J. Membrane Biol. 65, 155–174 (1982)

The Journal of
Membrane Biology

Topical Review

Electrogenic Proton Transport in Epithelial Membranes

Philip R. Steinmetz* and Olaf S. Andersen†

Departments of Medicine, University of Connecticut School of Medicine, Farmington, Connecticut 06032,
and University of Iowa College of Medicine, Iowa City, Iowa 52242,
and †Department of Physiology and Biophysics, Cornell University Medical College, New York, New York 10021

Summary. Certain polar epithelial cells have strong transport capacities for protons and can be examined *in vitro* as part of an intact epithelial preparation. Recent studies in the isolated turtle bladder and other tight urinary epithelia indicate that the apical membranes of the carbonic anhydrase-containing cell population of these tissues contain an electrogenic proton pump which has the characteristics of a proton-translocating ATPase. The translocation of protons is tightly coupled to the energy of ATP hydrolysis. Since the pump translocates protons without coupling to the movement of other ions, it may be regarded as an "ideal" electrogenic pump. The apparent simplicity of the functional properties has led to extensive studies of the characteristics of this pump and of the cellular organization of the secondary acid-base flows in the turtle bladder. Over a rather wide range of electrochemical potential gradients for protons ($\Delta\tilde{\mu}_H$) across the epithelium, the rate of H^+ transport is nearly linear with $\Delta\tilde{\mu}_H$. The formalisms of equivalent circuit analysis and nonequilibrium thermodynamics have been useful in describing the behavior of the pump, but these approaches have obvious limitations. We have attempted to overcome some of these limitations by developing a more detailed set of assumptions about each of the transport steps across the pump complex and to formulate a working model for proton transport in the turtle bladder that can account for several otherwise unexplained experimental results. The model suggests that the real pump is neither a simple electromotive force nor a constant current source. Depending on the conditions, it may behave as one or the other.

Key words proton pump · electrogenic pump · H^+-ATPase · turtle urinary bladder · epithelial transport · active transport

1. Introduction

Many epithelial membranes have the capacity to transport H^+ or OH^- ions. Gastrointestinal and urinary epithelia, in particular, contain a variety of ion pumps and exchangers which bring about acidification or alkalization of the luminal contents. Protons may be translocated by electroneutral processes, such as the passive H^+/Na^+ counter transport which occurs in "leaky" epithelia, notably the small intestine and the proximal tubule of the kidney (Murer, Hopfer & Kinne, 1976), or by an exchange pump such as the electroneutral H^+, K^+-ATPase proposed for the gastric mucosa (Sachs, Spenney & Lewin, 1978; Forte, Machen & Obrink, 1980). Alternatively, protons may be transported with their charge by an active process that is not directly coupled to the translocation of any other ion. Considerable evidence has accumulated that such an electrogenic proton pump is responsible for urinary acidification in a number of "tight" urinary membranes. Examples are the epithelia of the urinary bladders of the fresh water turtle, *Pseudemys scripta* (Steinmetz, Omachi & Frazier, 1967; Al Awqati, Mueller & Steinmetz, 1977), and the toad, *Bufo marinus* (Ludens & Fanestil, 1972), and, probably, segments of the collecting tubule of the mammalian kidney (Stoner, Burg & Orloff, 1974; Koeppen, 1980).

Our knowledge of these transport systems has greatly increased in recent years. For a discussion of the coupled transport mechanisms for protons the reader is referred to a number of excellent treatises (Sachs et al., 1978; Forte et al., 1980; Aronson, 1981). For this topical review we will focus on the properties of electrogenic proton transport in epithelial cell membranes. Since the most extensive studies have been done in the turtle urinary bladder, we will emphasize the explorations of electrogenic proton transport in this model. The advantage of this model is that it permits a number of major simplifications. Firstly, the turtle bladder can be studied as a flat epithelium *in vitro* between two bulk solutions so that the polarity of the epithelial cells is maintained (Ussing & Zerahn, 1951). The proton pump is located at the most accessible surface, i.e., the luminal membrane, of the epithelium where the problem of unstirred fluid layers can be minimized. Secondly, the active translocation of protons across the luminal membrane is tightly coupled to me-

* *Present address:* Department of Medicine, University of Connecticut School of Medicine, Farmington, Connecticut 06032.

0022-2631/82/0065-0155 $04.00

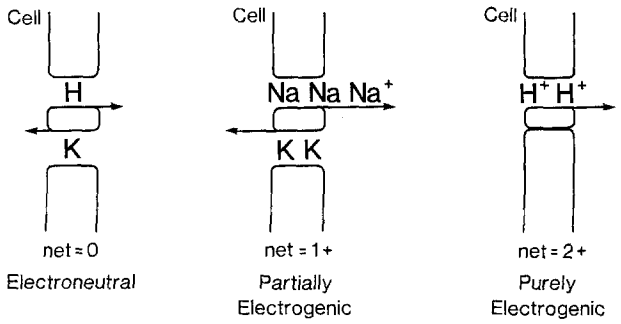

Fig. 1. Schematic representation of charge transfer by three common classes of active ion pumps which are driven by ATP hydrolysis

tabolism and not directly dependent on the movement of other ions. The H^+ pump, therefore, is functionally simpler than other ion pumps in epithelia. Furthermore, the passive H^+ permeability of the turtle bladder is so low that net H^+ transport rates can be used directly to study the behavior of the active component of H^+ transport. (The rate of transport may be measured directly by pH stat titration or indirectly as the short-circuit current after active sodium transport is abolished.) Thirdly, the turtle bladder is capable of transporting protons at appreciable rates during anaerobiosis and, thereby, permits exploration of the characteristics of metabolic coupling under these simplified conditions.

Several recent studies in the turtle bladder (Dixon & Al Awqati, 1979, 1980; Steinmetz, Husted & Mueller, 1980*b*; Steinmetz, Husted, Mueller & Beauwens, 1981) suggest that the acidification pump is a proton-translocating ATPase and shares many of the characteristics of the H^+-ATPases of fungal plasma membranes. These developments make it timely to review the electrophysiological and biochemical studies of this epithelial preparation and to present a simple kinetic model for the active step of proton transport. This working model accounts for the essential features of proton transport by the epithelium and may be applicable to other active transport processes.

2. Electrogenic and Electroneutral Ion Pumps

To define the scope of this review, we briefly describe some of the different ways an ion pump may transfer charge across a cell membrane. In Fig. 1 we depict three commonly considered types of ion pumps. In all three cases the translocation is directly coupled to ATP hydrolysis. In two of the examples the translocation consists of a *coupled* movement of two different ions in opposite directions.

Each complete cycle of the pump may be completely electroneutral as in the case of the H^+, K^+-ATPase that has been proposed for the parietal cells of gastric mucosa, based upon studies in vesicles from the luminal cell membrane (Sachs et al., 1978; Forte et al., 1980). In this case one K^+ enters the parietal cell for each H^+ secreted, and any electrophysiological correlates of the active transport depend upon the existence of secondary, passive pathways that recycle the K^+. If, for example, the passive K^+ efflux across the luminal cell membrane is exactly balanced by the active influx through the H^+, K^+-ATPase pathway, the active H^+ transport will be associated with a short-circuit current of appropriate sign and magnitude to account for the net H^+ secretion. This local recycling of K^+ will thus permit a pump that is intrinsically electroneutral to resemble an electrogenic pump.[1]

Each complete cycle of the pump need not, however, be completely electroneutral as illustrated by the Na^+, K^+-ATPase found in most animal cell membranes. In this case the cellular extrusion of $3\,Na^+$ is coupled to the uptake of about $2\,K^+$ (Thomas, 1972; Nielsen, 1979), such that the pump itself promotes a net charge movement across the membrane. But the measured short-circuit current fails again to reflect the intrinsic electrical characteristics of the pump. Part of the current will be generated directly by the active translocation steps, the remaining current is generated indirectly by the local recycling of K^+ (Koefoed-Johnsen & Ussing, 1958; Nielsen, 1979).

One may, lastly, find that the active translocation step is not coupled to the movement of any other ion species, as is the case for the H^+-ATPases from mitochondria and chloroplasts (Racker, 1976; Kagawa, Sone, Hirata & Yoshida, 1979), and, as we will discuss, for the H^+-ATPases from the plasma membranes of fungi and turtle bladder. In these cases the pump is purely electrogenic (Fig. 1).

3. Urinary Acidification by the Turtle Bladder, A Double Membrane Model

The overall process of urinary acidification by the turtle bladder may be described as the dissociation of H_2O into H^+ and OH^- coupled to the vectorial translocation of H^+ into the luminal compartment and of OH^- (or HCO_3^-) into the serosal compartment. The transport processes of urinary acidification therefore take place across two cell membranes in series and are best examined according to

[1] Local recycling of K^+ may be difficult to detect in epithelia with very deep infoldings in the "active" membrane. In gastric mucosa, for example, the structural complexity of the luminal membrane makes it difficult to exclude K^+ recycling in the intact epithelium.

a double membrane model first developed by Koefoed-Johnsen and Ussing (1958) for active sodium transport in the frogskin. In the simplest formulation, the transported ion is moved "uphill" across one of the cell membranes which has a low passive permeability to the ion and is moved "downhill" across the second cell membrane which has a high permeability to the ion. In the case of sodium transport the basolateral, or inner, cell membrane contains the pump; whereas the luminal, or outer, cell membrane contains channels which render it highly permeable to sodium. In the case of urinary acidification by the turtle bladder, the available evidence indicates that the H^+ pump is located at the luminal cell membrane while the efflux of HCO_3^- formed within the cellular compartment from OH^- and CO_2 occurs by facilitated diffusion via special protein sites in the basolateral cell membrane. To define the active and passive transport steps in the double membrane system, it is necessary to determine the electrochemical potential for protons in the cellular compartment under various conditions of transport. In the short-circuited turtle bladder, the overall pH is relatively high, near 7.4, even when H^+ secretion takes place against a steep pH gradient (Steinmetz, 1969). The intracellular electrical potential has been measured by Hirschhorn and Frazier (1971) in the short-circuited turtle bladder. The cell interior was electrically negative, by about 10 mV. More recent studies (Nagel, Durham & Brodsky, 1981) carried out with electrodes with smaller tip diameters suggest that the cell may be more negative, with potentials near 40 mV comparable to those of frogskin. The epithelial cells of the turtle urinary bladder are difficult to examine by microelectrodes. In addition to the technical limitations in obtaining the electrochemical profile for H^+ across the epithelium, there is the problem of getting the profile of the population of epithelial cells involved in H^+ secretion. Studies by Schwartz, Rosen and Steinmetz (1972) and by Husted, Mueller, Kessel, and Steinmetz (1981) indicate that the function of urinary acidification may be confined to a population of carbonic anhydrase-containing cells which constitute only 10 to 15 percent of the luminal surface area of the epithelium. The carbonic anhydrase-containing cells thought to be responsible for H^+ secretion have a distinctive appearance by scanning electron microscopy. As shown in Fig. 2, their luminal surface is characterized by prominent microplicae (Husted et al., 1981). It is presently not clear to which extent there may be cell-to-cell communication between these carbonic anhydrase-rich cells and the more common granular cells. If there were gap junctions between the different cell populations, this would provide for electrical and metabolic coupling (Loewenstein, 1979) and would facilitate the analysis of the transport compartments. The existence of gap junctions between the different surface epithelial cells, however, has not been established in the turtle bladder, although the studies of Dixon and Al Awqati (1980) and of Kelly, Dixon and Al Awqati (1980) on variations in epithelial cell ATP and ADP concentrations provide suggestive evidence for the existence of such junctions. Although the compartmentalization of the epithelium may interfere with the quantitative determination of the relevant electrochemical potential gradients for H^+, it should not affect the qualitative conclusion that the active step is at the luminal surface. This conclusion is further supported by studies indicating that the proton permeability of the turtle bladder is extremely low under ordinary conditions and that it can be increased by maneuvers which increase the H^+ permeability of the luminal cell membrane, such as the addition of amphotericin B to the luminal solution (Steinmetz & Lawson, 1970, 1971; Finn, Cohen & Steinmetz, 1977). The resistance to passive H^+ movement, therefore, resides primarily in the luminal cell membrane.

In Fig. 3 a schematic representation is given for the H^+ transport pathway across the epithelial cell of the turtle bladder. The active step is located at the luminal cell membrane which is assumed to be quite impermeable to passive H^+ movement. The second transport step is at the basolateral (or serosal) cell membrane in series with the H^+ pump. This step facilitates the efflux of alkali, primarily HCO_3^- formed within the cell from OH^- by a carbonic anhydrase-catalyzed reaction. The properties of this transport system at the serosal membrane are only partially understood. The efflux of HCO_3^- is inhibited by serosal addition of 4-acetamido-4′-isothiocyano-2,2′-disulfonic stilbene, SITS (Ehrenspeck & Brodsky, 1976; Cohen, Mueller & Steinmetz, 1978). SITS is best known as a potent inhibitor of anion exchange transport in red blood cells (Rothstein, Cabantchik & Knauf, 1976), but SITS inhibition of anion transport has also been reported in a variety of other tissues and in an anion-selective channel reconstituted into planar lipid bilayers from Torpedo electroplax membranes (Miller & White, 1980). In the turtle bladder the inhibition of H^+ transport by SITS is associated with increased alkalinity of the cell (Cohen et al., 1978). The inhibition, therefore, appears to be the consequence of decreased HCO_3^- permeability (or decreased effective H^+ permeability) of the serosal membrane. The SITS sensitivity of the HCO_3^- exit

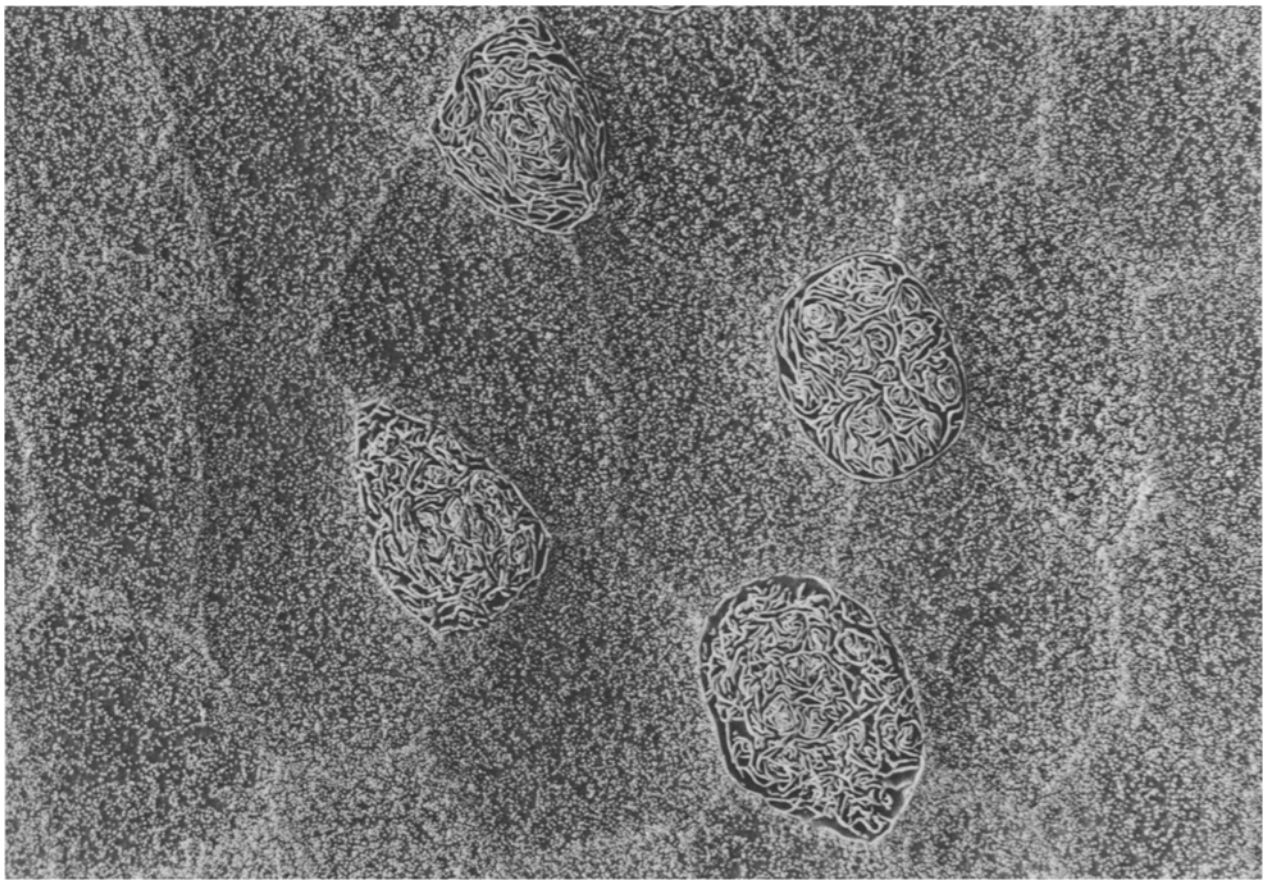

Fig. 2. Scanning electron micrograph of luminal surface of the turtle bladder. Note four cells with microplicae which represent carbonic anhydrase-rich cells and are thought to be responsible for active H^+ transport

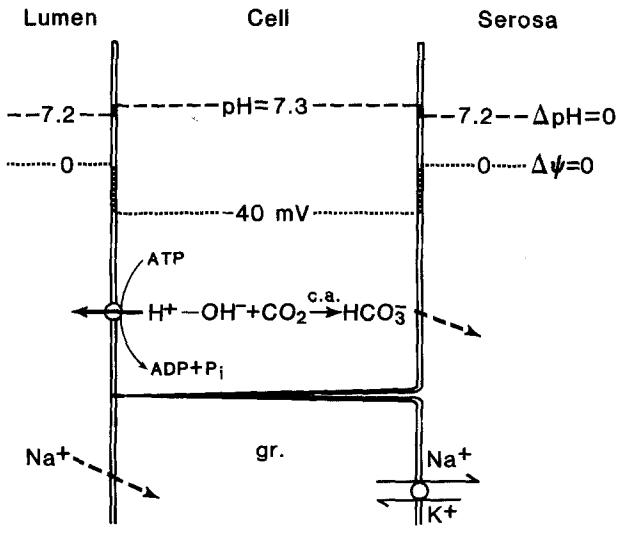

Fig. 3. Double-membrane model for active H^+ transport in turtle bladder epithelium. Approximate values for the electrochemical potential profile across the epithelium are given for the short-circuited preparation operating at half maximal transport. A proton pump is located at the luminal cell membrane. The exit of HCO_3^- across the basolateral cell membrane proceeds passively

step, by itself, does not clarify the precise manner in which HCO_3^- is translocated. Translocation might involve exchange of HCO_3^- for Cl^- with recycling of Cl^- via a separate conductive path or the conductive movement of HCO_3^- *per se*. Recent studies by Fischer, Husted and Steinmetz (1981) indicate that HCO_3^- efflux requires the presence of a counter anion such as Cl^- in the serosal compartment. They observed that in gluconate Ringer's the affinity of Cl^- is so high that serosal $[Cl^-]$ must be reduced below 0.2 mM to decrease H^+ secretion by 50%. These results are consistent with translocation by an exchange mechanism.

The high HCO_3^- permeability of the basolateral cell membrane has been utilized by Cohen and Steinmetz (1980) to vary the pH of the H^+ secreting cells by varying the $[HCO_3^-]$ of the serosal solution. Their studies suggest that changes in serosal pH cause similar changes in cell pH as long as serosal pH is altered by altering serosal $[HCO_3^-]$ or PCO_2, since the serosal cell membrane is highly permeable to both members of this buffer pair. The rate of active H^+ transport was found to be a sensitive

function of cell pH. Acute changes in cell pH cause immediate changes in the transport rate. In addition to these instantaneous effects on the pump rate, cell pH appears to affect the number of pump sites as judged from the amount of cell membrane exposed to the luminal solution. Husted et al. (1981) have shown that the luminal surface area of the carbonic anhydrase-containing cells is reduced dramatically when the cell interior is made alkaline. These changes are reversible and are demonstrable by scanning electron microscope in a time span of about an hour (Cohen, Husted, Mueller & Steinmetz, 1980). The relative rapidity of these morphologic changes is consistent with recent observations that stimulation of transport by CO_2 is associated not only with an increase in luminal membrane, but also with a disappearance of cytoplasmic vesicles (D.L. Stetson, *personal communication*), suggesting fusion and addition of vesicles to the luminal membrane.

In Fig. 3 cell pH is indicated as 7.3, a value at which the transport rate for H^+ is about half maximal. The available studies are certainly consistent with a double membrane model for urinary acidification in which the pump is located at the high resistance luminal membrane while the passive exit step (for HCO_3^-) is at the permeable basolateral cell membrane.

4. Electrogenicity of Proton Pump in Turtle Bladder

According to the double membrane model, urinary acidification by the turtle bladder may be described as the dissociation of H_2O. For each H^+ secreted into the luminal compartment, an OH^- (or HCO_3^-) appears in the serosal compartment (Steinmetz, 1967). This process is associated with the transfer of positive charge from the serosal to the luminal side of the bladder (Steinmetz et al., 1967). Thus, when net sodium transport is abolished by removal of sodium or the addition of ouabain or amiloride, the usual potential difference (PD), which is lumen negative with respect to serosa, is reversed to a lumen positive PD. The current required to nullify this reversed PD is equal to the rate of H^+ secretion measured simultaneously by pH stat titration. Studies by Schwartz (1976) and Husted, Cohen and Steinmetz (1979) indicate that this equality is preserved in a wide variety of experimental conditions.

These studies suggest but do not prove that the acidification pump is electrogenic. If the pump indeed operates in a purely electrogenic manner several of its characteristics can be predicted and tested. First, the translocation of H^+ by the pump should not be dependent on another cation for counter transport or an anion for cotransport. Steinmetz et al. (1967) have shown that H^+ secretion by the turtle bladder is not directly dependent on the transport of sodium or the presence of other ions such as potassium, calcium, or chloride. When sodium transport is inhibited by ouabain in the presence of exogenous CO_2 the rate of H^+ secretion by short-circuited bladders is completely unaffected (Steinmetz, 1974)[2]. As would be expected from an electrogenic pump, the rate of H^+ secretion is increased by the spontaneous PD (lumen negative) generated by sodium transport. In the open-circuited state, therefore, inhibition of sodium transport by ouabain causes a measurable decrease in H^+ secretion which represents indirect electrical coupling. Amiloride also causes a modest inhibition of H^+ secretion, even in the short-circuited state. This inhibition has been attributed to hyperpolarization of the luminal cell membrane (Husted & Steinmetz, 1979) and represents an indirect electrical coupling between Na^+ and H^+ transport.

The evidence that H^+ transport is not coupled directly to Na^+ transport is unambiguous in the turtle bladder and the toad bladder (Ziegler, Fanestil & Ludens, 1976) and probably applies also to the collecting duct (Stoner et al., 1974). A Na^+-H^+ antiport system, as described in the proximal tubule and certain other leaky epithelia, does not appear to contribute to the rate of acidification in these tight urinary epithelia.

H^+ secretion is not dependent on K^+ as it is in gastric mucosa. Although the turtle bladder has a small capacity for active K^+ absorption, this K^+ absorptive system is not coupled to H^+ secretion, but appears to depend on sodium and to represent a population of K^+-Na^+ exchange pumps at the luminal cell membrane (Husted & Steinmetz, 1981)[3].

H^+ secretion by the turtle bladder can proceed in the virtual absence of chloride, but the rate of secretion may be reduced as a function of the anion substituted for chloride. As discussed above, the HCO_3^- exit step at the basolateral cell membrane may involve exchange of HCO_3^- for another anion such as chloride. It should be noted that if basolateral HCO_3^- movement is mediated by an electroneutral system, there must be a second electrodiffusive pathway to allow recycling of the exchanged anion.

---

[2] The rate of active H^+ transport is dependent upon the presence of CO_2 (Schwartz & Steinmetz, 1971). In the absence of exogenous CO_2, ouabain reduces H^+ transport by reducing metabolic CO_2 production.

[3] K^+ absorption but not H^+ secretion is inhibited by luminal ouabain (Husted & Steinmetz, 1981).

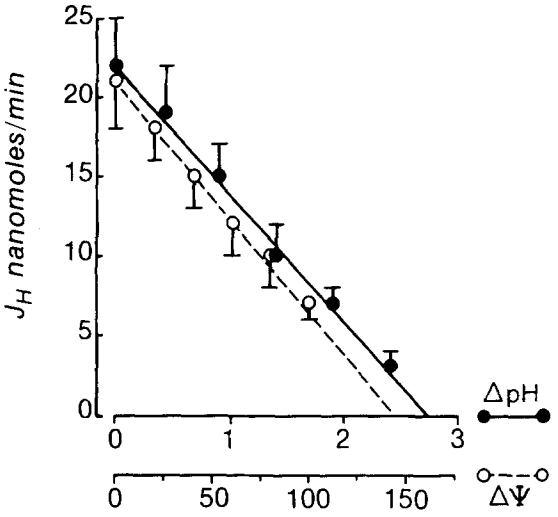

Fig. 4. Relation between H^+ transport (J_H) and $\Delta\Psi$ or $-\Delta$pH. Note the linear relation between J_H and imposed (chemical or electrical) potential differences and the close correspondence between a change in $\Delta\Psi$ and an equivalent change in $-\Delta$pH (summarized from data by Al Awquati et al., 1977)

Another prediction for an electrogenic pump is that there is a certain range over which the transport rate is voltage-sensitive. Studies in turtle bladder (Steinmetz, 1967) as well as toad bladder (Ziegler et al., 1976) have shown that the transport rate is influenced in a predictable manner by the PD across the epithelium. A lumen-negative voltage, as is generated by Na^+ transport, accelerates the rate of H^+ secretion, whereas an imposed lumen-positive voltage inhibits the rate. Al Awqati et al. (1977) have compared the inhibition of H^+ secretion by an opposing voltage, $\Delta\Psi$, with that caused by an opposing pH gradient, $-\Delta$pH (Fig. 4). Previous studies (Steinmetz & Lawson, 1970, 1971) had demonstrated that the rate of H^+ transport (J_H) decreases with decreasing luminal pH.

The change in J_H in response to a ΔpH or $\Delta\Psi$ is "instantaneous" within the admittedly rather poor time resolution of the experiments. When the luminal pH is decreased in steps of 0.5 pH units, transient responses associated with intracellular pH changes are usually not detectable. It is unlikely therefore that there are significant intracellular concentration changes, for ions other than H^+ and HCO_3^-, or other polarization phenomena associated with H^+ transport. The linear relationship expressed in Fig. 4 should, therefore, reflect the intrinsic properties of the H^+ transport system. The equivalence of inhibition by a $-\Delta$pH and $\Delta\Psi$ provides additional support for the conclusion that the pump is electrogenic.

The results of Steinmetz and Lawson (1971) and Al Awqati et al. (1977) suggest that the functional behavior of the H^+ transport system can be represented by a simple *linear equivalent circuit* comparable to the lumped circuit introduced by Ussing and Zerahn (1951) for the active Na^+ transport system in the frog skin (Fig. 5*A*). In the case of active H^+ transport the circuit can be simplified even further by setting the leak conductance equal to zero. This is possible because the passive H^+ fluxes are exceedingly small compared to the active fluxes (Steinmetz & Lawson, 1971) *and* because the coupling of H^+ transport to metabolism is very tight (Beauwens & Al Awqati, 1976; Steinmetz et al., 1981). Active H^+ transport can then be characterized by the conductance of the H^+ pathway, G_H, and by the apparent protonmotive force of the pump, *PMF'*. The *PMF'* is simply the (extrapolated) difference in transepithelial electrochemical potential for H^+, $\Delta\tilde{\mu}_H$, necessary to stop the pump. The use of equivalent circuit descriptions of membrane transport phenomena and, in particular, the dissection of epithelial lumped circuit parameters into their single membrane constituents is fraught with pitfalls (Schultz, Frizzell & Nellans, 1977). These problems are somewhat reduced when the leak conductance, as in our case, can be approximated to zero. The active transport pathway then may be expressed by the double-membrane circuit of Fig. 5*B*. The transepithelial potential difference, $\Delta\Psi$, can be broken down into the potential differences across the mucosal and basolateral cell membranes. $\Delta\Psi_m$ and $\Delta\Psi_b$, with the serosal solution as the reference for $\Delta\Psi_b$ and the cell interior as the reference for $\Delta\Psi_m$:

$$\Delta\Psi = \Delta\Psi_m + \Delta\Psi_b. \tag{1}$$

The transepithelial ΔpH can similarly be resolved into its components

$$\Delta\text{pH} = \Delta\text{pH}_m + \Delta\text{pH}_b. \tag{2}$$

The pH may be expressed in terms of the Nernst potential differences, E_H

$$E_H = (RT/F)\cdot\Delta\text{pH} \tag{3}$$

where R is the gas constant, T is the temperature in Kelvin and F is Faraday's constant. The "chemical" driving forces across each membrane can then be expressed as the sum of Nernst potentials across the membrane *plus* the electromotive force associated with active H^+ transport system in that membrane. The notation E' will be used to denote the sum of E and any active contributions. Hence:

$$E_H + \text{PMF} = E'_m + E'_b \tag{4}$$

where PMF denotes the electromotive force of the pump at the mucosal cell surface.[4]

When the system is in the steady state the H^+ flux can be expressed as:

$$I_H = G_b\cdot(E'_b - \Delta\Psi_b) = G_m\cdot(E'_m - \Delta\Psi_m) \tag{5}$$

where $I_H = F\cdot J_H$ or the current associated with the H^+ flux, G_b and G_m are the steady-state conductances of the basolateral and mucosal transport pathways. It should be noted that G_b and G_m *may* be quite complex functions of the $\Delta\Psi$'s and the H^+

[4] This notation has been convenient in studies of epithelial transport. We will use the term PMF as indicated despite some differences in terminology in other fields. Note that Eq. (4) implies that there is only one active H^+ transport system in the epithelium which is at the luminal cell membrane.

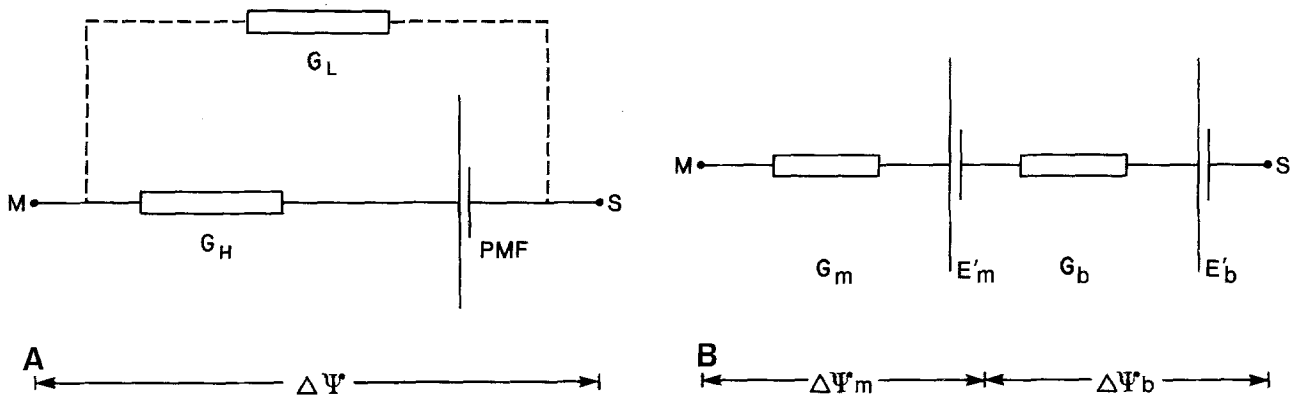

Fig. 5. Equivalent circuit description of active H^+ transport. (*A*): Lumped circuit in which *PMF* represents the electromotive force associated with the pump, G_H the conductance of the active pathway, and G_L the leak conductance, which in this case can be approximated to zero. (*B*): Detailed circuit diagram in which $G_L=0$ and in which the properties of the two cell membranes are considered. G_m represents the conductance of the pump at the mucosal (*m*) membrane and E_m the overall electromotive force associated with the pump; G_b and E'_b represent the passive conductance and the Nernst potential for H^+ at the basolateral (*b*) membrane. *M* = mucosal solution; *S* = serosal solution

concentrations in the three compartments. Such complications will not, however, affect the validity of Eq. (5) (Finkelstein & Mauro, 1963; Finkelstein, 1964). The overall conductance, G_H, is expressed as:

$$G_H=\frac{G_b\cdot G_m}{G_b+G_m}. \tag{6}$$

By combining Eqs. (1) through (6) we finally obtain the desired result:

$$I_H=G_H\cdot(E_H+\mathrm{PMF}-\Delta\Psi) \tag{7}$$

$$=G_H\cdot[\mathrm{PMF}-\Delta\Psi+(RT/F)\cdot\Delta\mathrm{pH}] \tag{8}$$

which expresses the rate of H^+ secretion in terms of the experimentally controllable variables $\Delta\Psi$ and ΔpH, and the intrinsic parameters G_H and PMF. G_H, therefore, does *not* represent the conductance of the active element itself. Only if $G_b \gg G_m$, such that the H^+ distribution across the basolateral cell membrane is fairly close to equilibrium, will G_H approximate G_m. In contrast, the PMF will always equal the PMF of the pump itself. It can, in particular, be concluded that the existence of a membrane potential difference is irrelevant when estimating the thermodynamic reversal potential of the H^+ pump in the intact bladder.

The simplicity of the equivalent circuit formalism, in particular of Eqs. (7) and (8), is appealing but also somewhat misleading. Firstly, if G_H itself is found to be constant over a wide variety of experimental conditions, this may not imply that G_m is constant. The only conclusion that can be drawn from such a finding is that the variations in G_m and G_b are such that the left-hand side of Eq. (8) is constant. Secondly, the possibility exists that the G_m (or G_m and G_b) vary in such a manner that one may observe several regions where there is a linear relation between $\Delta\Psi$ (or $-\Delta$pH) and J_H. If this is the case, and the "wrong" linear segment is analyzed in terms of Eq. (8), one may misjudge the magnitude of the PMF and of the active conductance. It is, therefore, preferable to characterize data such as those in Fig. 4 by an *apparent* PMF, PMF′ and an *apparent* G_H, G'_H, unless additional information is available to confirm that the extrapolated PMF′ really is the thermodynamic PMF for the transport system.

Finally, recent experimental observations indicate that the linear equivalent circuit may be applied only to a limited range of values. The relation between J_H and $\Delta\Psi$ (or $-\Delta$pH) loses its linearity when it is examined to the left of the ordinate in Fig. 4. J_H approaches a maximal value when the pH of the mucosal solution is increased from 7.5 to 8.5 (O.S. Andersen, A. Mueller, and P.R. Steinmetz, *unpublished observations*), or when cell pH is lowered from 7.3 (*see* example, Fig. 3) toward a pH of about 6.7 (Cohen & Steinmetz, 1980).

So even though there is ample evidence that the active transepithelial H^+ transport is mediated by a simple electrogenic pump, it is apparent that the equivalent circuit analysis does not provide a sufficient description of the kinetic and energetic aspects of H^+ transport through the active pathway. Alternative schemes, therefore, must be developed to describe the characteristics of the active transport system.

5. Biochemical Studies of Proton Pumps

Ion pumps can be classified in several ways. In the preceding sections, we have used functional criteria

to distinguish between an electrogenic and an electroneutral pump mechanism on the basis of the net charge transfer by the pump itself. In this section, we will use biochemical criteria to classify proton pumps on the basis of their energy source and their catalytic mechanism. The two major types of proton pumps are the redox pumps and the proton-translocating ATPases.

A. Redox Pumps

In a redox pump a series of oxido-reduction reactions occurs in a vectorially organized sequence across the membrane. For example, a substrate, RH_2, is oxidized by a cytochrome, Ct:

$$Ct^{++} + RH_2 \rightleftharpoons Ct + R + 2H^+. \quad (9)$$

The vectorial organization of the reaction sequence ensures that the H^+ appear on one side of the membrane, while the electrons remain on the other side where they react with an acceptor such as O_2:

$$2e + 1/2\,O_2 + H_2O \rightleftharpoons 2\,OH^-. \quad (10)$$

Such oxidation-reduction reactions have been defined in considerable detail for both mitochondria and chloroplasts (Racker, 1976; Mitchell, 1980), but there is no unambiguous evidence for the existence of a redox pump in the plasma membranes of a H^+ transporting epithelium. The strict O_2 dependence of H^+ secretion by the stomach (*see* Sachs et al., 1978) would certainly be consistent with the existence of a redox pump at the luminal cell surface, but recent studies by Forte et al. (1980), Reenstra, Lee & Forte (1980), and Sachs et al. (1978) have provided convincing evidence that the gastric mucosa contains a K^+-dependent H^+-translocating ATPase, and that this H^+, K^+-ATPase is responsible for H^+ secretion.

Indirect evidence has also provided some support for the existence of a redox pump in certain urinary epithelia. Dies and Lotspeich (1967) showed that the rat kidney responds to chronic acidosis with increased activity of the enzymes of the hexose monophosphate pathway. They postulated that this increased enzyme activity supported the increased rate of urinary acidification by a redox pump in the renal tubules. Norby and Schwartz (1978) observed in turtle urinary bladder that the rate of glucose metabolism by the pentose phosphate pathway is related to the rate of H^+ transport. The nature of the coupling between metabolism and pump rate remains, however, uncertain. Evidence against the existence of a redox pump is that the turtle bladder, in contrast to the gastric mucosa, is capable of secreting protons under anaerobic conditions (Schwartz & Steinmetz, 1977). Recent studies by Steinmetz, Husted and Mueller (1980*a*) have shown that the turtle bladder can sustain appreciable anaerobic secretion rates, if exogenous CO_2 is made available, and that J_H is tightly coupled to anaerobic glycolysis. This result by itself makes it unlikely that J_H is driven by a redox pump. Furthermore, Kelly et al. (1980) demonstrated coupling between J_H and the oxidation of a variety of substrates, glucose, butyrate, oleate, and β-OH-butyrate. It appears, therefore, that no metabolic pathway is exclusively coupled to H^+ transport. Aside from these considerations which fail to support a redox pump mechanism in urinary acidification, there are several new lines of evidence that directly indicate that the acidification pump of the luminal cell membrane of the turtle bladder is a H^+-translocating ATPase.

B. Plasma Membrane H^+-ATPases

It has taken many years to gather convincing evidence that plasma membranes of eukaryotic cells may contain a Mg^{++}-ATPase which translocates protons. Since such plasma membrane ATPases resemble mitochondrial H^+-ATPase, the burden of proof has been on the investigator making a case for their existence. Gastric and pancreatic physiologists, for example, have described an ATPase in homogenates of gastric mucosa and pancreatic tissue that was stimulated by HCO_3^- and other oxyanions (Kasbekar & Durbin, 1965; Milutinovic, Sachs, Haase & Schulz, 1977). It was suggested that such an anion-sensitive ATPase might be responsible for the transport of H^+ and, possibly, Cl^- and HCO_3^- in these tissues. Further investigation of this issue has shown, however, that oxyanion stimulation is not a unique characteristic of plasma membrane ATPases, but is also observed with mitochondrial H^+-ATPase. In many instances, the reported anion ATPase resulted from contamination of the plasma membrane fraction with mitochondria (Van Amelsvoort, de Pont & Bonting, 1977).

Several lines of evidence for the existence of a separate H^+-translocating ATPase in the plasma membrane have, nevertheless, been developed for certain eukaryotic cells. Forte and coworkers (Forte, Ganser, Beesly & Forte, 1975) observed an abundant phosphatase activity in gastric microsomes that was stimulated by K^+. Later this activity was shown to be a partial reaction for the gastric K^+-stimulated ATPase (Forte et al., 1980). This ouabain-insensitive, Na^+-independent K^+-ATPase has been demonstrated in the gastric mucosa of the frog (Ganser & Forte, 1973) and a variety of mammals (*see* Sachs et al., 1978). In contrast to the

HCO_3^- stimulation of ATPase, the K^+ stimulation *does not* occur in mitochondria. Studies in gastric vesicles by Lee, Simpson and Scholes (1974) and by Forte (1980) and Sachs (1978) and their coworkers have led to the characterization of this ATPase as a H^+, K^+-ATPase. These studies have also provided strong evidence for a function of this ATPase in gastric H^+ secretion. Gastric membrane vesicles form with the luminal surface inward, such that the interior of the vesicles is acidified when K^+ is available on the inside and ATP is added to the external solution. The coupling between H^+ uptake and K^+ extrusion appears to be one for one, and the pump has several other characteristics of an electroneutral pump (Sachs et al., 1978; Reenstra et al., 1980). Although our review is focussed on electrogenic proton transport, the gastric H^+ pump is of interest since it is a proton translocating ATPase that has been clearly distinguished from mitochondrial ATPase.

C. Electrogenic H^+-ATPases

The best evidence for the existence of an ATP driven electrogenic proton pump in the plasma membrane of an eukaryotic cell has been obtained in yeast (Matile, Moor & Mühlethaler, 1967; Delhez, Dufour, Thines & Goffeau, 1977; Dufour & Goffeau, 1978) and in *Neurospora crassa* (Slayman, Long & Lu, 1973; Scarborough, 1980). The functional and pharmacological characteristics of these plasma membrane ATPases are strikingly similar to the characteristics of the electrogenic H^+ pump in the turtle bladder. Their properties will, therefore, be reviewed briefly.

Neurospora crassa is particularly interesting because its cells are sufficiently large to permit intracellular potential and transmembrane current measurements (Slayman, 1965; Gradmann et al., 1978). It has thus been shown that the plasma membrane contains an ATP-dependent electrogenic pump, presumably H^+-transporting, that maintains a membrane potential of 150–200 mV (cell interior negative). Evidence for a H^+-translocating ATPase has been obtained in studies on inside-out plasma membrane vesicles prepared from *Neurospora* (Scarborough, 1980). In the presence of MgATP in the external medium these vesicles accumulate H^+ and generate an inside positive potential. The generation of a ΔpH and of a $\Delta\Psi$ exhibits identical saturation kinetics with respect to (MgATP) and shows parallel responses to pharmacological modifications with ATPase inhibitors. Since the H^+ accumulation was not directly dependent upon the transport of other ions, it represents the action of an electrogenic H^+ transport mediated by a H^+-ATPase.

Studies of fungal plasma membrane fragments have described a Mg^{++}-dependent ATPase in a variety of preparations. Matile et al. (1967) found that the plasma membrane of *Saccharomyces cerevisiae* contained such an enzyme, and that it differed from the mitochondrial ATPase in that it was oligomycin-insensitive. More recent studies in *Saccharomyces* (Willsky, 1979), in *Schizosaccharomyces pombe* (Dufour & Goffeau, 1978; Dufour, Bourty & Goffeau, 1980), and in *Neurospora crassa* (Bowman & Slayman, 1977; Dame & Scarborough, 1980) have confirmed the existence of a separate plasma membrane ATPase and have defined some of its properties. The plasma membrane ATPase of these eukaryotic cells is similar to the mitochondrial ATPase insofar as it is inhibited by dicyclohexylcarbodiimide (DCCD) and Dio-9, inhibitors of H^+ translocation through the hydrophobic channel portion (F) of mitochondrial ATPase. The plasma membrane ATPase differs, however, from the mitochondrial ATPase in many other respects: it is resistant to inhibition by oligomycin, but sensitive to vanadate, and its pH optimum is much lower ($\approx$6.7) than for the mitochondrial enzyme ($\approx$9). Upon purification this plasma membrane ATPase has a much simpler subunit structure than the mitochondrial enzyme (Delhez et al., 1977) and a phosphorylated intermediate has been isolated (Dame & Scarborough, 1980; Amory, Foury & Goffeau, 1980). These studies of the H^+-ATPase of fungal plasma membranes may be important for understanding epithelial proton transport, since epithelial membranes with electrogenic acidification mechanisms show strikingly similar pharmacological characteristics (*see* below).

D. Evidence for H^+-ATPase in Turtle Bladder

We have already reviewed the evidence that proton secretion by the turtle urinary bladder is electrogenic. Furthermore, since proton secretion is not dependent on the transport of other ions, the pump in turtle bladder like the one in *Neurospora* appears to be "purely electrogenic." The biochemical characteristics of the acidification pump in turtle bladder have been studied in a number of ways.

It has been known since the studies by Solinger et al. (1968), Bourgoignie et al. (1969), and Shamoo and Brodsky (1970) that the microsomal fraction of turtle bladder epithelial cells contain abundant Mg^{++}-ATPase activity, of which only a portion is stimulated by ($Na^+ + K^+$) or inhibited by ouabain. It has been difficult, however, to obtain good separation and purification of apical cell membranes in the turtle bladder. Heterogeneity of epithelial cells and contamination with mitochondrial Mg^{++}-

ATPase have hampered the preparation of membrane vesicles from the luminal membrane of the pertinent population of H^+ secreting cells. By means of free-flow electrophoresis, however, it is possible to obtain a membrane fraction that contains apical membrane as judged from the comigration of cyclic AMP-activated protein kinase and norepinephrine-sensitive adenylate cyclase and the absence of Na^+, K^+-ATPase (Brodsky et al., 1979). Similarly, a crude vesicle preparation of the luminal membrane obtained by a Ca^{++} precipitation technique revealed an abundance of Mg^{++}-ATPase with almost no ($Na^+ + K^+$)-stimulated ATPase (P.R. Steinmetz and R. Kaback, *unpublished observations*). Importantly, the bulk of the ATPase activity was oligomycin-insensitive. Before reasonably pure vesicles can be made from the apical membrane of the H^+ secreting cells, however, techniques must be developed to separate these these cells from the more abundant granular cells and to obtain sufficient quantities of apical membrane from them.

Fortunately, the turtle bladder has some unique characteristics which permit valuable simplifications when the intact epithelium is examined *in vitro*. It has, in particular, been shown that the rate of H^+ secretion, J_H, as measured by the reversed short-circuit current in ouabain-treated bladders, is tightly coupled to metabolism, J_r. This coupling to metabolism is, of course, the defining property of primary active transport systems, but it also provides a useful tool to study the characteristics of the pump.

The coupling between J_H and J_r has been observed in two different studies, in which J_H was varied experimentally by different maneuvers. In one study (Beauwens & Al Awqati, 1976), J_r was measured as the rate of ^{14}C-glucose oxidation, in the other (Steinmetz et al., 1981) J_r was measured as the rate of lactate production during anaerobiosis. Both studies indicate that the transport rate is tightly coupled to the metabolic driving reaction. This high degree of coupling is important for the later discussion of the biochemical nature of the pump.

The coupling between J_H and J_r is often described using the formalism of irreversible thermodynamics (Kedem & Caplan, 1965; Essig & Caplan, 1968; Beauwens & Al Awqati, 1976). The rate of H^+ secretion and of metabolism *coupled to H^+ transport* can then be expressed as

$$J_H = L_H \cdot (-\Delta\tilde{\mu}_H) + L_{Hr} \cdot A \tag{11}$$

and

$$J_r = L_{rH} \cdot (-\Delta\tilde{\mu}_H) + L_r \cdot A \tag{12}$$

where $\Delta\tilde{\mu}_H$ is the electrochemical potential difference for H^+ across the epithelium, and A is the affinity (the negative Gibbs free energy) of the reaction(s) coupled to the H^+ transport. The coefficients L_H and L_r act as phenomenological "conductances" that relate H^+ secretion and metabolism to their respective driving forces, while the coefficients L_{rH} and L_{Hr} relate the rate of one process to the driving force for the other process. The magnitude of these four coefficients will depend upon the composition of the different compartments through which the transport proceeds but should not vary with the magnitude of the driving forces *per se*.

As long as the system is very close to equilibrium ($A - \Delta\tilde{\mu}_H \leqq RT$), L_{Hr} and L_{rH} can be equated (Prigogine, 1961) and one can express the degree of coupling between the two processes unambiguously by the coupling coefficient, q

$$q = \frac{L_{Hr}}{\sqrt{L_r \cdot L_H}}. \tag{13}$$

It is also useful to define a stoichiometric coefficient, Z, as

$$Z = \sqrt{\frac{L_H}{L_r}} \tag{14}$$

which expresses the stoichiometry between metabolism and transport. The meaning of q and Z are most readily appreciated by eliminating A between Eqs. (11) and (12) and by expressing J_H as a function of $\Delta\tilde{\mu}_H$ and J_r:

$$J_H = (L_H - L_{Hr} \cdot L_{rH}/L_r) \cdot (-\Delta\tilde{\mu}_H) + \frac{L_{Hr}}{L_r} \cdot J_r \tag{15}$$

or

$$J_H = L_H(1 - q^2) \cdot (-\Delta\tilde{\mu}_H) + Z \cdot q \cdot J_r. \tag{16}$$

J_H will equal zero when:

$$(\Delta\tilde{\mu}_H)_{J_H=0} = \frac{L_{Hr}}{L_H} \cdot A \tag{17}$$

or using Eqs. (13) and (14) one finds that

$$(\Delta\tilde{\mu}_H)_{J_H=0} = (q/Z) \cdot A \tag{18}$$

when $q = 1.0$ it is, therefore, possible to express A as

$$A = Z \cdot (\Delta\tilde{\mu}_H)_{J_H=0} \tag{19}$$

or

$$A = Z \cdot F \cdot \mathrm{PMF}. \tag{20}$$

Eq. (20) and its companion

$$L_H = G_H \cdot F^2 \tag{21}$$

provide the connection between the irreversible thermodynamic description of the transport process and the equivalent circuit description, Fig. 5 and Eq. (8). Eq. (20) is of special interest since information about PMF (determined from an equivalent circuit analysis) and A (determined from a chemical analysis of epithelial cells) will provide an estimate of Z (Dixon & Al Awqati, 1980)[5]. This estimate is, however, subject to the considerable uncertainties involved in the estimate of the PMF.

The tight coupling between J_H and J_r can be used to identify the substrate for the pump and thus to obtain information about the biochemical identity

[5] The factor, F, in Eqs. (20) and (21) arises because the driving forces in the equivalent circuit formulation are expressed in volts, whereas they are expressed in joules/mole (or electron volts) in the description of irreversible thermodynamics. We will, however, adhere to standard conventions and express $\Delta\tilde{\mu}_H$ in millivolts.

of the pump. We have already seen how the pump appears to be stopped by a $\Delta\tilde{\mu}_H$ of about 180 mV (*see* Fig. 4). But this implies that a $\Delta\tilde{\mu}_H$ greater than 180 mV should reverse the proton flow through the pump and thus lead to the *production* of the substrate for the pump.

If the pump were a H^+-translocating ATPase, one might thus be able to demonstrate ATP synthesis by imposing a $\Delta\tilde{\mu}_H$ in excess of the reversal potential. This demonstration was achieved by Dixon and Al Awqati (1979). They showed that applying $\Delta\tilde{\mu}_H \gg 120$ mV by various combinations of a ΔpH and a $\Delta\Psi$, increased the ATP content of the epithelial cells. To eliminate all other ATP consuming and ATP generating reactions, they treated the bladders with ouabain, amiloride, sodium cyanide and iodoacetate. Under these conditions, the ATP levels increased as a function of the applied $\Delta\tilde{\mu}_H$. ATP generation was abolished when the ATPase inhibitors DCCD and oligomycin were added to the luminal solution. ATP generation was also abolished by low luminal concentrations of the protonophore dinitrophenol, presumably by collapsing the $\Delta\tilde{\mu}_H$ across the active transport pathway at the luminal cell membrane. These results provide considerable support for the hypothesis that the acidification pump of the turtle bladder is a H^+-translocating ATPase. Several issues remain, however, to be explored further. One of these deals with the resolution provided by this experimental approach. According to Husted et al. (1981) H^+ secreting cells are only a small population of all surface epithelial cells in turtle bladder. Does ATP formation occur only in this population of carbonic anhydrase containing cells? Or are all surface epithelial cells capable of contributing to the observed increases in ATP levels either directly or by diffusional exchange between the various cell populations? In particular, how well had mitochondrial ATP synthesis been excluded? The complete inhibition of the ATP synthesis by oligomycin is more consistent with a synthesis by the mitochondrial ATPase than by the cell membrane ATPase, as judged from the studies in yeast and neurospora as well as studies to be presented in turtle bladder. With respect to the issue of cell heterogeneity, Dixon and Al Awqati have shown in another study (1980) that in ouabain-treated bladders the average ATP level of the epithelial cells is inversely related to the H^+ transport rate. The ATP/ADP·P_i ratio increased when H^+ transport was reduced by lowering of the luminal pH from 7.4 to 5.0. Hence, in the absence of sodium transport, overall epithelial ATP levels are related to the transport rate of H^+.

The biochemical identity of the acidification pump can also be inferred from the transport characteristics found during anaerobic H^+ secretion. The turtle urinary bladder has the remarkable ability to transport protons in the absence of O_2. Schwartz and Steinmetz (1977) showed that anaerobic H^+ secretion required exogenous CO_2. In further studies an anaerobic preparation was developed that was capable of steady H^+ secretion for periods of 4 to 6 hr (Steinmetz et al., 1980*a*; 1981). The preparation was deoxygenated with N_2 and 1% CO_2 and maintained in a Ringer's solution containing 10 mM glucose. Under these anaerobic conditions O_2 was no longer detectable in the media, and H^+ secretion was not inhibited by sodium cyanide or dinitrophenol. During anaerobiosis, mitochondrial respiration is excluded as a source of energy for H^+ transport. The effects of ATPase inhibitors of the pump can thus be explored directly, without interference caused by inhibition of mitochondrial ATPase. Furthermore, since anaerobic H^+ transport is critically dependent on glucose, it is possible to examine the coupling between active H^+ transport and the rate of lactate production. (One ATP is produced for each lactate formed in the anaerobic metabolism of glucose.) J_H was measured as the reverse short-circuit current and by pH stat titration. Anaerobic J_H was markedly inhibited by addition of DCCD and Dio-9 to the luminal solution. Oligomycin, on the other hand, had no effect on anaerobic J_H at concentrations several times higher than required for inhibition of J_H in the presence of O_2. Vanadate, however, decreased J_H markedly under anaerobic as well as aerobic conditions if it was added to the serosal solution.

Table 1. Inhibitor characteristics of H^+-translocating ATPases

	Mitochondria	Plasma membranes	
		Fungi	Turtle bladder
Ouabain	−	−	−
DCCD	+	+	+
Dio-9	+	+	+
Oligomycin	+	−	−
Vanadate	−	+	+

As shown in Table 1, these inhibitor characteristics of the H^+-pump in the turtle bladder correspond to those observed for the fungal plasma membrane ATPase. They differ from those observed for the mitochondrial Mg^{++}-dependent H^+-ATPase in two important respects: the oligomycin resistance and the vanadate sensitivity. These differences may be determined by structural differences between the cell membrane enzyme and the mitochondrial enzyme. We have already alluded to the simpler sub-

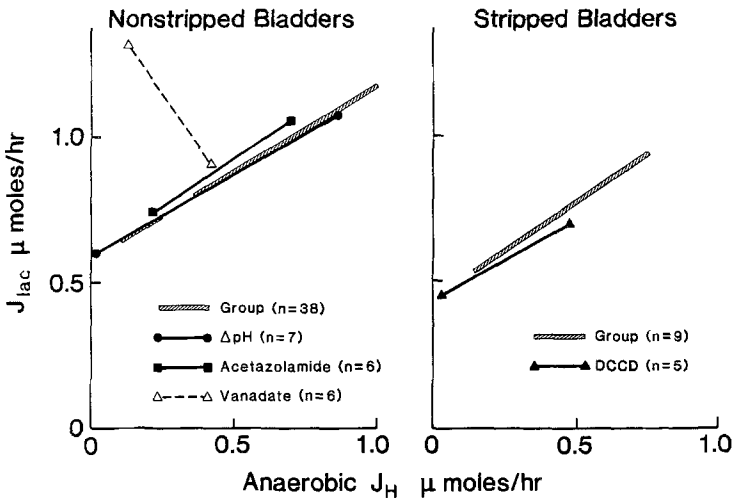

Fig. 6. The relation between lactate production (J_{lac}) and anaerobic H^+ transport (J_H) are shown in groups of bladders with different spontaneous transport rates (shaded lines). The effects of experimental maneuvers on J_{lac} and J_H are also shown (reproduced by permission of the Association of American Physicians: Steinmetz et al., 1980b)

unit structure of the former and to differences in the pH optimum. Another distinguishing feature may be that the plasma membrane ATPase forms a phosphorylated intermediate. The existence of a phosphorylated intermediate is interesting, especially for electrogenic H^+-ATPases of the fungal plasma membrane, since this ATPase does not translocate other cations and in fact has no ion requirement other than for Mg^{++} (and H^+). The recent studies by Dame and Scarborough (1980) and Amory et al. (1980) show that the H^+-ATPases of neurospora and yeast have a hydrolytic moiety with a molecular weight of about 100,000 and that this moiety is phosphorylated by ATP. This H^+-ATPase thus appears to hold an intermediate position between the metal cation translocating ATPases and mitochondrial H^+-ATPase. As judged from the electrophysiologic and metabolic studies, and from the inhibitor characteristics, a similar ATPase is present in the luminal cell membrane of the turtle bladder and possibly other "tight" urinary epithelia exhibiting electrogenic H^+ transport. Thus, acidification by the toad bladder is inhibited by DCCD and not by oligomycin (Fanestil & Park, 1980).

The studies on anaerobic H^+ transport in turtle bladder strengthen this interpretation by providing information about the coupling between J_H and anaerobic glycolysis, that is, ATP production. In Fig. 6 the rate of lactate production (J_{lac}) is plotted against anaerobic H^+ transport (J_H). The shaded lines show the relationship in two groups of ouabain-treated bladders with different spontaneous H^+ secretion rates. The slope of J_{lac} *vs.* J_H was 0.58 for the group of 38 bladders. The same slope was found in a smaller group of bladders in which the serosal muscle bundles and loose tissue layer were stripped off. Basal lactate production unrelated to H^+ transport is given by extrapolation to J_{lac} at $J_H = 0$. To examine the coupling between J_{lac} and J_H more closely, the two rates were measured simultaneously in individual bladders in which J_H was varied by applying pH gradients or by the addition of acetazolamide, DCCD, or vanadate. Figure 6 illustrates the average slopes, $\partial J_{lac}/\partial J_H$ for these experimental maneuvers. The slopes for the ΔpH, acetazolamide, and DCCD experiments (solid lines) were slightly above 0.5. Basal J_{lac} appeared to be unaffected by these maneuvers except for the vanadate experiments (dashed line) in which there was an apparent uncoupling[6]. It should, in particular, be noted that changes in $\Delta\tilde{\mu}_H$ did not affect the slope of the J_r *vs.* J_H relation. By comparison with Eq. (16) it can be seen that this implies that $(1-q^2)$ must be very close to zero – thus providing independent evidence for a very tight coupling between H^+ transport and metabolism.

E. H^+/ATP Stoichiometry

The H^+/ATP stoichiometry of a proton pump can be obtained directly by measuring the rate of active H^+ transport and the simultaneous rate of ATP hydrolysis. For a purely electrogenic pump the stoichiometry may also be estimated from the ratio $\Delta G'_{ATP}$/PMF but this approach is only valid when the efficiency of energy conversion is high. The studies of H^+ transport by the anaerobic turtle bladder revealed that slightly less than two protons are transported per lactate formed, or the hydrolysis of one ATP was coupled to the translocation of about two protons. This H^+/ATP stoichiometry of 2, based on the measurement of the two flows, is lower

[6] The actions of vanadate are complex. Vanadate may stimulate glycolysis under anaerobic as well as aerobic conditions by a mechanism not necessarily related to the rate of H^+ transport (*unpublished observations*).

than that obtained from the ratio of the two forces at zero flow by Dixon and Al Awqati (1980). Dixon and Al Awqati obtained a value of 3 by measuring $\Delta G'_{ATP}$, the apparent free energy of ATP hydrolysis, as an average value for all epithelial cells, and the apparent PMF from the ΔpH required to stop the pump. The difference between the two estimates remains to be clarified. The value of 2 of Steinmetz et al. (1981) could be in error to the extent that the measurements of the two flows were not accurate. The value of 3 of Dixon and Al Awqati (1980), on the other hand, depends upon the validity of the estimated PMF'. It would be too high if the estimate for the PMF of the H^+ pump in the H^+ secreting cell population were too low. As we will see (p. 171), this is a not unlikely possibility.

In *Neurospora*, Scarborough (1980) recently derived an approximate H^+/ATP stoichiometry of 2 from an estimate of H^+ transport and ATP hydrolysis in membrane vesicles. Warncke and Slayman (1980) estimated to stoichiometry in intact *Neurospora* cells from the estimated reversal potential of the pump and the amount of energy available from ATP hydrolysis under varied metabolic conditions. They arrived at a variable stoichiometry, ranging between 1 and 2, depending upon ATP availability.

6. A Working Model for H^+ Translocation by a H^+-ATPase

The discussions in the preceding sections have focused on the phenomenological description of the transepithelial H^+ flux and on the biochemical characteristics of the pump. These two aspects of the pump were related through the formalisms of nonequilibrium thermodynamics and equivalent circuit analysis. These approaches, however, have their limitations in resolving several of the important experimental questions. Thus, different protocols led to different estimates for the H^+/ATP stoichiometry, and the saturating behavior of the H^+ transport rate is not readily explained within this framework. It seems timely, therefore, to try to account for some of these results by making some reasonable inferences about the structure of the H^+ pump and by combining these with kinetic considerations to arrive at a working model of the H^+ pump in turtle bladder.

A. Structural Considerations

Little structural information exists about electrogenic H^+ pumps in epithelial membranes. Nevertheless, some relatively safe assumptions can be made. First, we may assume that H^+ transport is mediated by a polypeptide structure that spans the membrane as a channel-like conduit (Singer, 1974). We will assume, secondly, that the pump is a H^+-ATPase similar to the H^+-ATPase of the fungal plasma membrane and, thirdly, that its major structural features resemble those described for the bacterial, mitochondrial, and chloroplast H^+-ATPases. (These are the only membrane-bound ATPases where significant structural information is available (Baird & Hammes, 1979; Kagawa et al., 1979; Nelson, 1980).) According to this scheme, the pump is composed of two components, a catalytic unit that possesses the ATPase activity and thus provides the coupling between ATP hydrolysis and net H^+ translocation, and a hydrophobic channel that mediates the actual transmembrane movement of the H^+ (Fig. 7). These two subsystems are assumed to function independently of one another. One can thus visualize the proton movement as a two-step process: first the

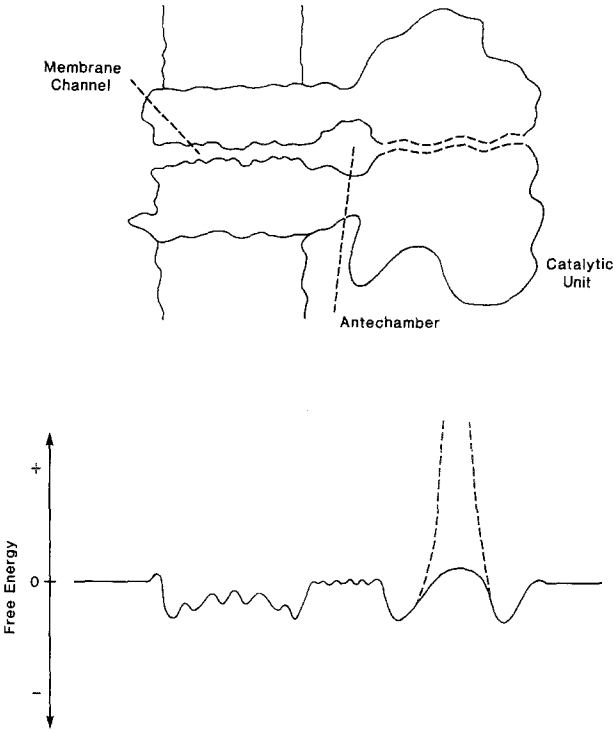

Fig. 7. Schematic representation of a proton pump. *Top*: The pump consists of three components: a membrane channel, an antechamber which serves as a buffer compartment, and the catalytic unit in which ATP hydrolysis is coupled to the translocation of H^+. The path for H^+ through the catalytic unit is illustrated by the dashed line. *Bottom:* The free energy profile for H^+ as it moves through the pump. The solid line represents the free energy profile for H^+ as it is actively translocated in the catalytic unit and as it moves through the antechamber and the membrane channel. The dashed profile at the level of the catalytic unit represents the barrier to H^+ leak in the catalytic unit. Perfect coupling between ATP hydrolysis and H^+ translocation implies an infinitely high barrier for the leak pathway

H^+ is translocated through the catalytic unit from the cytoplasm to an antechamber that connects the catalytic unit to the channel. This translocation will express the true *energetics* of the pump. The H^+ then moves through a hydrophobic channel with a finite resistance by an electrodiffusive mechanism. The free energy profile for H^+ as it moves through the system is illustrated schematically in the bottom half of Fig. 7.

It should be noted that this kind of scheme eliminates the need for a mobile element within the membrane. In the simplest version all conformational changes associated with the active transport step *per se* occur *within* the catalytic unit – which exhibits a kinetic pattern that is indistinguishable from that of a "mobile carrier." The assumption that the "extracellular" side of the catalytic unit is facing an antechamber at some distance from the luminal surface of the cell membrane, is easily justified for the ATP-synthesizing ATPases. However, a component similar to the F_o segment of mitochondrial ATPase has not yet been identified for the fungal plasma membrane ATPase. The molecular reality of a separate transmembrane channel, therefore, remains uncertain. This should not, however, affect the basic feature of the model: that the actual ATP-driven translocation step only proceeds over a fairly short distance of the total pathway and that the two aqueous phases communicate with the ATP-dependent segment by channel-like structures. These may be *bona fide* voids in the protein structure, produced by the geometrical properties of the various subunits or, alternatively, virtual channels without permanent structure through which H^+ moves as a function of rapid conformational fluctuations in the protein (Lalowicz & Weber, 1973; Case & Karplus, 1979; Frauenfelder & Petsko, 1980).

B. Kinetic Considerations

A comparison of the equivalent circuit description for active H^+ transport (Fig. 5*B*) with the structural model for the pump (Fig. 7*A*) suggests immediately a possible biophysical interpretation for both G_m and the PMF: If H^+ translocation can proceed much faster through the catalytic unit than through the membrane-bound channel, then the rate of active H^+ transport will be limited by the resistive properties of the channel, expressed through G_m, and by the energetics of the catalytic step, expressed through the PMF. This picture, which is almost certainly oversimplified, is useful in illustrating how active H^+ transport can be decomposed into a number of elementary steps distributed along the transport path. In the remainder of this section, we describe a simple version of this model in which both the catalytic unit and the transmembrane channel may pose a finite resistance to H^+ movement. A more detailed treatment will appear elsewhere (O.A. Andersen & P.R. Steinmetz, *in preparation*).

We assume that H^+ translocation through each of the two subsystems is intrinsically independent of the state of the other subsystem. The rate of H^+ translocation through the channel segment is thus determined only by the difference in H^+ concentration and electrical potential across it, and by its intrinsic permeability properties. The rate of H^+ translocation through the catalytic unit is likewise determined by the H^+ concentrations at its reactive interfaces, the electrical potential difference across it, the cellular concentrations of ATP, ADP, and inorganic phosphate, P_i, as well as the rate constants for interconversion among its various states. Even though these two subsystems are assumed to be independent of each other, they must operate under the constraint that the H^+ concentration[7] in the intermediary compartment (the antechamber) will determine the stationary rate of H^+ translocation through both units.

H^+ translocation through the catalytic unit is ultimately coupled to the breakdown or synthesis of ATP. This coupling is presumably rooted in conformational changes in the catalytic unit. It seems likely that the overall process will involve a fairly large number of intermediate steps, including a phosphorylated intermediate which eventually may be identified. At the present time, it seems reasonable to neglect such complexities and focus upon a particular four-step scheme for H^+ transport. As depicted in Fig. 8, H^+ translocation may be mediated by negatively charged groups, for example, carboxylates that are located in the wall of a "channel" that stretches through the catalytic unit. These groups can face towards either of the two reactive surfaces of the catalytic unit and react with H^+ in the cytoplasm or antechamber, respectively. The neutral complex thus formed can freely "translocate" from one position to the other. The translocation of the negatively charged carboxylate groups, on the other hand, are presumed to depend upon ATP synthesis or breakdown (Boyer, 1975). The actual physical movement of the carboxylate groups may be quite limited, as long as it is ensured that a particular group cannot be simultaneously accessible to both of the

[7] The "concentration" of H^+ in the antechamber is a virtual concentration related to the time-averaged probability of finding a H^+ in this part of the channel, and to the buffer capacity of the groups that line the channel wall.

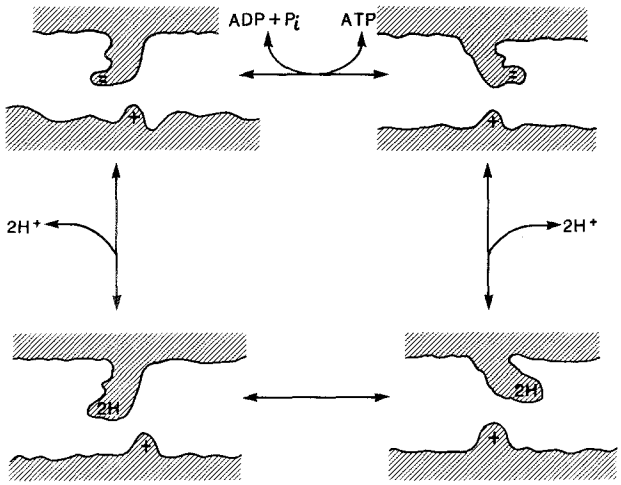

Fig. 8. A possible scheme for the active translocation reactions (after Boyer, 1975). The reactions are assumed to occur in a void in the catalytic unit. The translocating groups denoted by two negative charges can face towards the right or left of the barrier for H^+ leak depicted by the positive charge in the wall of the void. Protons can react with the translocating groups in either configuration to form $E''H_2$ and $E'H_2$, respectively; the neutral complex EH_2 can "translocate" freely across the barrier associated with the positive charge. The "translocation" of the unloaded E^{--} group, however, proceeds only if it is coupled to ATP hydrolysis (or synthesis)

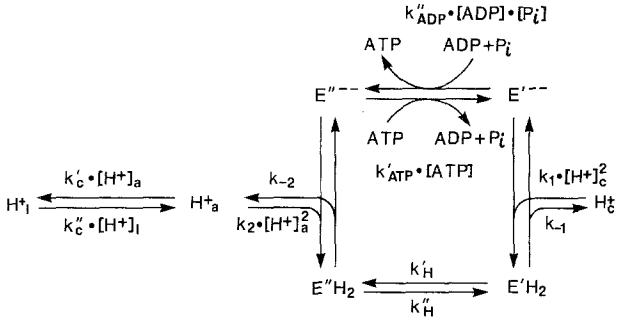

Fig. 9. Reaction scheme for active H^+ transport. The horizontal segment at left depicts H^+ translocation through the transmembrane channel, the square segment at right depicts the reactions in the catalytic unit. Note how the two segments are intrinsically independent of each other, but coupled through the variations in $[H^+]_a$, the H^+ concentration in the antechamber. $[H^+]_l$ and $[H^+]_c$ are the luminal and cellular H^+ concentrations, respectively. [ATP], [ADP] and $[P_i]$ refer to concentrations in the cell. All other rate constants are defined by the graphs. Note that any transmembrane potential difference is incorporated into the rate constants (for the present model in k'_c, k''_c, k'_{ATP} and k''_{ADP}). The association constants for E' and E'', K_1 and K_2, are defined as k_1/k_{-1} and k_2/k_{-2}, respectively

aqueous phases. The critical H^+-translocating groups, therefore need not be in actual contact with either of the two aqueous compartments. The communication with the reactive interfaces can be visualized to be mediated through the channel that winds through the catalytic unit. Nonspecific leakage of H^+ through the catalytic unit is prevented by one or more positive charges lining the wall of the channel.

The transport of H^+ through the pump probably occurs along a series of coordination sites (a sequence of energy wells and energy barriers), by rapid jumps from one energy minimum to the next; the jump rate in a particular direction will depend upon the height of the adjacent barrier that the H^+ must cross. This picture is, of course, similar to the description of ion movement through narrow channels (*see*, e.g., Andersen & Procopio, 1980). There is a fundamental difference, however: at some point in the transport pathway through the pump the H^+ translocation is tightly coupled to conformational changes of the channel. It is necessary, therefore, to consider not only the translocation of the H^+, but also the movement of the carboxylate groups. It is this coupled movement of H^+ and carboxylate across the permeability barrier (exemplified by the fixed positive charge that distinguishes the present scheme from a simple channel mechanism. The *kinetic* description of the tightly coupled H^+ transport through the catalytic unit is, in fact, identical with that of a conventional mobile carrier (*see*, e.g., Wilbrandt & Rosenberg, 1961; Läuger & Stark, 1970; Hladky, 1979). The overall H^+ transport, however, is not describable by a simple mobile carrier formalism, because the H^+ translocation through the catalytic unit is coupled to the translocation through the transmembrane channel.

The kinetic transitions of this system are illustrated in Fig. 9. The equations associated with the diagrams are solved in the Appendix. The final expression for the H^+ transport rate for a stoichiometry of $2H^+/ATP$ is:

$$J_H = \frac{2\cdot\{K_1\cdot[H^+]_c^2\cdot k'_H\cdot k'_{ATP}\cdot[ATP] - K_2\cdot(J_H/k'_c+(k''_c/k'_c)\cdot[H^+]_1)^2\cdot k''_H\cdot k''_{ADP}\cdot[ADP]\}}{\{k'_{ATP}\cdot[ATP]\cdot(K_1\cdot[H^+]_c^2\cdot((k'_H+k''_H)/k_{-2}+1)+1+k''_H/k_{-2}+k'_H/k_{-1}) + k''_{ADP}\cdot[ADP]\cdot[P_i]\cdot(K_2\cdot(J_H/k'_c+(k''_c/k'_c)\cdot[H^+]_1)^2\cdot((k'_H+k''_H)/k_{-1}+1)+1+k''_H/k_{-2}+k'_H/k_{-1}) + K_2\cdot(J_H/k'_c+(k''_c/k'_c)\cdot[H^+]_1)^2\cdot(k''_H+(k'_H+k''_H)\cdot K_1\cdot[H^+]_c^2)+K_1\cdot k'_H\cdot[H^+]_c^2\}}. \quad (22)$$

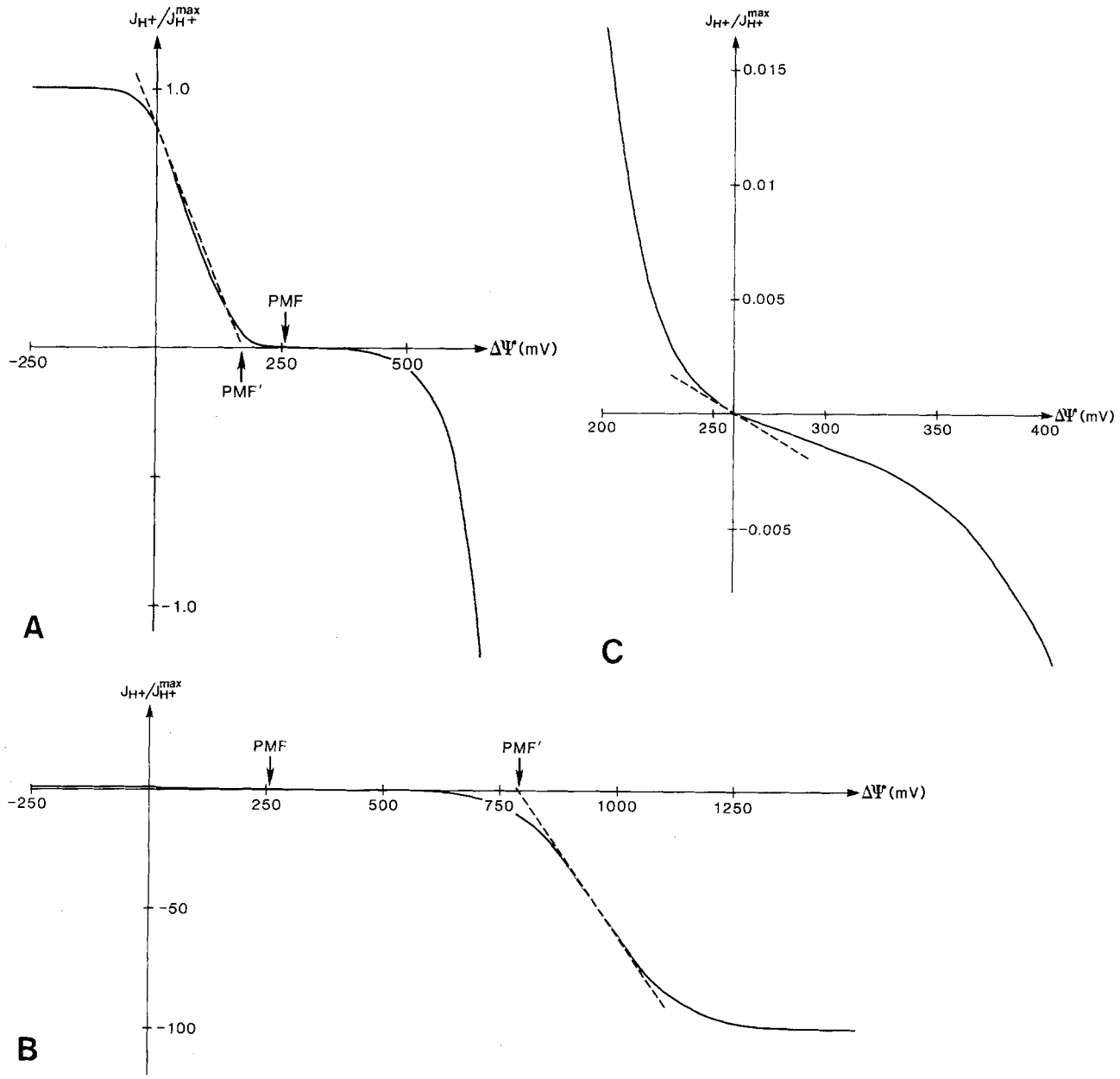

Fig. 10. Theoretical J_H *vs.* $\Delta\Psi$ characteristic for the proposed H^+ pump. *Abscissa:* transmembrane potential (reference is serosal solution). *Ordinate:* J_H/J_H^{max}. The relative values of the various rate constants are: $k_c=1$, $k_{ATP}=k_{-1}=k_{-2}=10^4$, $k'_H=k''_H=300$ (all in sec^{-1}). $\Delta G_{ATP}=-50$ kJ/mol, $K_1=K_2=1$, and $[H^+]_i=[H^+]_c=0.1$. $\Delta\Psi$ is assumed to be distributed equally across the transmembrane channel and the catalytic unit. (*A*): $-250\,mV \leqq \Delta\Psi \leqq +650\,mV$. The full curve is the theoretical J_H *vs.* $\Delta\Psi$ characteristic; the arrow denotes the position of the reversal potential of the pump; the true PMF = +259 mV at 25 °C. The interrupted line is drawn by eye, to illustrate that the J_H *vs.* $\Delta\Psi$ characteristic can be approximated by a straight line over an extended potential range. Note the extrapolated intercept with the abscissa, the apparent PMF, is only +173 mV or about 2/3 of the true PMF. (*B*): $-250\,mV \leqq \Delta\Psi \leqq +1500\,mV$. The scale on the ordinate is diminished by a factor 100 to permit visualization of the reversed (mucosal-to-serosal) J_H. The interrupted line is drawn by eye to emphasize that the theoretical characteristic again can be approximated by a straight line over an extended potential span. Note that the intercept of this line with the abscissa, the apparent PMF for the reversed current, again differs significantly from the true PMF. (*C*): $+200\,mV \leqq \Delta\Psi \leqq +400\,mV$. The scale on the ordinate is magnified 100-fold relative to *A* to visualize the J_H *vs.* $\Delta\Psi$ relation in the vicinity of the reversal potential. The full line is the theoretical characteristic. The interrupted line is drawn by eye to emphasize the very narrow potential range where the characteristic can be approximated by a straight line!

This equation expresses how J_H varies as a function of the cellular and luminal H^+ concentrations and the transmembrane potential, albeit that J_H must be obtained as the root of a cubic equation. In practice J_H is, therefore, most conveniently obtained by numerical approximation. The relation between J_H and $\Delta\Psi$ is illustrated in Fig. 10 for a particular set of rate constants and a relatively simple voltage-

dependence of the rate constants (*see* Appendix). Figure 10*A* and *B* illustrate the behavior observed over a large potential range ($-250\,\text{mV} \leqq \Delta\Psi \leqq +1500\,\text{mV}$), while Fig. 10*C* illustrates the potential dependence of J_H in a fairly narrow potential range around the reversal potential.

These graphs contain several interesting features. The most exciting of these is the *S*-shaped relationship between J_H and $\Delta\Psi$, and the existence of *three* separate potential regions where there exists a fairly linear relation between J_H and $\Delta\Psi$. There is, as expected, a fairly narrow region around the reversal potential where J_H is linear with $\Delta\Psi$ (*see* Fig. 10*C*). Somewhat unexpected is the finding of two additional, much more extensive, regions where J_H is an approximately linear function of $\Delta\Psi$. This behavior is most pronounced for the segment to the left of the PMF (*see* Fig. 10*A*). These latter two linear segments are important because the linear behavior extends almost to the baseline ($J_H=0$). It is therefore possible to misinterpret either of them, in particular the leftmost, to reflect the behavior of the H^+ pump in a region around the reversal potential – where this latter parameter, the apparent PMF, is estimated by extrapolation of the linear segment to $J_H=0$. As illustrated in Fig. 10*A*, this estimate may differ considerably from the true PMF defined by J_H being exactly zero.

The true PMF can be obtained from Eq. (22) upon setting $J_H=0$, in which case we have that:

$$K_1 \cdot [H^+]_c^2 \cdot k'_H \cdot k'_{ATP} \cdot [ATP] = K_2 \cdot (k''_c/k'_c)^2 \cdot [H^+]_1^2 \cdot k''_H \cdot k''_{ADP} \cdot [ADP] \cdot [P_i] \quad (23)$$

or by separating out the H^+ concentration and potential terms, one finds that:

$$\left\{ \frac{[H^+]_1 \cdot \exp\left(\frac{F \cdot \Delta\Psi}{RT}\right)}{[H^+]_c} \right\}^2 = \frac{K_1 k'_H \cdot k_{ATP}}{K_2 \cdot k''_H \cdot k_{ADP}} \cdot \frac{[ATP]}{[ADP] \cdot [P_i]}. \quad (24)$$

If the coupling between H^+ transport and metabolism (ATP breakdown/synthesis) is perfect, one can also describe the condition of $J_H=0$ as the thermodynamic equilibrium for the pump, in which case

$$2 \cdot PMF = -\Delta G_{ATP} = -\left(\Delta G^o_{ATP} + RT \cdot \ln\left\{\frac{[ADP] \cdot [P_i]}{[ATP]}\right\}\right). \quad (25)$$

A comparison of Eqs. (24) and (25) shows that:

$$G^o_{ATP} = -RT \cdot \ln\left\{\frac{K_1 \cdot k'_H \cdot k_{ATP}}{K_2 \cdot k''_H \cdot k_{ADP}}\right\} \quad (26)$$

which describes the thermodynamic constraint that exist upon the kinetics of H^+ movement through the catalytic unit.

The existence of linear segments far away from the PMF is a fairly common feature of kinetic models of active transport in which the active translocation steps are rate limiting (Rottenberg, 1973; Gradmann, Nansen & Slayman, 1981; Andersen & Steinmetz, *in preparation*). The extent of the linearity will, of course, depend upon the specific values of rate constants and especially on the voltage-dependence of the various rate constants. If the movement of the H^+ or of the carboxylate group is a diffusion-like movement over a fairly smooth barrier, rather than a jump across a sharp barrier, one finds that the voltage-dependence of the rate constants become less pronounced (Andersen & Fuchs, 1975; Andersen, 1978*a*, *b*). All three linear segments in the J_H *vs.* $\Delta\Psi$ plot (Fig. 10) will then be more extensive. The linear segments, far away from the reversal potential of the pump, are particularly noticeable when ΔG_{ATP} is large. With such large values the apparent PMF (PMF′) may differ substantially from the real PMF. Thus, in Fig. 10*A*, PMF′ is approximately two thirds of the PMF. The stoichiometry of the pump as calculated from PMF′ according to Eq. (20), therefore, would be off by a factor of 1.5. This is, of course, a special case, but it is noteworthy that the apparent stoichiometry would be about 3, which is the value derived by Dixon and Al Awqati (1980).

The biophysical basis for this behavior seen in Fig. 10 is, of course, that the turnover of the pump is exceedingly slow in the vicinity of the PMF, such that a measurable pump rate is observed only when $\Delta\Psi$ is very far from the *PMF*. One may thus have a hyperbolic *sine* relation between $\Delta\Psi$ and J_H in the vicinity of the PMF (*see* Fig. 10*C*). But large distances from the PMF one will observe a finite transport rate that is determined by other, voltage-independent, rate constants. It is this interplay between voltage-dependent and voltage-independent rate constants in the catalytic unit and H^+ transport through the transmembrane channel that produces the extended linear segments in the J_H *vs.* $\Delta\Psi$ plot.

As $\Delta\Psi$ departs even further from the PMF, one will finally observe that the J_H reaches a constant, voltage- and ATP-independent value, the magnitude of which is given by:

$$J_H^{max,f} = \frac{2 \cdot K_1 [H^+]_c^2 \cdot k_{-2} \cdot k'_H}{K_1 \cdot [H^+]_c^2 (k'_H + k''_H + k_{-2}) + k_{-2} + k''_H + k'_H k_{-2}/k_{-1}} \quad (27)$$

or

$$J_H^{max,r} = \frac{2 \cdot k_{-1} \cdot k''_H}{k'_H + k''_H + k_{-1}}. \quad (28)$$

Where Eq. (27) applies in the cell-to-lumen direction and Eq. (28) applies in the lumen-to-cell direction. The precise form of these limiting equations depends upon the assumptions made for the voltage-dependence of the various rate constants. The asymmetry between the expressions for $J_H^{max,f}$ and $J_H^{max,r}$ occurs because the H^+ concentration in the antechamber will increase as $\Delta\Psi$ increases, if part of $\Delta\Psi$ falls across the transmembrane channel. It is thus possible to use a potential difference to increase the H^+ concentration at a critical position (in the antechamber) – and to ensure complete saturation of the reversed H^+ transport! It is very important to note, however, that the pump in any case will behave as a constant-current source at very large potential excursions (*see also* Finkelstein, 1964; Gradmann, 1978; Läuger, 1979; Gradmann et al., 1981).

Such saturation of J_H has, in fact, been observed by Cohen and Steinmetz (1980) who found that J_H approached a maximal rate as the cellular pH was decreased to a value near 6.7. Similarly increasing the luminal pH above 7.4 or applying a lumen-negative transepithelial PD will lead to saturation of J_H (Andersen et al., *unpublished observations*). Under these physiologic conditions the H^+ pump begins to resemble a constant-current (zero conductance) source.

The proposed model is qualitatively consistent with the experimental data obtained on H^+ transport by the turtle bladder. The experimental data, however, are clearly insufficient to determine the various rate constants and can only support a preliminary model. Among the deficiencies of the model, we note that only four states are allowed for the catalytic unit and that only a single transition is assumed to be voltage-dependent. A more complete description must include the various intermediate states and a more realistic assignment of the voltage-dependence of the rate constants. Furthermore, we have ignored all saturation phenomena in the transmembrane channel and in the ATP/ADP reactions. Experiments with simple channels, such as the gramicidin A channel (*see* Finkelstein & Andersen, 1981, for a recent review) and with the *Neurospora* H^+-ATPase (Bowman & Slayman, 1979) indicate that these phenomena should be taken into account before a model can be considered adequate. In addition, we have assumed that the "concentration" of H^+ in the antechamber can be varied without any effects on the charge density in this region — that is, without any changes in the electrostatic potential across the channel and the catalytic unit. Experiments with hydrophobic ions in lipid bilayer membranes have clearly demonstrated that significant potential changes may be produced by such localized charge accumulations (Andersen et al., 1978).

Despite these shortcomings, the simplicity of the scheme and its ability to account for a number of otherwise unexplained experimental results make it an attractive working model. It suggests that the real pump is neither a constant-current source nor a simple electromotive force. Depending upon the experimental protocols, it may appear as one or the other.

Finally, a detailed understanding of the proton pump must await biochemical identification and purification of the pump complex in the plasma membrane. Since the H^+ pumps appear to be localized in the apical membranes of only a minority of epithelial cells, this goal has yet to be reached for any of the electrogenic proton pumps of tight epithelial membranes.

This work was supported by grants AM 30693 and GM 21342 from the National Institutes of Health. Dr. Steinmetz was the recipient of a Josiah Macy, Jr., Faculty Award during part of this work. Dr. Andersen is an Irma T. Hirschl Career-Scientist Awardee.

References

Al Awqati, Q., Mueller, A., Steinmetz, P.R. 1977. *Am. J. Physiol.* **233(6)**:F502–F508

Amory, A., Foury, F., Goffeau, A. 1980. *J. Biol. Chem.* **255**:9353–9357

Andersen, O.S. 1978a. *In*: Renal Function. G.H. Giebisch and E.F. Purcell, editors. pp. 71–99. Josiah Macy, Jr., Foundation, New York

Andersen, O.S. 1978b. *In*: Membrane Transport in Biology. G.H. Giebisch, D.C. Tosteson, and H.H. Ussing, editors. Vol. 1, 369–446. Springer-Verlag, New York

Andersen, O.S., Feldberg, S., Nakadomari, H., Levy, S., Mc Laughlin, S. 1978. *Biophys. J.* **21**:35–70

Andersen, O.S., Fuchs, M. 1975. *Biophys. J.* **15**:795–830

Andersen, O.S., Procopio, J. 1980. *Acta Physiol. Scand. Suppl.* **481**:27–35

Aronson, P.S. 1981. *Am. J. Physiol.* **240**:F1–F11

Baird, B.A., Hammes, G.G. 1979. *Biochim. Biophys. Acta* **549**:31–53

Beauwens, R., Al Awquati, Q. 1976. *J. Gen. Physiol.* **68**:421–439

Bourgoignie, J., Klahr, S., Yates, J., Guerra, L., Bricker, N.S. 1969. *Am. J. Physiol.* **217**:1496–1503

Bowman, B.J., Slayman, C.W. 1977. *J. Biol. Chem.* **252**:3357–3363

Bowman, B.J., Slayman, C.W. 1979. *J. Biol. Chem.* **254**:2928–2934

Boyer, P.D. 1975. *FEBS lett.* **58(1)**:1–6

Brodsky, W.A., Cabantchik, Z.I., Davidson, N., Ehrenspeck, G., Kinne-Saffran, E.M., Kinne, R. 1979. *Biochim. Biophys. Acta* **556**:490–508

Case, D.A., Karplus, M. 1979. *J. Mol. Biol.* **132**:343–368

Cohen, L.H., Husted, R.F., Mueller, A., Steinmetz, P.R. 1980. *Clin. Res.* **28**:532A

Cohen, L.H., Mueller, A., Steinmetz, P.R. 1978. *J. Clin. Invest.* **61**:981–986

Cohen, L.H., Steinmetz, P.R. 1980. *J. Gen. Physiol.* **76**:381–393
Dame, J.B., Scarborough, G.A. 1980. *Biochemistry* **19**:2931–2937
Delhez, J., Dufour, J.P., Thines, D., Goffeau, A. 1977. *Eur. J. Biochem.* **79**:319–328
Dies, F., Lotspeich, W.D. 1967. *Am. J. Physiol.* **212**:61–71
Dixon, T.E., Al Awqati, Q. 1979. *Proc. Natl. Acad. Sci. USA* **76 (7)**:3135–3138
Dixon, T.E., Al Awquati, Q. 1980. *J. Biol. Chem.* **255(8)**:3237–3239
Dufour, J.P., Bourty, M., Goffeau, A. 1980. *J. Biol. Chem.* **255 (12)**:5735–5741
Dufour, J.P., Goffeau, A. 1978. *J. Biol. Chem.* **253(19)**:7026–7032
Ehrenspeck, G., Brodsky, W.A. 1976. *Biochim. Biophys. Acta* **419**:555–558
Essig, A., Caplan, S.R. 1968. *Biophys. J.* **8**:1434–1457
Fanestil, D.D., Park, C.S. 1980. *Fed. Proc.* **39**:738
Finkelstein, A. 1964. *Biophys. J.* **4**:421–440
Finkelstein, A., Andersen, O.S. 1981. *J. Membrane Biol.* **59**:155–171
Finkelstein, A., Mauro, A. 1963. *Biophys. J.* **3**:215–237
Finn, J.T., Cohen, L.H., Steinmetz, P.R. 1977. *Kidney Int.* **11**:261–266
Fischer, J.L., Husted, R.F., Steinmetz, P.R. 1981. *Clin. Res.* **29**:687A
Forte, J.G., Ganser, A., Beesly, R., Forte, T.M. 1975. *Gastroenterology* **69**:175–189
Forte, J.G., Machen, T.E., Öbrink, K.J. 1980. *Annu. Rev. Phys.* **42**:111–126
Frauenfelder, H., Petsko, G.A. 1980. *Biophys. J.* **32**:465–483
Ganser, A.L., Forte, J.G. 1973. *Biochim. Biophys. Acta* **307**:169–180
Gradmann, D. 1978. *J. Membrane Biol.* **44**:1–24
Gradmann, D., Hansen, U.P., Long, W.S., Slayman, C.L., Warncke, J. 1978. *J. Membrane Biol.* **39**:333–367
Gradmann, D., Hansen, U.P., Slayman, C.L. 1981. *Curr. Top. Membr. Transp. (in press)*
Hirschhorn, N., Frazier, H.S. 1971. *Am. J. Physiol.* **220**:1158–1161
Hladky, S.B. 1979. *Curr. Top. Membr. Transp.* **12**:53–164
Husted, R.F., Cohen, L.H., Steinmetz, P.R. 1979. *J. Membrane Biol.* **47**:27–37
Husted, R.F., Mueller, A.L., Kessel, R.G., Steinmetz, P.R. 1981. *Kidney Int.* **19**:491–502
Husted, R.F., Steinmetz, P.R. 1979. *J. Pharmacol. Exp. Ther.* **210**: 264–268
Husted, R.F., Steinmetz, P.R. 1981. *Am. J. Physiol.* **241**:F315–F321
Kagawa, Y., Sone, N., Hirata, H., Yoshida, M. 1979. Structure and function of H^+-ATPase. *J. Bioenerget. Biomembr.* **11**:39–78
Kasbekar, D.K., Durbin, R.P. 1965. *Biochim. Biophys. Acta* **105**:472–482
Kedem, O., Caplan, S.R. 1965. *Trans. Faraday Soc.* **61**:1897–1911
Kelly, S., Dixon, T.E., Al Awquati, Q. 1980. *J. Membrane Biol.* **54**:237–243
Koefoed-Johnsen, V., Ussing, H.H. 1958. *Acta Physiol. Scand.* **42**:298–308
Koeppen, B.M. 1980. Ph.D. Thesis. University of Illinois at Urbana-Champaign, Urbana (Il.)
Lakowicz, J.R., Weber, G. 1973. *Biochemistry* **12**:4171–4179
Läuger, P. 1979. *Biochim. Biophys. Acta* **552**:143–161
Läuger, P., Stark, G. 1970. *Biochim. Biophys. Acta* **211**:458–466
Lee, J., Simpson, G., Scholes, P. 1974. *Biochem. Biophys. Res. Commun.* **60(2)**:825–832
Loewenstein, W.R. 1979. *Biochim. Biophys. Acta* **560**:1–65
Ludens, J.H., Fanestil, D.D. 1972. *Am J. Physiol.* **223**:1338–1344
Matile, P., Moor, H., Mühlethaler, K. 1967. *Arch. Mikrobiol.* **58**:201–211
Miller, C., White, M.M. 1980. *Ann. N.Y. Acad. Sci.* **341**:534–548
Milutinovic, S., Sachs, G., Haase, W., Schulz, I. 1977. *J. Membrane Biol.* **36**:253–280
Mitchell, P. 1980. *Ann. N.Y. Acad. Sci.* **341**:564–584
Murer, H., Hopfer, U., Kinne, R. 1976. *Biochem. J.* **154**:597–604
Nagel, W., Durham, J.H., Brodsky, W.A. 1981. *Biochim. Biophys. Acta* **646**:77–87
Nelson, N. 1980. *Ann. N.Y. Acad. Sci.* **358**:25–35
Nielsen, R. 1979. *Acta Physiol. Scand.* **107**:189–191
Norby, L.H., Schwartz, J.H. 1978. *J. Clin. Invest.* **62**:532–538
Prigogine, I. 1961. Introduction to Thermodynamics of Irreversible Processes. (2nd ed.) Interscience, New York
Racker, E. 1976. A New Look at Mechanisms in Bioenergetics. Academic Press, New York
Reenstra, W., Lee, H.C., Forte, J.G. 1980. *In*: Hydrogen Ion Transport in Epithelia. pp. 155–164. Elsevier/North Holland, Amsterdam —New York
Rothstein, A., Cabantchik, Z.I., Knauf, P. 1976. *Fed. Proc.* **35**:3 – 10
Rottenberg, H. 1973. *Biophys. J.* **13**:503–511
Sachs, G., Spenney, J.G., Lewin, M. 1978. *Physiol. Rev.* **58**:106–173
Scarborough, G. 1980. *Biochemistry* **19**:2925–2931
Schultz, S.G., Frizzell, R.A., Nellans, H.N. 1977. *J. Theor. Biol.* **65**:215–229
Schwartz, J.H. 1976. *Am. J. Physiol.* **231**:565–572
Schwartz, J.H., Rosen, S., Steinmetz, P.R. 1972. *J. Clin. Invest.* **51**:2653–2662
Schwartz, J.H., Steinmetz, P.R. 1971. *Am. J. Physiol.* **220**:2051–2057
Schwartz, J.H., Steinmetz, P.R. 1977. *Am. J. Physiol.* **233(2)**: F145–F149
Shamoo, Y.E., Brodsky, W.A. 1970. *Biochim. Biophys. Acta* **203**:111–123
Singer, S.J. 1974. *Annu. Rev. Biochem.* **43**:805–833
Slayman, C.L. 1965. *J. Gen. Physiol.* **49**:69–92
Slayman, C.L., Long, W.S., Lu, C.H. 1973. *J. Membrane Biol.* **14**:305–338
Solinger, R.E., Gonzalez, C.F., Shamoo, Y.E., Wyssbrod, H.R., Brodsky, W.A. 1968. *Am. J. Physiol.* **215**:249–260
Steinmetz, P.R. 1967. *J. Clin Invest.* **46(10)**:1531–1540
Steinmetz, P.R. 1969. *J. Clin. Invest.* **48(7)**:1258–1265
Steinmetz, P.R. 1974. *Physiol. Rev.* **54**:890–956
Steinmetz, P.R., Husted, R.F., Mueller, A. 1980a. *In*: Hydrogen Ion Transport in Epithelia. I. Schulz, G. Sachs, J.G. Forte, and K.J. Ullrich, editors. pp. 59–68. Elsevier/North Holland Biomedical, Amsterdam — New York
Steinmetz, P.R., Husted, R.F., Mueller, A. 1980b. *Trans. Assoc. Am. Phys.* **93**:289–294
Steinmetz, P.R., Husted, R.F., Mueller, A., Beauwens, R. 1981. *J. Membrane Biol.* **59**:27–34
Steinmetz, P.R., Lawson, L.R. 1970. *J. Clin. Invest.* **49**:596–601
Steinmetz, P.R., Lawson, L.R. 1971. *Am. J. Physiol.* **220**:1573–1580
Steinmetz, P.R., Omachi, R.S., Frazier, H.S. 1967. *J. Clin. Invest.* **46(10)**:1541–1548
Stoner, L.C., Burg, M.B., Orloff, J. 1974. *Am. J. Physiol.* **227**:453–459
Thomas, R.C. 1972. *Physiol. Rev.* **52**:563–594
Ussing, H.H., Zerahn, K. 1951. *Acta Physiol. Scand.* **23**:110–127
VanAmelsvoort, J.M.M., De Pont, J.J.H.H.M., Bonting, S.L. 1977. *Biochim. Biophys. Acta* **466**:283–301
Warncke, J., Slayman, C.L. 1980. *Biochim. Biophys. Acta* **591**:224–233
Wilbrandt, W., Rosenberg, T. 1961. *Pharmacol. Rev.* **13**:109–183
Willsky, G.R. 1979. *J. Biol. Chem.* **254(9)**:3326–3332
Ziegler, T.W., Fanestil, D.D., Ludens, J.H. 1976. *Kidney Int.* **10**:279–286

Received 17 March 1981; revised 25 August 1981

Appendix

Let W', W'_H, W'' and W''_H denote the probability that the catalytic unit exists in state E'^{--}, $E'H_2$, E''^{--} and $E''H_2$, respectively. The transitions among these four catalytic states illustrated in Fig. 9 are then described by the following set of equations:

$$\frac{dW'}{dt} = -(k_1 \cdot [H^+]_c^2 + k''_{ADP} \cdot [ADP] \cdot [P_i]) \cdot W' + k_{-1} \cdot W'_H + k'_{ATP} \cdot [ATP] \cdot W'' \quad (A1)$$

$$\frac{dW'_H}{dt} = -(k_{-1} + k'_H) \cdot W'_H + k_1 \cdot [H^+]_c^2 \cdot W' + k''_H \cdot W''_H \quad (A2)$$

$$\frac{dW''_H}{dt} = -(k_2 \cdot [H^+]_a^2 + k'_{ATP} \cdot [ATP]) \cdot W'' + k_{-2} \cdot W''_H + k''_{ADP} \cdot [ADP] \cdot [P_i] \cdot W' \quad (A3)$$

$$\frac{dW''_H}{dt} = -(k_{-2} + k''_H) \cdot W''_H + k_2 \cdot [H^+]_a^2 \cdot W'' + k'_H \cdot W'_H \quad (A4)$$

and

$$1 = W' + W'_H + W'' + W''_H \quad (A5)$$

In the steady state

$$\frac{dW'}{dt} = \frac{dW'_H}{dt} = \frac{dW''}{dt} = \frac{dW''_H}{dt}. \quad (A6)$$

The potential dependence of the H^+ transport is contained in the potential-variation of the various rate constants. The flux through the catalytic unit is obtained by taking any three of Eqs. (A1)–(A4) and Eqs. (A5) and (A6) and solving for J_H:

$$K_1 = k_1/k_{-1} \quad (A11)$$

and

$$K_2 = k_2/k_{-2} \quad (A12)$$

to denote the association constants of the translocation sites in the catalytic unit.

To proceed further we must assign voltage dependence to some of the rate constants and specify how these rate constants vary with potential. One must also specify how much of the potential difference, $\Delta\Psi$, falls across the transmembrane channel and how much across the catalytic unit, $\Delta\Psi_1$ and $\Delta\Psi_2$, respectively, where

$$\Delta\Psi = \Delta\Psi_1 + \Delta\Psi_2. \quad (A13)$$

A minimal requirement is that k'_c, k''_c, k'_{ATP}, and k''_{ADP} are voltage-dependent. We consider the simplest case of voltage-dependence, which is that observed with sharp, symmetrical barriers, and write:

$$k'_c = k_c \cdot \exp(-F \cdot \Delta\Psi_1/2RT), \quad (A14)$$

$$k''_c = k_c \cdot \exp(F \cdot \Delta\Psi_1/2RT), \quad (A15)$$

$$k'_{ATP} = k_{ATP} \cdot \exp(-F \cdot \Delta\Psi_2/RT), \quad (A16)$$

$$k''_{ADP} = k_{ADP} \cdot \exp(F \cdot \Delta\Psi_2/RT). \quad (A17)$$

We use this convention throughout this article. It should be noted that irrespective of the assumed voltage dependence:

$$k''_c/k'_c = \exp\left(\frac{F\Delta\Psi_1}{RT}\right). \quad (A18)$$

$$J_H = \frac{2 \cdot (k_1 \cdot [H^+]_c^2 \cdot k'_H \cdot k_{-2} \cdot k'_{ATP} \cdot [ATP] - k_2 \cdot [H^+]_a^2 \cdot k''_H \cdot k_{-1} \cdot k''_{ADP} \cdot [ADP] \cdot [P_i])}{\{k'_{ATP} \cdot [ATP] \cdot ((k'_H + k''_H + k_{-2}) \cdot k_1 \cdot [H^+]_c^2 + k_{-1} \cdot k_{-2} + k_{-1} \cdot k''_H + k_{-2} \cdot k'_H) + k''_{ADP} \cdot [ADP] \cdot [P_i] \cdot ((k'_H + k''_H + k_{-1}) \cdot k_2 \cdot [H^+]_c^2 + k_{-1} \cdot k_{-2} + k_{-1} k''_H + k_{-2} \cdot k'_H) + k_{-1} \cdot k''_H k_2 \cdot [H^+]_a^2 + k_{-2} \cdot k'_H \cdot k_1 \cdot [H^+]_c^2 + (k'_H + k''_H) \cdot k_1 \cdot [H^+]_c^2 \cdot k_2 \cdot [H^+]_a^2\}}. \quad (A7)$$

Eq. (A7) expresses J_H through the catalytic unit in terms of the potential difference across the unit and the H^+ concentrations in the cell and the antechamber. To express J_H in terms of measurable parameters, we proceed as follows:

$$J_H = k'_c \cdot [H^+]_a - k''_c \cdot [H^+]_l \quad (A8)$$

in which $[H^+]_a$ and $[H^+]_l$ are the H^+ concentrations of the antechamber and the lumen. $[H^+]_a$ is thus expressed as:

$$[H^+]_a = J_H/k'_c + (k''_c/k'_c) \cdot [H^+]_l \quad (A9)$$

By substitution of Eq. (A9) into Eq. (A7) we finally arrive at the desired expression for J_H in terms of measurable parameters:

$$J_H = \frac{2 \cdot \{K_1 \cdot [H^+]_c^2 \cdot k'_H \cdot k'_{ATP} \cdot [ATP] - K_2 \cdot (J_H/k'_c + (k''_c/k'_c) \cdot [H^+]_l)^2 \cdot k''_H \cdot k'_{ADP} \cdot [ADP] \cdot [P_i]\}}{\{k'_{ATP} \cdot [ATP] \cdot (((k'_H + k''_H)/k_{-2} + 1) \cdot K_1 \cdot [H^+]_c^2 + 1 + k''_H/k_{-2} + k'_H/k_{-1}) + k''_{ADP} \cdot [ADP] \cdot [P_i] \cdot (((k'_H + k''_H)/k_{-1} + 1) \cdot K_2 \cdot (J_H/k'_c + (k''_c/k'_c) \cdot [H^+]_l)^2 + 1 + k''_H/k_{-2} + k'_H/k_{-1}) + K_2 \cdot (J_H/k'_c + (k''_c/k'_c) \cdot [H^+]_l)^2 \cdot (k''_H + (k'_H + k''_H) \cdot K_1 \cdot [H^+]_c^2) + K_1 \cdot [H^+]_c^2 \cdot k'_H\}} \quad (A10)$$

where we have used the abbreviations