Secondary active transport: Introductory remarks

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For a long time it was a matter of debate how the energy for concentrative or active transport is provided by living systems. According to the type of energetic coupling one can distinguish between primary and secondary active processes. Primary active transport means that by chemiosmotic coupling between an exergonic chemical reaction (such as hydrolysis of ATP) and transport the free energy generated by the chemical reaction is used for concentrative transport (such as Na⁺/K⁺-ATPase or H⁺-ATPase). In secondary transport the energy stored in those concentration gradients is used in osmoosmotic coupling (co- or countertransport) for concentrative transport of other solutes. To discriminate between these two types of energization is not easy as the historical development of the concept of secondary active transport, especially of the Na⁺-solute cotransport systems, illustrates.

History

The evolution of our present knowledge and ideas on cotransport of cations, as Na⁺ or H⁺, with organic or inorganic cotransportates as the mechanism for secondary active transport, can be divided into two main periods. During the first period several crucial experimental and conceptual prerequisites for formulating the hypothesis on cotransport as energy converter (Crane’s Hypothesis) were developed. During the second period Crane’s Hypothesis was critically tested. In addition it was generalized to apply to various biological systems.

The state of the knowledge at the end of the first period was well documented at the transport congress in Prague in 1960 [1]. In hindsight, it seems strange that at the eve of two of the most fruitful hypotheses in membrane bioenergetics (Mitchell’s Chemiosmotic Hypothesis and Crane’s Cotransport Hypothesis) at least some of the participants of that meeting had a feeling of stagnation of membrane research, as was expressed as follows: “My own opinion is that we have come to something like a standstill in our transport studies.” [1, p. 505]

The second period seems to have started in April 1962 with Crane’s hypothesis of cotransport between Na⁺ and glucose as driving process for secondary active accumulation of glucose in the intestine [2], a concept valid up to the present day without major modifications. (An appendix to the paper presented by Crane during the Prague meeting gives a short preliminary formulation of the hypothesis [3].) During the second period the hypothesis was extended and critically tested. In 1963 Mitchell applied it to protons as driving ions [4]. Important aspects of the development were summarized in 1977 by Crane [5]. The following description of the early history of the cotransport concept is mainly based on this review.

The first observation that sodium chloride is important for the reabsorption of sugars in the small intestine was published at the beginning of this century by the Scottish physiologist Reid [6, 7], as mentioned by Goldschmidt in 1921 [8]. In the following years Reid’s observations seem to have been forgotten until the sodium-dependence of glucose resorption was rediscovered in 1958 by Riklis and Quastel [9]. Later similar results were obtained by other authors with various techniques. An important step forward was the demonstration by Crane and his collaborators that the sodium-dependent transport process is located at the luminal side of the epithelium [10]. Various authors showed that during the transport process the sugars were not altered chemically, whereas up to the 1950s most authors explicitly or implicitly had assumed sugar resorption to be a vectorial chemical reaction.

The importance of sodium and potassium for active transport of amino acids was described as early as 1952 by Christensen and Riggs [11]. Christensen and his group continued to study this question during the 1950s, but interpreted their results as countertransport between amino acids and potassium. The authors explicitly dismissed the possibility that amino acid accumulation in Ehrlich cells was due to cotransport with sodium [12]. This idea may be considered to be an extension of the concept of exchange-diffusion or preloading effect as postulated for glucose transport in erythrocytes [13, 14] or amino acids by Ehrlich cells [15].

Christensen’s ideas on the potassium gradient as a driving force were in line with Fleckenstein’s hypothesis that the energy stored in a potassium and/or sodium gradient were used to energize muscle contraction [16]. This concept turned out to be incorrect, but is nonetheless important for the development of the theory of secondary active processes, as it represents the first attempt to invoke an ion gradient as energy source. In the meantime, osmo-mechanic coupling was indeed shown for microorganism, which use the proton gradient to energize flagellar and ciliar motion [17].

One disadvantage of the studies mentioned by Christensen was that in the early experiments of the group, steady state accumulations (1 to 4 hrs incubation) were measured and the amino acid concentrations used were quite high (up to 40 mM), which might have provoked osmoregulatory reactions of the
cells. During the Prague Congress Heinz mentioned some results clearly speaking against countertransport between glycine and potassium in Ehrlich cells [1, p. 501]. The first unequivocal evidence that glycine transport is sodium dependent was shown by Kromphardt et al [18] in 1963 after publication of Crane’s hypothesis.

Further conceptional prerequisites were the development of the concept of primary active transport of sodium and the identification of the Na/K-ATPase as sodium pump. The primary ion pump were identified after Ussing in 1947 had described transepithelial active sodium transport [19]. This work was based on the investigations of Huf in 1935 [20]. Skou [21], Glynn [22] and Post et al [23] isolated the Na/K-ATPase from the cytoplasma membrane and identified it as catalyzing active sodium transport out of cells.

Furthermore, the carrier model for transport was developed and described mathematically as it was first formulated by Widdas in 1952 [14]. In 1957 Patlak showed, in a not much noticed paper [24], that the kinetic predictions by the model of a mobile carrier are also applicable to gated pores. Therefore, in the following the term carrier is meant to include such gate mechanisms.

In 1960 Crane discussed coupling between sugar resorption and sodium transport as one of several possibility for energization of sugar transport [25]. The formulation of secondary active transport driven by cotransport between sodium and glucose theory by Crane [2] strongly stimulated further development of ideas about active transport of organic solutes. Soon afterwards many other accumulative transport processes were found to be sodium dependent. The state of the knowledge at the end of the 1960s about those processes was reviewed by Schultz and Curran [26]. Sodium-dependent sugar transport was found in epithelia of various vertebrate species, while nonepithelial sugar transport was neither accumulative nor sodium dependent. Sodium-dependent amino acid transport was found in epithelial as well as in nonepithelial tissues and in single cells. For some other organic or inorganic solutes sodium-dependency was also shown. In the meantime many other sodium-driven secondary active transport processes were found; the most complex seems to be the loop diuretics-sensitive Na⁺-K⁺-2Cl⁻ cotransport [27, 28], which is involved in volume regulation and transepithelial salt and water transport.

Of eminent importance was that Mitchell in 1963 extended Crane’s ideas, and postulated that in microorganisms the same mechanisms are operating, but that instead of sodium, protons serve as driver ions [29–31]. Today for many procaryotic and eucaryotic microorganisms cotransport processes (mostly named symport, following Mitchell’s nomenclature) are described. The transport kinetic description for proton-dependent symport is analogous to that of sodium-dependent cotransport.

Wilson and Lin formulated the attractive hypothesis that the sodium-cotransport processes during evolution arose from proton-dependent ones [32]; however, since sound information on the structure of cotransporters is still lacking, these ideas must remain speculative, but they give an interesting explanation for the observation that in the absence of sodium a potentially dependent transport of glucose is observed in renal brush border membrane vesicles [33]. It could be, that as some type of phylogenetic reminescence the transporter can also accept protons instead of sodium, if sodium concentration is low. The hypothesis by Wilson that Na⁺-solute cotransport has evolved from H⁺-solute cotransport is supported by the behavior of the H⁺ (Na⁺)-melibiose cotransporter in E. coli [34]. This transport system cotransports the sugar either with protons or with sodium. There are mutants with reduced affinity for protons and increased affinity for sodium so that functionally the system changes from proton-dependence to sodium-dependence. This transport system may illuminate the evolution from proton- to sodium-dependence.

Based on studies of Kedem and Caplan [35] on degree and efficiency of coupling, Heinz [36] developed a quasichemical formulation for the degree of coupling, assuming coupling with fixed stoichiometry between the two cotransportates from uncoupled fluxes, and suggested an experimental protocol to determine the degree of coupling.

For cotransport, detailed kinetic models were developed. The first one was published by Curran et al [37]; the authors assumed an ordered reaction for the binding of the two cotransportates to the carrier, amino acid binding first followed by sodium. In addition they assumed equal permeation probabilities for the various loaded and unloaded carrier species. The model was extended by various authors, especially by Eddy [38], who made no assumption on the order of binding of sodium and cotransportate. In addition he discussed the possibility that potassium was countertransported, but assumed equal rate constants for the transfer of the various loaded and unloaded carrier species. Giving up the restriction of equal rate constants Heinz, Geck and Wilbrandt [39] formulated a more general model, taking into account the possibility of an asymmetric behavior of the transport system. The authors showed that there were two different possibilities to cause energetic coupling: by positive cooperativity for binding of the two cotransportates (affinity effect) or by different velocities of the carrier species (velocity effect). The influence of membrane potential as part of the driving force on the transport kinetic parameters was later implemented quantitatively by Geck and Heinz [40].

The intensive thermodynamic description and kinetic characterization of cotransport was provoked by some experimental results that seemed incompatible with secondary active cotransport with sodium as the mechanism for accumulative uptake of solutes into cells or across epithelia. These critical tests were mainly performed with respect to amino acid transport in Ehrlich cells. Eddy and his collaborators [36] had shown by a series of studies that in energy depleted cells an accumulative transport of amino acids takes place as long as there is an inwardly directed sodium gradient. Steady state accumulation of amino acids is inversely correlated to the concentration ratio for sodium. In addition the authors could show that along with amino acid transport, additional sodium is transported into the cell and potassium out of it. They interpreted these results as amino acids being cotransported with sodium and countertransported against potassium. However, especially in cells which were not energy depleted, the energy available from the sodium and potassium gradients seemed to be insufficient to explain the amino acid accumulation by the co- and countertransport model. Especially Schafer and Jacquez [41] and Schafer and Heinz [42] have shown that in sodium rich, potassium-depleted cells amino acid accumulation was correlated to the gradients
of secondary active transport. This seemed to indicate that in ATP-rich cells amino acid transport is mainly primary active, whereas secondary active transport may serve as an emergency system activated under situations when energy supply is insufficient for primary active transport. These calculations were based on the assumption that membrane potential is predominantly a potassium-diffusion potential, until Pietrzyk, Geck and Heinz [43] showed that especially in sodium-rich, potassium-depleted cells the electrogenic Na⁺/K⁺-pump generates a pump potential which contributes significantly to membrane potential and therefore to the driving force for cotransport. The seeming incongruities between driving force and amino acid flux could thus be removed in term of cotransport. In addition it was shown that membrane potential influences amino acid transport [44], and that in ATP-rich cells coupling between sodium and amino acid transport also takes place. These investigations solved all discrepancies between the hypothesis and experimental results and strongly supported secondary active transport at least for this experimental system. The arguments against the gradient hypothesis vanished almost completely.

By micropuncture studies several authors could characterize different various cotransport systems, especially in epithelia [reviewed in 45]. Detailed investigations of the potential dependence of cotransport processes were also performed for plant cells, which show cotransport of sugar and amino acids with protons. These studies measuring current-voltage dependencies gave interesting information for details of cotransport mechanism. We shall not discuss this interesting field here; some review articles on this topic should be consulted for more information [reviewed in 46, 47].

For epithelial cells such current-voltage dependencies were determined using the patch-clamp technique [48—50]. To study transport processes independently of interfering metabolism membrane vesicles were very helpful. The first experiments go back to Kaback, who succeeded in isolating closed vesicles that formed spontaneously from E. coli membranes. These were to become very useful for transport studies [51].

Later, Hopfer et al [52] succeeded in preparing vesicles from brush border membranes. This technique was further developed by various other groups [53] and is now one of the standard procedures for studying transport processes, especially in epithelia, since it is possible to prepare vesicles from brush border membranes as well as from basolateral membranes, and to distinguish between processes taking place at two sides of an epithelial cell.

In characterization of the protein catalyzing a cotransport reaction the investigations on the proton-lactose-cotransporter (lac-permease) were most successful. For this protein primary and secondary structure and arrangement in the membrane are almost completely known. Furthermore, by nucleotide-specific mutations more detailed information on the transfer mechanism could be obtained [54]. Of the sodium-dependent cotransport system most authors concentrate on the characterization of the sodium-glucose cotransporter. As a functional test for a successful isolation, the protein isolated is incorporated into liposomes and cotransport determined [reviewed in 55, 56].

Although it is accepted by most experts that for most solutes secondary active transport is the mechanism of accumulative uptake, there are still some open questions, especially concerning the mode of energization and the role of membrane potential in kinetics and energetics. These two questions will be dealt with in the following.

**Energetic coupling in secondary active transport**

So far, it seems that most systems of secondary active transport operate through symport, with Na⁺, H⁺, or both. The few systems of antiport with K⁺ described, such as the glucose-transport in various tissues, appear to be tightly associated with Na⁺ symport: their functioning requires, or is strongly promoted, by the presence of Na⁺, though not a Na⁺ gradient. This appears to indicate that in those cases both symport and antiport are operated by the same translocator system. Such a combination of modes may provide interesting insights into the mechanism of secondary active transport in general. In view of the great analogy between symport and antiport these two modes will be treated here together as two opposite and complementary expressions of the same principle of coupling. Secondary active transport requires energetic coupling between the two flows concerned, that of the driver ion and that of the substrate. Obviously, this coupling occurs with considerable efficiency, but its molecular mechanism is still not understood. As mentioned before, two distinct kinds of effects of the driver ion on the translocator have been postulated to be instrumental for such coupling: velocity effects and affinity effects [39], respectively depending on whether the velocity of the translocator or the affinity of the latter for the substrate is modified. As each of these effects by itself could account for energetic coupling, the question arises as to which of them is at work in a particular system.

The function of these effects was discussed in detail by Heinz, Geck and Wilbrandt [39] under the usual assumption that the overall transport rate is limited by the translocation of the translocator sites through the barrier (quasi equilibrium). The relationship between energetic coupling and the magnitudes of the various transport parameters can be expressed for symport and antiport by the following ratio, which is independent of electrical potentials and of asymmetry:

\[ R = \frac{r_{ab} \cdot (p_{ab} \cdot p_0)/(p_a \cdot p_b)}{p_{ab}} \]

where \( r_{ab} \) is the cooperativity coefficient with respect to the binding of the substrate (a) and the corresponding driver ion (b) to the translocator, and \( p_0, p_a, p_b \), and \( p_{ab} \) are the permeation probabilities (velocity constants) for unloaded carrier, carrier-substrate complex, carrier-ion complex, and ternary complex, respectively.

Symport requires that \( R \) be greater than unity, and antipot that \( R \) be smaller than unity. For \( R \) equal to unity, no energy transfer is possible, but the ion (b) may have a catalytic influence on the transfer of the substrate (a).

A velocity effect comes about whenever the ratio of the rate coefficients differs from unity. In the simplest case all \( p \) may be similar in magnitude with the exception of one (critical coefficient), which by its deviation would suffice to raise the ratio...
above unity in symport, or to depress it below unity in antiport. An affinity effect would result from cooperativity between substrate and driver ion for binding to the translocator: To effect coupling, it must be positive in symport ($r_{ab} > 1$) and negative in antiport ($r_{ab} < 1$).

Few attempts have been made so far to estimate the magnitude of any parameter in the above expression, for any transport system. But from scattered data in the literature the following information may be extracted. Experimentally, velocity effects should be revealed by transeffects of the driver ion on the rate of substrate flow under zero trans-conditions. Under those conditions the substrate on the transside is absent or so low that cooperativity effects can be neglected. It was found that in systems driven by symport with $Na^+$, such as the transport of sugars and amino acids across renal or intestinal brush border membranes, the initial substrate flow is strongly inhibited if $Na^+$ is added to the transside (transinhibition), as shown by Aronson and Sacktor [57] for renal brush border vesicles, and subsequently by other authors for numerous systems. We may conclude here a true (negative) velocity effect, in that binding of $Na^+$ retards the substrate-free translocator ($p_b < p_a$). This effect, though not directly promoting substrate translocation, tends to obstruct an internal leak pathway (slipping), and thereby to provide some energetic coupling between substrate flow and $Na^+$ flow.

By contrast, in systems known to be driven also by antiport with $K^+$, such as the transport of glutamate in renal and intestinal brush border [58—60], and of hydroxytryptamine in blood platelets [61], the initial substrate influx was found to be strongly stimulated by $K^+$ added to the transside (transstimulation). With the same reservation mentioned with respect to the transinhibition by $Na^+$, we may also conclude that there is a velocity effect, in that the binding of $K^+$ accelerates the substrate-free translocator ($p_a > p_b$). In contrast to the $Na^+$ effect in symport, the effect of $K^+$ in antiport directly promotes a transport effective step, and thereby provides some energetic coupling. Accordingly, in each symport and antiport energetic coupling can, at least in part, be accounted for by such a velocity effect.

That these velocity effects are not the only effects to account for the coupling in these systems can be revealed by equilibrium exchange, which should not depend on the mobilities of the substrate-free translocator species, and hence not on the above velocity effects.

In the few pertinent experiments reported so far, equilibrium exchange has been found to be stimulated by $Na^+$ (on both sides) in systems with $Na^+$ symport, such as glucose transport and glutamate transport in renal brush border membranes [62], but to be inhibited by $K^+$ (on both sides) in a system known to have $K^+$ antiport, such as that of glutamate in the same tissue [63].

These findings are consistent with affinity effects that is, with positive cooperativity between $Na^+$ and substrate in symport, and with negative cooperativity between $K^+$ and substrate in antiport. They do not, however, prove this, but merely indicate that the pathway of the ternary complex with $Na^+$ in symport is expanded ($r_{ab} > p_a$) and that the pathway of the ternary complex with $K^+$ in antiport is restricted ($r_{ab} < p_a$). In other words, without detailed kinetic analysis we cannot distinguish between the cooperativity effects and the corresponding velocity effects.

Against these additional velocity effects concerning the ternary complexes, several lines of arguments can be:

1. Lack of plausibility. These additional velocity effects imply some improvable assumptions. For instance $Na^+$, while strongly reducing the mobility of the empty translocator, would have to do the exact opposite in the presence of bound substrate, namely accelerate it. Vice versa, $K^+$, while strongly raising the mobility of the empty translocator, also does the opposite in the presence of substrate, namely inhibit it. In other words, we would have to postulate that the same substrate strongly accelerates the translocator in the presence of $Na^+$, but just as strongly retards it in the presence of $K^+$.

2. Kinetic evidence. A more valid distinction between velocity and cooperativity parameters may be based on Michaelis-Menten kinetics of equilibrium exchange. According to the model analyzed by Heinz, Geck and Wilbrandt [39] the following equation holds for equilibrium exchange of the substrate $a$:

$$J(\rho)_{ee} = \max(J_{\rho}^{ee,o}) \times \left( \frac{1 + (p_{ab}/p_a) * r_{ab} * \beta}{1 + \alpha + \beta + r_{ab} * \alpha * \beta} \right) \times \left( \frac{P_{ab} < Pa}{P_{ab} > Pa} \right)$$

with $\alpha = \frac{a}{K_a}$ and $\beta = \frac{b}{K_b}$

Accordingly the maximum equilibrium exchange should be:

$$\max(J_{\rho}^{ee}) = \max(J_{\rho}^{ee,o}) \times \left( \frac{1 + (p_{ab}/p_a) * r_{ab} * \beta}{1 + r_{ab} * \beta} \right)$$

which should increase with increasing $\beta$ if $p_{ab} > p_a$, whereas the Michaelis constant

$$(K_m)_{ee} = \frac{1 + \beta}{1 + r_{ab} * \beta}$$

should decrease with increasing $\beta$ if $r_{ab} > 1$.

For antiport the relationships are quite similar, except that the Michaelis contrast should increase with increasing $\beta$ if $r_{ab} < 1$.

For $Na^+$-linked glucose transport in renal brush border membrane, the maximum rate of equilibrium exchange was found rather independent of $Na^+$, indicating that no pronounced additional velocity effect plays a role ($p_{ab} \approx p_a$). The Michaelis constant of the same exchange rate, however, decreases with increasing $Na^+$ indicating that the cooperativity coefficient ($r_{ab}$) exceeds unity [64].

For glutamate $K^+$ antiport in renal brush border vesicles it was found that $(K_m)_{ee}$ increases with rising $K^+$, indicating negative cooperativity for glutamate and $K^+$ binding.

3. Evidence from binding studies. It has been found for the same glucose transport system that the affinity of the glucose site for phlorizin, known to bind to the same site, is markedly increased in the presence of $Na^+$ [65, 66]. This increase in affinity appears to apply also to glucose, which in the presence of $Na^+$ competes more strongly with phlorizin than it does in the presence of $Na^+$ [67]. The mentioned arguments support a cooperativity effect in this particular system, while making a (second) velocity effect unlikely. Whether the analogous considerations also apply to other systems of secondary transport is not known.

So far, experimental evidence, though not yet complete, seems to point towards a rather simple and unifying mechanism
that involves both a velocity and an affinity effect in each symport and antiport. The velocity effects result from the inhibition of the substrate-free translocator by a symporting ion, such as by Na⁺ in Na⁺-linked symport, and in the acceleration of the substrate-free translocator by an antiporting ion, such as by K⁺ in K⁺-linked antiport. The velocity effect appears to serve a different function in each transport mode: to obstruct a leakage pathway in symport, but to promote a transport effective pathway in antiport. The affinity effects result from cooperativity between substrate and driver ion for binding to the translocator. They also appear to serve a different function in each transport mode: to expand a transport effective pathway in symport, but to constrict a leakage pathway in antiport.

This combination of one velocity effect with one affinity effect in each mode, while being consistent with all experimental observation, is amply sufficient to account for effective energetic coupling. The velocity and the affinity effect reinforce each other, that is, their joint effect on coupling is greater than the sum of each acting alone. The assumption of a second velocity effect, modifying the mobility of the fully loaded translocator species, is neither necessary nor supported by experimental evidence.

Electrogenicity and electrosensitivity

As secondary active transport involves cationic driver ions, it is often associated with net movement of charges between the bulk compartments. For the number of charges thus translocated per substrate Turner has introduced the term "charge stoichiometry" [68]. It can be expressed by the following equation:

\[ Z = z_a + n_b * z_b - n_c * z_c \]

where \( z_a \) is the charge of the substrate particle, \( z_b \) and \( z_c \) that of the symporting (b) and of the antiporting (c) ion, respectively. \( n_b \) and \( n_c \) are the number of symporting and antiporting ions, respectively, transported per substrate particle.

Clearly, the charge of the empty translocator does not enter charge stoichiometry, but may, under special conditions, affect the electrosensitivity of the system, as will be discussed in this review. A system that owes its operation to such net charge translocation responds to a change in transmembrane potential, or that, vice versa, by its operation induces a potential change is called "electrogenic" or "rheogenic". Such electrical effects have often been used to obtain information about the detailed stoichiometry of the system, for instance, about how many cations are transported with a given substrate. Before going into details, we would like to semantically define certain concepts that, owing to nonuniform terminology in the literature, have caused some confusion.

First, there is the alternative "electrogenic" versus "rheogenic". For plausible reasons, the former has been recommended to be used for ion pumps only, and the latter for passive and secondary active transport. This recommendation, however, is widely disregarded, both terms being used synonymously (with clear preference for "electrogenic"). Furthermore, the electrophysiologists use "rheogenic" in a different sense, namely to indicate a constant current source. We, therefore, recommend to drop the term "rheogenic" altogether and to use the term electrogenic for both kinds of active transport, but to specify it as "primary electrogenic" for ion pumps, and "secondary electrogenic" for secondary active transport.

Second, there is the alternative "electrogenic" versus "electrosensitive"; the former means to indicate actual net movement of charge (non-zero charge stoichiometry) and the latter to describe the mere observation that a given transport rate responds to a change in electrical potential. This distinction is recommendable, since a charge translocation does not always manifest itself by electrosensitivity, nor does electrosensitivity, always warrant a net translocation of charge. Unfortunately, it has become customary to call any system "electrogenic" whose transport rate responds to a change in membrane potential, with the often erroneous implication of a net movement of charge.

Electrogenicity gives the desired information on the stoichiometry between driver ion(s) and substrate only to the extent that it reveals the above defined charge stoichiometry (Z), of the overall reaction, or better, of a single translocation step, for instance, of the substrate translocating one. As to the overall charge stoichiometry, there are several methods to estimate it, but many of them yield dubious results or at best crude approximations. The most reliable method would be to directly measure the current flow associated with the substrate flow under voltage clamp conditions. This method, however, is hardly possible with vesicles and cells. Another rather safe method is to study the transport rate very closely to thermodynamic equilibrium, that is, under conditions when the notation of irreversible thermodynamics is permitted.

Steady state conditions such as static head or level flow are bound to give values that are too low owing to leakages, the more so the farther the steady state distribution is removed from equilibrium. Since, however, the expected Z is presumably an integer, the obtained values may often give a useful approximation. Several practicable procedures for this purpose have been devised by Turner [68].

As to the charge stoichiometry of special translocation steps, initial rate studies may give some useful information [69]. Since most studies of electrogenicity have been carried out under initial rate conditions, a somewhat more detailed treatment of the problems inherent in this method seems appropriate. It should be pointed out that "initial rate" in this context is usually meant to approximate a "zero-trans" steady state. Such would require that the incubation time is short enough to keep substrate and driver ion on a negligible level of the transside, but long enough to ensure steady state conditions for the carrier distribution.

With respect to the overall charge stoichiometry, initial rate studies may be even more misleading than those carried out under the conventional steady state conditions (static head and level flow), because any relationship between electrosensitivity and charge stoichiometry is highly accidental, and may be entirely missing. For instance, there are truly electrogenic systems, that is, those with non-zero charge stoichiometry, whose initial rate under certain conditions does not show any potential dependency at all. Their electrogenicity appears to be "masked", as might happen whenever the overall rate is determined by an electroneutral intermediate step [37]. In such a case electrogenicity can be revealed (unmasked) only after
changing the experimental conditions so as to increase the rate limitancy of the charge-translocating intermediate step [61]. Also the opposite is possible, that a nonelectrogenic system, having an overall charge stoichiometry of zero, becomes electroosensitive under conditions when a charge-translocating intermediate is rate limiting. It appears that zero-trans conditions are particularly susceptible to electrical potential effects that are unrelated to charge stoichiometry. They may result from changes in structure-dependent permeability parameters, or from potential effects on rate and redistribution of a charge bearing translocator, etc. Such effects, which could be called "catalytic", may obscure the (true) electrogenicity of the system. Another difficulty with initial rate studies concerns the quantitative assessment of the effective potential, as will be discussed below.

On the other hand, as mentioned before, initial rate studies may be advantageous in some respect: Under suitable conditions, they may give some information on the charge stoichiometry of certain translocating steps, to the extent that the rate limitancy of these steps can be influenced at will. For instance, at maximally increased rate limitancy of the substrate translocating step, the relative electrogenicity of the substrate translocating step, the relative electrogenicity of the overall transport could reveal the charge stoichiometry of this step. It does not make any difference by which means maximal rate limitancy is accomplished, by reducing the flow density of the substrate bearing pathway, or by enhancing the flow density of the substrate-free "return" pathway [57].

The magnitude of the effective potential difference

The effective driving potential across the barrier proper is not necessarily equal to the overall membrane potential measurable between the adjacent bulk solutions. It is usually implied that in electrotransport both rate and electrogenicity are determined by the translocation step, rather than by the binding and release reaction between the binding site and the transported solutes. Such would require that these reactions are not only very fast as compared to the translocation, but also potential-independent. Whereas the former is generally taken for granted on good grounds, the latter is subject to debate. It implies that the binding sites interact directly with their ligands dissolved in the bulk solutions, that is, that they move through the whole distance between the membrane interfaces. It is, however, considered more likely that they move only through the much smaller thickness of the "barrier proper", and hence can be reached by their ligands only through "access channels" along which the potential must drop somewhat. Only if these channels are of low resistance (low field channels) can their potential drop be negligible. If they are, however, specific (high field channels), their potential drops may significantly reduce the effective driving potential and make the binding and release reactions potential-dependent [70].

Special problems concerning the effective driving potential arise in studies with initial rate or in the zero-trans state. Since these states are thermodynamically undefined, the potential effective here can only crudely be estimated. It is usually understood to be only a fraction (p) of the potential difference across the barrier, namely the potential drop between the cis side of the barrier and the "point of transition", is a plane within the barrier, in which the translocator passes from the cis to the transposition, and which presumably indicates the peak of the free energy of activation [71, 72]. Usually the location of this plane is not known, but is estimated to be close to the middle of a symmetrical barrier (p = 0.5). But even this fraction is not fully available to the transport unless the charge-translocating step is fully rate-limiting, that is, unless its "fractional rate limitancy" is unity. Otherwise, the above effective potential difference has to be further corrected for the fractional rate limitancy (p). There may, however, sometimes be ways to estimate this factor with fair approximation or to keep it close enough to unity by the experimental conditions.

Conclusion

Our present knowledge on transport processes in bacteria, plants and animals, can be postulated by the following transport kinetic dogma:

There are no primary active transport processes but for some cations. Other accumulative transport is always secondary active via cotransport with sodium or protons (and/or countertransport with potassium).

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