the principle of detailed balancing.

The mathematical model: We assume that V and V_e remain constant. The equations describing the model when two amino acids are present are then:

$$C_0 = x_e + x_i + y_e + y_i + z_e + z_i. \tag{1}$$

$$\frac{dx_e}{dt} = -(k_{-1} + \alpha_1 c_{e1} + \gamma_1 c_{e2})x_e + k_1 x_i + \beta_1 y_e + \delta_1 z_e. \tag{2}$$

$$\frac{dy_e}{dt} = \alpha_1 c_{e1} x_e - (k_2 + \beta_1) y_e + k_{-2} y_i. \tag{3}$$

$$\frac{dy_i}{dt} = \alpha_2 c_{i1} x_i + k_2 y_e - (k_{-2} + \beta_2) y_i. \tag{4}$$

$$\frac{dz_e}{dt} = \gamma_1 c_{e2} x_e - (k_3 + \delta_1) z_e + k_{-3} z_i. \tag{5}$$

$$\frac{dz_i}{dt} = \gamma_2 c_{i2} x_i + k_3 z_e - (k_{-3} + \delta_2) z_i. \tag{6}$$

$$\frac{V_e}{A} \frac{dc_{e1}}{dt} = -k_{p1}(c_{e1} - c_{i1}) + \beta_1 y_e - \alpha_1 c_{e1} x_e. \tag{7}$$

$$\frac{V}{A} \frac{dc_{i1}}{dt} = k_{p1}(c_{e1} - c_{i1}) + \beta_2 y_i - \alpha_2 c_{i1} x_i. \tag{8}$$

$$\frac{V_e}{A} \frac{dc_{e2}}{dt} = -k_{p2}(c_{e2} - c_{i2}) + \delta_1 z_e - \gamma_1 c_{e2} x_e. \tag{9}$$

$$\frac{V}{A} \frac{dc_{i2}}{dt} = k_{p2}(c_{e2} - c_{i2}) + \delta_2 z_i - \gamma_2 c_{i2} x_i. \tag{10}$$

The equations applicable when only substrate "1" is present can be obtained by setting $z_e = z_i = c_{e2} = c_{i2} \equiv 0$.

Such a system of ordinary differential equations presents great analytical difficulties because of the non-linear terms. Furthermore, the initial conditions on x_e and x_i are unknown except for the constraint $x_e + x_i = C_0$ at $t = 0$. It is difficult to see how sensitive the solutions are to changes in the initial values of x_e and x_i. However, it is more important to determine whether the carrier models *could* give the same type of phenomena as are found experimentally in active transport and exchange diffusion experiments. The detailed kinetics are of secondary importance, particularly since these models are quite simplified. We consider only the carrier-mediated flux, since the flux due to simple permeability is usually quite small in comparison to carrier-mediated flux. Let $F_T(1)$ represent the active transport flux when only substrate 1 is added to the cell suspension and let $F_X(1)$ represent the carrier-mediated flux of substrate 1 when the cells have been preloaded with substrate 2. We wish to know whether the carrier models can give $F_X(1) > F_T(1)$ and what the effects of inhibitors are on $F_X(1) - F_T(1)$ and $F_X(1)/F_T(1)$ when $F_X(1) > F_T(1)$.

Although the equations cannot be solved as they are, they can be solved for the stationary-state fluxes. In analogy with the derivation of the Michaelis-Menten equations of enzyme kinetics, we obtain the stationary-state fluxes for conditions corresponding to those at the start of the uptake experiments. This is equivalent to performing the hypothetical experiments of keeping c_{e1} and c_{i2} constant and $c_{e2} = c_{i1} \equiv 0$ and solving for the stationary state. Furthermore, it should be stressed that except for possibly some special initial conditions, these fluxes represent upper bounds for the initial fluxes in the experiments. Under these conditions,

the stationary-state equations corresponding to equations (1) to (10) reduce to equations (11) to (16).

$$x_e + x_i + y_e + y_i + z_e + z_i = C_0.$$ (11)

$$-(k_{-1} + \alpha_1 c_{e1})x_e + k_1 x_i + \beta_1 y_e + \delta_1 z_e = 0.$$ (12)

$$\alpha_1 c_e x_e - (k_2 + \beta_1)y_e + k_{-2} y_i = 0.$$ (13)

$$k_2 y_e - (k_{-2} + \beta_2)y_i = 0.$$ (14)

$$-(k_3 + \delta_1)z_e + k_{-3} z_i = 0.$$ (15)

$$\gamma_2 c_{i2} x_i + k_3 z_e - (k_{-3} + \delta_2)z_i = 0.$$ (16)

Aside from the tedious algebra these are readily solved to give equations (17) to (20) when only substrate 1 is present.

$$F_T(1) = \beta_2 y_i = \frac{\beta_2 k_1 k_2 \alpha_1 c_{e1} C_0}{\Delta_1}.$$ (17)

$$\Delta_1 = A + B\alpha_1 c_{e1}.$$ (18)

$$A = (k_1 + k_{-1})[k_2\beta_2 + k_{-2}\beta_1 + \beta_1\beta_2].$$ (19)

$$B = [k_2(k_1 + \beta_2) + k_1(k_{-2} + \beta_2)].$$ (20)

It is immediately apparent that if the stationary state is rapidly attained this should be a good approximation for the initial transport flux; F_T gives the expected saturation behavior in c_{e1}.

The solution obtained when substrates 1 and 2 are present under the conditions c_{e1} and c_{i2} constant, $c_{e2} = c_{i1} \equiv 0$, is given by:

$$F_X(1) = \frac{\beta_2 k_2 \alpha_1 c_{e1} C_0 \{k_1 k_3 \delta_2 + k_1 \delta_1(k_{-3} + \delta_2) + \delta_1 k_{-3}\gamma_2 c_{i2}\}}{\Delta_2}.$$ (21)

$$\Delta_2 = D + E\alpha_1 c_{e1} + \gamma_2 c_{i2}[F + G\alpha_1 c_{e1}].$$ (22)

$$D = [k_3\delta_2 + \delta_1(k_{-3} + \delta_2)]A.$$ (23)

$$E = [k_3\delta_2 + \delta_1(k_{-3} + \delta_2)]B.$$ (24)

$$F = (k_3 + k_{-3} + \delta_1)(k_{-1}k_2\beta_2 + k_{-1}k_{-2}\beta_1 + k_{-1}\beta_1\beta_2) + \\ k_{-3}\delta_1[k_2\beta_2 + \beta_1(k_{-2} + \beta_2)] \quad (25)$$

$$G = (k_3 + k_{-3} + \delta_1)k_2\beta_2 + k_{-3}\delta_1(k_2 + k_{-2} + \beta_2).$$ (26)

The difference between the two fluxes is given by the equation

$$F_X(1) - F_T(1) = \frac{\beta_2 k_2 \alpha_1 c_{e1} \gamma_2 c_{i2} C_0}{\Delta_2 \Delta_1} [\delta_1 k_{-3} - k_1(k_3 + k_{-3} + \delta_1)] \times \\ [k_2\beta_2\alpha_1 c_{e1} + k_{-1}[k_2\beta_2 + \beta_1(k_2 + \beta_2)]]. \quad (27)$$

Since all the rate constants and concentrations in equation (27) are positive, $F_X(1)$ is greater than $F_T(1)$ when

$$\delta_1 k_{-3} - k_1(k_3 + k_{-3} + \delta_1) > 0.$$ (28)

Finally, the ratio of the two fluxes is given by the equation

$$\frac{F_X(1)}{F_T(1)} = \frac{[k_1 k_3 \delta_2 + k_1 \delta_1(k_{-3} + \delta_2) + \delta_1 k_{-3}\gamma_2 c_{i2}]\Delta_1}{k_1 \Delta_2}. \tag{29}$$

Thus, all the carrier models can give $F_X(1) > F_T(1)$ under the conditions imposed by equation (28). To distinguish between the different carrier models, we assume $F_X > F_T$ and seek what effect metabolic inhibitors would have on $F_X - F_T$ and F_X/F_T when the two amino acids in the exchange diffusion experiment are different and when they are the same except for an isotopic label. It will be assumed that the rate constants for a labeled amino acid are the same as for the unlabeled. The effect of metabolic inhibitors will be to decrease the rate constant which is linked to cell metabolism in the various models. Thus, in the model of active transport of free carrier, the linkage to cell metabolism increases k_1 to give $k_1 > k_{-1}$. The effect of a metabolic inhibitor is to make k_1 decrease and approach k_{-1} in value. Carrying out the above program we obtain the results given in Table 1.

TABLE 1

RELATIVE EFFECT OF METABOLIC INHIBITORS ON F_X AND F_T WHEN $F_X > F_T$

* If all diffusion parameters are much less than the association and dissociation rate constants so that equilibrium constants can be used.
† Decrease if $\beta_1 k_2 - k_1(2k_2 + \beta_1) > 0$, otherwise increase.
‡ Not determinable unless relative rates of change of β_2 and δ_2 are known.

First, it should be noted that equation (28) reduces to the same conditions for models 1, 3, and 4 in order for $F_X(1) > F_T(1)$. Secondly, only the model of active transport of free carrier predicts an increase in F_X/F_T and $F_X - F_T$ in all cases when a metabolic inhibitor acts. However, the directional changes in the second model could not be determined in hetero-exchange diffusion. Furthermore, the signs of $d[F_X(1)/F_T(1)]/dk_2$ and $d[F_X(1) - F_T(1)]/dk_2$ could not be determined in the isoexchange case for the model of active transport of carrier-substrate complex. However, these can be determined if one assumes that the reaction rates are much faster than the diffusion rates so that equilibrium exists between carrier and substrate at each of the surfaces of the cell membrane. For this case, metabolic inhibitors decrease $F_X(1) - F_T(1)$ and $F_X(1)/F_T(1)$. By continuity, we see that

this must also be true for some range of parameter values where the equilibrium assumption is less tenable. For the association reaction model, metabolic inhibitors give a decrease in $F_X(1)/F_T(1)$ and $F_X(1) - F_T(1)$ in the isoexchange case. In hetero-exchange, $F_X(1)/F_T(1)$ and $F_X(1) - F_T(1)$ decrease if $\beta_1 k_2 - k_1(2k_2 + \beta_1) > 0$ and increase if $\beta_1 k_2 - k_1(2k_2 + \beta_1) < 0$. The former condition implies $F_X(2) > F_T(2)$ if the roles of the two amino acids are switched, whereas the latter gives $F_X(2) < F_T(2)$. Again for the dissociation reaction model, metabolic inhibitors decrease $F_X(1) - F_T(1)$ and $F_X(1)/F_T(1)$ in the isoexchange situation, but the corresponding changes in the hetero-exchange situation were not obvious from the expressions obtained.

It should be emphasized that the results obtained are strictly applicable only to the stationary state which would be obtained if we could maintain the initial conditions which apply to measurements of initial uptake fluxes. Nonetheless, they demonstrate the *theoretical* possibility of obtaining $F_X > F_T$ with carrier models. This so-called pseudostationary state approximation has been useful in chemical kinetics and has been basic to the derivation of the equations of enzyme kinetics. Since the amino acid active transport system is quite similar kinetically to enzyme systems and since, in analogy with the latter, the amount of carrier present is probably far less than the amount of substrate, the stationary state fluxes may be fairly good approximations to the measured "initial" uptake fluxes. Even if this is not true, we can expect that at least the directional changes due to inhibitors will be the same for initial uptake fluxes as for the corresponding stationary-state fluxes. For this reason, the effects of inhibitors have been considered primarily in terms of the directional changes in the fluxes. The induction period in amino acid active transport is certainly less than one minute. Thus, the pseudostationary state solutions should at the least be a good approximation to the kinetics after the process has gone on for a few minutes.[11] However, with two amino acids present, even the pseudostationary-state equations are difficult to solve for the kinetics in the general case where we do not impose the restrictions that c_{e1}, c_{e2}, c_{i1}, and c_{i2} remain constant.

The meager data available[6] on the effects of inhibitors on F_X and F_T fit in best with the model of active transport of free carrier. Obviously, more measurements on the effects of inhibitors on F_X/F_T and $F_X - F_T$, particularly in isoexchange experiments, are needed.

The difference between the model of active transport of free carrier and the other models may be seen on a physically more intuitive basis by examination of Figures 2 and 3. Let us call the closed cycle in Figure 2 the active transport loop and the outer closed cycle in Figure 3, involving both substrates, the exchange diffusion loop. Then, it is only in the model of active transport of free carrier that the metabolically linked step does not occur in the exchange diffusion loop. The flux $F_T(1)$ is obtained by operation of the active transport loop alone, whereas $F_X(1)$ is obtained by the simultaneous operation of the active transport loop and the exchange diffusion loop. Since only that part of $F_X(1)$ contributed by the active transport loop can be decreased by an inhibitor and even that can be decreased no more than can $F_T(1)$, it is obvious why $F_X(1) - F_T(1)$ and $F_X(1)/F_T(1)$ cannot decrease. In each of the other three models, two steps in the exchange diffusion loop are linked to metabolism. One of these steps is in the direction of the flux

being determined, whereas the other opposes this flux. Thus, the effect of an inhibitor will depend on the relative changes in the two parameters involved. It would appear that the predominant effect is to decrease $F_X - F_T$ and F_X/F_T, although this must be qualified somewhat for the model of active transport of carrier-substrate complex, because the findings are applicable only when carrier and substrate are near equilibrium.

In conclusion, the use of the stationary-state approximation with carrier models gives results which check adequately with experimental data on the initial uptake fluxes in active transport and exchange diffusion. The data on the directional effects of inhibitors on these fluxes can also be fitted by the carrier models and the inhibitor studies may provide the data required to differentiate among the various types of carrier models. The mathematical solutions corresponding to the experimental steady-state have not been considered; the algebraic equations involved are so cumbersome it is difficult to see the forest for the trees. The results on competition also have not been considered. It is obvious from the structure of the models that the carrier models can give competition for uptake; it is not obvious that they can give an increase in initial uptake flux of one amino acid when another is present, as has been found with Ehrlich ascites cells. Unfortunately, even with the pseudostationary-state approximation, the equations for this case remain difficult; it would probably be simpler to try a computer solution for this case. We conjecture that the amino acids which at low concentrations give an increased initial uptake flux of another amino acid in a competition experiment will be those which give an increased initial uptake of that amino acid in an exchange diffusion experiment.

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