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THE OVERSHOOT PHENOMENON IN COTRANSPORT

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Based on simplified equations, the overshoot curve experimentally observed with Na^+-linked cotransport of neutral substrate (sugars or amino acids) has been simulated by computer. The approach is in principle similar to that of previous approaches (Weiss, S.D., McNamara, P.D. and Segal, S. (1981) J. Theor. Biol. 91, 597–608), but more general; in particular, it includes the effect of electrical membrane potential difference, and the quantitative relationship between height of peak and certain transport parameters, such as maximum rate, dissociation constant of ternary complex, electric charge of translocator, respectively. In addition, it tests two alternative models with respect to the rate-determining step: the translocation, on the one hand, and the association/dissociation of the ligands at the translocator site, on the other. The major findings are the following: (1) An overshoot can be obtained similar to that usually found experimentally, provided that maximum rate and affinity between translocator and transport of solute exceed certain minimum values. (2) The overshoot effect with Na-linked cotransport is enhanced by a negative membrane potential (inside relative to outside) and decreased by a positive potential. In the first case, the peak is higher and occurs faster. In the latter case, the peak is lower and delayed. (3) The effect of an electric potential difference on the overshoot curve does not depend appreciably on the charge of the empty translocator, except if the translocation of the latter is strongly rate-limiting. (4) To obtain an overshoot curve, it is not necessary that the translocation step be rate-limiting, contrary to what has been postulated previously (Läuger, P. (1980) J. Membrane Biol. 57, 163–178).

The verification of secondary active transport, especially its distinction from primary active transport in whole cells and tissues, is hampered by the interferences of metabolic activity, of intracellular compartmentalization and of sequestration in cellular organelles. To avoid these interferences, vesicular preparations of membraneous material have been used, such as are formed directly from cellular envelopes, or are reconstituted by the incorporation of transport-active proteins into liposomes.

The absence of metabolism, though an advantage in one respect, is a disadvantage in another; namely, without ion pumps, cotransport cannot be studied under convenient steady-state conditions. The required electrochemical potential gradient of the driving ion will soon dissipate towards equilibrium. Hence, cotransport can manifest itself only by transient phenomena, such as an increase in the initial rate of the uptake of the test solute and, in particular, the 'overshoot' phenomenon, i.e., a transient accumulation of the test solute above its finite equilibrium distribution, which is amply described in the literature [2]. Neither of these by itself proves cotransport. The initial rate may also rise, for instance, if the added salt for some reason increases the passive permea-

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A glossary of terms used appears after the Appendix.

bility of the cell membrane to the test solute. The overshoot phenomenon, on the other hand, may be mimicked by other side effects, such as transient swelling of the vesicles, or repolarization of the membrane [3]. In the following treatment, it is assumed that such effects have been excluded and that the overshoot is real, i.e., represents the transient rise of the (electrochemical) activity of test solute inside the vesicle above its final equilibrium value.

The shape of the overshoot curve has mostly been interpreted intuitively, and little is known about relationships betwen characteristic details of this curve and the basic parameters of the assumed mechanism of cotransport, such as maximum rate, affinity between ligands and translocator sites, relative mobility of loaded and unloaded translocator, etc. A previous investigation in this direction has been made by Weiss et al. [4] based on an 'affinity effect', assuming that by the binding of Na^+ to the translocator site, the affinity for the substrate is greatly enhanced. The present attempt is basically similar, but has been extended to include the effect of electrical potential on rheogenic cotransport, and modulation of this effect by the electric charge of the (unloaded) translocator itself. This is important in view of many experimental approaches to study such electrical effects in order to determine the degree and direction of rheogenicity of the cotransport.

The electrical potential, whenever it enters such experiments, is also transient, whether it is produced by anion replacement of the sodium salt or by K^+ diffusion potential in the presence of valinomycin. For the present purposes, we consider only the first alternative, in which the decay of the electrical potential is directly linked to the decay of the chemical potential, depending on the difference of mobility between cation and anion. The implementation of a K^+ diffusion potential on the other hand, would require information on the kinetics of the K^+ exit, and hence make the approach more complicated without adding new insight into the principle.

As a model for our calculations, we selected the simplest form of a cotransport system as has been described and analyzed before [5]. Though it is most easily interpreted in terms of a mobile carrier, it does not depend on such an assumption and would equally apply to a gated channel, for instance of the Patlak-Läuger type [6,7].

The model consists of two compartments (a small one, representing the intravesicular space and a large one, representing the suspending medium) and a separating membrane that mediates cotransport between a (driven) neutral solute (A) and a (driver) ion (B). The following simplifying assumptions are made: (1) The test solute (A) enters the vesicle only by cotransport, i.e., in the form of a 'ternary complex' with a 'mediator' (C) and the driver ion (B). (2) The driver ion (B) is present as a monovalent binary salt whose concentration greatly exceeds that of test solute. It enters the vesicle mainly by (electroneutral) nonmediated diffusion, with negligible contribution of cotransport. As a consequence, the concentration of B inside and outside can be taken throughout to stand for the corresponding concentrations of the free salt. (3) The intravesicular volume is so small as compared to the extravesicular one, that the outside concentrations of test solute and salt can be treated as constant. (4) Changes in intravesicular volume during the experiment are negligible.

Of these four assumptions, No. 4 is the least secure. Whereas the vesicular volume may be found approximately the same before and after the uptake experiment, transient changes of this volume cannot be excluded. At the present time, little useful evidence is available to this point, probably due to technical difficulties. The rapid initial entry of the sodium salt is likely to cause temporary swelling of the vesicles. Such volume changes are unlikely to fundamentally alter the uptake curves, except that some delay rather than depression of the overshoot peak is to be expected on the basis of Fig. 3.

As to the rate-limiting step of the overall process, we consider only the two extreme cases: limitation by the translocation across the barrier (case I), and limitation by the association and dissociation steps in the interface (case II) (see Appendix). Since, under analogous conditions, these two cases do not give fundamentally different results, it is expected that this will also hold for more realistic systems in which neither the association or dissociation nor the translocation is exclusively rate-limiting.

The overshoot phenomenon in case II depends on the stringent condition that equilibrium is approached only by the completely loaded (ABC) and the unloaded form (C) of the carrier. The assumption that the incompletely loaded translocators (AC and BC) are also near equilibrium, is incompatible with effective cotransport and an overshoot curve cannot be obtained [8].

As mentioned before, the concentration of the sodium salt greatly exceeds that of the substrate (100 mM as compared to 0.1 mM), so that the entry of Na^+ as part of the ternary complex in cotransport can be neglected as compared to the unmediated entry as free Na^+, which, like that of any binary salt, can be treated like that of a single neutral solute, i.e., as a first-order reaction. Assuming that at time zero the sodium salt is at the outside only ($y = 0$), the entry will follow the equation:

$$y = 1 - e^{-\alpha t} \tag{1}$$

y is the ratio of inside over outside concentration of B (b''/b'), and α is a function of the individual permeabilities of the ions according to the equation:

$$\alpha = \frac{2P_+ \cdot P_-}{P_+ + P_-} \cdot \frac{1}{W_c} = \frac{2}{(r+1)} \frac{P_+}{W_c} \tag{2}$$

P_+ and P_- are the individual permeabilities of cation and anion, respectively. As the P values usually refer to protein weight, they must be divided by W_c, the volume to protein ratio, in order to refer to concentrations.

The model equations, which follow from these assumptions are given in the Appendix. When the carrier translocation is rate-limiting, Eqns. A-6a,b describe the vesicle uptake, and when binding is rate-limiting, Eqns. A-11a,b apply. These differential equations have been formulated as a centered finite difference scheme and solved implicitly at successive time steps. Numerical accuracy was verified by refining the integration mesh. Some of the results have been presented in preliminary form [1].

Results and Discussion

In order to simulate a typical experimental curve, we may select from the great number of experimental overshoot curves described in the literature the one presented by Murer and Hopfer (Ref. 9, Fig. 5) on D-glucose uptake by isolated intestinal brush-border microvilli. For this purpose, some of the parameters listed in the glossary have been estimated as follows (Table I):

TABLE I

MODEL PARAMETERS

P_+	2ml/g per min	b'	100.0 mM
$P_0 c_T$	10 μmol/g per min	r	1
K_{ab}	20 mM^2	ρ	1
W_c	3 ml/g	k_{-ab}	10 μmol/g per min
a'	0.1 mM		

The rate coefficient of the entry of sodium salt has been derived on the presumption that the activity ratio of the substrate between inside and outside ($x = a''/a'$) at the peak is equal to the inverse of the corresponding activity ratio of the driver cation ($1/y = b'/b''$) [3]. In the above-mentioned curve, the peak of the ratio x was reached after about 1 min at a value of about 2, i.e., the intracellular substrate level was about twice the final equilibrium value. Hence, y is 1/2 after 1

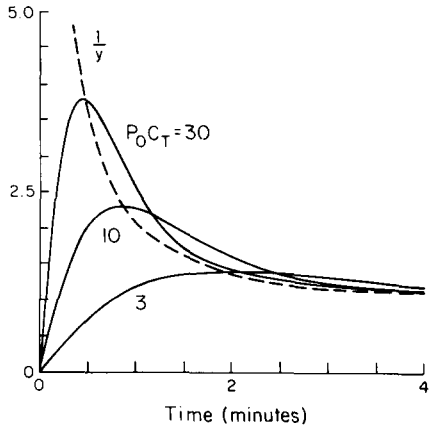

Fig. 1. Uptake curve at varying rate coefficient of translocation. Ordinate: x, the distribution ratio inside/outside of the driven solute (a''/a') (————), and of $1/y$, the inverse of the corresponding ratio of the driver ion (b'/b'') (- - - - -), respectively. Translocation is rate-limiting (case I, Eqn. A-6a of Appendix). Parameters as in Table I, except that P_0C_T varies between 3 and 30 μmol/g per min. No electrical potential difference ($r = 1$). It is seen that no overshoot of x occurs if P_0C_T is 3 μmol/g per min. The curve of $1/y$ intercepts all curves of x at their peak, i.e., the maximum accumulation is attained when $x = 1/y$.

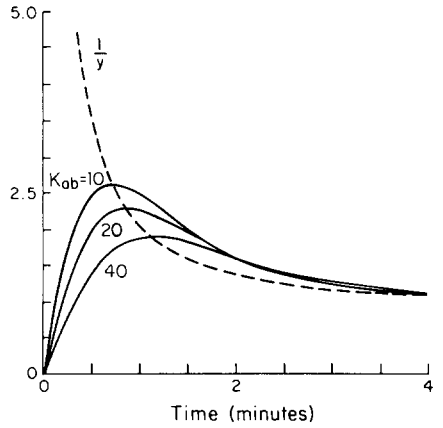

Fig. 2. Uptake curve of substrate at varying dissociation constant (K_{ab}). Same as Fig. 1, except that P_0C_T is constant, but K_{ab}, the overall dissociation constant of the ternary complex is varied between 10 and 40 mM^2. As expected, the overshoot peak of x is higher the smaller K_{ab}. Again, the curve of $1/y$ passes through the peak of each accumulation curve.

min from Eqn. 1 and P_+ can be derived to be about 2.0 ml/g per min. The parameter P_0C_T has been estimated on the basis of an average maximum initial rate of various cotransport systems to be about 10 μmol/g per min, unless otherwise stated. The overall dissociation constant of the ternary complex with respect to both solutes (K_{ab}) has been derived from available saturation curves with respect to various substrates, and after adjusting it to the product of both solute activities, is taken to be about 20 mM^2, unless stated otherwise. This takes into account that the sodium concentration is initially about 100 mM as compared to 0.1 mM of the substrate.

The differential equations underlying the curves described in the following are developed in the Appendix, following in essence the procedure outlined previously [5].

As seen in Fig. 1, these parameters yield overshoot curves which with respect to height and timing of the peak closely resemble the experimental curve. It should be observed from the development of the model equations (Appendix) that in the absence of an electrical potential, i.e., if the mobility of Na^+ equals that of the associated anion ($r = 1$), the electrical charge of the empty carrier has no influence. Fig. 1 shows that P_0c_T has to be greater than 3 μmol/g per min for overshoot to occur. The peaks of overshoot, whenever they occur, lie always on the decay curve of the Na^+ activity ratio $(1/y) = (b'/b'')$.

In Fig. 2, the dissociation constant of the ternary complex, K_{ab}, is varied: the smaller the K_{ab} the higher and earlier is the peak, whereas at $K_{ab} = 40$ hardly any overshoot occurs. In Fig. 3, the volume wt. ratio of the vesicles (W_c) is varied. The height of the peak is not affected but its occurrence is delayed by increasing W_c. In Fig. 4, the ratio of the rate coefficient of fully loaded over empty

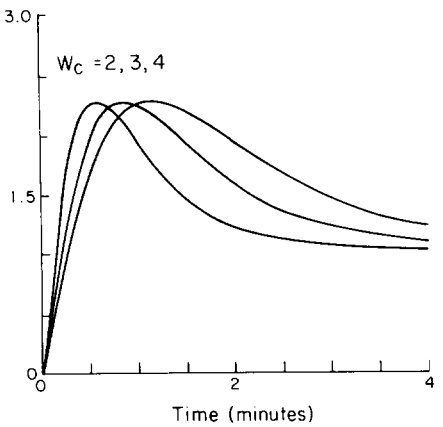

Fig. 3. Uptake curve at varying volume-protein ratio (W_c) of vesicles. Same as Figs. 1 and 2, except that P_{ab} and K_{ab} are constant whereas the W_c varies between 2 and 4 ml/g. The height of the peak remains the same but the time of its occurrence is delayed by increasing W_c.

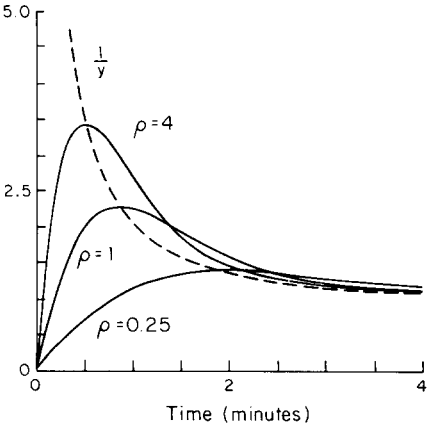

Fig. 4. Uptake curve at different rate coefficients for loaded and empty translocator. Same as Figs. 1 and 2, except that the ratio of the rate coefficients of loaded and unloaded translocator, P_{ab}/P_0 (ρ), is varied between 0.25 and 4. It is seen that no overshoot occurs if ρ is as small as 0.25, i.e., if loaded carrier moves more slowly than the empty one.

translocator (ρ) at constant P_0 is varied. As expected, the peak becomes higher and appears earlier with increasing ρ.

In the following, an electrical potential difference is introduced (Figs. 5–7) by 'anion replacement,' i.e., by choosing sodium salts with anions whose permeabilities are higher ($r < 1$) or lower ($r > 1$) than that of Na^+. Assuming that the electrical potential is at any time determined by the distribution ratio, y, of the salt between intra- and extravesicular space, the electrical potential will be greatest at the beginning and will decay with the salt gradient towards zero at the final equilibrium. There are two ways of expressing the electrical potential difference of its e-function at a given distribution ratio (y) of the salt: by the Henderson equation as applied to a single binary salt [10].

$$\Delta\psi = -\frac{r-1}{r+1} \cdot \frac{RT}{F} \ln y$$

$$\text{or } \xi = y^{-\frac{r-1}{r+1}} \tag{3a}$$

and by the Goldman-Hodgkin equation [11]:

$$\Delta\psi = -\frac{RT}{F} \ln \frac{yr+1}{y+r}$$

$$\text{or } \xi = \frac{yr+1}{y+r} \tag{3b}$$

R, T, and F stand for gas constant, absolute temperature and Faraday constant, respectively, $\Delta\psi$ is the electrical potential difference, ξ is the electrochemical activity coefficient ($e^{-F\Delta\psi/RT}$).

If y is higher than 0.3, the two equations give similar values of $\Delta\psi$, differing by less than 5%. With decreasing y, the disagreement becomes larger. At very low y, there are considerable discrepancies between potential difference values and hence between the initial rates of uptake in the presence of an electric potential difference, depending on which of the two equations is being used (Fig. 5). For a negative potential difference ($r = 0.25$), the Henderson equation predicts higher values than does the Goldman-Hodgkin equation (upper part), whereas at a postive potential difference ($r = 4$), the reverse is true (lower part). In the latter case, the initial rate is even 0, according to the Henderson equation, but finite according to the Goldman-Hodgkin equation. It is customary in such cases to consider the Goldman-Hodgkin equation to be more trustworthy than the Henderson equation.

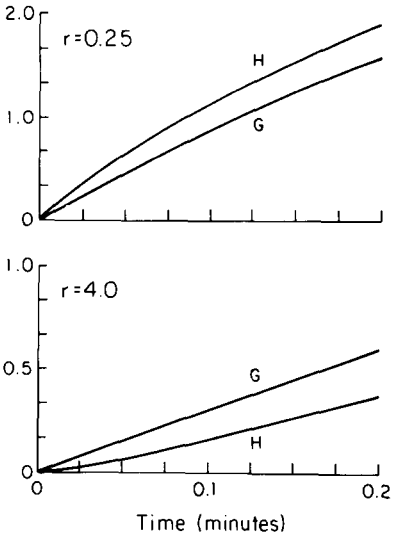

Fig. 5. Initial rate of uptake. Plotting as in Figs. 1 through 4, but for shorter time-spain and based on Eqn. A-7. Parameters as in Table I, except that electrical potential difference is introduced by 'anion replacement', i.e., by changing r to 0.25 (upper part) or 4.0 (lower part), respectively. Eqns. (A-7a, b). The electrical potential difference is derived by both the Henderson equation (H) and the Goldman-Hodgkin equation (G). At (inside) negative potential difference ($r = 0.25$), the former equation gives higher values than the later one, whereas at positive potential difference ($r = 4.0$), the reverse is true. In the latter case, the initial rate is even zero with the Henderson equation, but finite with the Goldman-Hodgkin equation.

In the presence of electrical potentials, the time-course of substrate uptake may depend somewhat on the electric charge of the empty translocator site. At present only two possibilities are considered, the empty site being either neutral ($z_c = 0$) or negative ($z_c = -1$). In Na^+-linked cotransport of a neutral substrate, the loaded site would become positive in the first, but neutral in the second case. In either case, a negative potential difference enhances and a positive potential difference depresses overshoot (Fig. 6). With equal mobilities of both species ($\zeta = 1$), the difference is very small (Fig. 7, lower curve). The small difference seen is due to the fact that the concentrations of the solutions are above the half-saturation value (K_m),

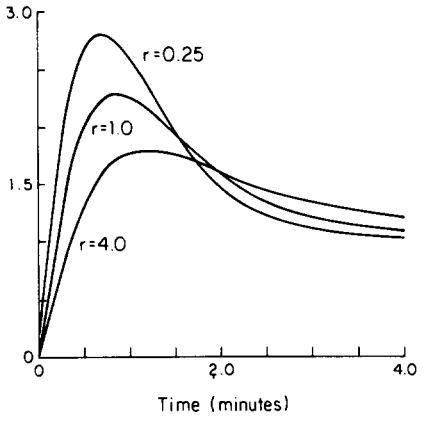

Fig. 6. Electrical potential difference on overshoot curve. Plotting as in Fig. 1, parameters as in Table I, except that an electrical potential difference is introduced by 'anion replacement' ($r \neq 1$). The electrical potential difference is derived by the Goldman-Hodgkin equation (Eqn. 3b), but using the Henderson equation does not change the peak of the overshoot curve appreciably. The empty carrier is treated as neutral ($z_c = 0$).

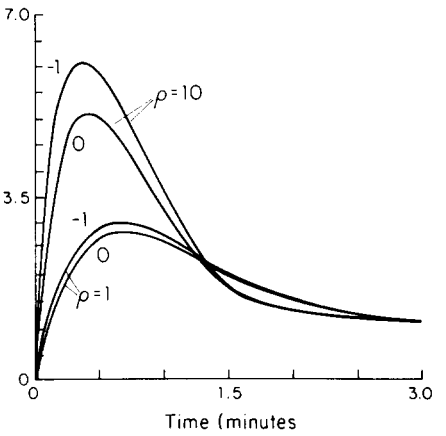

Fig. 7. Effect of charge of empty translocator on overshoot. Plotting and parameters as in Fig. 6, for (inside) negative potential difference only ($r = 0.25$), but for both uncharged ($z_c = 0$) and charged ($z_c = -1$), each at $\rho = 1$ and $\rho = 10$. The potential difference is derived by the Henderson equation (Eqn. 3a), but using the Goldman-Hodgkin equation (Eqn. 3b) instead has little influence on the results. It is seen that at $\rho = 1$, the difference between charged and uncharged carriers with respect to the height of the peak is very small, but markedly enhanced if loaded and unloaded carriers have greatly different mobilities ($\rho \gg 1$). This is because the empty translocator is rate-limiting and, therefore, if charged, more strongly affected by potential difference.

the number of unloaded sites exceeds that of loaded ones. Hence, the movement of the latter limits the rate and, if it carries a (positive) charge, is thus more sensitive to the electric potential difference. Increasing the concentrations of the ligands reverses this effect (not shown). At a very high value of ρ, the overall rate is determined by the unloaded site, so the effect of electric potential difference is appreciably stronger if this site carries the (negative) charge (Fig. 7, upper curve). Increasing the ligand concentrations above K_m would increase this effect still further (not shown).

Fig. 8 refers to the model of case II in which the interaction between the translocator and dissolved ligands determines the rate. The translocation is assumed to be so fast that for each translocator species, the loaded and unloaded one, there is quasi-equilibrium distribution between the two interfaces of the barrier. It is seen that under the assumptions made that if the incompletely loaded translocators species (AC and BC) either do not form to an appreciable extent or cannot penetrate the barrier, overshoot does occur as in the model of case I and is also dependent on the electrical potential. It should be emphasized that if the incompletely loaded species are also allowed to equilibrate rapidly, cotransport becomes ineffective, and as a consequence no overshoot can be expected [8].

Raising the stoichiometry of Na^+/solute from one to two does not significantly change the shape of the overshoot curve, except that the peaks are considerably higher and that effects of electrical potential are stronger (Fig. 9). Furthermore, the peak is no longer on the decay curve of the inverse ratio of the electrochemical activities of the driver ion but on that of the square of this ratio.

In summary, the curves obtained by the computer simulations resemble those obtained in biological experiments. Provided that the underlying assumptions are valid, the overshoot curves obtained experimentally can thus be used as strong evidence in favor of cotransport. The effects predictable from the superimposed electrical potential, for instance by 'anion replacement', i.e., by choosing anions of lower or higher permeability in the sodium salt, give qualitative information about the presence and orientation of rheogenicity of the cotransport.

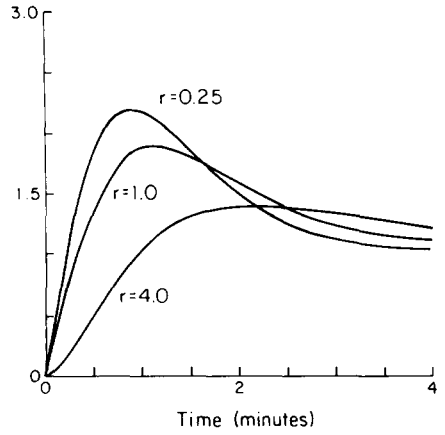

Fig. 8. Uptake curves if binding and unbinding of ligands to carrier rather than translocation is rate-limiting (case II). Plotting as in Fig. 1. For reasons of simplicity it is assumed that the distribution between the two states of the translocator sites, outward-oriented or inward-oriented, respectively, are at quasi-equilibrium, whereas association and dissociation between solutes and translocation sites are not (Eqn. A-11a). Electrical potential difference is introduced as in Fig. 6, and derived through the Goldman-Hodgkin equation (Eqn. 3b), assuming that $z_c = 0$. It is seen that also under these conditions overshoot curves are obtained similar to those under the condition of rate-limiting translocation, provided, however, that the incompletely loaded translocator species (binary complex) do not penetrate the barrier.

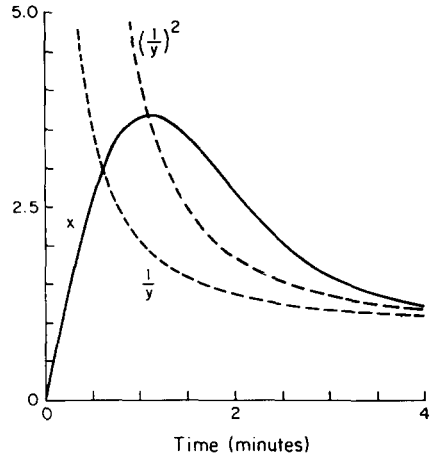

Fig. 9. Uptake curve at stoichiometry 2:1 with respect to sodium solute: Plotting as in Fig. 1. The flux equation is essentially that of case I for the uncharged carrier (A-6a), except for the replacement of b' and b'' by b'^2 and b''^2, respectively, and of $\xi^{1/2}$ by ξ. Parameters as in Table I, except that K_{ab} has been adjusted by the factor 100. The curve $1/y^2$ refers to the square of the inverse distribution square of the driver ion. It is seen that the curve $1/y^2$ rather than that of $1/y$ intercepts with the x-curve at the overshoot peak.

There remain still several additional questions that one would like to answer for any cotransport under investigation, such as:

(1) Is the cotransport of the velocity type or the affinity type?

(2) What is the stoichiometry between Na^+ and these translocated substrates?

(3) What is the charge of the empty carrier?

(4) How do the velocity coefficients of loaded and unloaded translocator relate to each other (ρ)?

(5) What are the 'standard parameters' of the transport system; i.e., the maximum initial rate and the apparent Michaelis constant?

(6) What is the absolute value of the rate coefficient of the loaded or unloaded carrier, respectively?

There is no easy quantitative way to answer any such question from a single overshoot curve, for example, from the height of the peak, the time of its occurrence and from the initial rate. More information might be available by many overshoot curves of the same system under varying conditions.

Acknowledgement

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Appendix

Case I: Carrier translocation is rate-limiting

The following simplifying assumptions are made:

(1) The association and dissociation reaction between empty carrier (C) and ligand solutes (A, B) are very fast, as compared with the translocation of the carrier species, that the former can be treated as if they were always in equilibrium (quasi-equilibrium):

$$\frac{a' \cdot b' \cdot c'}{abc'} = K_{ab'} \qquad \frac{a'' \cdot b'' \cdot c''}{abc''} = K_{ab''} \tag{A-1}$$

(2) The sum of all carrier species per g vesicle remains the same at all times (c_T). The numbers of binary complexes (ac and bc) are so small that they can be neglected. This implies a strong 'affin-

ity effect', i.e., a strong cooperative effect between the two solutes in forming the ternary complex. If we instead assumed that the binary complexes do form to some extent, we have to invoke also a velocity effect, i.e., by assuming that these do not translocate, the equations would become somewhat more complicated but not different in principle. Hence:

$$c' + c'' + abc' + abc'' = c_T \tag{A-2}$$

(3) Only the fully loaded and the empty carrier species translocate appreciably, and the translocation of all carrier species in the one direction is always about equal to that in the other direction. Hence for $z_c = 0$:

$$P'_{ab} \cdot abc' \xi^{1/2} + P'_0 \cdot c' = P''_{ab} \cdot abc'' \xi^{-1/2} + P''_0 \cdot c'' \tag{A-3a}$$

and for $z_c = -1$:

$$P'_{ab} \cdot abc' + P'_0 \cdot c' \xi^{-1/2} = P''_{ab} \cdot abc'' + P''_0 \cdot c'' \xi^{1/2} \tag{A-3b}$$

It should be added that this equality is strictly true only to the extent that the sum of all carrier species on each side remains the same, as cannot be if $\rho \neq 1$ in the transient state; under such a condition, the distribution of the translocator sites between the interfaces is subject to changes, as a function of the redistribution of solutes between the two phases. We feel justified, however, that these changes in carrier distribution are small enough and occur fast enough to be neglected in Eqns. A-3a and A-3b.

(4) The system is symmetric, i.e.,

$$P'_0 = P''_0 = P_0;\ P_{ab'} = P_{ab''} = P_{ab} = P_0;\ K_{ab'} = K_{ab''} = K_{ab} \tag{A-4}$$

The net movement of A, neglecting any leakage is

$$J_a = \frac{da''}{dt} \cdot W_c = P_{ab}\left(abc' \xi^{1/2} - abc'' \xi^{-1/2}\right), \text{ for } z_c = 0 \tag{A-5a}$$

$$\text{or } J_a = \frac{da''}{dt} \cdot W_c = P_{ab}\left(abc' - abc''\right), \text{ for } z_c = -1 \tag{A-5b}$$

Combining Eqns. A-1–5 we obtain:

at $z_c = 0$ (uncharged carrier) (A-6a)

$$\frac{da''}{dt} = \frac{c_T}{W_c} \cdot \left[\rho P_0 \left(a' \cdot b' \cdot \xi^{1/2} - a'' \cdot b'' \cdot \xi^{-1/2}\right) K_{ab}\right]$$
$$\cdot \left\{2K_{ab}^2 + \left(\rho \xi^{1/2} + 1\right) K_{ab} \cdot a' \cdot b'\right.$$
$$+ \left[\left(\rho \xi^{-1/2} + 1\right) K_{ab}\right.$$
$$\left.\left. + \rho\left(\xi^{1/2} + \xi^{-1/2}\right) a' \cdot b'\right] a'' \cdot b''\right\}^{-1}$$

at $z_c = -1$ (charged carrier)

$$\frac{da''}{dt} = \frac{c_T}{W_c}\left[\rho P_0 \left(a' \cdot b' \cdot \xi^{1/2} - a'' \cdot b'' \cdot \xi^{-1/2}\right) K_{ab}\right]$$
$$\cdot \left\{\left(\xi^{1/2} + \xi^{-1/2}\right) K_{ab}^2 + \left(\rho + \xi^{1/2}\right) K_{ab} a' \cdot b'\right.$$
$$\left. + \left[\left(\rho + \xi^{-1/2}\right) K_{ab} + 2a' \cdot b'\right] a'' \cdot b''\right\}^{-1} \tag{A-6b}$$

Initial rate of cotransport

$$z_c = 0 \quad J_a^0 = \frac{\rho P_0 c_T a' \cdot b' \cdot \xi^{1/2}}{2K_{ab} + \left(\rho \xi^{1/2} + 1\right) a' \cdot b'} \tag{A-7a}$$

$$z_c = -1 \quad J_a^0 = \frac{\rho P_0 c_T a' \cdot b' \cdot \xi^{1/2}}{\left(\xi^{1/2} + \xi^{-1/2}\right) K_{ab} + \left(\rho + \xi^{1/2}\right) a' \cdot b'} \tag{A-7b}$$

Case II: Association and dissociation reactions are rate limiting

The following simplifying assumptions are made:

(1) The translocation of the empty and the fully loaded carier species (*c, abc*) is fast as compared to the association and dissociation reactions, that they can be treated as being in equilibrium between both interfaces:
Hence, for $z_c = 0$ we can set:

$$\frac{abc''}{abc'} = \xi \tag{A-8a}$$

and

$$\frac{c''}{c'} = 1$$

and for $z_c = -1$:

$$\frac{abc''}{abc'} = 1$$

$$\frac{c''}{c'} = \xi^{-1} \tag{A-8b}$$

(2) As in case I, the total of carrier species per g vesicle is constant (c_T) and the existence of binary complexes (AC and BC) is negligible

$$c' + c'' + abc' + abc'' = c_T$$

(3) There is a steady-state with respect to the (rate-limiting) association and dissociation reactions in that what enters the barrier on the one side is equal to that which leaves it on the other side: hence for either $z_c = 0$ or $z_c = -1$ we set

$$k_{ab} \cdot (c' \cdot a' \cdot b' + c'' \cdot a'' \cdot b'') = k_{-ab} \cdot (abc' + abc'') \qquad \text{(A-9)}$$

As in case I, this equality strictly holds only as long as the ratio abc/c within the barrier does not change. Again, though this ratio during the transient state is subject to some change, we feel justified to assume that these changes are small enough and occur slow enough not to appreciably upset the above equality.

(4) As in case I, the system is symmetrical.

The net movement of A (J_a), neglecting outer leakage is

$$J_a = k_{-ab} abc'' - k_{ab} \cdot a'' \cdot b'' \cdot c'' \qquad \text{(A-10)}$$

Combining Eqns. 6–8 we obtain

$z_c = 0$ (uncharged carrier)

$$\frac{da''}{dt} = k_{-ab} \cdot \frac{c_T}{(1+\xi) W_c} \cdot \frac{a' \cdot b' \xi - a'' \cdot b''}{a' \cdot b' + a'' \cdot b'' + 2K_{ab}} \qquad \text{(A-11a)}$$

$z_c = -1$ (charged carrier)

$$\frac{da''}{dt} = k_{-ab} \cdot \frac{c_T}{2W_c} \cdot \frac{a' \cdot b' \xi - a'' \cdot b''}{a' \cdot b' \xi + a'' \cdot b'' + (1+\xi) K_{ab}} \qquad \text{(A-11b)}$$

Glossary

a', a''	outside and inside activity of (neutral) substrate (A), respectively (mM)
b', b''	outside and inside activity of (positive monovalent) driver ion (B), respectively (mM)
P_0	rate coefficient of translocation of empty translocator site (min^{-1})
$P_{ab} = \rho P_0$	rate coefficient of translocation of fully loaded translocator site (min^{-1})
K_{ab}	dissociation constant of ternary complex (abc) $\frac{a' \cdot b' \cdot c'}{abc'} = \frac{a'' \cdot b'' \cdot c''}{abc''}$ (mM^2)
c_T	total amount of translocator sites per g protein (μmol/g)
W_c	vesicular volume per g protein (ml/g)
$\Delta\psi$	electrical potential difference across vesicular membrane (mV)
ξ	= electrochemical activity coefficient $\xi = e^{-\frac{F\Delta\psi}{RT}}$
z_c	charge of translocator site
k_{ab}, k_{-ab}	rate coefficients of formation and dissociation of ternary complex, respectively (ml/g per min)
r	P_+/P_- = ratio of cation over anion permeability of the sodium salt (B)
ρ	P_{ab}/P_0 = ratio of rate coefficient of loaded translocator over that of unloaded carrier.

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