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A General Channel Model Accounts for Channel, Carrier, Countertransport and Cotransport Kinetics

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Abstract. In this work we propose a unifying model of mediated membrane transport, based upon the idea that the integral membrane proteins involved in these processes operate via complex channel mechanisms. In the first part, we briefly review literature about the structural aspects of membrane transporters. We conclude that there is a substantial amount of evidence suggesting that most membrane proteins performing transport are embodied with channel-like structures that may constitute the translocation paths. This includes cases where the phenomenological transport kinetics do not correspond to the classical channel behavior. In the second part of this article we introduce the general channel model of mediated transport and employ it to derive specific examples, like simple one- or two-ligand channels, water-ligand channels, simple carriers, co- and counter-transport systems and more complex water-ligand carriers. We show that, for the most part, these particular cases can be obtained by the application of the techniques of diagram reduction to the full model. The necessary conditions for diagram reduction reflect physical properties of the protein and its surroundings.

Key words: Carriers — Channels — Kinetic models — Membrane transport — Diagram reduction

Introduction

The protein-mediated transport of ionic and non-ionic ligands across membranes represents one of the crucial properties of cells. Understanding the kinetic, thermodynamic, structural and mechanistic aspects of this type of processes has therefore constituted a major goal of biological research. By the late seventies, the available structural and functional evidence supported the view that mediated transport across membranes occurred via one of two basic mechanisms: channel or carrier (Heinz, 1978; Läuger, 1980; Schultz, 1980). This idea emerged largely from studies performed in relatively simple experimental systems. In particular, the membrane-binding antibiotics gramicidin and valinomycin were, respectively, recognized as the canonical representatives of the channel and carrier categories. The kinetic diagrams describing the two mechanisms embody the fundamental difference between them: while channels simultaneously offer ligand binding sites at the two compartment openings, the binding site or sites of a carrier can only be available from one compartment at a time (Schultz, 1980; Stein, 1986; Andersen, 1989). This fundamental difference underlies the kinetic criteria developed to distinguish between the two mechanisms (Lieb & Stein, 1974a; 1974b). Although most of the physiological processes of membrane transport were already considered to be significantly more complex than the ones mediated by the simpler ionophores, the finding of kinetic properties consistent with the channel and carrier behaviors led authors to adopt the simpler models to describe their more involved situations. It soon became evident that ionic channels have a structure similar to that of ionophores of the channel type, basically consisting of an inner hydrophilic path connecting the two compartments separated by the membrane (Urry, 1971; Hladky & Haydon, 1972; Bamberg, Apell & Alpes, 1977; Toyoshima & Unwin, 1988; Hille, 1992). However, the complex biological carriers most likely would not operate via the mobile mechanism characteristic of ionophores of the valinomycin type (Szabo, Eisenman & Ciani, 1969; Krasne, Eisenman &

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Szabo, 1971; Stark et al., 1971; Eisenman, Krasne & Ciani, 1975). Instead, in order to perform facilitated diffusion, a physiological carrier should produce ligand translocation by means of conformational changes of the protein (Heinz, 1978; Schultz, 1980; Stein, 1986; West, 1997). A major conceptual breakthrough was the proposal, by Läuger (1980; 1984), that the typical channel and carrier behaviors of the physiological transport systems could be considered to represent limit cases of a general model of membrane transport. In Läuger's unifying conception the basic structure involved in membrane transport is a channel fluctuating between conformational states. The simplest case of a two-conformational, single-site and single-ligand channel model was thus demonstrated to exhibit carrier properties in the presence of particular profiles of the fluctuating activation energy barriers for ligand binding from the two compartments (Läuger, 1980). During the last twenty years several complex processes of mediated transport have indeed been suggested to operate via channel-like mechanisms, including cases of facilitated diffusion of organic ligands, like sugars or aminoacids, and of primary active transport of ions (*see* references in Hernández & Fischbarg, 1994a; Su et al., 1996; Hernández, Fischbarg, & Vera, 1996). Keeping with the line of unifying reasoning, more recent theoretical analyses conclude mat there actually is a dissociation between the kinetic and structural concepts of carriers and channels. Thus, the typical carrier and channel kinetic behaviors (Lieb & Stein, 1974a; 1974b) can both be exhibited by processes characterized by carrier and channel mechanisms of diverse complexity (Hernández, 1998; 2001). In essence, these results strongly suggest that there may be a general kinetic design of the processes of mediated transport, of sufficient structural complexity and properties to generate most types of kinetic transport behaviors, from simple typical channels and carriers to the most complex processes of facilitative and active transport.

The purpose of this work is to propose a general kinetic model of mediated transport, based upon the idea that the integral membrane proteins operate via complex inner hydrophilic channels that may undergo structural and functional modifications as a consequence of conformational transitions of the protein. The model proposed here should basically be considered to represent a generalization of the original unifying model introduced by Läuger (1980). In the first part of this work we briefly review relevant literature about structural studies of integral membrane proteins involved in transport. We conclude that there is a significant amount of recent evidence suggestive of the existence of inner channel structures in many membrane transport proteins and of their possible participation in the transport processes. In the second part, we propose the general channel model of mediated transport and derive particular cases. Basically, the proposed general mechanism consists of a channel capable of binding water and other ionic or non-ionic ligands and subject to conformational modifications. For illustrative purposes, we consider here the simplest case of a two-site, two-conformational single-file channel capable of binding water and other ligands. We explicitly study cases of passive transport and of secondary active transport. As previously stated (Hill, 1977), the coupling of some transitional steps to chemical reactions would underlie the existence of primary active transport. We show that the simplest case of the general model considered here can generate diverse kinetic behaviors, which range from elementary water- or ligand-only channels or carriers to complex models of facilitated diffusion or of secondary active transport, where water and/or diverse ligands may interact. In most situations, the particular kinetic behaviors result from the application of the techniques of diagram reduction (Hill, 1977) to the general model. The procedures of diagram reduction have been used for the study of the kinetic properties of diverse transport processes (Hansen et al., 1981; Hernandez & Fischbarg, 1992; Hernández, 2001; Hernández & Valle Lisboa, 2004). The particular conditions of model reduction reflect the physical and physicochemical properties characteristic of a specific transport protein and its surroundings.

Results and Discussion

Channel-like Structures in Transport Proteins

For many years studies of the structure of membrane proteins faced a formidable methodological challenge (Branden & Tooze, 1991). The difficulties in deriving plausible structural models for the great majority of these proteins did not allow, in most cases, detailed interpretations of the processes of mediated membrane transport in mechanistic terms. This circumstance has delayed progress, particularly for the case of proteins performing carrier-like transport. Still, for some examples of this type of proteins such as the lactose permease (Collins, Permuth & Brooker, 1989), the facilitative glucose transporter Glut1 (Mueckler et al., 1985; Fischbarg et al., 1990) and the metal-tetracycline/H^+ antiporter (Tamura et al., 2001), approximate structural models were proposed using indirect methods. The common idea emerging from these models was that these proteins have a water-filled or water-permeable channel serving as the basic structure for the substrate transport pathway. As mentioned above (*see* Introduction) this proposal was basically in agreement with functional evidence obtained for many different processes of membrane transport, ranging from facilitative transport of organic molecules to active primary transport

of ions (*see* references in Hernández & Fischbarg, 1994a; Su et al., 1996; Hernández, Fischbarg, & Vera, 1996), strongly suggestive of the existence of inner channel-like pathways for the ligand translocation. In this respect it is noteworthy to emphasize the concept that the highly complex membrane proteins performing primary active transport of ions also most likely operate via channel mechanisms. This seems to be remarkably so for the case of the calcium pump (Antoine, Pinet & Coulombe, 2001) and particularly for the sodium pump, where a myriad of functional studies suggesting the existence of inner channels (Apell, Borlinghaus & Läuger, 1987; Stürmer et al., 1991; Gadsby, De Weer & Rakowski, 1993; Hilgemann, 1994; Holmgren & Rakowski, 1994; Wuddel & Apell, 1995; Redondo, Fiedler & Scheiner-Bobis, 1996; Peluffo & Berlin, 1997; Holmgren et al., 2000; De Weer, Gadsby & Rakowski, 2001) is supported by some structural evidence (Guennoun & Horisberger, 2000; 2002). Recently, more direct evidence supporting the general channel concept has become available as the crystallographic structures of two 12-helical transporters of *E. coli* have been communicated, namely, the glycerol-3-phosphate transporter (Huang et al., 2003) and the lactose permease (Abramson et al., 2003). In addition, a projected structure has been advanced for a bacterial oxalate transporter (Heymann et al., 2001), which also suggests the existence of an inner channel-like structure. We may conclude that the ongoing structural studies of membrane proteins performing mediated transport seem to confirm the concept that, independently of the nature of the particular process of mediated transport considered, ligand translocation occurs via inner protein channels.

General Channel Model and Particular Examples

The general channel model of mediated transport is characterized by the following properties:

i) Ligand translocation occurs through an inner channel of the integral membrane protein that performs the mediated transport
ii) The channel is permeable to water. Hence, the ligand may interact with water molecules inside the channel
iii) The channel may not be absolutely selective. Hence, different ligands may traverse the channel
iv) The channel is subject to structural and functional modifications as a consequence of conformational transitions of the protein
v) Under particular conditions the kinetic diagram describing the transport process can be reduced, as a consequence of the existence of transient intermediate states and/or of rapid equilibrium between neighboring states
vi) Diagram reduction occurs as a consequence of intrinsic properties of the transport system — the membrane protein and its lipid and aqueous surroundings — and/or of particular ambient conditions (e.g., large water or ligand activities in the compartments separated by the membrane)
vii) Some of the protein transitions may be coupled to a downhill source of free energy, such as a chemical reaction. The system may thus be capable of performing primary active transport

Figure 1 shows a particular simple example of the general channel model. This case, to be utilized here to discuss the basic concepts of this study, consists of a two-conformational single-file channel that offers two internal binding positions, which can be occupied by two different ligands. In some cases, one of the ligands can be water. The model of Fig. 1 is thus embodied with the properties i) to iv) described above. In what follows we show that, by the application of different conditions of diagram reduction [properties (v) and (vi)], the general model of Fig. 1 can yield diverse kinetic submodels of passive or secondary active transport. We do not consider here the case of the incorporation, into the kinetic diagram, of features resulting in primary active transport [property (vii)], a matter thoroughly discussed elsewhere (Hill, 1977; Läuger, 1984). As a reference for this work, in Appendix I we summarize the basic aspects of the procedures of diagram reduction, as developed by Hill, (1977). For this purpose, we utilize the simple reactional schemes depicted in Fig. 2. As discussed previously (Hill 1977; Hernández, 2001; Hernández & Valle Lisboa, 2004)), it is important to emphasize here that the reduction procedure does not imply any modification in the fundamental mechanism of action of the transporter. That is, for the case of the general channel model shown in Fig. 1, its reduction to yield the particular diagrams shown in Figs. 3–10 below can only be considered to represent a means to reveal specific kinetic behaviors under particular conditions, and not as the transformation of a molecular mechanism into another. For all of the reduced models, the underlying mechanism of transport remains the general one described in Fig. 1. It must also be noted here that since the purpose of this work is to illustrate how the standard kinetic behaviors can represent particular examples of a general mechanism, we do not perform here the detailed kinetic analysis of the particular cases considered below. Examples of the detailed application of the reduction technique to derive simple kinetic behaviors from complex kinetic diagrams can be found in our previous work (Hernández & Fischbarg, 1992; Hernández, 2001; Hernández & Valle Lisboa, 2004).

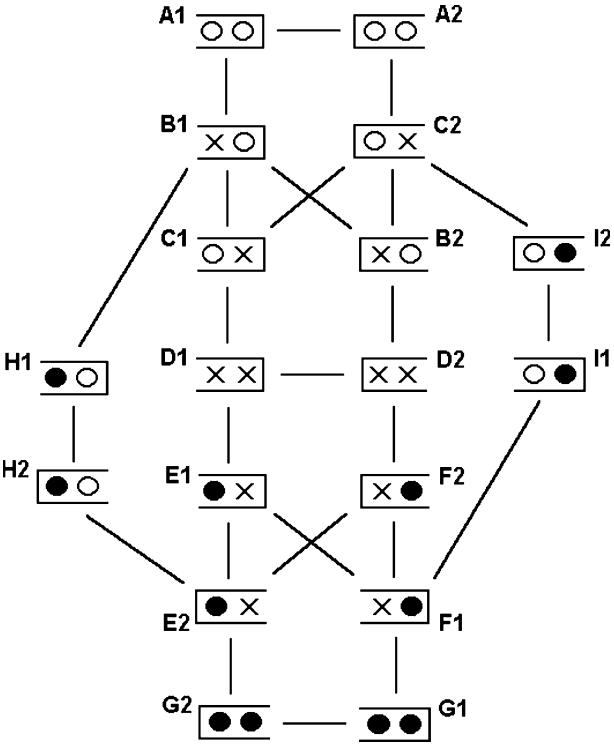

Fig. 1. State diagram describing an example of the general channel model of mediated transport. The diagram corresponds to a two-conformational, two-site single-file channel performing transport of two different ligands (represented by the empty and filled circles, respectively). In some cases, the empty circles can represent water molecules (*see* Figs. 4–6 and 8 below). In each conformation state the channel is accessible to the ligands from only one of the compartments. For the example shown in Fig. 1, the diagram includes all the possible states and connections between them. The solid lines represent actual reversible transitions. Here and in the following figures, the binding of ligands from the two compartments is not explicitly shown. Al, A2, ... , I1, I2, are the intermediate states. X: vacant positions.

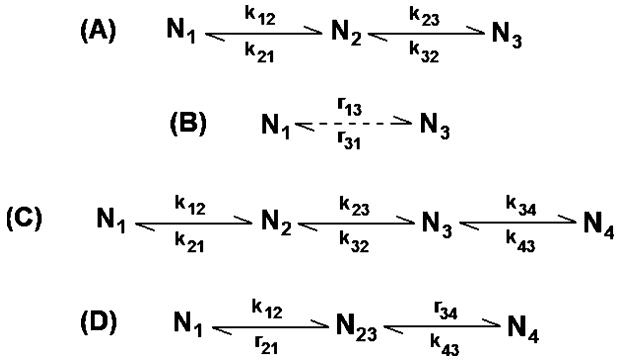

Fig. 2. (*A*) Linear path of sequential transitions connecting states N_1 and N_3 via the intermediate state N_2. The k's are rate constants. (*B*) Reduced scheme obtained from the one shown in Fig. 2*A* under the condition that state N_2 is a transient intermediate. Here and subsequently a transition represented by a broken line indicates the existence of a transient intermediate. The k's are original rate constants; the r's are reduced rate constants (*see* Appendix I). (*C*) Linear path of sequential transitions connecting states N_1 and N_4 via the intermediate states N_2 and N_3. The k's are rate constants. (*D*) Reduced scheme obtained from the one shown in Fig. 2*C* under the condition that a rapid equilibrium is always established between states N_2 and N_3. The double subscript in state N_{23} indicates that it is composed of two substates in rapid equilibrium between them (i.e., N_2 and N_3). The k's are original rate constants; the r's are reduced rate constants (*see* Appendix I).

Multi-ligand, Single-file Channel

If rapid equilibrium always exists between the two conformational states of the channel, independently of whether the channel is occupied or not, the diagram of Fig. 1 can be reduced to the one shown in Fig. 3. The single transitions of this model are governed by original and reduced constants (Appendix I). Since the double transitions do not involve the binding of any species, they can easily be condensed into single transitions. The reduced model shown in Fig. 3 is certainly equivalent to the one corresponding to a two-site single-file channel that does not experience any conformational transition. In this respect it is interesting to emphasize here that, as discussed previously (Hill, 1977), the recognition of a particular macromolecular state is a consequence of the time scale of the experimental procedure employed. Hence, a state can be composed of several substates in rapid equilibrium between them, not identifiable by the experimental procedures utilized. As a consequence of this, it might not be possible to establish whether the model of Fig. 3 already describes the basic mechanism of operation of the transporter (which would then correspond to a "rigid" channel) or whether it instead represents a particular approximate case (i.e., obtained by diagram reduction) of the general model of Fig. 1. Still, the model of Fig. 1 can in turn represent a reduced version of a more complex underlying mechanism involving a larger number of substates. The model of Fig. 3 (or analogous single-file models including a larger number of internal binding sites) has been employed, for instance, as an alternative to interpret ion-ion interactions in potassium (Hille & Schwarz, 1978), gramicidin (Urban, Hladky & Haydon, 1980) and sodium (Daumas & Andersen, 1993) channels, and to describe ion-coupled transport of non-ionic ligands (Su et al., 1996).

Particular Cases of Single-File Channels

Several simpler models can be derived from the one of Fig. 3. The ones shown in Figs. 4*A* and 4*B* correspond to cases where the channel predominantly binds one of the two species. In particular, Fig. 4*A* describes a situation where the double-occupancy state is considered to participate in the transport kinetics. This can be the case, for instance, of a transported ligand present at a high concentration in the two compartments, such as water (Hernández & Fischbarg, 1994b). The diagrams shown in Fig. 4*A* and 4*B* also represent classical models for the study

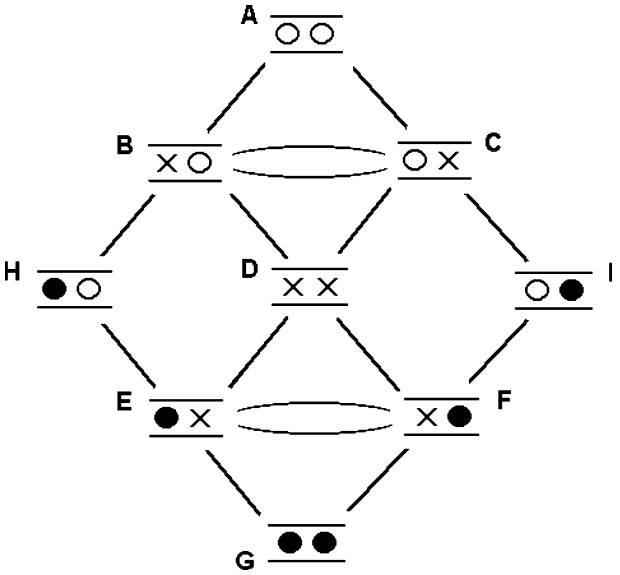

Fig. 3. Reduced model obtained from the general model of Fig. 1 for the case that rapid equilibrium is established between corresponding conformational states (e.g., rapid equilibrium between states *A*1 and *A*2 of the model in Fig. 1 yields state *A* of the present model, etc.). The solid lines represent transitions resulting from the establishment of rapid equilibrium between intermediate states (*cf.* Fig. 2). For each ligand, the double line representing the transition between the single-occupancy states corresponds to the two original paths for ligand movement inside the channel (*cf.* Fig. 1).

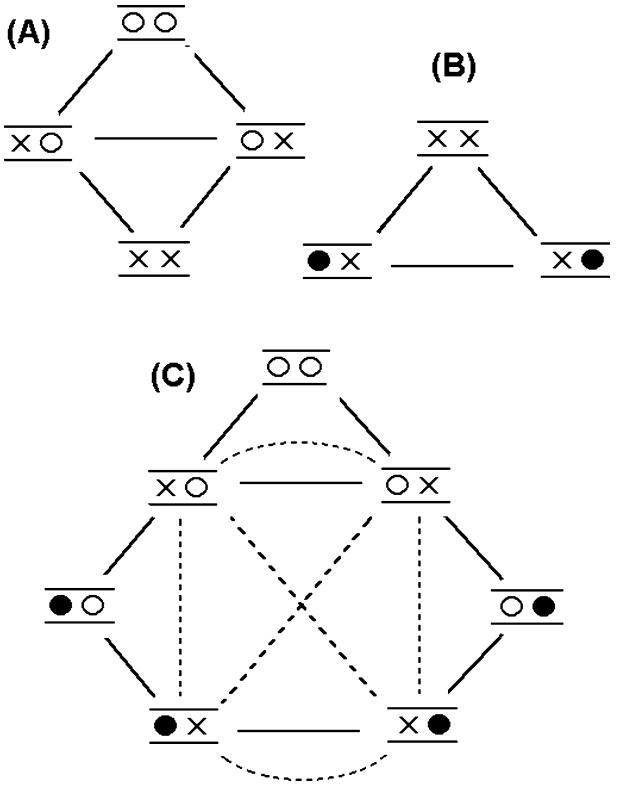

Fig. 4. Diagrams derived from the model of Fig. 3 for the case that the open circles represent water molecules. The diagrams represent water- (*A*) and ligand-only (*B*) channels and a water-ligand channel (*C*). The two simpler models are obtained by assuming that the channel is only capable of exclusively binding water (*A*) or ligand (*B*). Also, the water channel can function under near-saturating conditions (*A*) whereas die ligand channel always operates far from saturation (*B*). The model of Fig. 4*C* combines these two latter conditions. The solid lines represent the corresponding transitions of the original model of Fig. 3; the double lines of this figure have been condensed into single lines. The broken lines represent reduced transitions, obtained from the corresponding original paths of Fig. 3 by assuming that state *D* (the fully vacant state) is a transient intermediate (e.g., as a consequence of very high water activities in the compartments separated by the membrane, *see* main text).

of the basic kinetic properties of ion translocation through single-file ionic channels (Stein, 1986; Andersen, 1989). In the model shown in Fig. 4*C* the channel can bind the two ligands, one of them is present at high concentrations (e.g., water) while the other is not. The fully vacant state is a transient intermediate as a consequence, for instance, of the predominance of the intermediate states occupied by the abundant ligand. A still simpler version of the diagram shown in Fig. 4*C* can be obtained if these reduced steps are negligible. The resulting diagram (Fig. 5*A*) represents the simplest example of a single-file model employed to interpret permeability properties of ionic (Kohler & Heckmann, 1979; Schumaker & MacKinnnon, 1990) and water (Hernández & Fischbarg, 1992) channels. To be noted, the models shown in Figs. 4*C* and 5*A* exhibit carrier-like kinetics for the less abundant ligand (Hernández, 1998). If the single-vacancy states of the model of Fig. 5*A* represent transient intermediates, this model can be reduced to the one shown in Fig. 5*B*, which now represents a channel model for the less abundant ligand (*cf.* Fig. 4*B*). This situation also represents a particular case of a channel-like behavior exhibited by a reduced carrier mechanism (Stein, 1986; Hernández, 2001). A more complex version of the model shown in Fig. 5*B* (i.e., considering more positions inside the channel and occupancy by more than one ion) has been recently employed to interpret the role of the selectivity filter in the conduction properties of the KcsA K^+ channel (Morais-Cabral, Zhou & MacKinnon, 2001).

Gated Channel

As a final example of models derived from the one of Fig. 3, in the diagram shown in Fig. 6*A* the channel can bind both ligands, but they are mutually excluding. Again, the fact that one of them is significantly more abundant (e.g., the case of water) dictates the inclusion of the corresponding fully-occupied state only for it. In principle, this more concentrated ligand could dominate the overall transport process performed by the channel. Still, a very high affinity of the channel for the less abundant ligand would nevertheless determine the

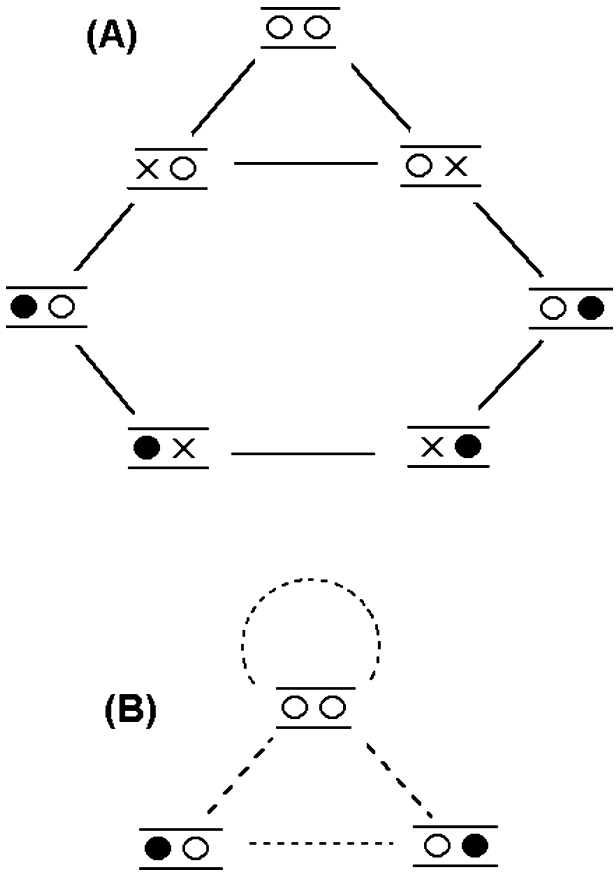

Fig. 5. Particular models obtained from the water-ligand channel model of Fig. 4*C*, for the case that the reduced transitions are negligible (*A*) and that, in addition, the states having vacancies are transient intermediates (*B*). Again, the solid lines represent transitions conserved from the model of Fig. 3, whereas the broken lines correspond to reduced transitions. Note that these models imply the basic assumptions that water and ligand are close-to and far-from saturation, respectively (*cf.* Fig. 4). The models shown in this figure exhibit carrier-like kinetic behavior for the ligand (Hernández, 1998). The model of Fig. 5*B* represents a particular case of a ligand carrier that exhibits channel-like transport kinetics (Stein, 1986; Hernández, 2001).

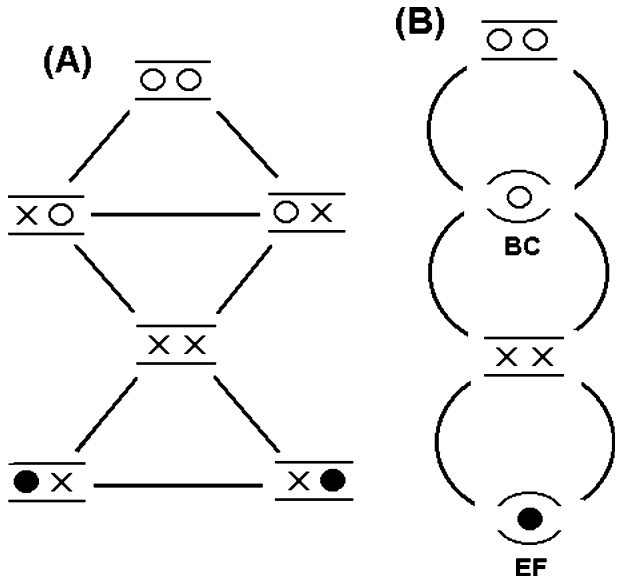

Fig. 6. Models obtained from the diagram of Fig. 3, for the case of water (*empty circles*) and ligand (*filled circles*) transport. (*A*) This model assumes that there is mutual exclusion of water and ligand, a far-from-saturation regime for the ligand and a near-saturation condition for water. In spite of these conditions, a very high affinity of the channel for the ligand allows effective ligand transport (*see* main text). (*B*) Similar assumptions to the model of Fig. 6*A* and, in addition, rapid equilibrium between the states having vacancies (*B–C* and *E–F*, Fig. 3) to yield the condensed states *BC* and *EF* (Fig. 6*B*).

existence of significant transmembrane fluxes of it. Fig. 6*B* shows a reduced version of the model of Fig. 6*A*, for the case that the states having each ligand in either inner binding position establish a rapid equilibrium between them. If the more abundant ligand is water and the less abundant ligand is an inorganic ion, the model of Fig. 6*B* can represent a simple model of gating kinetics (Aidley & Stanfield, 1996), where the water-occupied states would correspond to non-conducting channel states.

Single-ligand, Two-conformational Channel

The model shown in Fig. 7 is directly obtained from the complete original one, depicted in Fig. 1, by assuming that the channel can only bind one of the two ligands. The model of Fig. 7 was proposed as an alternative to interpret contradictory kinetic results about facilitative sugar transport (Hernández, Fischbarg & Vera, 1996). For this model it can be demonstrated that the single-occupancy region of the diagram (i.e., corresponding in the figure to the region limited by the broken lines) exhibits a steady-state kinetic behavior indistinguishable from that of a four-state simple carrier model (Hernández, Fischbarg & Vera, 1996; Hernández, 1998). For the case of glucose transporters, the simple carrier model has been repeatedly utilized to describe experimental evidence (Lowe & Walmsley, 1986; Walmsley, 1988; Wheeler & Whelan, 1988). However, the totality of the available kinetic evidence about facilitative sugar transport cannot be accounted for by the employment of a single simple carrier model (*see* references in Stein, 1986; Carruthers, 1990). It is noteworthy that a channel mechanism, such as the one depicted in Fig. 7, can account for these discrepancies and may therefore constitute a plausible alternative to describe the kinetics of facilitative sugar transport (Hernández, Fischbarg & Vera, 1996; Hernández, 1998). The glucose transporter thus represents an example of a transport system possibly characterized by a complex underlying mechanism, although capable of yielding some experimental behavior compatible with a relatively simple model. The general model shown in Fig. 1, of which the model in Fig. 7 represents a particular case, may thus result helpful as a basis to

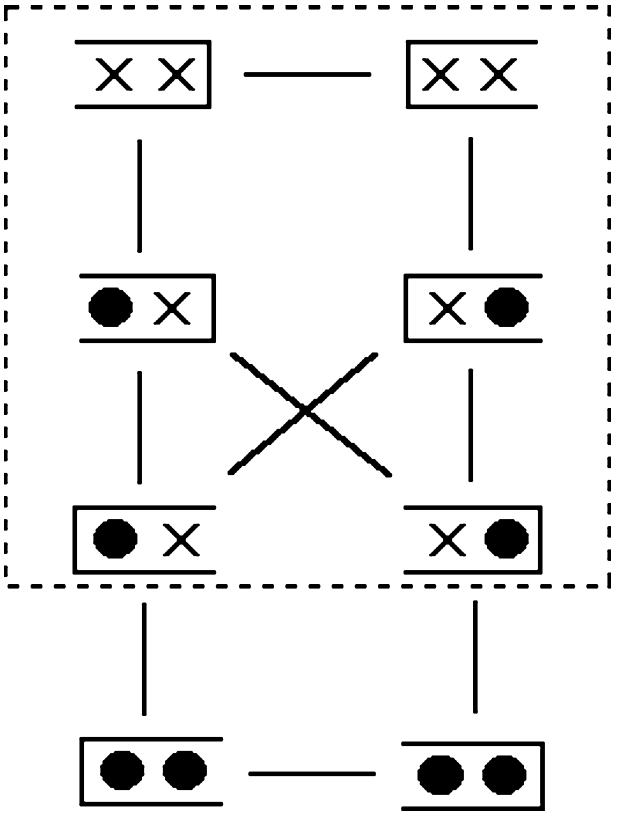

Fig. 7. Model obtained from the original diagram of Fig. 1 by assuming that only one ligand (e.g., the one represented by the black circles) can bind the channel. The submodel contained inside the broken lines corresponds to the single-occupancy regime; it exhibits carrier-like kinetic behavior (Hernández, Fischbarg & Vera, 1996; Hernández, 1998).

interpret experimental evidence of transport processes that cannot be easily understood in terms of simple standard models, as is the case of facilitative sugar transport.

Multi-Ligand, Two-Conformational Channel

The model shown in Fig. 8*A* is also directly derived from the original one (Fig. 1). In this case, a far-from-saturation regime is assumed for one of the ligands and a near-saturation condition for the other one (e.g., water). The resulting model (Fig. 8*A*) represents a particular case of a general model utilized to study the transport properties of two-conformational single-file pores permeable to water and another ligand (Daumas & Andersen, 1993). It can be demonstrated that the model of Fig. 8*A* and, correspondingly, its reduced version shown in Fig. 8*B*, exhibit simple carrier-like kinetic behavior for the less abundant ligand (Hernández, 1998).

Countertransport

By assuming that there is mutual exclusion of the two ligands inside the channel and a far-from-saturation

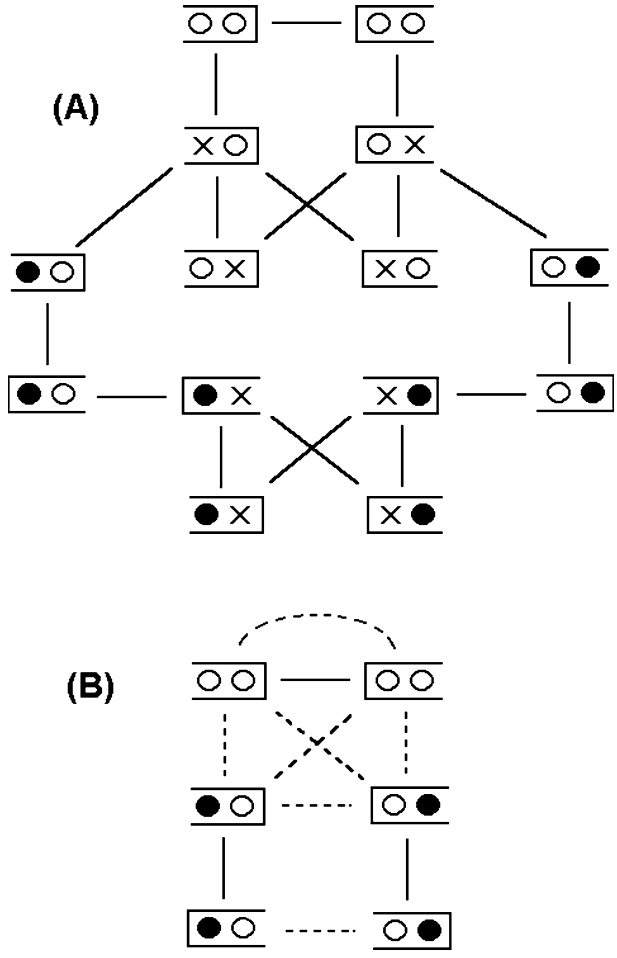

Fig. 8. Models obtained from the diagram of Fig. 1, for the case that the open circles represent water molecules and for discernible conformational transitions. (*A*) In this case, there is a far-from-saturation regime for the ligand and a near-saturation condition for water. This latter condition determines that the fully vacant states have negligible probabilities (*cf*. Figs. 4*C* and 5*A*); they have therefore been excluded from the diagram. (*B*) Similar assumptions to the model of Fig. 8*A* and, in addition, that the states having vacancies are transient intermediates. The models shown here exhibit carrier-like kinetic behavior for the ligand (Hernández & Fischbarg, 1994a; Hernández, 1998). The solid lines represent transitions conserved from the original model of Fig. 1; the broken lines represent reduced transitions.

regime for both, the model of Fig. 1 yields the simpler model shown in Fig. 9*A*. A still simpler version can be obtained if, in addition, there is rapid equilibrium between the ligand-binding conformational states opened to the same compartment (Fig. 9*B*). This latter model corresponds to the classical one employed to represent countertransport of two different ligands (Stein, 1986; Andersen, 1989; Denner, Heinrich & Bernhardt, 1993). For the countertransport process described by the model shown in Fig. 9*B*, the phenomenological coupling and stoichiometric ratios depend on the rate constants governing the transition between states D1 and D2.

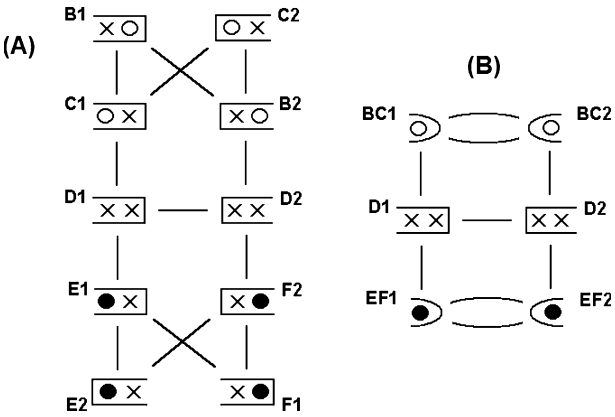

Fig. 9. (*A*) Model obtained from the diagram of Fig. 1 for the case of two different ligands (*empty* and *filled circles*). The model assumes mutual exclusion of the two ligands inside the channel and a far-from-saturation regime for both. (*B*) Assumptions similar to the model of Fig. 9*A* and, for each ligand, rapid equilibrium between the ligand-binding conformational states opened to the same compartment (*B*1–*C*1, *B*2–*C*2, *E*1–*F*1 and *E*2–*F*2, Fig. 9*A*) to yield the condensed states *BC1*, *BC2*, *EF1* and *EF2* (Fig. 9B). The models shown here describe countertransport of the two ligands (*see* main text).

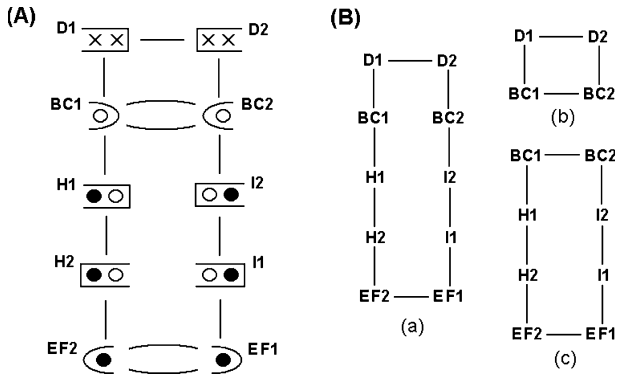

Fig. 10. (*A*) Model obtained from the diagram of Fig. 1 for the case of two different ligands (*empty and filled circles*). The model assumes that the second ligand (*filled circles*) can bind to the transporter only when the first ligand (*empty circles*) is already bound to it. Both ligands operate under far-from-saturation conditions. It is also assumed that, for each ligand, there is rapid equilibrium between the conformational states opened to the same compartment (*B1–C1*, *B2–C2*, *E1–F1* and *E2–F2*, Fig. 1) to yield the condensed states *BC1*, *BC2*, EF1 and *EF2* (Fig. 10*A*). (*B*) Cycles (*a*, *b* and *c*) contained in the diagram of Fig. 10*A*. The model shown here describes cotransport of the two ligands (*see* main text and Appendix II).

Cotransport

For the last example to be considered here, the case of cotransport, some restrictive conditions allow one to derive me model of Fig. 10 from the complete original one shown in Fig. 1. In this case, one of the ligands (e.g., the one represented by black circles in Fig. 10) can bind to the transporter only when the other ligand (represented by the open circles) is already bound to it. The two ligands are under far-from-saturation conditions. The model further assumes that for each ligand, there is rapid equilibrium between the conformational states opened to the same compartment. We demonstrate here that the resulting single-file model of Fig. 10 may exhibit cotransport properties (Appendix II). In this case, the basic mechanism determining cotransport is more complex than the ones considered for the case of single-site mobile carriers (Turner, 1981; Stein, 1986). This is an inevitable consequence of the fact that, in the present model, the operating device is a unique single-file channel shared by the two participating species. The model of Fig. 10 is also different from other proposed mechanisms of cotransport, such as the consecutive mechanism (Läuger, 1987), where ternary complexes (i.e., presenting simultaneous binding of the two ligands to the transporter) do not occur.

The particular example of the general channel model depicted in Fig. 1 can be considered to be relatively simple from a biological, though not from an analytical, point of view, since it has only considered the existence of two binding sites inside the channel and two basic conformational states. Still, as shown here, it is capable to generate a great variety of kinetic behaviors that include most of the standard types of mediated membrane transport. For some of the particular models derived here, other specific properties of transport systems may be accounted for by the inclusion of additional states, like modifier sites (Denner, Heinrich & Bernhardt, 1993), transition states (Krupka, 1989a; 1989b; 1990) or more non-conducting states for the case of ionic channels (Hille, 1992; Aidley & Stanfield, 1996).

Conclusions

A survey of recent literature permits to conclude that there is a significant amount of evidence suggestive of the presence of channel-like structures in many different integral membrane proteins performing facilitative and active transport of ionic and non-ionic ligands. This experimental evidence, together with analytical results from theoretical studies of kinetic models of mediated transport, suggests that a unified vision of this type of processes may be proposed, based upon the existence of a common channel mechanism. Keeping with this line of thought, we have proposed here a general channel model that is capable of generating all of the standard kinetic models of membrane transport. This is achieved, for the most part, by the application of the procedures of reduction of kinetic diagrams. The particular reduction conditions may, in principle, be interpreted in terms of specific properties of the transport proteins and their interacting lipid and aqueous media. The main theoretical conclusion of this work is therefore

that the unifying conception for the processes of membrane transport, originally developed by Läuger (1980) to cover simple cases of channels, carriers and pumps, can indeed be extended to account for diverse, more complex transport processes.

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Appendix I

Reduction of Kinetic Diagrams

The basic methodological aspects of the reduction of kinetic diagrams were developed by Hill (1977). As a reference for the present work we summarize here the fundamentals of the procedures of diagram reduction, both for the cases of the existence of transient intermediate states and of rapid equilibrium between neighboring states.

Transient Intermediate State

We shall refer here to the linear sequence of transitions shown in Fig. 2*A*. In order to derive expressions for the reduced rate constants we assume that the total membrane density of the transporter (N_T, expressed, for instance, in mol cm^{-2}) is conserved, i.e.:

$$N_T = N_1 + N_2 + N_3 = \text{constant}, \tag{A1}$$

where N_i ($i = 1, 2, 3$) is the membrane density of the ith state of the transporter.

If state N_2 (Fig. 2*A*) is a transient intermediate,

$$p_2 \ll p_1, p_3, \tag{A2}$$

where p_i is the probability (frequency) of the ith state in the complete ensemble ($p_i = N_i/N_T$) Relation (A2) is fulfilled if

$$k_{23}, k_{21} \gg k_{12}, k_{32}. \tag{A3}$$

The system of rate equations governing the reactional scheme of Fig. 2*A* is

$$\begin{aligned} dN_1/dt &= -k_{12}N_1 + k_{21}N_2 \\ dN_2/dt &= k_{12}N_1 - (k_{21}+k_{23})N_2 + k_{32}N_3 \\ dN_3/dt &= k_{23}N_2 - k_{32}N_3 \end{aligned} \tag{A4}$$

From Eqs. (A1)–(A2), $dN_2/dt \sim 0$. Therefore, from Eqs. (A4), N_2 is always approximately given by

$$N_2 = (k_{12}N_1 + k_{32}N_3) / (k_{21} + k_{23}) \tag{A5}$$

Replacing Eqs. (A4) with (A5), we obtain the system of rate equations corresponding to the reduced model (Fig. 2*B*):

$$\begin{aligned} dN_1/dt &= -r_{13}N_1 + r_{31}N_3 \\ dN_1/dt &= r_{13}N_1 - r_{31}N_3, \end{aligned} \tag{A6}$$

where the reduced rate constants r_{13} and r_{31} are given by

$$\begin{aligned} r_{13} &= k_{12}k_{23} / (k_{21} + k_{23}) \\ r_{31} &= k_{32}k_{21} / (k_{21} + k_{23}) \end{aligned} \tag{A7}$$

Rapid Equilibrium Between Neighboring States

The kinetic scheme shown in Fig. 2*C* can be reduced if, for instance, a rapid equilibrium is established between states N_2 and N_3. For this to occur, the necessary condition is

$$k_{23}, k_{32} \gg k_{12}, k_{21}, k_{34}, k_{43}. \tag{A8}$$

The system of rate equations governing the kinetic scheme shown in Fig. 2*C* is

$$\begin{aligned} dN_2/dt &= -k_{12}N_1 + k_{21}N_2 \\ dN_2/dt &= k_{12}N_1 - (k_{21}+k_{23})N_2 + k_{32}N_3 \\ dN_3/dt &= k_{23}N_2 - (k_{32}+k_{34})N_3 + k_{43}N_4 \\ dN_4/dt &= k_{34}N_3 - k_{43}N_4. \end{aligned} \tag{A9}$$

Since rapid equilibrium always takes place between N_2 and N_3:

$$k_{23}N_2 = k_{32}N_3. \tag{A10}$$

We define:

$$N_{23} = N_2 + N_3 \tag{A11}$$

From Eqs. (A10)–(A11),

$$\begin{aligned} N_2 &= k_{32}N_{23} / (k_{23} + k_{32}) \\ N_3 &= k_{23}N_{23} / (k_{23} + k_{32}). \end{aligned} \tag{A12}$$

Replacing these expressions into Eqs. (A9), we obtain the system of rate equations corresponding to the reduced model (Fig. 2*D*):

$$\begin{aligned} dN_1/dt &= -k_{12}\,N_1 + r_{21}\,N_{23} \\ dN_{23}/dt &= k_{12}\,N_1 - (r_{21}+r_{34})\,N_{23} + k_{43}\,N_4 \\ dN_4/dt &= r_{34}\,N_{23} - k_{43}\,N_4. \end{aligned} \tag{A13}$$

where state N_{23} is made up of substates N_2 and N_3, and where the reduced rate constants r_{21} and r_{34} are given by

$$\begin{aligned} r_{21} &= k_{32}\,k_{21}/(k_{23}+k_{32}) \\ r_{34} &= k_{23}\,k_{34}/(k_{23}+k_{32}). \end{aligned} \tag{A14}$$

Appendix II

A Single-File Model of Cotransport

We analyze here the general steady-state properties of the diagram shown in Fig. 10*A*, that describes the transport of ligands L (*empty circles*) and M (*filled circles*) between the compartments e (on the right side of the channel) and i (on the left side of the channel). With the aid of the graphic algorithm (Hill, 1977), the corresponding cycle fluxes J_a, J_b and J_c (Fig. 10*B*) are given by

$$\begin{aligned} J_a &= (N\Pi_a/\Sigma)\,(L_e^2\,M_e - L_i^2\,M_i) \\ J_b &= (N\Sigma_b\,\Pi_b/\Sigma)\,(L_e - L_i) \\ J_c &= (N\Sigma_c\,\Pi_c/\Sigma)\,(L_e\,M_e - L_i\,M_i). \end{aligned} \tag{B1}$$

In Eqs. (B1), Σ is the sum of all the directional diagrams of the model, and Σ_j and Π_j ($j = a, b, c$) are the sums of all the appendages to the corresponding cycle (j) and the products of all the true rate constants of the jth cycle in either direction, respectively. L_e, L_i, M_e and M_i are the concentrations of L and M in compartments e and i, respectively. The general forms of Eqs. (B1) are a consequence of the detailed balance restriction. To be noted, the cycle fluxes have been considered to be positive in the clockwise direction (*see* Fig. 10B).

The net transmembrane fluxes of L and M (J_L and J_M, positive in the $e \rightarrow i$ direction) are given by

$$\begin{aligned} J_L &= 2J_a + J_b \\ J_M &= J_a + J_c. \end{aligned} \tag{B2}$$

The phenomenological stoichiometric ratio r is given by

$$r = J_L/J_M = (2J_a + J_b)/(J_a + J_c). \tag{B3}$$

We now consider a particular case. Let the following relation hold:

$$(M_e/M_i) \gg (L_i/L_e)^2 \gg 1. \tag{B4}$$

As a consequence of relation (B4), J_a and J_c are positive and J_b is negative. Hence, in this case, $r \leq 2$. The particular values $r = 2$ and $r = 1$ are obtained when $J_b = J_c = 0$ and when $J_a + J_b = J_c$, respectively.

Under the condition given by (B4) the system is performing secondary active transport of L coupled to the passive transport of M in the same direction (i.e., $e \rightarrow i$). Hence, the model of Fig. 10*A* is capable of performing cotransport of L and M and to exhibit different values of the experimental stoichiometric ratio. These depend upon the particular values of the rate constants governing the kinetics. In this way, the model can provide a basis to interpret the mechanistic aspects of many different types of cotransport systems, including some characterized by stoichiometric ratios different than one, such as the Na^+:K^+:2Cl^- or the Na^+:2HCO_3^- cotransporters.

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