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COUPLING IN SECONDARY ACTIVE TRANSPORT

ACTIVATION OF TRANSPORT BY CO-TRANSPORT AND/OR COUNTER-TRANSPORT WITH THE FLUXES OF OTHER SOLUTES

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SUMMARY

The transport of a solute may be activated by coupling to the fluxes of other solutes *via* co-transport and/or counter-transport. This coupling may stimulate the transport rate of the former and, under certain conditions, also contribute energy for the active accumulation. The coupling may occur in different ways, either by increasing the affinity of the carrier to the transported solute or by increasing the velocity of the carrier–solute complex, or by both effects at the same time. Each of these effects can be conceived as occurring either directly, in that the co- or counter-substrate alters the properties of the carrier to which it is bound, or indirectly, in a quasi-allosteric way by shifting the equilibrium between two conformations of the carrier in favor of that with the higher affinity or velocity, respectively. These possibilities have been treated kinetically in a more general way, taking into account all the mentioned possible effects. From the general formula two selected simplified model types are derived: the affinity type and the velocity type. Each of these types has been subdivided in a direct and a quasi-allosteric variant. It is found that both variants of the affinity type would predict that only the K_m of the main transport is changed by the activation. Hence the affinity type in its pure form only applies to a limited number of experimentally investigated cases. Of the velocity-type models both variants would account for an increase in maximum velocity. But only the direct type would under rather stringent conditions explain an active accumulation, whereas the quasi-allosteric variant could not account for any accumulation at all. In that case the activation is merely catalytic, not energetic. In addition, a few combinations of velocity and affinity effects are considered and also a special model with three conformational modifications is presented. Each of these will account for a change in both K_m and v_{max}, as has usually been found in the systems so far studied. The differential effect of co- and counter-transport effects is evaluated for most of these.

INTRODUCTION

As for other solutes, the flux of amino acids into and across cells is stimulated by Na^+ present on the cis side and inhibited by Na^+ on the trans side (see reviews

by CHRISTENSEN[1]; and see ref. 2). K^+ seems to have the inverse effect, inhibiting amino acid net influx from the cis side and stimulating it from the trans side[3]. Experimental evidence is consistent with the view that this "activation" of transport is due to a "co-transport" between Na^+ and amino acids, and, less certain, to a "counter-transport" between amino acid and K^+ (ref. 4)*. The transport of the amino acids, to the extent that it is thus driven by the ECP-gradients of the alkali ions, is therefore "secondary active", *i.e.* secondary to an active transport of the ions[5].

Co- and counter-transport entail an energetic coupling between fluxes, in this case between the flux of the amino acid, on the one hand, and the parallel flux of Na^+ and the antiparallel flux of K^+, on the other hand. It is generally assumed that this coupling involves the formation of ternary complexes between amino acid, Na^+, and the hypothetical carrier (X). The mere formation of such a complex *per se*, however, does not necessarily lead to coupling. For that effect binding of Na^+ or K^+ to X must profoundly modify some properties of the latter, *e.g.* its affinities for the amino acid or its mobility. Such modifications can be conceived of as coming about directly or indirectly[6]. In the first case the modifying ion will, by its mere attachment to the carrier, directly and reversibly alter the latter with respect to its affinity and/or mobility properties. In the second case the carrier is supposed to occur in two or more different conformational states with different mobility and/or affinity properties. If these states rapidly equilibrate with each other, and if a modifying ion binds preferentially to one of them, such a binding will shift the equilibrium in favor of this state, thus indirectly changing the transport properties of the overall system. Because of its similarity to the well-known allosteric effects in enzyme systems, this effect will be called "quasi-allosteric"**.

Before studying the various possible modifications and their kinetic characteristics separately and in more detail, we shall start with a more general treatment, using a model in which all the above mentioned modifications may occur together. In this treatment we disregard the ionic nature of the two effectors, Na^+ and K^+, and call them "co-effector" and "counter-effector", respectively, whereas the transported amino acid will be referred to as "transportee". Most appropriate for such a treatment, according to common views on mediated transport, appears to be a model with a carrier freely mobile in but restricted to, the transport region. Each carrier molecule has two different binding sites, one for the transportee (A) and one for either the co-effector (B) or the counter-effector (C). Attachment of B to X at its proper site increases the affinity of X for A and/or the mobility of the carrier–transportee complex (XA), whereas attachment of C to its proper site will do the opposite, *i.e.* decrease the affinity of X for A and/or increase the mobility of X over XA. The carrier is assumed to occur in two or more (N) conformational states (X_i, $i = 1, 2, \ldots N$) which almost instantaneously equilibrate with each other so that the concentration

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* The terms co-transport and counter-transport are used here phenomenologically without implying any specific molecular mechanism.

** In contrast to allosteric enzyme systems, no quaternary structure of the carrier is implied in the present treatment of indirect modification, since a quaternary structure is neither necessary for the basic concept nor is it indicated by experimental evidence. To emphasize this difference, we use here the term "quasi-allosteric".

ratios of the unloaded states (L_i) can be treated as constants*. The affinities for A, B, and C, and also the mobility coefficients of the carrier species may be different for each state. No assumption is necessary that the transport region is symmetrical*. In other words, the equilibrium constants (Li), the permeability constants (P_{1x}, P_{2x}, *etc.*), and the affinity constants with respect to A, B, and C of a distinct conformational state need not be the same on both sides of the region. However, in order not to violate the second law of thermodynamics, the different parameters of the two sides must be related in such a way that with equal activities of A, B, and C on both sides of the region the net fluxes of each of these species vanish. The other conditions are as usual:

(a) The movement of the carrier species across the region limits the rate of the overall process; in other words, the carrier species in the boundaries of the region are treated as being always in equilibrium with their ligands of the adjacent phases**.

(b) The total number of all carrier molecules, free and loaded, in the two membrane faces remains constant (X_T).

(c) The turnover of the carrier transport is fast enough, and the observation time long enough, to allow that a steady state can be assumed at all times. In other words, the net movement of the total of the carrier species across the region can be taken as o***.

(d) No gradients of carrier species other than perpendicular to the surfaces of the transport region are considered.

The following notations are used:

a, b, c are the activities of transportee (A), co-effector (B), and the counter-effector (C), respectively, in the bulk phases.

x_1, x_2 ... x_n are the concentrations of different conformational states of the free carrier (X) in the boundaries of the region.

ax_i, bx_i, cx_i, abx_i, acx_i, *etc.* are the concentrations of the possible binary and ternary complexes between the carrier and its ligands;

p_{io}, p_{ia} are the mobilities of the carrier species X_i, AX_i, *etc.* within the transport region;

L_i *etc.* are the equilibrium ratios for the unloaded carrier modifications.

K_{ia} *etc.* are the dissociation constants of the various complexes between carrier and substrates;

* If, on the other hand, the assumption is made that the transition between the conformations is slower than the translocation of the carrier species', one obtains a flux equation which can be split formally into several Michaelis–Menten terms, as will be shown in a subsequent publication by one of us (P.G.). The conclusions drawn from the present derivations will, however, remain fundamentally valid.

** This assumption was made for the sake of simplicity. It seems also justified since the energetic coupling between the fluxes tends to vanish unless the mobility of the carrier species' within the transport region limits the rates of the overall fluxes. As can be shown by a simple calculation, given in APPENDIX, the maximal flux ratio of the substrate (A) becomes unity if the rates of dissociation and association between carrier and ligands become small enough as compared to the movements of the carrier species across the transport region.

*** If the various carrier species have different mobilities, changes in the ligand concentration on either side of the membrane will cause a redistribution of total carrier within the membrane, thus transiently disturbing the steady state. During this transient period assumption (c) would clearly not hold, until a new steady state is reached. Since, however, the cycling time of most carriers is probably in the order of milliseconds, such transient disturbances must escape detection during the observation times hitherto used in transport studies. Hence assumption (c) can be taken as valid in the present context.

f_b, f_c are the factors by which the affinity of the carrier for the transportee (A) is changed by B or C, respectively.

The superscript prime (') and double prime (") refer to the left and the right side of the region, respectively.

To characterize the influence of the effectors B and C on the transport of A, the following "standard" parameters and their dependence on the concentrations of B and C will be derived:

(1)The maximal rate of the unidirectional influx of A, $(J_a)_{max}$.

(2) The apparent Michaelis constant of the above flux of A (K_m).

(3) The maximal flux ratio (f_{max}) of A at equal and very small concentrations of a A on both sides. This value is identical with the maximal accumulation ratio attained in the steady state, *i.e.* at the flux ratio of 1.

THE GENERAL MODEL*

The carrier may occur in N different conformational states. Hence the following species of the carrier and its complexes may be present:

$x'_1 \ldots x'_N$; $ax'_1 \ldots ax'_N$; $bx'_1 \ldots bx'_N$; $cx'_1 \ldots cx'_N$;
$abx'_1 \ldots abx'_N$; $acx'_1 \ldots acx'_N$;
$x''_1 \ldots x''_N$; $ax''_1 \ldots ax''_N$; $bx''_1 \ldots bx''_N$; $cx''_1 \ldots cx''_N$;
$abx''_1 \ldots abx''_N$; $acx''_1 \ldots acx''_N$.

The conformational states of the unloaded carrier are in equilibrium with each other, L_i being the equilibrium constants:

$$L'_i = \frac{x'_i}{x'_1}; \qquad L''_i = \frac{x''_i}{x''_1}; \qquad L'_1 = L''_1 = 1 \tag{1}$$

The transportee (A) and the effectors (B and C) are bound to the carrier according to mass action law:

$$\frac{a' \cdot x'_i}{ax'_i} = K'_{ia}; \qquad \frac{b' \cdot x'_i}{bx'_i} = K'_{ib}; \qquad \frac{c'_i \cdot x'_i}{cx'_i} = K'_{ic};$$

$$\frac{a' \cdot b' \cdot x'_i}{abx'_i} = K'_{iab} = K'_a \cdot K'_b / f'_b, \tag{2}$$

$$\frac{a' \cdot c' \cdot x'_i}{acx'_i} = K'_{iac} = \frac{K'_a \cdot K'_c}{f'_c},$$

and analogous for the " side.

The unidirectional mobility constants of the various species are

p'_{io}; p'_{ia}; p'_{ic}; p'_{iab}; p'_{iac}; p'_{ib};
p''_{io}; p''_{ia}; p''_{ib}; p''_{ic}; p''_{iab}; p''_{iac};

for

x'_i; ax'_i; bx'_i; cx'_i; abx'_i; acx'_i;
x''_i; ax''_i; bx''_i; cx''_i; abx''_i; acx''_i,

respectively.

* Readers more interested in the applications than in the somewhat mathematically involved general treatment may immediately proceed to p. 451 (THE REDUCED MODELS).

Since the total amount of carrier species per unit transport region is constant:

$$\sum_{i=1}^{N} x'_i + \sum_{i=1}^{N} ax'_i + \sum_{i=1}^{N} bx'_i + \sum_{i=1}^{N} cx'_i + \sum_{i=1}^{N} abx'_i + \sum_{i=1}^{N} acx'_i + \sum_{i=1}^{N} x''_i +$$
$$+ \sum_{i=1}^{N} ax''_i + \sum_{i=1}^{N} bx''_i + \sum_{i=1}^{N} cx''_i + \sum_{i=1}^{N} abx''_i + \sum_{i=1}^{N} acx''_i = X_T \qquad (3)$$

Since the system is assumed to be in the steady state:

$$\sum_{i=1}^{N} p'_{io} \cdot x'_i + \sum_{i=1}^{N} p'_{ia} \cdot ax'_i + \sum_{i=1}^{N} p'_{ib} \cdot bx'_i + \sum_{i=1}^{N} p'_{ic} \cdot cx'_i + \sum_{i=1}^{N} p'_{iab} \cdot abx'_i +$$
$$+ \sum_{i=1}^{N} p'_{iac} \cdot acx'_i = \sum_{i=1}^{N} p''_{io} \cdot x''_i + \sum_{i=1}^{N} p''_{ia} \cdot ax''_i + \sum_{i=1}^{N} p''_{ib} \cdot bx''_i +$$
$$+ \sum_{i=1}^{N} p''_{ic} \cdot cx''_i + \sum_{i=1}^{N} p''_{iab} \cdot abx''_i + \sum_{i=1}^{N} p''_{iac} \cdot acx''_i. \qquad (4)$$

The unidirectional fluxes of A, B, and C are:
for A

$$J'_a = \sum_{i=1}^{N} p'_{ia} \cdot ax'_i + \sum_{i=1}^{N} p'_{iab} \cdot abx'_i + \sum_{i=1}^{N} p'_{iac} \cdot acx'_i$$
$$J''_a = \sum_{i=1}^{N} p''_{ia} \cdot ax''_i + \sum_{i=1}^{N} p''_{iab} \cdot abx''_i + \sum_{i=1}^{N} p''_{iac} \cdot acx''_i$$
$$J^N_a = J'_a - J''_a$$

for B

$$J'_b = \sum_{i=1}^{N} p'_{ib} \cdot bx'_i + \sum_{i=1}^{N} p'_{iab} \cdot abx'_i$$
$$J''_b = \sum_{i=1}^{N} p''_{ib} \cdot bx''_i + \sum_{i=1}^{N} p''_{iab} \cdot abx''_i \qquad (5)$$
$$J^N_b = J'_b - J''_b$$

for C

$$J'_c = \sum_{i=1}^{N} p'_{ic} \cdot cx'_i + \sum_{i=1}^{N} p'_{iac} \cdot acx'_i$$
$$J''_c = \sum_{i=1}^{N} p''_{ic} \cdot cx''_i + \sum_{i=1}^{N} p''_{iac} \cdot acx''_i$$
$$J^N_c = J'_c - J''_c$$

Introducing Definitions 1 and 2 into Eqns. 3 and 4

$$x'_1 \left\{ \sum_{i=1}^{N} L'_i + \sum_{i=1}^{N} \frac{L'_i \cdot a'}{K'_{ia}} + \sum_{i=1}^{N} \frac{L'_i \cdot b'}{K'_{ib}} + \sum_{i=1}^{N} \frac{L'_i \cdot c'}{K'_{ic}} + \sum_{i=1}^{N} \frac{L'_i \cdot f'_{ib} \cdot a' \cdot b'}{K'_{ia} \cdot K'_{ib}} + \right.$$

$$+\sum_{i=1}^{N}\frac{L'_i\cdot f'_{ic}\cdot a'\cdot c'}{K'_{ia}\cdot K'_{ic}}\Bigg\}+x''_1\Bigg\{\sum_{i=1}^{N}L''_i+\sum_{i=1}^{N}\frac{L''_i\cdot a''}{K''_{ia}}+\sum_{i=1}^{N}\frac{L''_i\cdot b''}{K''_{ib}}+\sum_{i=1}^{N}\frac{L''_i\cdot c''}{K''_{ic}}+ \quad (6)$$

$$+\sum_{i=1}^{N}\frac{L''_i\cdot f''_{ib}\cdot a''\cdot b''}{K''_{ia}\cdot K''_{ib}}+\sum_{i=1}^{N}\frac{L''_i\cdot f''_{ic}\cdot a''\cdot c''}{K''_{ia}\cdot K''_{ic}}\Bigg\}=X_T$$

and

$$x'_1\Bigg\{\sum_{i=1}^{N}L'_i\cdot p'_{io}+\sum_{i=1}^{N}\frac{L'_i\cdot p'_{ia}\cdot a'}{K'_{ia}}+\sum_{i=1}^{N}\frac{L'_i\cdot p'_{ib}\cdot b'}{K'_{ib}}+\sum_{i=1}^{N}\frac{L'_i\cdot p'_{ic}\cdot c'}{K'_{ic}}+$$

$$+\sum_{i=1}^{N}\frac{L'_i\cdot f'_{ib}\cdot p'_{iab}\cdot a'\cdot b'}{K'_{ia}\cdot K'_{ib}}+\sum_{i=1}^{N}\frac{L'_i\cdot f'_{ic}\cdot p'_{iac}\cdot a'\cdot c'}{K'_{ia}\cdot K'_{ic}}\Bigg\}=$$

$$x''_1\Bigg\{\sum_{i=1}^{N}L''_i\cdot p''_{io}+\sum_{i=1}^{N}\frac{L''_i\cdot p''_{ia}\cdot a''}{K''_{ia}}+\sum_{i=1}^{N}\frac{L''_i\cdot p''_{ib}\cdot b''}{K''_{ib}}+\sum_{i=1}^{N}\frac{L''_i\cdot p''_{ic}\cdot c''}{K''_{ic}}+ \quad (7)$$

$$+\sum_{i=1}^{N}\frac{L''_i\cdot f''_{ib}\cdot p''_{iab}\cdot a''\cdot b''}{K''_{ia}\cdot K''_{ab}}+\sum_{i=1}^{N}\frac{L''_i\cdot f''_{ic}\cdot p''_{iac}\cdot a''\cdot c''}{K''_{ia}\cdot K''_{ic}}\Bigg\}$$

In order to simplify the notation we introduce the following abbreviations:

$$x'_1\sum_{i=1}^{N}L'_i=x' \qquad \frac{\sum_{i=1}^{N}L'_i}{\sum_{i=1}^{N}\frac{L'_i}{K'_{ia}}}=K'_a$$

$$\frac{\sum_{i=1}^{N}L'_i}{\sum_{i=1}^{N}\frac{L'_i}{K'_{ib}}}=K'_b$$

$$\frac{\sum_{i=1}^{N}L'_i}{\sum_{i=1}^{N}\frac{L'_i}{K'_{ic}}}=K'_c$$

$$\frac{\sum_{i=1}^{N}\frac{L'_i\cdot f'_{ib}}{K'_{ia}\cdot K'_{ib}}\sum_{i=1}^{N}L'_i}{\sum_{i=1}^{N}\frac{L'_i}{K'_{ia}}\sum_{i=1}^{N}\frac{L'_i}{K'_{ic}}}=f'_b \quad (8)$$

$$\frac{\sum_{i=1}^{N}\frac{L'_i\cdot f'_{ic}}{K'_{ic}\cdot K'_{ia}}\sum_{i=1}^{N}L'_i}{\sum_{i=1}^{N}\frac{L'_i}{K'_{ia}}\sum_{i=1}^{N}\frac{L'_i}{K'_{ic}}}=f'_c$$

$$\sum_{i=1}^{N} \frac{L'_i \cdot p'_{io}}{L'_i} = p'_o \qquad \frac{\sum_{i=1}^{N} \frac{L'_i \cdot p'_{ia}}{K'_{ia}}}{\sum_{i=1}^{N} \frac{L'_i}{K'_{ia}}} = p'_a$$

Hence Eqns. 5, 6, and 7 will be reduced to Eqns. 9, 10, and 11.

$$\begin{aligned}
J'_a &= p'_a \cdot ax' + p'_{ab} \cdot abx' + p'_{ac} \cdot acx' \\
J''_a &= p''_a \cdot ax'' + p''_{ab} \cdot abx'' + p''_{ac} \cdot acx'' \\
J'_b &= p'_b \cdot bx' + p'_{ab} \cdot abx' \\
J''_b &= p''_b \cdot bx'' + p''_{ab} \cdot abx'' \\
J'_c &= p'_c \cdot cx' + p'_{ac} \cdot acx' \\
J''_c &= p''_c \cdot cx'' + p''_{ac} \cdot acx''
\end{aligned} \tag{9}$$

$$\begin{aligned}
&x' \cdot \left(1 + \frac{a'}{K'_a} + \frac{b'}{K'_b} + \frac{c'}{K'_c} + \frac{f'_b \cdot a' \cdot b'}{K'_a \cdot K'_b} + \frac{f'_c \cdot a' \cdot c'}{K'_a \cdot K'_c}\right) + \\
&+ x'' \cdot \left(1 + \frac{a''}{K''_a} + \frac{b''}{K''_b} + \frac{c''}{K''_c} + \frac{f''_b \cdot a'' \cdot b''}{K''_a \cdot K''_b} + \frac{f''_c \cdot a'' \cdot c''}{K''_a \cdot K''_c}\right) = X_T
\end{aligned} \tag{10}$$

and

$$\begin{aligned}
&x' \left(p'_o + \frac{p'_a \cdot a'}{K'_a} + \frac{p'_b \cdot b'}{K'_b} + \frac{p'_c \cdot c'}{K'_c} + \frac{p'_{ab} \cdot f'_b \cdot a' \cdot b'}{K'_a \cdot K'_b} + \frac{p'_{ac} \cdot f'_c \cdot a' \cdot c'}{K'_a \cdot K'_c}\right) = \\
&= x'' \left(p''_o + \frac{p''_a \cdot a''}{K''_a} + \frac{p''_b \cdot b''}{K''_b} + \frac{p''_c \cdot c''}{K''_c} + \frac{p''_{ab} \cdot f''_b \cdot a'' \cdot b''}{K''_a \cdot K''_b} + \frac{p''_{ac} \cdot f''c \cdot a'' \cdot c''}{K''_a \cdot K''_c}\right)
\end{aligned} \tag{11}$$

Eqns. 10 and 11 are abbreviated as follows

$$x' \cdot p'_o \cdot U' + x'' \cdot p''_o \cdot U'' = X_T \tag{10a}$$

$$x' \cdot p'_o \cdot V' = x'' \cdot p''_o \cdot V'' \tag{11a}$$

U', V', U'', V'' replacing the expressions in parentheses divided by p'_o or p''_o, respectively. Hence

$$\begin{aligned}
U' &= \frac{1}{p'_o} + \frac{a'}{p'_o \cdot K'_a} + \frac{b'}{p'_o \cdot K'_b} + \cdots \\
V' &= 1 + \frac{p'_a \cdot a'}{p'_o \cdot K'_a} + \frac{p'_b \cdot b'_,}{p'_o \cdot K'_b} + \cdots
\end{aligned} \tag{12}$$

U'' and V'' are analogous.

We now solve Eqns. 10a and 11a for x' and x'' and obtain

$$x' = \frac{p''_o \cdot V''}{N} \tag{13a}$$

$$x'' = \frac{p'_o \cdot V'}{N} \tag{13b}$$

$$N = p'_o \cdot p''_o \cdot (U' \cdot V'' + U'' \cdot V')/X_T \tag{14}$$

The net fluxes may now be given as follows

$$J_a^N = \left\{ \left(\frac{p'_a \cdot a'}{K'_a} + \frac{p'_{ab} \cdot f'_b \cdot a' \cdot b'}{K'_a \cdot K'_b} + \frac{p'_{ac} \cdot f'_c \cdot a' \cdot c'}{K'_a \cdot K'_c} \right) p''_o \cdot V'' - \right.$$
$$\left. - \left(\frac{p'' \cdot a''}{K''_a} + \frac{p''_{ab} \cdot f''_b \cdot a'' \cdot b''}{K''_a \cdot K''_b} + \frac{p''_{ac} \cdot f''_c \cdot a'' \cdot c''}{K''_a \cdot K''_c} \right) p'_o \cdot V' \right\} \Big/ N \tag{15}$$

$J^N{}_b$ and $J^N{}_c$ are analogous.

According to the second law of thermodynamics the net flux of A has to vanish if the activities, or here the concentrations of A, B, and C are equal on both sides of the membrane. It follows from Eqn. 15 that under such conditions

$$\left(\frac{p'_a \cdot a'}{K'_a} + \frac{p'_{ab} \cdot f'_b \cdot a' \cdot b'}{K'_a \cdot K'_b} + \frac{p'_{ac} \cdot f'_c \cdot a' \cdot c'}{K_a \cdot K_c} \right) \cdot p''_o \cdot V'' =$$
$$= \left(\frac{p''_a \cdot a''}{K''_a} + \frac{p''_{ab} \cdot f''_b \cdot a'' \cdot b''}{K''_a \cdot K''_b} + \frac{p''_{ac} f''_c \cdot a'' \cdot c''}{K''_a \cdot K''_c} \right) \cdot p'_o \cdot V' \tag{16}$$

Since this equation must hold for any concentration of A, B, or C, the coefficients of a, b, c, and of any combination thereof must be zero; to verify this we rearrange Eqn. 16 to collect all of each combination of variables as a, ab, ac, ab^2, ac^2, and abc, in separate terms. Making the parameters of each of these terms equal to zero, we obtain the following relationships between the parameters of velocity (p_i) and affinity (K_i) at one side and those at the other side of the membrane:

$$\frac{p'_o}{p'_a} \cdot K'_a = \frac{p''_o}{p''_a} \cdot K''_a = \bar{K}_a$$

$$\frac{p'_o}{p'_b} \cdot K'_b = \frac{p''_o}{p''_b} \cdot K''_b = \bar{K}_b$$

$$\frac{p'_o}{p'_c} \cdot K'_c = \frac{p''_o}{p''_c} \cdot K''_c = \bar{K}_c \tag{17}$$

$$F_b = f'_b \cdot \frac{p'_{ab} \cdot p'_o}{p'_a \cdot p'_b} = f''_b \cdot \frac{p''_{ab} \cdot p''_o}{p''_a \cdot p''_b}$$

$$F_c = f'_c \cdot \frac{p'_{ac} \cdot p'_o}{p'_a \cdot p'_c} = f''_c \cdot \frac{p''_{ac} \cdot p''_o}{p''_a \cdot p''_c}$$

which allow us to replace asymmetric parameters by side-independent ones ($\bar{K}_a$, $\bar{K}_b$, $\bar{K}_c$, F).

We may now use the normalized concentrations of A, B, C, namely

$$\bar{\alpha}' = \frac{a'}{\bar{K}_a}; \qquad \bar{\alpha}'' = \frac{a''}{\bar{K}_a} \tag{18a}$$

$$\bar{\beta}' = \frac{b'}{\bar{K}_b}; \qquad \bar{\beta}'' = \frac{b''}{\bar{K}_b} \tag{18b}$$

$$\bar{\gamma} = \frac{c'}{\bar{K}_c}; \qquad \bar{\gamma}'' = \frac{c''}{\bar{K}_c} \tag{18c}$$

Substituting these expressions in Eqns. 9 and 10, and making $(U' \cdot V'' + U'' \cdot V')/X_T = R$ we obtain

$$\begin{aligned} V' &= (1 + \bar{\alpha}' + \bar{\beta}' + \bar{\gamma}' + F_b \cdot \bar{\alpha}' \cdot \bar{\beta}' + F_c \cdot \bar{\alpha}' \cdot \bar{\beta}') \\ V'' &= (1 + \bar{\alpha}'' + \bar{\beta}'' + \bar{\gamma}'' + F_b \cdot \bar{\alpha}'' \cdot \bar{\beta}'' + F_c \cdot \bar{\alpha}'' \cdot \bar{\beta}'') \\ R &= \Bigg((1 + \bar{\alpha}' + \bar{\beta}' + \bar{\gamma}' + F_b \cdot \bar{\alpha}' \cdot \bar{\beta}' + F_c \cdot \bar{\alpha}' \cdot \bar{\gamma}') \cdot \\ &\quad \cdot \left(\frac{1}{p''_o} + \frac{\bar{\alpha}''}{p''_a} + \frac{\bar{\beta}''}{p''_b} + \frac{\bar{\gamma}''}{p''_c} + \frac{F_b \cdot \bar{\alpha}'' \cdot \bar{\beta}''}{p''_{ab}} + \frac{F_c \cdot \bar{\alpha}'' \cdot \bar{\gamma}''}{p''_{ac}} \right) + \\ &\quad + (1 + \bar{\alpha}'' + \bar{\beta}'' + \bar{\gamma}'' + F_b \cdot \bar{\alpha}'' \cdot \bar{\beta}'' + F_c \cdot \bar{\alpha}'' \cdot \bar{\gamma}'') \cdot \\ &\quad \cdot \left(\frac{1}{p'_o} + \frac{\bar{\alpha}'}{p'_a} + \frac{\bar{\beta}'}{p'_b} + \frac{\bar{\gamma}'}{p'_c} + \frac{F_b \cdot \bar{\alpha}' \cdot \bar{\beta}'}{p'_{ab}} + \frac{F_c \cdot \bar{\alpha}' \cdot \bar{\gamma}'}{p'_c} \right) \Bigg) \Bigg/ X_T \end{aligned} \tag{20}$$

The further procedure may be simplified by introducing resistences (R) instead of the velocity coefficients (p) used so far. These resistances are not to be confused with thermodynamic resistances. The latter relate the flow of matter to a potential difference, the present ones to a concentration (or activity) difference. The present resistances are not simply analogous to electrical resistances either. For instance, the addition of several R terms does not necessarily represent an arrangement in series. Hence, the various notations of R in the following should simply be taken as formal aids o simplify the derivation.

We can split the R of Eqn. 20 into two terms:

$$R = R'_{(o)} + \bar{\alpha}' \cdot R'_{(a)} \tag{21}$$

which are

$$\begin{aligned} R'_{(o)} &= R'^{(o)}_{(o)} + \bar{\beta}' \cdot R'^{(b)}_{(o)} + \bar{\gamma}' \cdot R'^{(c)}_{(o)} \\ R'_{(a)} &= R'^{(o)}_{(a)} + F_b \cdot \bar{\beta}' \cdot R'^{(b)}_{(a)} + F_c \cdot \bar{\gamma}' \cdot R'^{(c)}_{(a)} \end{aligned} \tag{22}$$

and, furthermore,

$$\begin{aligned} R'^{(o)}_{(o)} = R^{(oo)}_{(oo)} + \bar{\gamma}'' \cdot R^{(oo)}_{(oc)} + \bar{\beta}'' \cdot R^{(oo)}_{(oo)} + \bar{\alpha} \cdot R^{(oo)}_{(oa)} . + . F_b \cdot \bar{\alpha}'' \cdot \bar{\beta}'' \cdot R^{(ab)}_{(oa)} + \\ + . F_c \cdot \bar{\alpha}'' \cdot \bar{\gamma}'' \cdot R^{(oc)}_{(oa)} \end{aligned} \tag{23}$$

and, finally

$$R\binom{oo}{oo} = \left(\frac{1}{p'_o} + \frac{1}{p''_o}\right) \Big/ X_T \qquad R\binom{bo}{oo} = \left(\frac{1}{p'_b} + \frac{1}{p''_o}\right) \Big/ X_T$$
$$R\binom{ob}{oo} = \left(\frac{1}{p'_o} + \frac{1}{p''_b}\right) \Big/ X_T \qquad R\binom{bo}{ao} = \left(\frac{1}{p'_{ab}} + \frac{1}{p''_o}\right) \Big/ X_T \text{ etc.} \tag{24}$$

Introducing (21) into the following equations of the unidirectional fluxes:

$$J'_a = \bar{\alpha}' \cdot (1 + F_b \cdot \bar{\beta}' + F_c \cdot \bar{\gamma}') \cdot V''/R$$
$$J''_a = \bar{\alpha}'' \cdot (1 + F_b \cdot \bar{\beta}'' + F_c \cdot \bar{\gamma}'') \cdot V'/R$$
$$J'_b = \bar{\beta}' \cdot (1 + F_b \cdot \bar{\alpha}') \cdot V''/R \qquad J''_b = \bar{\beta}'' \cdot (1 + F_b \cdot \bar{\alpha}'') \cdot V'/R \tag{25}$$
$$J'_c = \bar{\gamma}' \cdot (1 + F_c \cdot \bar{\alpha}') \cdot V''/R \qquad J''_c = \bar{\gamma}'' \cdot (1 + F_c \cdot \bar{\alpha}'') \cdot V'/R$$

We obtain the "standard parameters"

$$(J'_a)_{max} = (1 + F_b \cdot \bar{\beta}' + F_c \cdot \bar{\gamma}') \cdot V''/R(_a) \tag{26}$$

$$(K_m)_a = \bar{K}_a \cdot R'(_o)/R'(_a) \tag{27}$$

$$f_{max} = \frac{(1 + F_b \cdot \bar{\beta}' + F_c \cdot \bar{\gamma}') \cdot (1 + \bar{\beta}'' + \bar{\gamma}'')}{(1 + \bar{\beta}' + \bar{\gamma}') \cdot (1 + F_b \cdot \bar{\beta}'' + F_c \cdot \bar{\gamma}'')} \tag{28}$$

$$(\partial J_b'/\partial J_a')_{b,c} = \frac{\bar{\beta}' \cdot \{F_b - R'(_a)/R'(_o)\}}{1 + F_b \cdot \bar{\beta}' + F_c \cdot \bar{\gamma}'} \tag{29}$$

THE REDUCED MODELS

The resulting equations and parameters are too involved for an intuitive appreciation of the various activation effects on the transport process. We shall therefore deduce from the general model selected boundary models, in each of which the possible modification effects are reduced to one. This procedure seems also realistic since not all the conceivable activating effects are likely to contribute to the overall effect to an appreciable extent.

The following "reduced" models will be considered[6]:

(I) Affinity-type models, *i.e.* those in which the effectors modify only the affinity of the carrier for the transportee, without changing its mobility coefficient.

(II) Velocity-type models, *i.e.* those in which the effectors modify only the mobility of the carrier, without changing its affinity for the transportee.

(III) Mixed-type models, *i.e.* those in which two kinds of effects are combined.

Since, as has been mentioned before, both modifying effects can be conceived of as being (a) direct and (b) quasi-allosterical, each of the above types will be subdivided accordingly into a direct and an indirect (quasi-allosteric) variant. In order to characterize kinetically the resulting six reduced models we shall again use the corresponding "standard parameters" as to their kinetic responses to the presence or absence of an appropriate effector gradient. In order to keep the equations simple and to obtain maximal effects, we arbitrarily postulate in each case that the co-

effector (B) be present on the cis side only, and the counter-effector present on the trans side only, the terms "cis" and "trans" referring to the transport direction of the transportee (A), *i.e.* from left (') to right (''). Instead of the straight concentrations of A, B, and C we use the so-called "normed" concentrations, *i.e.* the concentrations relative to the dissociation constants of the respective complexes with X. The constants (K) used in these terms are meant to be the "true" dissociation constants, valid for the carrier species concerned and for the particular side, in contrast to the equalized constants ($\bar{K}$) as used in the equations of the general model. The kinetic parameters, $(J_a{}^o)_{max}$ and $K_m{}^o$ refer to the initial net flux, assuming zero concentration of the transportee (A) on the trans side ($a'' = 0$), so that trans-effectors are excluded.

(I) AFFINITY-TYPE MODELS

(*a*) *The direct (one-component) subtype*

We maintain the following assumptions: (1) the carrier (X) has two binding sites, one for the transportee (A) and another one for either one of the two effectors, B and C; (2) the binding of the co-effector (B) to X increases the affinity of the latter for A by the factor f_b, whereas the binding of the counter-effector (C) decreases this affinity by the factor f_c. For thermodynamic reasons each of these effects must be reciprocal, hence the binding of A to X must increase the affinity of the latter for B and decrease that for C by the same factor. For reasons of simplicity, we assume that the mutual effects between A and C are so strong that the complex ACX can be neglected. On the other hand, the assumptions of different mobilities (p) of the resulting carrier species, and of the existence of one or more conformational states (components) of X are abandoned. The standard parameters of the model reduced in this way, under the condition that B is present on the cis side only, and C on the trans side only (b'', $c' = 0$), are the following:

$$(J'^{o}_{a})_{max} = \frac{X_T \cdot p_o}{2}$$

$$K_m^o = K_a \cdot \frac{1 + \beta'}{1 + f_b \cdot \beta'}$$

$$f_{max} = \frac{1 + f_b \cdot \beta'}{1 + \beta'} \cdot \frac{1 + \gamma''}{1 + f_c \cdot \gamma''}$$

It is seen that the co-effector (B) decreases K_m but does not appear in the maximal initial influx. The *trans*-concentration of C appears in none of the flux parameters but in the maximum accumulation ratio. In other words, the counter-effector does not activate the unidirectional flux, but contributes to the increase of the final accumulation ratio reached.

If the carrier loaded with transportee (XA) has a velocity different from that of the carrier without A, an assumption often made in order to explain "trans" effects, the maximum rate and the apparent Michaelis constant have to be expanded as follows:

$$(J_a^o)_{max} = \frac{\rho_a}{1 + \rho_a} \cdot X_T \cdot p_o$$

$$K_m = K_a \cdot \frac{2}{1+\rho_a} \cdot \frac{1+\beta'}{1+f_b\beta'}$$

$$f_{max_i} = \frac{1+f_b\cdot\beta'}{1+\beta'} \cdot \frac{1+\gamma''}{1+f_c\cdot\gamma''}$$

ρ_a being the factor by which the carrier species' loaded with A (XA, XAB) are faster than the A-free species (X, XB). The maximal flux ratio is not affected by ρ_a. The statement that B affects the K_m only, and does not appear in $(J_a^o)_{max}$ still holds. Trans-effects, *i.e.* homo-exchange between A' and A'', which are to be expected if $\rho_a \neq 1$, will not appear here, since the above parameters refer to the initial net fluxes.

(*b*) *The indirect or quasi-allosteric (two-component) variant*

The assumption is maintained that the carrier occurs in two conformational states (components) which are in instantaneous equilibrium with each other, the equilibrium constant being L. The assumption that binding of one ligand to X *per se* changes the affinity for the other is abandoned (f_b and $f_c = 1$). So are the assumptions that the various species of the two carrier modifications have different mobilities. For reason of simplicity we assume that the one state, let us say X_1, has an affinity only for both A and B, each at its proper site, whereas X_2 has an affinity for C only. Obviously the combination of the carrier with A or B, or both, shifts the equilibrium towards X_1, whereas that with C does the opposite. The model shows the features of an affinity-type model. The standard parameters, on condition that B is present on side 1 only, and C on side 2 only, are the following:

$$(J_a^o)_{max} = \frac{X_T\cdot p_1}{2}$$

$$K_m = K_a \cdot \frac{1+L+\beta'}{1+\beta'}$$

$$f_{max} = \frac{(1+L)\cdot(1+\beta')}{1+L+\beta'} \cdot \frac{1+L\cdot(1+\gamma'')}{L+1}$$

Also here the co-effector (B) does not influence the maximal initial influx, whereas C appears only in the final distribution ratio. It is noteworthy that the activation by B is the greater, the higher L, *i.e.* the higher the equilibrium ratio of X_2 over X_1, whereas for C the opposite is true.

If we again introduce the assumption that the loading of the carrier with A increases its velocity by the factor ρ_a, we obtain the following parameters, in analogy to Model Ia:

$$(J_a^o)_{max} = \frac{(1+\rho_a\cdot L+\rho_a\cdot L\cdot\gamma'')}{2+L\cdot(1+\rho_a)+L\cdot(1+\rho_a)\cdot\gamma''}\cdot p_1 X_T$$

$$K_m = K_a\cdot\left(1+L\cdot\frac{1+\rho_a+2\rho_a\cdot L+2\rho_a\cdot L\cdot\gamma''}{[2+\rho_a\cdot L+L+L\cdot(1+\rho_a)\cdot\gamma'']\,[1+\beta']}\right)$$

$$f_{max} = \frac{(1 + \rho_a \cdot L)\cdot(1 + \beta')}{1 + \rho_a \cdot L + \beta'} \cdot \frac{1 + \rho_a \cdot L + \rho_a \cdot L \cdot \gamma''}{1 + \rho_a \cdot L}$$

A special case of the affinity type models obtains if the two ligands, A and B, combine with the carrier X only in a given order, *e.g.* first A, then B. The equations derived for the affinity effect would still hold except that the dissociation constant between the free carrier and the second ligand and consequently f_b, would be infinite. Accordingly the dissociation constant between the carrier and the first ligand (A) becomes zero after the second ligand has been bound. This circumstance would introduce some simplifications into the equations.

(II) THE VELOCITY TYPE MODELS

(*a*) *The direct (one-component) variant*

We abandon the assumptions that the binding of any ligand to the carrier (X) changes the affinity of the latter for any other ligand and that there are different conformational states. On the other hand we maintain the assumption that the binding of B to X imparts the latter a higher mobility. The binding of C to X must also alter the velocity of X, but in order to support the effect of B, this altering effect of C must differ from that of B in a specific way, as will be clear from analyzing the standard parameters. The standard parameters, as derived from the usual conditions (b'', $c' = 0$) are

$$(J_a^o)_{max} = X_T \cdot \frac{(p_a + p_{ab}\cdot\beta')\cdot(p_o + p_c\cdot\gamma'')}{(p_o + p_a) + (p_{ab} + p_o)\cdot\beta' + (p_a + p_c)\cdot\gamma'' + (p_{ab} + p_c)\cdot\beta'\cdot\gamma''}$$

$$K_m = K_a \cdot \frac{2p_o + (p_o + p_c)\cdot\gamma'' + (p_o + p_b)\cdot\beta' + (p_b + p_c)\cdot\beta'\cdot\gamma''}{(p_a + p_o) + (p_a + p_c)\gamma'' + (p_o + p_{ab})\cdot\beta' + (p_{ab} + p_c)\cdot\beta'\cdot\gamma''}$$

$$f_{max} = \frac{p_a + p_{ab}\cdot\beta'}{p_o + p_b\cdot\beta'} \cdot \frac{p_o + p_c\cdot\gamma''}{p_a + p_{ac}\cdot\gamma''}$$

Both β' and γ'' appear in all parameters In order to study the conditions under which they are effective we investigate best the effect of B alone, *i.e.* at $\gamma'' = 0$;

$$(J_a^o)_{max}^{\gamma''=0} = X_T \cdot \frac{p_o\cdot(p_a + p_{ab}\cdot\beta')}{p_o + p_a + (p_o + p_{ab})\cdot\beta'}$$

$$K_m^{\gamma''=0} = K_a \cdot \frac{2p_o + (p_o + p_b)\cdot\beta'}{p_o + p_a + (p_o + p_{ab})\cdot\beta'}$$

$$f_{max}^{\gamma''=0} = \frac{p_a + p_{ab}\cdot\beta'}{p_o + p_b\cdot\beta'} \cdot \frac{p_o}{p_a}$$

The influx is obviously severely limited by p_o Thus the stimulating effect of β' can never be greater than by the factor 2, unless $p_a < p_o$, *i e.* unless loading the empty carrier by A reduces its velocity. Most striking is the result that no accumulation

occurs, unless $(p_o \cdot p_{ab})/(p_a \cdot p_b) > 1$. In other words, the co-effector B accelerates the loaded carrier (XA) to a greater extent than the empty one. Since this relationship is reciprocal, the binding of A must also accelerate the XB complex to a greater extent than the empty carrier (X). In order to produce an efficient stimulation of the initial influx of A, the binding of A should even slow down the empty carrier, as has been mentioned above. Hence an effective stimulation of both influx and accumulation requires that the binding of A has an effect on the mobility of X opposite to the effect it has on the mobility of XB.

If we now investigate the influence of γ'' on the standard parameters independent of B, *i.e.* at $\beta' = 0$, we find that for a synergetic effect C'' must also accelerate the carrier, but in a fashion inverse to that in which β' does: it must have a stronger effect on the mobility of X than on that of XA; accordingly, γ'' will contribute to the stimulation of $(J^o{}_a)_{max}$ only if $p_c > p_o$, and to the maximum flux ratio only if $(p_a \cdot p_c)/(p_o \cdot p_{ac}) > 1$; γ'' will decrease K_m if $p_c > p_o$ and $p_a < p_o$ or if both relations are reversed.

(*b*) *The quasi-allosteric (two-component) variant*

In analogy to the two component affinity-type model we may devise one on a pure velocity basis. We assume again two modifications of the carrier, X_1 and X_2, of which only X_1 has an appreciable mobility. The substrate A may combine equally to either of them, whereas B combines only (or preferentially) with the mobile form, X_1. Obviously the effect of B would be to shift the equilibrium towards the mobile form without necessarily changing the affinity for A, as shown by the following "standard parameters" under the usual conditions:

$$(J^o_a)_{max} = \frac{X_T p_1 (1 + \rho \cdot L + \beta')\left(1 + \frac{\rho \cdot L}{1 + \rho L}\gamma''\right)}{2(1 + L) + \left(1 + \frac{1 + L}{1 + \rho \cdot L}\right)\cdot\beta' + \left(1 + \frac{(1 + L)\cdot\rho}{1 + \rho \cdot L}\right)\cdot\gamma'' + \frac{(1 + \rho)\cdot L}{1 + \rho \cdot L}\cdot\beta'\cdot\gamma''}$$

$$K^o_m = K_a$$

$$f_{max} \cdot = 1$$

The maximal initial influx is increased by both B' and C''. The apparent K_m should not be changed by B. It is noteworthy, however, that no accumulation will occur with this model, no matter how asymmetric the distribution of B and C may be. Hence this model could account only for a catalytic but not for an energetic effect of the effector gradients.

Since an accumulating effect of Na^+ gradients on amino acid transport is generally observed, this model does not fit the experimental findings without further assumptions, such as implementing additional affinity effects which will be considered in MIXED-TYPE MODELS of the next chapter.

(III) MIXED-TYPE MODELS

In the following models the effects of affinity- and velocity-type models are combined. For example, it is assumed that the ligand B would increase the affinity for A as well as the velocity of the carrier–substrate complex.

Model IIIa

We shall first consider the direct (one-component) variety of this model type, *i.e.* that which combines the properties of Model Ia and IIa. The standard parameters derived for this model are

$$(J_a^o)_{max} = X_T \cdot p_o \cdot \frac{(1 + f_b \cdot \rho_b \cdot \beta')(1 + \rho_c \cdot \gamma'')}{(1 + \rho_a) + (1 + \rho_a \cdot \rho_b) \cdot f_b \cdot \beta' + (\rho_c + \rho_a) \cdot \gamma'' + (\rho_c + \rho_a \cdot \rho_b) \cdot f_b \cdot \beta' \cdot \gamma''}$$

$$K_m = K_a \cdot \frac{2 + (1 + \rho_b) \cdot \beta' + (1 + \rho_c)\gamma'' + (\rho_c + \rho_b) \cdot \beta' \cdot \gamma''}{(1 + \rho_a) + (\rho_c + \rho_a)\gamma'' + (1 + \rho_a \cdot \rho_b) \cdot f_b \cdot \beta' + (\rho_c + \rho_a \cdot \rho_b) \cdot f_b \cdot \beta' \cdot \gamma''}$$

$$f_{max} = \frac{1 + f_b \cdot \rho_b \cdot \beta'}{1 + \rho_b \cdot \beta} \cdot \frac{1 + \rho_c \cdot \gamma''}{1 + f_c \cdot \rho_c \cdot \gamma''} \qquad f_b > 1 > f_c$$

As seen from the parameters listed, this model fits the observations made with most Na^+-activated transport systems. The co-effector B changes both the flux rate and the apparent Michaelis constant, and allows an energetic coupling, as indicated by an accumulation effect. The opposing gradient of the counter-effector (C), if present alone, increases the maximum influx if $\rho_c > 1 \cdot K_m$ is decreased if $\rho_a > 1 > \rho_c$ or if $\rho_c > 1 > \rho_a$.

Model IIIb

The quasi-allosteric (two-component) variety of the mixed-type model combines Models Ib and IIb. Of the two conformational states (X_1 and X_2) X_1 has the higher affinity for A and the higher velocity. B is assumed to combine with X_1 only. C, on the other hand, has a greater affinity for X_2 than for X_1. The standard parameters under the usual conditions ($b'' = 0$; $c' = 0$):

$$(J_a^o)_{max} = \frac{(1 + \rho_a \cdot L + f_b\beta')p_1 X_T}{2(1 + L) + \dfrac{(2 + \rho_a \cdot L + L) \cdot f_b \cdot \beta'}{1 + \rho_a \cdot L}}$$

$$K_m^o = K_a \frac{2(1 + L) + \dfrac{2 + L + \rho_a \cdot L}{1 + L} \cdot \beta'}{2(1 + L) + \dfrac{2 + L + \rho_a \cdot L}{1 + \rho_a \cdot L} \cdot f_b\beta'}$$

$$f_{max} = \frac{\left(1 + \dfrac{f_b}{1 + \rho_a \cdot L} \cdot \beta'\right) \cdot \left(1 + \dfrac{\rho_a \cdot L}{1 + \rho_a \cdot L} \cdot \gamma''\right)}{\left(1 + \dfrac{1}{1 + \rho_a \cdot L} \cdot \beta'\right) \cdot \left(1 + \dfrac{L \cdot f_c \cdot \rho_c}{f_b \cdot (1 + \rho_c \cdot L)} \cdot \gamma''\right)}$$

Also in this model, B on the cis side increases the maximum influx, decreases K_m, and causes an accumulation of A. On the other hand, C on the trans side, while barely acting on $(J_a)_{max}$ and on K_m, augments the accumulation of A. The terms containing γ'' are therefore missing in $(J^o a)_{max}$ and K_m. The overall transport rate is strongly limited by the mobility of the unloaded carrier, which is mostly in the less mobile

state (X_2). Accordingly, the accelerating effect of B on the cis side is small, perhaps smaller than is compatible with experimental observations. To overcome this shortcoming, one would have to assume that C, while being attached to X_2 would impart to this modification a higher mobility. Such an effect, however, would not fit in with the quasi-allosteric mechanism prevailing in this model. To overcome this difficulty, one may consider a three component system which will be shortly described in the following paragraph.

Model IIIc: the three-component system

This system consists of three conformational modifications (X_1, X_2, X_3) in equilibrium with each other. We assume that X_1 and X_3 are mobile, whereas X_2 is not. We further assume that X_1 and X_2 have, for simplicity reason, an equal affinity for A, whereas only X_1 has an affinity for B, and only X_3 has an affinity for C. This model, although derived from the general model, is very complicated so that little is gained from presenting explicitly the standard parameters. B (cis) has a stimulating effect on the maximum influx of A as well as an decreasing effect of K_m. It also causes accumulation. C markedly augments these effects.

DISCUSSION

Many attempts have been made to formally relate the activating effects of the electrolyte ions on transport processes in terms of the "gradient hypotheses", most thoroughly and extensively by EDDY *et al.*[8], by EDDY[9,10] and by SCHULTZ AND CURRAN[2] in their recent review. A clear separation and juxta-position of the two possible models of activation, namely through affinity and through velocity, has usually not been made. Sometimes a velocity effect has been tacitly introduced by the assumption that only the ternary complex (XAB) passes the barrier. This assumption is not tenable without a second assumption, namely that also the empty carrier, X, or the carrier loaded with the counter-effector (XC) passes the barrier rapidly. A model of this kind, offered by INUI *et al.*[11], did not consider the problem of carrier redistribution, and hence implicitly assumed that the total carrier concentration remains constant and equal on each side, an assumption clearly conflicting with the assumption that the velocities of the different carrier complexes are highly different from each other. The activation of given flux by others, as in co- and counter-transport, is generally considered to involve a ternary complex between the carrier and the mutually interacting passengers. It will be clear from the foregoing derivations that the formation of such a complex, in order to lead to mutual activation of the passenger fluxes involved, must be accompanied by distinct modifying effects of the ligands on the properties of the complex. Two effects appear to be relevant in this respect, namely that on the affinity of the carrier towards its ligands and that on the velocity of the carrier or its complexes. Each of these two may by itself under suitable conditions give rise to co-transport or counter-transport.

In order to study the specific modifying effects mentioned above and the conditions under which these may lead to coupling by co- and counter-transport, we have started with a general model, in which the two effects are present at the same time. The model furthermore provides for the existence of different conformational states of the carrier, which are in equilibrium with each other. This provision permits the

possibility that affinity and/or velocity effects may be produced indirectly, *i.e.* simply by shifting the equilibrium distribution between these states towards a more mobile or more strongly binding state, a mechanism which we have called "quasi-allosteric". Finally, the usual assumption of symmetry has been omitted, so that the various parameters important for transport and activation may be different on each side of the membrane. Such asymmetry will for obvious thermodynamic reasons not lead *per se* to accumulation of the solutes concerned, but may nevertheless cause rectification or valve-effects, so that the equilibrium distribution may be reached faster from the one side than from the other one. Such asymmetry effects may explain various peculiar phenomena in facilitated diffusion[7], but are not of particular importance in the present context.

In all other respects the general model corresponds to the conventional ones applied for similar purposes. In order to characterize the various possible activating modifications we have chosen the three standard parameters: (1) the maximal unidirectional flux, (2) the apparent Michaelis constant, and (3) the maximal flux ratio at equal and minimal concentration of transportee on both sides of the region. The behaviour of these parameters in response to the existence or nonexistence of substantial effector gradients are given in equations. The symbols in these equations are too involved to allow an immediate and intuitive evaluation of the various activating effects. But simplified models, in which only one or two of the modifying effects are present at a time, with correspondingly "reduced" parameters have been derived. This derivation has been carried out for a set of such reduced models, the so-called "pure" types, *i.e.* with velocity effects only, or affinity effects only, and a few mixed types in which a special velocity effect is combined with a special affinity effect.

It is seen that the affinity type in its direct form (one-component system) is essentially the model suggested by Crane for the transport of glucose across the small intestine of hamster[5]. The pure affinity type, *i.e.* that without any effect of B or C on the mobility, predicts that the effectors only alter the apparent Michaelis constant, K_m, leaving the maximal rate unchanged. This prediction would be true even if the assumptions of quasi-equilibrium between carrier and its ligands in the boundary faces of the transport region were abandoned. This kind of model would obviously fit only a few of the described cases, such as the already mentioned transport of glucose across the epithelial layer of the small intestine of the hamster[5] and of the transport of alanine across the mucosal border of the epithelial cells of the rabbit ileum[12-16].

The quasi-allosteric affinity type (two-component system) behaves very much like that above, especially in that only the apparent Michaelis constant is affected. The activation is the greater the higher the value of L, *i.e.* the higher the concentration of the noncombining modification (X_2). These observations would be fundamentally the same if the assumption of equilibrium between the two modifications were abandoned. It is interesting that with an asymmetric membrane, for which L' is not equal to L'', valve-like effects can be expected in that the equilibrium distribution of A is reached faster in the one than in the other direction. In the velocity type model (IIa, one-component) the flux rate is activated in any case, in which the attachment of B to the carrier increases its velocity. Accumulation of A, however, occurs only under very restricted conditions, namely if the accelerating effect of B on the complex A is greater than that on the unloaded carrier, X. Moreover the accumulating effect is

even strengthened, if the attachment of B to the unloaded carrier X reduces its rate. In other words the final accumulation is the greater the more the ratio $(p_{ab} \cdot p_o)/(p_a \cdot p_b)$ exceeds unity. The mobility of the free carrier must be greater than that of the binary carrier–substrate complexes (XA and XB), if the maximal initial rate is to be accelerated more than 2-fold. The gradient of C inside out, on the other hand, will increase the maximum influx of A only if $p_c > p_o$, and the accumulation only if $(p_a \cdot p_c)/(p_o \cdot p_{ac}) > 1$. It is interesting that under such conditions both the maximal unidirectional flux and the apparent Michaelis constant (K_m) are altered by the effector. The change of the latter value is, however, small and may hardly be noticeable. The model would account for observations made for a few systems, such as the transport of glucose in the epithelial cell of the rabbit ileum[12].

Model IIb, the quasi-allosteric (two-component) variant of the velocity-type models, permits the acceleration of the maximal unidirectional flux (v_{max}) by the effector B, whereas the Michaelis constant remains unchanged. No accumulation of A, however, is possible with this model, no matter how asymmetric the distributions of B and/or C may be. Obviously the coupling between the flux of A and that of B and C is merely catalytic, and does not allow a transfer of energy, Hence it does not fit any systems in which accumulation of the transportee actually occurs.

In most biological transport systems so far described, the effectors Na^+ and K^+ act by changing simultaneously the maximal flux and the apparent K_m. Since none of the previously mentioned "pure" types of the reduced models seems to apply to those cases, mixed types of models are therefore generally preferred, *i.e.* models in which effectors alter both affinity and velocity simultaneously.

Of special interest may be a three-component system, which has been devised in order to overcome shortcomings of the quasi-allostgeric variant of the pure velocity type model. A third conformational state (X_3) is invoked which combines with C only, not with A and B. If X_3 is mobile enough, an accumulative effect on A is produced by the combined action of B and C, apart from stimulation of the maximal initial influx of A, and a decrease of the apparent Michaelis constant. The model would predict a trans-stimulatory effect of cellular C on the unidirectional influx of A. Such, however, has not been demonstrated for the unidirectional influx of glycine into Ehrlich ascites cells, this influx being almost entirely independent of cellular K^+ within wide concentration ranges[17]. Whether other transport systems are in better agreement with this model remains to be seen.

The above derived models show that an activation of transport by an effector gradient can be explained by an affinity effect as well as by a velocity effect. They also show that the affinity-type models are more plausible and less strained by *ad hoc* assumptions than are the pure velocity type models. Since, however, in most experimentally investigated cases both Michaelis parameters are altered during the activation, it seems unlikely that the above models in their "pure" form occur in most systems. The quasi-allosteric variants of the different model types often explain the findings as well as do the direct variants. Special experiments will have to be designed to distinguish between the two possibilities.

APPENDIX

In the following, kinetic equations are derived for the effect on the flux of a substrate (A) exerted by an effector (B) under the condition that the association and

dissociation reactions between the ligands (A, B) and the carrier, rather than the mobility of the carrier species', determines the transport rate. In order to keep the derivation simple we chose as an example a symmetric one-component system, *i.e.* the carrier (X occurs in only one conformational state, and the mobility constants are the same in either direction.

Obviously, a distinction between an affinity effect and a velocity effect of the effector is meaningless if the mobility of the carrier species' is no longer rate limiting. It also follows that under the present conditions the distribution of each carrier species can be considered as in quasi-equilibrium within the transport region, so that

$$x' = x'' = x; \quad ax' = ax'' = ax; \quad bx' = bx'' = bx; \quad abx' = abx'' = abx \tag{1}$$

Assuming, as in the main treatment, that the amount of total carrier within the transport region is conserved, we get

$$x' + x'' + ax' + ax'' + bx' + bx'' + abx' + abx'' = X_T \tag{2}$$

The steady-state conditions for the carrier species' are:

$$\begin{aligned}
\frac{a'\cdot x' + a''\cdot x''}{ax' + ax''} &= \frac{k_{-oa}}{k_{+oa}} = K_a \\
\frac{b'\cdot x' + b''\cdot x''}{bx' + bx''} &= \frac{k_{-ob}}{k_{+ob}} = K_b \\
\frac{b'\cdot ax' + b''\cdot ax''}{abx' + abx''} &= \frac{k_{-ab}}{k_{+ab}} = \frac{K_b}{f_b} \\
\frac{a'\cdot bx' + a''\cdot bx''}{abx' + abx''} &= \frac{k_{-ba}}{k_{+ba}} = \frac{K_a}{f_b}
\end{aligned} \tag{3}$$

The flux of A is:

$$\begin{aligned}
J_a &= k_{+oa}\cdot a'\cdot x' - k_{-oa}\cdot ax' + k_{+ba}\cdot a'\cdot bx' - k_{-ba}\cdot abx' \\
&= -k_{+oa}\cdot a''\cdot x'' + k_{-oa}\cdot ax'' + k_{+ba}\cdot a''\cdot bx'' + k_{-ba}\cdot abx''
\end{aligned} \tag{4}$$

or

$$\begin{aligned}
2J_a = {} & k_{+ao}\cdot(a'\cdot x' - a''\cdot x'') - k_{-oa}\cdot(ax' - ax'') \\
& + k_{+ba}\cdot(a'\cdot bx' - a''\cdot bx'') - k_{-ba}\cdot(abx' - abx'')
\end{aligned} \tag{5}$$

Combining Eqns. 1, 2, 3, and 5, we obtain

$$J_a = \frac{X_T}{4}\cdot\frac{(\alpha' - \alpha'')\cdot\left(k_{-oa} + k_{-ba}\cdot f_b\cdot\dfrac{\beta' + \beta''}{2}\right)}{1 + \dfrac{\alpha' + \alpha''}{2} + \dfrac{\beta' + \beta''}{2} + f_b\cdot\dfrac{\alpha' + \alpha''}{2}\cdot\dfrac{\beta' + \beta''}{2}} \tag{6}$$

From (6) we can now extract the standard parameters:

$$(J_a^o)_{max} = \frac{X_T}{2} \cdot \frac{k_{-oa} + k_{-ba} \cdot f_b \cdot \frac{(\beta' + \beta'')}{2}}{1 + f_b \cdot \frac{(\beta' + \beta'')}{2}} \tag{7}$$

$$K_m^o = 2K_a \cdot \frac{1 + \frac{(\beta' + \beta'')}{2}}{1 + f_b \cdot \frac{(\beta' + \beta''_{''})}{2}} \tag{8}$$

$$f_{max} = 1 \tag{9}$$

From (9) it is obvious that no accumulation is possible under the present conditions. In other words, if the mobility of the carrier species' within the transport region is not rate limiting, the model does not permit an energetic coupling between the fluxes of A and B. These fluxes can only be coupled "catalytically", in that J_{max} and K_m of the flux of A are affected by the co-effector B, as follows from Eqns. 7 and 8. These results, though derived for the most simple one-component system, are fundamentally similar for the general system.

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