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Performance and Conservation of Osmotic Work by Proton-Coupled Solute Porter Systems

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Introduction

According to the chemiosmotic coupling conception of oxidative and photosynthetic phosphorylation systems,[1–3] the hydrogen and electron carriers of the respiratory chain and photoredox chain are looped across the non-aqueous (proton-insulating) M phase of the coupling membrane in such a way that redox activity along the chain is accompanied by the translocation of protons from one side of the membrane to the other, generating a protonmotive force of some 200 to 300 mV between the aqueous (proton-conducting) phases on either side. Thus, the primary physiological function of the respiratory chain and photoredox chain systems of mitochondria, chloroplasts and microorganisms is regarded as the provision of a source of power in the form of the proton current that can be used by appropriate systems plugged through the M phase of the coupling membrane.[4–7]

Owing to the relatively widespread interest of biochemists in the mechanism of reversal of the ATPase reaction in oxidative and photosynthetic phosphorylation systems, and owing to the relative importance attached to the elucidation of the fundamental mechanism of oxidative and photosynthetic phosphorylation,[8–9] the special energetic role of the reversible proton-translocating ATPase as a user of the proton current generated by the respiratory chain and photoredox chain systems has tended to monopolize attention.

In the present paper, my object is to emphasize the physiological importance of proton-coupled solute porter systems, plugged through the coupling membrane, the function of which depends on the use of the proton current to raise or lower the concentration of specific solutes in the inner aqueous phase of the cell or organelle relative to the concentration in equilibrium with that in the outer aqueous phase (or environmental medium). Such systems may obviously be energetically important in microorganisms that grow in dilute nutrient solutions, because the atomic constituents of the media are at a much lower average concentration than that of the cell constituents. Under these

circumstances, nutrient assimilation obviously requires both the chemical work involved in the bond exchanges of intermediary metabolism and the osmotic work involved in bringing the assimilated chemicals from their low concentrations in the media to their relatively high concentrations in the organism. Under other circumstances, when the distinction between the chemical work and the osmotic work involved in chemical synthesis or intermediary metabolism in this type of system is not so obvious, the conservation of osmotic work by the solute porters is nevertheless fundamentally important in the overall efficiency of energy transduction; and it is the purpose of the present article to help to explain how the diffusion reactions of solute translocation and exchange between the two aqueous compartments separated by the coupling membrane participate in the partially reversible reactions of energy transduction. The general methods and nomenclature used here follow those introduced previously.[7,10–12]

Those who are concerned with the strategy of research may find it interesting to note that although the concept of proton-coupled solute translocation in bacteria, mitochondria and chloroplasts was introduced 10 years ago,[1,4,5] and reasons were given for regarding the much-studied but little understood β-galactoside translocation system of *Escherichia coli* as a prototype[5] (see Fig. 1), this simple and unifying concept was not investigated experimentally in the established schools of bacterial membrane transport.[13–16] Meanwhile, students of energy metabolism, who found themselves in the field of membrane-transport more by accident than by design, encouraged by the pioneering work from Chappell's laboratory,[17,18] confirmed the existence of proton-linked porter systems in mitochondria[19–23] and chloroplasts[9,24,25] and began to characterize the solute specificity and stoichiometry of these systems in detail (e.g. refs. 26–30). This work recently began to encourage students of bacterial metabolism to undertake similar studies which, especially in the laboratories of Hamilton[31,32] and Harold[33,34] have yielded results that are fundamentally similar to those obtained with mitochondria. However, some microbiologists, notably Kaback,[35–39] prefer to assume that the coupling between respiratory chain activity and the transport of sugars and amino acids, observed in certain bacteria, is due to hypothetical respiratory chain components that act as specific solute carriers. A penetrating commentary was given by Greville[8] on the strategy of postulating chemical coupling intermediates to produce explanations such as those deployed by Kaback.[35–39] Greville pointed out that, compared with their chemiosmotic counterparts, "such explanations are less precise and more hypothetical, since they involve postulated but undiscovered entities ...". But he went on to suggest that the hypothetical chemical coupling intermediates might nevertheless be spared Occam's Razor because "in these days beards and dialogue are characteristics of youth

and iconoclasm". I detect, however, that beards may now be going out of fashion, especially amongst young students of bioenergetics who prefer their dialogue not to be obscured by extraneous growths. Therefore I propose to sharpen the Razor, as best I can, in the light of recent work in my laboratory.[40]

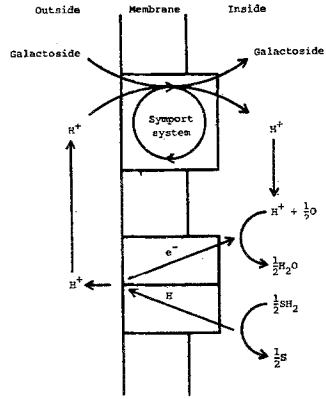


Figure 1. Diagram of cyclic coupling suggested between proton-translocating redox system and a β-galactoside-H^+ symport system in the plasma membrane of *E. coli* (from ref. 5). This diagram does not represent the stoichiometric relationships between the proton current and the redox and β-galactoside flows.

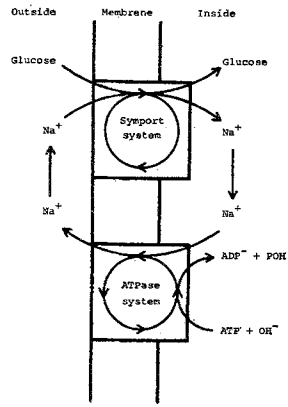


Figure 2. Diagram of cyclic coupling suggested between the cation-translocating ATPase, and the glucose-Na^+ symport system catalysing translocation of glucose across the intestinal mucosa (from ref. 5). The translocation of K^+ by the ATPase is omitted from this diagram for simplicity, and the stoichiometric relationships between the flows are not represented.

Although special attention is devoted to the proton-linked solute porter systems in the present article (because there are reasons for thinking that they may be as ubiquitous as the respiratory chain, the photoredox chain and the proton-translocating ATPase complex, with which they are associated[7]), other solute porter systems, such as that illustrated in Fig. 2 (from ref. 5), are also mentioned

in order to illustrate broadly the general molecular mechanisms whereby the chemicomotive forces of vectorial metabolism are associated with the osmotic forces of solute electrochemical potential gradients. It is emphasized that, as long as certain fundamental properties of thermodynamically natural systems are not lost sight of,[4] the transmission of power through trains of solute molecules interacting by way of specific solute porters may conveniently be understood by regarding the solute porters not as abstract "transducers of energy", but as physically real miniature engines, having specifically articulated parts, designed (by natural selection) to couple the flow of one species of solute particle to that of another.[5]

The availability of several recent reviews on solute porter systems[7, 19–25, 31, 33, 34] makes it unnecessary to catalogue the numerous solute-specific porter activities that have been identified in recent years, and enables me to concentrate attention on a few selected topics.

"Osmotic Coupling" Compared with "Chemical Coupling"

The fact that Wang could recently state in an authoritative treatise on electron transport and oxidative phosphorylation[41] that the coupling mechanisms of the chemiosmotic type that I have proposed are less attractive than mechanisms involving chemical intermediates "because they are electrochemically all equivalent to the inefficient concentration cells" shows that it is still not generally appreciated that specific porter-catalysed osmotic reactions, which involve secondary and ionic bond interchanges across a coupling membrane separating two aqueous phases, may occur as reversibly as the more familiar specific enzyme-catalysed reactions, which involve covalent bond interchanges between chemical reactants and resultants. Therefore, I should like to make it particularly clear that there is no fundamental difference energetically between the reactions usually observed by chemists, which involve covalent bond exchanges, and the reactions usually observed by students of transport, which involve secondary and ionic bond exchanges—provided that appropriate catalysts are available to facilitate and channel the reactions in predetermined ways, minimizing the dissipation of available energy by the effective frictional resistance impeding the flow or interchange of chemicals in the required reactions, and minimizing the escape of chemicals (and available energy) in side-reactions.[12]

According to the rationale of vectorial metabolism and chemiosmotic coupling that I have endeavoured to foster,[4, 5] non-radiative thermodynamic energy transduction can occur only by means of the diffusion of trains or groups of material particles that move (with conserved impulse and momentum) under the influence of forces transmitted by local electrical and chemical interactions between them. Thus, a

biochemically satisfactory understanding of the coupling between solute translocation reactions and the group translocation reactions of metabolism, or between one solute translocation reaction and another, can be obtained relatively simply in terms of the coupling between the flows of the chemical particles thermodynamically involved in these processes, all of which (in the form of the actual thermally mobile species) must move down their electrochemical potential gradients. This formulation of the "energetic coupling" problem is helpful to the biochemist because it explicitly focuses attention on the actual thermally mobile species involved in solute and group translocation and on the coupled flows of solutes and chemical groups channelled by the catalytic carrier and enzyme systems. It is, of course, particularly relevant that the actual thermally mobile species through which the forces and flows of energy transduction are transmitted include conformationally mobile enzymes and catalytic carriers and their substrate or passenger complexes. This was originally impressed upon me while studying a highly specific system catalysing tightly coupled exchange of phosphate for phosphate or of phosphate for arsenate across the plasma membrane of *Staphylococcus aureus*,[42–44] which has recently become of more topical interest because it appears to resemble a system catalysing the net translocation of phosphoric acid across the cristae membrane of mitochondria.[45]

Owing to the sensitivity of the staphyloccal phosphate translocation system to phenyl mercuric acetate, it was possible to estimate a maximum value for the absolute translocation rate and, by appropriate temperature studies, to obtain a minimum value for the entropy of activation, which accounted for at least 17,700 cal/mol in a total heat of activation of 37,400 cal/mol. The kinetics of this system thus resembled that of reversible protein denaturation and suggested that the translocation of phosphate involved a large conformation change in the translocater system in the membrane.[43,44]

The exchange translocation of phosphate for phosphate or of phosphate for arsenate by this system was remarkably tightly coupled, as shown by the fact that the net flux of phosphate (or of phosphate + arsenate) was not normally more than 1% of the exchange flux under non-metabolizing conditions, even when the system was operating well below its apparent dissociation coefficient for phosphate (and arsenate) on the outer side of the membrane.[43,44] It was therefore suggested[46] that, owing to the mutual satisfaction of valencies, the mobility of a translocater across a membrane may be as dependent upon its occupation by the specific passenger species as the translocation of the passenger species across the membrane may be dependent on combination with the specific translocater. I originally assumed that the tight coupling and high specificity of the translocation of phosphate by the staphylococcal system probably required the formation of a covalent compound

with the translocater,[47] but it later became evident that this need not necessarily be the case.[1,5]

The chemiosmotic theory focused attention on the components coupling the flows of particles in the biochemical systems catalysing solute, group and electron translocation.[5] Particular significance was therefore attached to the tentative suggestions by Christiansen[48,49] and by Crane,[50] that the transport of amino acids and sugars might be coupled to the translocation of K^+ ions or Na^+ ions in the opposite or in the same direction respectively by specific carriers in the membrane. The same significance was attached to my own postulate[1] that the membranes of bacteria, mitochondria and chloroplasts contain specific systems catalysing tightly coupled exchange-diffusion of cations against H^+ ions and anions against OH^- ions. These systems evidently represented a new class of translocation catalyst corresponding to the exchange diffusion type of system originally postulated by Ussing[51] and Widdas[52] for coupling the translocation of chemically *analogous* solutes, but differing in that they were supposed to catalyse the sym- or anti-coupled translocation of chemically and sterically *unrelated* solutes, as exemplified by the suggested prototype symport systems for the coupled translocation of sugars with H^+ or Na^+ illustrated in Figs. 1 and 2 (from ref. 5).

In order to promote the precise description of coupled solute translocation reactions, it was suggested that non-coupled solute translocation should be described as uniport* and that anti- and sym-coupled solute translocations should be described as antiport and symport respectively. The general mechanism of catalysis of solute uniport, antiport and symport reactions is illustrated in terms of the operation of a cyclically mobile carrier (or carrier centre,[10] analogous to the active centre of an enzyme), represented by X, in Figs. 3, 4 and 5 (from ref. 5). These diagrams take account of the fact that the solutes involved in specifically catalysed translocation reactions through lipid membranes separating aqueous phases exist as the hydrates (written SW_L, AW_L, BW_L and SW_R, AW_R, BW_R in the left and right aqueous phases respectively) and that translocation across the lipid phase generally requires disengagement of the solute from its hydration shell by exchange of valencies with the carrier (or carrier centre) X, specific thermal mobility of the solute-X complex across the non-aqueous phase of the membrane, and re-engagement of the solute with its hydration shell so that it is released from X on the other side of the membrane.[5] The catalysis of uniport (Fig. 3) arises from the specific mobility of both unoccupied X and of the SX complex across the porter system at given concentrations of S in the aqueous phases. The catalysis of A-B symport (Fig. 5) likewise arises from the specific

* Monoport was originally suggested,[5] but was withdrawn in favour of uniport[10] for the sake of linguistic consistency.

mobility of both unoccupied X and of the inclusive complex ABX across the porter system at given concentrations of A and B in the aqueous phases; but the tightness of coupling depends on the extent to which the flux of A and B passing through the partial complexes AX

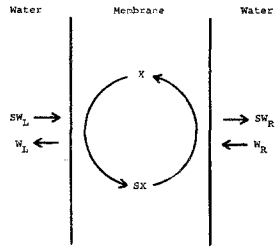


Figure 3. Diagram of uniport system for translocation of molecules or ions S combining with a carrier X in a lipoprotein membrane system between aqueous phases L and R in which S exists as the hydrate SW_L and SW_R (from ref. 5).

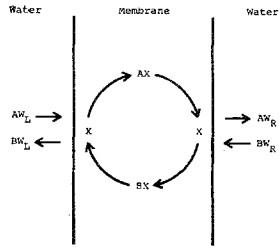


Figure 4. Diagram of antiport system for molecules or ions A and B competing for carrier X in a lipoprotein membrane system between aqueous phases in which A and B exist as hydrates WA and WB. The symbols L and R denote the aqueous phases (from ref. 5).

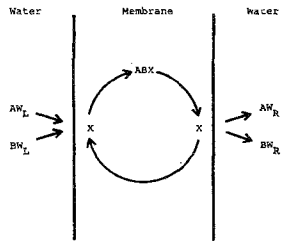


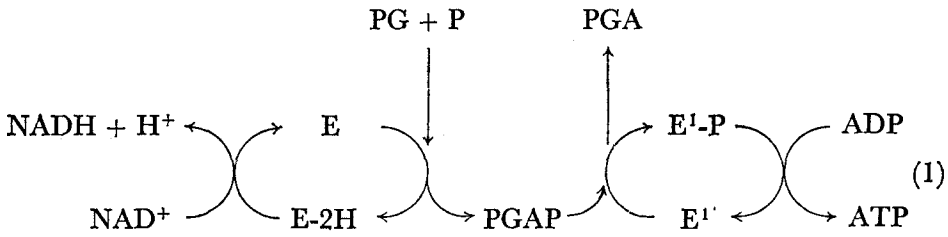
Figure 5. Diagram of symport system for molecules or ions A and B combining synergistically with carrier X. Other conventions as in Fig. 4 (from ref. 5).

and BX across the porter is minimized, compared with the flux of A and B passing through the inclusive ABX complex.[10] In the case of A/B antiport (Fig. 4) catalysis of translocation depends on the specific mobility of both AX and BX; but the tightness of coupling depends on the extent to which the flux of unoccupied X is minimized, compared with the flux of A and B passing through the AX and BX complexes.[10]

The rate of any reaction, such as that catalysed by a symporter or antiporter, along a given diffusion pathway is dependent upon the concentrations of the species actually mobile through the degrees of freedom constituting the pathway; and since, according to the Maxwell–Boltzmann law, the concentration of a given state of a system is relatively small if its potential energy is large compared with the thermal energy kT, it follows that a pathway that it may be evolutionarily desirable to close, because it corresponds to a side-reaction and causes uncoupling (such as that involving unoccupied X translocation in A/B antiport or AX and BX translocation in A-B symport), can be selected against by mutations causing it to correspond to states of the system having relatively high potential energy compared with states along the preferred, tightly-coupled, translocation pathway[12] (such as those involving translocation of AX and BX in A/B antiport or those involving translocation of XAB and X in A-B symport).

My object has been to emphasize, as stated before,[5, 12] that the meaning of ("energetic") coupling in this context is nothing more than the mutual dependence of the flows of A and B due to their passage as exclusive or inclusive complexes (AX and BX or ABX) specifically through the antiporter or symporter system, so that work can be performed by the transmission of forces and flows through the train of molecules of A and B undergoing the coupled translocation through the membrane.

It is instructive to compare the mechanism of "osmotic coupling" described for the symporter of Fig. 5 with the classical substrate-level mechanism of "chemical coupling" associated with 3-phosphoglyceraldehyde oxidation, with which we are, perhaps, more familiar. As discussed elsewhere,[12, 53] the coupling between the oxidation of 3-phosphoglyceraldehyde (PG) to 3-phosphoglycerate (PGA) and the phosphorylation of ADP through the reactions catalysed by 3-phosphoglyceraldehyde dehydrogenase (E) and 3-phosphoglycerate kinase (E^1) can conveniently be summarized by the following abbreviated equation:



(1)

where PGAP stands for 1,3-diphosphoglycerate and E-2H and E^1-P stand for the respective enzyme-substrate-cofactor transitional complexes.

Each cycle of changes in the reactions represented by equation (1) corresponds to the flow of specific chemical groups through pathways determined by the interactions between the substrates and cofactors and the active centre regions of the enzymes E and E^1; and the tightness of coupling between the redox reaction and the phosphorylation reaction depends on the extent to which the movements of the chemical components are prescribed by the closely articulated enzyme-substrate-cofactor complexes so that the uncoupling effects of side-reactions are minimized. In particular, coupling between the production of PGA by the oxidation of PG and the production of ATP by the phosphorylation of ADP is attributable to the coupling between the flow of PGA out of the oxidation reaction and the flow of phosphoryl into the phosphorylation reaction because the PGA and phosphoryl are linked together in the inclusive intermediate PGAP.

There is a strict correspondence between the coupling function of the flow of the intermediate PGAP in the "chemically coupled" reactions of equation (1) and the coupling function of the flow of the intermediate ABX in the "osmotically coupled" reactions of Fig. 5. The fact that PGAP is a chemical compound, in which the components undergoing coupled flow are united by a strong covalent bond (with free energy of activation for uncatalysed exchange or dissociation $\gg kT$), need not produce an effectively tighter coupling in the "chemically coupled" system than can be achieved through the intermediate ABX in the "osmotically coupled" system, because strong bonding may occur between the components of ABX (with free energy of activation for uncatalysed exchange or dissociation $\gg kT$) by the co-operation of a number of secondary bonds. Indeed, components of ABX may well be linked covalently. For example, the net translocation of H^+ with $H_2PO_4^-$ catalysed by the phosphoric acid porter may involve the linkage of H^+ and $H_2PO_4^-$ in the covalent intermediate H_3PO_4. Moreover, the H_3PO_4 undergoing net translocation in the reaction catalysed by the phosphoric acid porter could conceivably be linked covalently to functional groups in the porter.[7,47] As far as the tightness of "energy-coupling" is concerned, it is irrelevant what type of bonding is responsible for prescribing the flow of a given type of particle or what type of bonding causes the flow of one species of particle to be reciprocally dependent upon (coupled to) the flow of another species of chemical particle. The relevant factor is that the bonding prescribing the coupled group transfers or solute translocations involved in the "chemically coupled" or "osmotically coupled" systems should be such as to provide free energies of activation of exchange or dissociation that are large compared with kT, so that thermally activated dissociation or exchange is minimized, and the forces and flows can be efficiently transmitted between the two sets of particles without appreciable slip. Thus, both in enzyme-catalysed group-transfer reactions (or

group-translocation reactions) and in porter-catalysed solute-translocation reactions, the uniqueness of the flow process (of chemical groups or of solutes) that is catalysed, which we customarily describe as the substrate specificity, may be taken to depend on the same type of condensed complex, with certain precisely specified conformational transitions corresponding to the chemical or translocation reaction. It would appear, therefore, that there is no reason for supposing that porter-catalysed solute-translocation reactions need be any less substrate-specific or any less well-coupled to one another than their enzymically-catalysed group-transfer counterparts.[12]

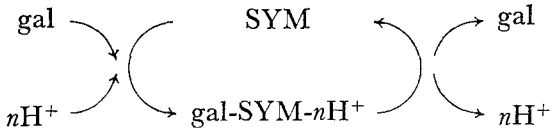
The reader will, I hope, be tolerant of the fact, reflected in this discussion, that the development of the solute porter concept originally owed much to inference from general principles, and tended to proceed somewhat ahead of the more detailed experimental knowledge needed to show that evolution actually exploited the physicochemical options thought to be available. At all events, as mentioned in the introductory section, the recent intensive studies of the highly specific and tightly-coupled proton-linked porter systems catalysing the translocation of anionic and cationic substrates in mitochondria, and studies on similar systems in bacteria and chloroplasts that are beginning to be undertaken by a few pioneers, provide very substantial experimental support for the proton-coupled solute porter concept that I have described as part of the chemiosmotic theory of energy transduction. The statement[41] that the chemiosmotic coupling mechanisms are "electrochemically all equivalent to the inefficient concentration cells" is therefore not a valid passport for the chemist, studying energy metabolism, who is anxious to provide against the accident of being landed inadvertently in the field of membrane-transport.

The Proton-Coupled β-Galactoside Porter System of Escherichia coli *as a Model for Proton-Linked Solute Translocation*

The experimental observations that West and I recently described[40] provide strong support for the proposition[5] that the specific uptake and accumulation of β-galactosides in *Escherichia coli* is primarily catalysed by a porter system, the general principle of which is illustrated in Fig. 1. In this type of system the specific solute porter catalysing the translocation of the solute is conceived as being physically and chemically separate from the proton-translocating respiratory chain system (or the proton-translocating ATPase system or pyrophosphatase system) which provides the proton current required for net solute uptake. Therefore the properties of the proposed β-galactoside-H^+ symporter can be investigated, uncomplicated by metabolic coupling, provided that the experimental conditions are arranged so that there is little or

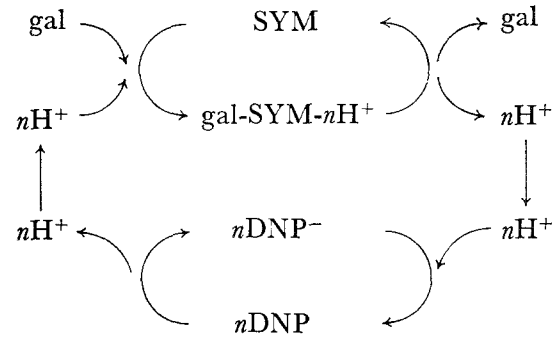
no respiratory or ATPase (or pyrophosphatase) activity. In practice, such conditions are not particularly difficult to achieve.

The β-galactosides, such as lactose, are ideal substrates for studies of proton-coupled translocation because, owing to their lack of net charge and acid/base properties near pH 7, linkage between their translocation and the translocation of H^+ ions across the coupling membrane may be recognized by the corresponding translocation of acid equivalents and positive charge across the membrane, in accordance with the following type of scheme:



(2)

where SYM represents the translocater centre[10] of the proposed β-galactoside-proton symporter, gal stands for β-galactoside, and n is a stoichiometric coefficient. Thus, the translocation of β-galactoside would be coupled to the creation of a pH difference and a membrane potential across the membrane. According to this mechanism, the presence of specific proton conductors, such as DNP or azide, should uncouple the system by permitting the recirculation of H^+ ions back across the membrane, as illustrated by the following equation, in which 2,4-dinitrophenol is represented as the proton conductor in protonated (DNP) and deprotonated (DNP^-) forms:



(3)

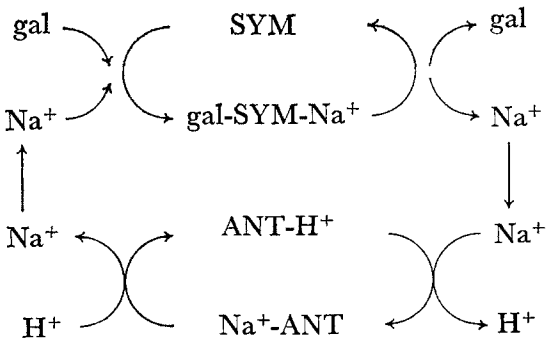
Under these circumstances the β-galactoside should be able to equilibrate freely (although only by the same specific porter) across the membrane—as observed.[54] The proton-coupled mechanism of β-galactoside uptake described by equation (2) was, in fact, originally suggested[5] because it provided such a simple explanation of the observed *specific* equilibration of β-galactoside, in presence of proton-conducting reagents.

The experimental observations of the experimental paper[40] demonstrate the four main coupling relationships between the translocation of H^+ and β-galactoside described by equation (2), as follows:

1. Diffusion of β-galactoside down its concentration gradient by an NEM-sensitive reaction is coupled to the translocation of acid equivalents in the same direction.
2. Diffusion of β-galactoside down its concentration gradient across the membrane by an NEM-sensitive reaction is coupled to the translocation of positive charge in the same direction, and the number of positive charges translocated across is equal to the number of acid equivalents translocated.
3. Diffusion of H^+ ions down a pH gradient across the membrane requires the presence of β-galactoside in an NEM-sensitive reaction.
4. Diffusion of H^+ ions down an electrical potential gradient across the membrane requires the presence of β-galactoside in an NEM-sensitive reaction.

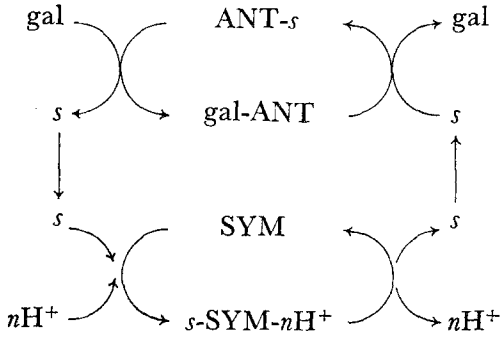
The value of n was not precisely determined, but these and previous observations by West[55] indicate that it is probably 1.

The translocation of β-galactoside through the NEM-sensitive system was found not to be directly coupled to the translocation of certain other ion species, such as Na^+ or K^+, as might have been inferred from Stock and Roseman's speculative interpretation of their experiments[56] showing that melibiose accumulation in actively metabolizing *Salmonella typhimurium* is affected by the presence of Na^+. Therefore, although there might well be a tightly-coupled Na^+/H^+ antiporter in the membrane of *E. coli*[7] as in mitochondria[57] and in *Streptococcus faecalis*,[58,59] the lack of direct coupling between Na^+ (or K^+) translocation and β-galactoside translocation showed that there was no β-galactoside-Na^+ symporter or β-galactoside-K^+ symporter and eliminated the following type of possibility:



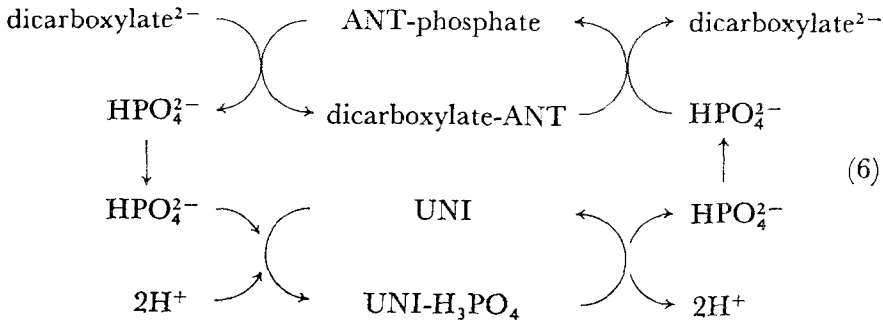
(4)

It was still conceivable, however, that some other intermediary solute (s) could have been involved in the observed coupling between β-galactoside translocation and proton translocation according to the following general type of mechanism:



(5)

In this type of mechanism, β-galactoside translocation would initially involve a specific β-galactoside/s antiporter, and coupling with proton translocation would involve a specific s-nH$^+$ symporter. This possibility deserves serious consideration because it corresponds to a system known to be responsible for proton-linked dicarboxylate uptake in mitochondria, which depends upon the cyclic involvement of phosphate, according to the following type of process:



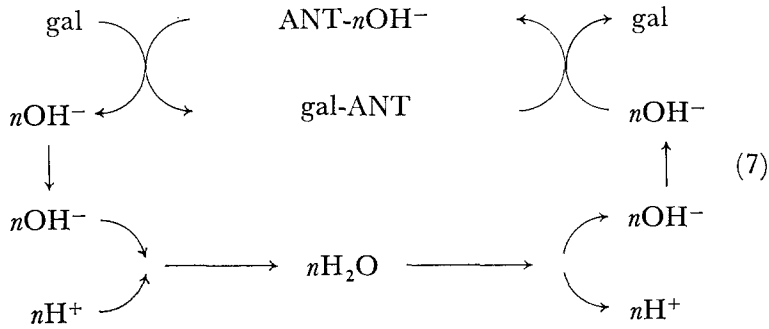
(6)

In this case, it has been shown that a specific dicarboxylate/phosphate antiporter catalyses the strictly coupled antiport represented by the upper part of equation (6), while a separate phosphoric acid uniporter with translocater centre represented by UNI (or a corresponding phosphate/hydroxyl ion antiporter) catalyses the net phosphate-proton symport represented in the lower part of the equation.[19,26,27,60] It is

noteworthy that the mitochondrial dicarboxylate/phosphate antiporter (in which I have represented the translocater centre as -ANT-) has separate binding sites for phosphate and dicarboxylate although there is a functional linkage that prevents both being occupied simultaneously.[60] Thus, there is an experimentally well-documented precedent for the suggestion that the observed proton-linked β-galactoside translocation could proceed via an antiport reaction with a chemically unrelated solute *s*, as represented in equation (5).

As our experiments[40] were done with suspensions of whole bacteria (which contained considerable quantities of endogenous solutes including phosphate), they were not helpful in eliminating possible anionic candidates for the role of *s* in equation (5). However, Kaback and co-workers[35–37] studied the specific β-galactoside translocation process in membrane vesicles from *E. coli* under conditions where the endogenous solutes were largely removed. Since he observed good respiration-linked β-galactoside uptake in the virtual absence of endogenous solutes, but routinely used a phosphate buffer, we can eliminate all except phosphate and hydroxyl ion from our considerations of a possible solute intermediary, *s*, in the observed proton-linked β-galactoside translocation.

As shown in the following equation:



(7)

the possible involvement of the OH^- ion in the proton-coupled β-galactoside translocation through a β-galactoside/hydroxyl ion antiporter is a somewhat special case because lipid membranes have a relatively high permeability to H_2O.

In general, hydroxyl ion antiport is difficult to distinguish from proton symport because, as illustrated by equation (7), the net (proton-coupling) effect of solute/OH^- antiport is the same as that of solute-H^+ symport, because no additional solute porter is required for H_2O translocation. As discussed elsewhere,[2, 11, 12] it is conceptually convenient, for this reason, to describe all porters catalysing proton or hydroxyl ion symport or antiport as proton-coupled. But it is, neverthe-

less, biochemically important to distinguish between the alternative intermediary mechanisms of achieving the overall proton-coupled translocation through porters (or solutes) reacting with H^+ ions or OH^- ions.

To summarize: the experimental evidence at present available supports the proposition that specific β-galactoside translocation is coupled to net proton translocation by a proton-coupled porter system, which could be a β-galactoside-proton symporter, a β-galactoside/OH^- antiporter, or a β-galactoside/phosphate antiporter together with a phosphate-proton symporter.

The uptake of β-galactoside through the plasma membrane of *E. coli* is normally linked to respiratory activity in whole cells;[14] and the recent important experimental work of Kaback and co-workers[35–37] shows that it is also linked to the activity of the respiratory chain in membrane vesicles from which most of the soluble cytoplasmic solutes have been discharged. Therefore, there can no longer be any reasonable doubt that, as indicated, for example, by earlier observations of Kashket and Wilson[61] and of Pavlasova and Harold,[61] the specific uptake of β-galactoside is not directly coupled to the metabolism of ATP or other known metabolic intermediates, such as phosphoenolpyruvate. What, then, is the mechanism of coupling between redox activity through the respiratory chain and the translocation of β-galactosides through the specific catalytic system which is well known to catalyse β-galactoside equilibration across the membrane in presence of proton-conducting reagents,[14] and which we have characterized[40] as a catalyst of net β-galactoside-proton symport?

During respiratory chain activity in *E. coli*, as in other bacteria[4, 63, 64] and as in mitochondria,[66–68] protons are translocated outwards through the membrane. This has been observed, not only in intact *E. coli*,[40] but also in the membrane vesicle type of preparation used by Kaback and co-workers to demonstrate the respiration-linked uptake of β-galactoside and other solutes.[69] It is particularly noteworthy that Reeves[69] found that the proton translocation associated with respiration was directed outwards across the vesicle membrane, as our observations subsequently showed[40] was the case in the intact bacteria. These experimental observations directly support the proton-coupled chemiosmotic type of mechanism of β-galactoside uptake described, in principle, in Fig. 1—irrespective of the detailed biochemical mechanism of the proton-translocating respiratory chain and of the proton-coupled β-galactoside porter system.

According to Kaback and co-workers,[35–38] the transport systems in the isolated membrane vesicles of *E. coli*, which catalyse the concentrative uptake of β-galactosides, a wide variety of amino acids, galactose, arabinose, glucuronate, glucose-6-phosphate, manganese and potassium (in presence of valinomycin) are each coupled primarily to a

membrane-bound flavin-linked D-lactic dehydrogenase; and the site of energy coupling is between the primary dehydrogenase and cytochrome b_1. It was further suggested[36,38] that the simplest conception that accounts for the experimental data is a model requiring that the β-galactoside-specific M protein, and the transport-specific "carriers" for the other solutes listed, are electron transfer intermediates, which undergo reversible oxidation-reduction, between D-lactic dehydrogenase and cytochrome b_1. Thus, for each transport system there should be a redox component between D-lactic dehydrogenase and cytochrome b_1, which has a binding site for that particular transport substrate. As with many similar theories of respiratory "energy-coupling" presented in the past,[8,20,22] no direct biochemical evidence for the proposed intermediates or for their special affinities and specific mechanism of participation in the energy-transduction process was provided.

One of the most significant observations in the extensive and painstaking measurements of Kaback and co-workers was that the proton-conductors DNP and carbonyl cyanide *m*-chlorophenylhydrazone caused rapid efflux of accumulated β-galactosides (and other specifically accumulated solutes) from the membrane vesicle preparations,[35–38] as from intact bacteria. But it was not explained how these reagents were supposed to affect the hypothetical redox reactions or specific carrier functions of the proposed energy-transducing intermediates in the respiratory chain system between D-lactic dehydrogenase and cytochrome b_1. Likewise, no explanation was given for the observation that the specific K^+-conductor valinomycin inhibited β-galactoside uptake by the vesicles in K^+-containing media; nor was it explained how valinomycin was supposed to participate in the hypothetical K^+-pumping units of the respiratory chain complex between D-lactic dehydrogenase and cytochrome b_1.

With regard to the interpretation of Kaback and co-workers that the translocation of β-galactoside coupled to the respiratory chain is dependent on a particular "site of energy coupling" between D-lactic dehydrogenase and cytochrome b_1, the experimental observations of Barnes and Kaback[35] showed that, with various respiratory substrates, the rates of lactose translocation into the vesicles did not run parallel to the rates of oxidation of the substrates; and it was stated that D-lactate was the more effective substrate. However, it is instructive to compare the quantitative effectiveness of the different substrates that were used in terms, not only of the rate of lactose uptake relative to that observed with D-lactate, but also in terms of the ratio (lactose uptake rate)/(oxygen uptake rate) for each substrate compared with the same ratio with D-lactate. These two indices calculated, as percentages of the values for D-lactate, from the data of Barnes and Kaback,[35] are as follows, the latter index being put in brackets:

D-lactate, 100 (100); DL-α-hydroxybutyrate, 58 (264);

succinate, 40 (24); L-lactate, 40 (146); NADH, 6·7 (8·2).

With vesicles prepared from bacteria grown on complex media, the rate of lactose uptake given by oxidation of α-glycerophosphate or formate was described as being about the same as with succinate—i.e. about 40% of the rate with D-lactate. It is noteworthy that fairly good β-galactoside uptake rates (i.e. not less than 40% of the rate with D-lactate) were given by all the substrates listed except NADH; and that when the respiratory rates with each substrate were taken into account, as shown by the bracketed numbers, DL-α-hydroxybutyrate and L-lactate were 264 and 146% as effective respectively as D-lactate. Therefore it is difficult to see why D-lactate, or the part of the respiratory chain associated with D-lactate oxidation, should be singled out as having a special relationship with the system catalysing β-galactoside translocation. The unreality of this interpretation is further emphasized by the fact that in studies on vesicles from *Staphylococcus aureus* Short, White and Kaback[39] were led to the conclusion that "amino acid transport in *S. aureus* is catalysed by mechanisms similar to those found in *E. coli* with the exception that α-glycerol phosphate, rather than D-lactate, is the primary electron donor".

The exceptionally low rate of β-galactoside uptake observed during NADH oxidation by the vesicles from *E. coli*[35] is not surprising, and would indeed be expected, because it is known that while the other "substrates" listed (see above) normally gain access to the respiratory chain system of intact bacteria from the outer medium and are normally oxidized rapidly, this is not the case with NADH. Access of NADH to the sites of the NADH dehydrogenase on the inner side of the membrane, where it is normally oxidized, presumably requires evertion, lysis or partial disorganization of the membrane. Thus, the considerable rate of NADH oxidation observed in the *E. coli* membrane vesicle preparations should be attributed to everted or disorganized membranes; and NADH would not be expected to act as a good "substrate" for proton-translocating respiratory chain activity capable of producing the outwardly-directed translocation of protons and the resultant inwardly-directed protonmotive force needed to drive β-galactoside uptake into vesicles of the same polarity as the intact cells.

Whatever may be the detailed biochemistry of the proton-translocating respiratory chain and of the proton-coupled β-galactoside porter system in *E. coli*, the observed activities of these two systems provide the basis for the simple chemiosmotic type of mechanism of β-galactoside accumulation illustrated in principle in Fig. 1, and discussed in more detail in a recent review.[7] This type of mechanism enables us to account for the specific uncoupling action of the proton-conducting reagents, such as DNP, in terms of the known specific

biochemical property of these reagents. Likewise, the inhibitory effect of valinomycin (in K^+-containing media) on β-galactoside uptake can be explained by its known biochemical property of conducting K^+ ions across the membrane and collapsing the electric potential component of the protonmotive force driving β-galactoside uptake.

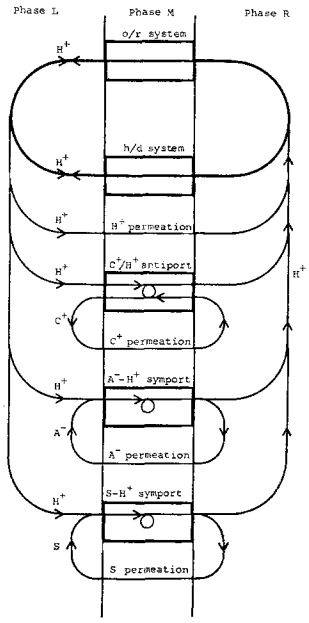


Figure 6. Composite proton circuit diagram illustrating coupling between metabolism and transport as it is thought to occur in mitochondria and in certain prokaryotic cells (after refs. 2, 5 and 7). Translocation of protons through oxido-reduction (o/r system) is shown poised against proton translocation through the reversible ATPase (h/d system). For simplicity, the oxidoreduction reactants for the o/r system and the hydrodehydration reactants (i.e. ATP, ADP, POH and H_2O) for the h/d system have been omitted from the diagram. Dissipation of part of the proton current occurs by the translocation of H^+ through substrate-specific antiporter systems for certain cations C^+ (e.g. Na^+) and symporter systems for certain anions A^- (e.g. phosphate or Krebs cycle acids) and neutral substrates S (e.g. sugars or neutral amino acids). Some of the proton-coupled porter systems may be complex, as explained in the text. The rate of dissipation of the proton current through the porter-coupled reactions in the steady state is dependent on anion, cation and neutral substrate permeation as indicated. The symbols A^- and C^+ do not denote the valency of the anions and cations and the stoichiometry of translocation is not indicated for A^-, C^+ or S.

The explanations given by Kaback and co-workers[35–39] to justify a chemical coupling mechanism of respiration-linked uptake of β-galactosides and many other solutes in *E. coli* and in other bacteria require complex *ad hoc* assumptions which have no direct biochemical foundation. The proposed chemically coupled model provides only a relatively vague account of the "energy-coupling" mechanism in the respiratory chain and does not satisfy the criterion of relative simplicity which Kaback and co-workers have themselves laid down.[36] Therefore I suggest that, since the edge of Occam's Razor has been sharpened,[40]

so that it can leave a clean chemiosmotic profile,[7] we should apply Occam's Razor to the superfluous growth of hypothetical energy-transducing solute-carrying chemical intermediates attributed to the respiratory chain system.

Figure 6 shows a composite proton circuit diagram (from ref. 7) representing the cation/proton antiport (C^+/H^+ antiport) and anion-proton symport (A^--H^+ symport) systems of mitochondria[2] and the S-H^+ symport system catalysing the proton-coupled translocation of the non-ionic solute S which is based on the β-galactoside-proton symport system of *E. coli*.[5] It should be noted that phase L corresponds to the outer aqueous medium and phase R to the enclosed aqueous phase of the vesicles of the chemiosmotically coupled system. In the light of the foregoing discussion I would re-emphasize[7] the usefulness of exploring the possibility that the chemiosmotic theory, illustrated by the diagram of Fig. 6, may be found to be generally applicable to bacteria as well as to mitochondria and chloroplasts. The upper part of Fig. 6 represents the primary group-translocation systems (the respiratory chain or photoredox chain, and the ATPase or pyrophosphatase) coupling proton translocation to oxidoreductive (o/r) and hydrolytic or hydrodehydrative (h/d) metabolism. The lower part of the diagram illustrates the use of the proton current: (1) for the *extrusion* of certain cations C^+, such as Na^+ and Mg^{2+}; (2) for the *uptake* of certain anions A^-, such as phosphate, sulphate, acidic amino-acids and Krebs cycle acids; and (3) for the *uptake* of neutral substrates S, such as sugars and neutral amino-acids. The symbols A^- and C^+ in the diagram do not denote the valency of the anions and cations, and the stoichiometry of proton-linked translocation is not indicated for A^-, C^+ and S. The dependence of the poise of such proton-coupled solute porter systems on the stoichiometry and electrogenicity of the overall translocation reaction at given values of the electric component

$$\Delta\psi = \psi_L - \psi_R$$

and the chemical component

$$-Z\Delta pH = -(2{\cdot}303\,RT/F)(pH_L - pH_R)$$

of the total protonmotive force* Δp has been described in detail elsewhere,[11, 12] and is discussed briefly in the next section. Meanwhile it is noteworthy that the *non-electrogenic* classes of C^+/H^+ antiport and A^--H^+ symport reactions are particularly important both in practice and in theory, and that the *electrogenic* S-H^+ symport reactions which constitute the other most important class of translocation reactions should be taken to include, not only the translocation reactions causing uptake of non-ionic solutes, such as sugars, but also those causing uptake of solutes such as basic amino-acids that can normally exist as cations because of protonation of an electrically neutral species.

* The total protonmotive force Δp is conventionally reckoned in mV, Z then being about 60 at 25°.

For simplicity of presentation, Fig. 6 illustrates the coupling of the overall translocation reactions with the proton current and does not include the intermediary circulating solutes and their specific antiporters, such as phosphate and the specific solute/phosphate antiporters, and L-malate (see equation (6)) and the specific L-malate/tricarboxylate antiporter, known to couple the translocation of certain solutes with the translocation of protons in mitochondria.[7,19,26–29] As a matter of fact, circulating intermediaries, such as phosphate, were first suggested in the case of translocation reactions in bacteria,[47] but it is not yet known to what extent they may be involved in bacterial systems. These circulating intermediary solutes do, of course, affect dissipation of the proton current by non-specific permeation of the intermediaries through the M phase of the membrane, in addition to the dissipation indicated in the case of the species C^+, A^- and S in Fig. 6; but they do not affect the stoichiometry of the overall reactions under tightly coupled conditions—i.e. when the proton-current dissipation through the non-specific permeation reactions is a small fraction of the total proton current.

A recent discussion of the Na^+-linked solute translocation reactions in the small intestine by Semenza[70] implies the existence of a sodium circuit system similar to the proton circuit system described by Fig. 6.

It is, perhaps, particularly appropriate that the β-galactoside-proton symport system of *E. coli* should be recognized as a model for the further extension of knowledge of solute porter systems in bacteria because, as Kepes[14] has pointed out, the β-galactoside transport system of *E. coli* has received intensive study in the past and has come to be considered as a "classical" type of system, especially with regard to its genetic determination, which has been investigated with much ingenuity, following the initiative of the Paris school.[71]

The discussion of energetic aspects of proton-coupled solute porter systems in bacteria is made more interesting by the fact that the initial step in the metabolism of glucose (and some other sugars) in *E. coli* and other bacteria depends on a phospho-enolpyruvate phosphotransferase system, discovered by Kundig, Roseman and co-workers,[72,73] which catalyses the accumulation of glucose-6-phosphate (and other corresponding phosphorylated sugars) in the inner aqueous phase.[16,72–78] Evidence has been obtained in support of the proposition that the sugar-specific phosphotransferase component (called Enzyme II) of this system catalyses the translocation of the "glucose-6-" group (or other corresponding specific sugar group) inwards across the membrane where it is accepted by a phosphoryl (or phosphate) group from an intermediary phosphoryl-carrier protein (called HPr) and accumulates as glucose-6-phosphate (or other specific sugar phosphate) in the inner aqueous phase. Thus, Roseman[73] drew the conclusion "that most sugars penetrate bacterial membranes by group translocation,

mediated by the respective Enzyme II for each sugar. The sugar is phosphorylated as it is transported, and the phosphate is derived from phospho-HPr". After further intensive studies in several laboratories, a detailed mechanism was suggested by Kaback[16] with the object of explaining how "a *vectorial phosphorylation* of the sugar would be accomplished, producing a group translocation type of transport system"; and arguments were advanced by Kaback[16] in favour of general mechanisms of this type.

The mechanism of sugar-group translocation suggested by Roseman[73] and by Kaback[15,16] apparently corresponds, in general principle, to the type of system described by Fig. 7.[79] For the case of "glucose" uptake by the phospho-enolpyruvate phosphotransferase system, E would represent the glucose-specific phosphotransferase

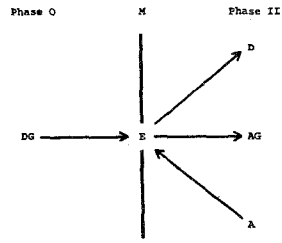
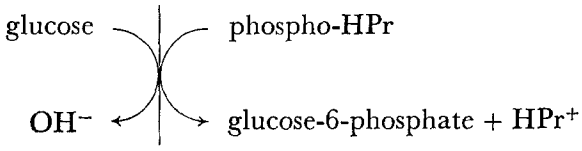


Figure 7. Diagram of group translocation system for the group G donated by the group D and accepted by the group A. The aqueous phases O and II are separated by the membrane M in which the group-transfer enzyme E is orientated so that DG can only communicate with the active centre from phase O, and D, AG, and A can only communicate from phase II (from ref. 79).

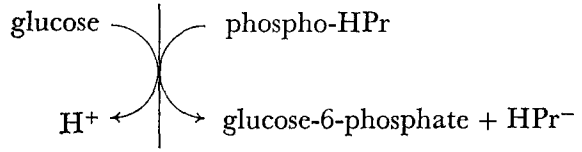
Enzyme II, DG would represent glucose (made up of a donor group D and the "glucose-6-" group undergoing transfer), and the acceptor group A would stand for the phosphoryl group (or phosphate group) of phospho-HPr which accepts the "glucose-6-" group and forms AG, representing glucose-6-phosphate. It has not so far been made clear, however, in the case of the phospho-enolpyruvate phosphotransferase system, precisely what sugar group is supposed to be translocated in the overall reaction or what other group or groups may also be translocated at the same time. Using the description "glucose-6·" to refer to the electrically neutral group remaining after removal of one OH group from the carbon atom in position 6 of glucose, the question arises, referring to Fig. 7, which of the following possible pairs of constituent groups might, for example, represent G and D: glucose-6· and OH·; glucose-6$^+$ and OH$^-$; glucose-6-O· and H·; glucose-6-O$^-$ and H$^+$. According to the system shown in Fig. 7, both the component groups G and D of the substrate DG would be translocated across the membrane, and the overall translocation reaction (i.e. of glucose) would not be affected by the above alternative possibilities. However,

according to other group-translocation schemes that have been considered,[5] the group G might be translocated from left to right, while the group D passed back to the left. This latter type of group translocation reaction may be of practical interest, particularly if the reaction splitting the substrate DG into its constituent groups proceeds heterolytically,[5] i.e. if, for glucose translocation, G and D were, for example, to represent glucose-6^+ and OH^- or glucose-6-O^- and H^+.

The processes corresponding to glucose-6^+ translocation or glucose-6-O^- translocation can conveniently be described in the following notation, introduced for this purpose:[5]

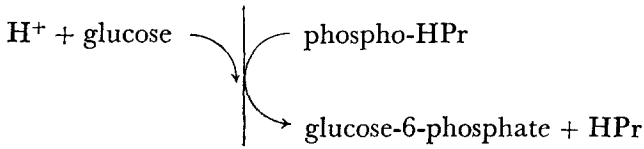


(8)



(9)

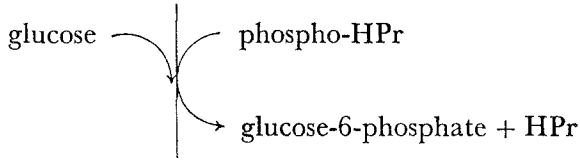
These possibilities, which, it will be noted, include the equivalent of (electrogenic) proton translocation across the membrane, depend on the chemical potentialities of the substrate DG (glucose), of the acceptor A (phosphoryl or phosphate) and of the carrier of A (HPr). There are additional possibilities of solute translocation, that may be associated with group translocation, depending on ligand-binding properties of E—by analogy with the binding and translocation of Na^+ and K^+ in the $3Na^+/2K^+$ antiporter ATPase during hydrolysis of ATP. The following hybrid group-translocation and solute-translocation type of reaction would not, for example, be inconceivable in view of the precedent set by the $3Na^+/2K^+$ antiporter ATPase:[5, 7, 10, 12]



(10)

The foregoing considerations are intended to illustrate two main points. The first is that the possible group-translocation process involved in the phospho-enolpyruvate phosphotransferase system

requires further analysis in order to define it explicitly and unequivocally. The second is that, in view of the existence of a considerable electric potential and pH difference across the membrane of certain (if not all) bacteria, the energetics of the putative sugar group translocation reactions would be dependent upon their possible electrogenicity and protonogenicity illustrated, for example, by equations (8) to (10). In particular, it is interesting to note that the reaction shown in equations (8) or (10) would be equivalent energetically to the two-stage process involving: (i) the uptake of the sugar in a sugar-H^+ symport reaction; and (ii) the phosphorylation of the sugar by a phosphotransferase working at the same phosphate potential as that of the phospho-enolpyruvate phosphotransferase system. It is also noteworthy that if the phosphotransferase were physically positioned on the inner side of a glucose uniporter in the plasma membrane, so that glucose molecules gaining access by the uniporter would enter a closed microscopic phase leading only to the active centre of the phosphotransferase, the following reaction, equivalent to that shown in Fig. 7 would result:



(11)

Alternatively, if there were the same type of association between a glucose-H^+ symporter and the phosphotransferase, the reaction of equation (10) would result. These possibilities illustrate the close interrelationships that are possible between enzymes catalysing group translocation and porters catalysing solute translocation,[79] especially since entry of a substrate to the active centre of an enzyme may be through a region of the polypeptide complex of the enzyme that acts like a solute porter.

Energy Transduction by Solute Porters

When I postulated the occurrence of systems catalysing anion/OH^- antiport (or anion-H^+ symport) and cation/H^+ antiport in organelles catalysing oxidative and photosynthetic phosphorylation by a chemiosmotic type of mechanism,[1] I had in mind that such "exchange diffusion" systems were required to maintain osmotic stability by using the pH component $-Z\Delta pH$ of the total protonmotive force for translocating the anions and cations back across as fast as they tended to diffuse through the relatively ion-impermeable M phase of the coupling membrane under the influence of the electric potential component $\Delta\psi$ of the total protonmotive force. Thus, these proton-coupled solute

porters were conceived as catalysing electrically neutral overall translocation reactions, in which the net work done would be the balance of the work done by the hydrogen ions moving down their chemical potential gradient represented by $-Z\Delta pH$, and that done on the solute anions or cations being moved up their corresponding chemical potential gradient.[1,2,3,10–12] Using the "p" notation, as in pH, to denote $-\log_{10}$ (chemical activity) of any solute, the equilibrium distribution of anion A^{n-} or cation C^{n+} achieved only through electrically neutral A^{n-}-nH^+ symport or C^{n+}/nH^+ antiport respectively is as follows:

$$-\Delta pA = n\Delta pH \tag{12}$$

$$\Delta pC = n\Delta pH \tag{13}$$

where Δ means the value of the given variable in phase L (or outside the vesicle) minus that in phase R (or inside the vesicle). On the other hand, the equilibrium of the same anion and cation species achieved only through diffusion of the ions A^{n-} and C^{n+} across the M phase is given by the Nernst or Donnan equilibrium relationship as follows:

$$-\Delta pA = n\Delta\psi/Z \tag{14}$$

$$\Delta pC = n\Delta\psi/Z \tag{15}$$

The total protonmotive force Δp is given by

$$\Delta p = \Delta\psi - Z\Delta pH \tag{16}$$

Thus, when the total protonmotive force Δp is zero,

$$\Delta pH = \Delta\psi/Z \tag{17}$$

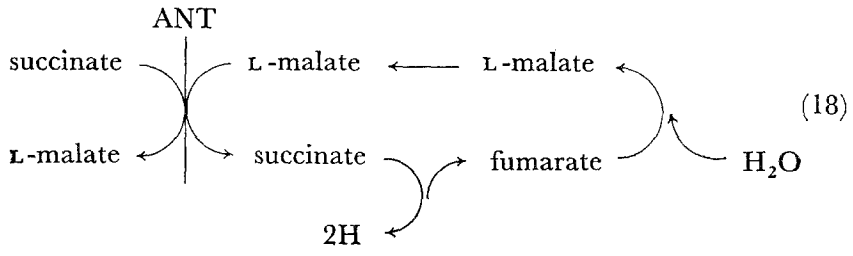
and it will be noted, by substituting $\Delta\psi/Z$ for ΔpH in equations (12) and (13), that under this special condition, when Δp is zero, the distribution of the anionic and cationic species A^{n-} and C^{n+} is independent of whether A^{n-} and C^{n+} equilibrate across the M phase by electrogenic ionic diffusion, or by non-electrogenic A^{n-}-nH^+ symport or C^{n+}/nH^+ antiport. However, comparing equations (12) with (14) and equations (13) with (15), it can be seen that when Δp is not zero, the species A^{n-} and C^{n+} circulate across the M phase, passing one way (as the ions) down their ionic electrochemical potential gradient and the other way down the chemical potential gradient of the A^{n-}-nH^+ or C^{n+}/nH^+ porter complexes, as illustrated in Fig. 6.

As mentioned above, this circulatory process was conceived as having a regulatory function. In mitochondria and bacteria (and possibly also in whole chloroplasts) where the pH of the inner aqueous phase containing metabolic enzymes must be regulated at a point not far from neutrality, the C^{n+}/nH^+ antiport systems deplete the $-Z\Delta pH$ component of Δp by lowering the internal concentration of cations other than H^+ and thereby maintain a large proportion of Δp in the form of $\Delta\psi$ and minimize osmotic swelling. A high Na^+/H^+ antiport

activity has been observed in mitochondria[57,80] and in *Streptococcus faecalis*,[59] and a relatively low K^+/H^+ antiport activity has also been observed in mitochondria.[57] The relative abundance of K^+ compared with Na^+ in mitochondria and bacteria may well be attributable to the lower rate of K^+/H^+ antiport.[7,59,81] The circulation of the anionic solutes may also be considered to contribute to the regulatory function described for the cations because the A^{n-}-nH^+ symport systems deplete the $-Z\Delta pH$ component of Δp by raising the internal concentration of anions other than OH^- and thus help to maintain a large proportion of Δp in the form of $\Delta\psi$. The relationship shown in equation (12), which is characteristic of electrically neutral anion-proton symport, has been nicely confirmed by Palmieri and co-workers[82,83] in the case of phosphate and phosphate-linked Krebs cycle anions in mitochondria; and extensions of these important studies indicate that this relationship applies also to other anions undergoing proton-linked translocation.[21,26–30]

Regarded in the regulatory context discussed here, the anion and cation circulation would not be expected to constitute a major pathway of energy transduction, because the permeability of the M phase to the anion and cation species normally involved in these circulations is low, and only a small proportion of the total proton current available from metabolism would be dissipated.[2,3,11] In this context the proton-linked porter systems—especially the C^{n+}/nH^+ antiporters—may be regarded as being analogous to bilge pumps on a ship.

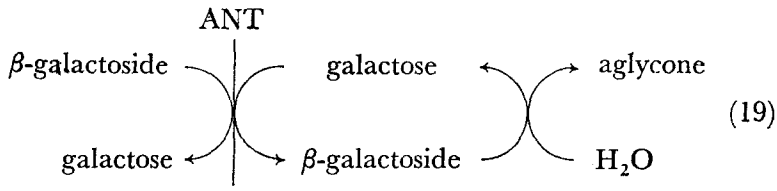
Apart from their regulatory role, the non-electrogenic A^{n-}-nH^+ symport systems fall into the class of proton-coupled substrate uptake systems of which the β-galactoside-proton symporter is the prototype.[5] Osmotic work must, of course, be done during the uptake and concentration of substrates by the proton-coupled porter systems. However, as discussed earlier in a more general context,[47] and as pointed out by Chappell[19] in the case of specific succinate/L-malate antiport accompanying succinate oxidation in mitochondria, the osmotic work required to bring in a substrate may be done by the exit of its metabolic product, thus:



(18)

where ANT denotes the succinate/L-malate antiporter in the M phase.

Observations on the kinetics of β-galactoside translocation and hydrolysis in *E. coli* reviewed by Kepes[14] indicate that the β-galactoside porter system can catalyse β-galactoside/galactose antiport, thus:



(19)

In this case, since a separate system for galactose translocation has been identified, which has similar properties to the β-galactoside-proton symport system,[13–16, 38, 71] the β-galactoside/galactose antiport reaction might be catalysed either by the β-galactoside-specific component of the β-galactoside-proton symport system, or by this component coupled by the proton current to a similar galactose-specific component of the putative galactose-proton symport system. At all events, the analogy between equations (18) and (19) is physiologically interesting because it indicates a general mechanism by which the proton-coupled porter systems can act as osmotic work transducers, enabling osmotic work to be transferred from an end-product passing down an osmotic gradient to a required metabolite passing up an osmotic gradient. This compensatory type of process would not be expected to operate in the same way, however, under conditions of rapid assimilation, as it would during steady-state (or near steady-state) metabolism; and in this respect, considerable differences may be anticipated between bacterial solute porter systems and the corresponding solute porter systems in mitochondria and chloroplasts.

The relationship between the distribution of the solute S and the components of the total protonmotive force when S equilibrates across the membrane only by S-nH$^+$ symport is as follows:

$$-\Delta \mathrm{pS} = n(\Delta \mathrm{pH} - \Delta\psi/Z) \qquad (20)$$

Thus, whereas the greater part of the work done by the proton current in carrying an anion inwards through an electrically neutral A^{n-}-nH$^+$ symport system is used in overcoming the electric repulsion of the anion by the membrane potential $\Delta\psi$ (negative inside), and only a small fraction of the work is left to produce a higher concentration of the anion inside than outside (see equation (12)), no electric work is done in carrying the electrically neutral solute S inwards through the electrogenic S-nH$^+$ symport system, and comparatively very large concentration ratios of S may therefore occur if equilibrium is reached. For example, if $n = 1$ and Δp is 240 mV, the solute S would be about 10,000 times more concentrated in the inner aqueous phase than in the

outer medium at equilibrium. Actual concentration ratios of 2000 times and 10,000 times have been recorded for DNP-sensitive lactose[84] and galactose[54] uptake respectively in respiring *E. coli*. These relatively enormous concentration ratios would be expected to cause osmotic swelling that would lead to damage or lysis of the membrane unless the external concentration of the solute being accumulated was less than 100 μM. In the light of these considerations it seems significant that the respiratory stimulation observed by Kepes[14] in suspensions of *E. coli* on adding 1 mM phenyl-β-D-thiogalactoside or 0·5 mM methyl-β-D-thiogalactoside did not last only during uptake of the β-galactoside, but continued, and was thus characteristic of an uncoupling phenomenon.

To conclude, it is interesting to note that the proton-coupled porter systems may participate significantly in the overall energy transduction process required for assimilation and organic synthesis, because, as the proton-translocating ATPase system probably translocates $2H^+$ ions per ATP molecule hydrolysed,[8] the osmotic and electric work that can be done towards the assimilatory process in proton-coupled solute translocation is equivalent to half the free energy of hydrolysis of ATP per proton used in the solute translocation reaction.

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