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Reconstitution of a Proton Pump from Gastric Mucosa

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Summary. Purified vesicular fractions from hog gastric mucosa have been incorporated into phosphatidyl serine bilayers. In the presence of MgATP on one side and symmetrical Na_2SO_4 solutions, a short circuit current (SCC) away from that side is observed increasing exponentially with time, while the corresponding open circuit potential (OCP) is maintained constant for >30 min. In K_2SO_4 solutions the SCC time course is essentially unchanged, but the OCP falls to almost zero after 15–20 min. In Na – K gradient there is a similar SCC away from the K-side whose exponential rate is increased by ATP added to both sides. The time course of these events depends only on the time from the formation of the black film. These results are interpreted as showing: (1) There is an ATP-driven proton pump generating a constant potential E_H in series with a time dependent conductance $g_H \propto e^{kt}$. (2) There is a shunting K-conductance $g_K \propto e^{k't}$. (3) In the presence of ATP $k' > k$. (4) This time dependence is due to thickness changes in the bilayer. A model relates these results to those obtained with the intact vesicles.

The microsomal or plasma membrane fraction of dog or hog gastric mucosal homogenates take up H^+ into an intravesicular space with the addition of ATP (Lee, Simpson & Scholes, 1974; Sachs *et al.*, 1976*a*, *b*). Redox substrates are neither oxidized by the purified membrane fraction nor show any observable effects on ion distributions (Sachs *et al.*, 1976*b*). The uptake of H^+ and simultaneous efflux of K^+ (Sachs *et al.*, 1976*a*) seems to be a property of the K^+-ATPase (Ganser & Forte, 1973) which is 40-fold enriched in the purified membranes. Much of the evidence relating to this H^+/cation exchange suggests that the pump mechanism is neutral or, if electrogenic, then isopotential (Lee *et al.*, 1974; Sachs *et al.*, 1976*a*, *b*). Although techniques are available for potential measurements in vesicles and for short circuiting vesicles using a combination of ionophores or lipid permeable ions (Skulachev, 1976), measurements of conductance in vesicles is considerably more difficult. Data using ionophores to dissipate H^+ gradients or to alter rates of H^+ uptake or K^+

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efflux suggested that proton conductance was predominant in these membranes and that the inherent K^+ or Cl^- conductance was low. Cation exchange efflux studies provided evidence for a cation-selective path corresponding to sequence *V* (Eisenman, 1961) in the nonenergized membrane. A similar selectivity was obtained for H^+ uptake dependent on ATP and for ATPase activity and these data suggest the possibility of a cation conductance pathway, although from the above, of low magnitude. The low conductance of artificial bilayers may allow a more exact analysis of these properties.

This work reports on the conductance properties of planar bilayers following incorporation of the gastric vesicles and on the electrical events associated with ATP-induced transport in the artificial system.

Materials and Methods

Hog gastric membrane vesicles were prepared as described in detail elsewhere (Saccomani *et al.*, 1976). Briefly, this involved separation of the vesicular fraction of the gastric homogenate on a ficoll-sucrose density gradient (Fig. 1) and the lighter of the 2 membrane peaks was used in these studies. The anodic peak from free flow electrophoretic fractionation of this peak (Fig. 2) contains the highest activity of the K^+-ATPase (Saccomani *et al.*, 1976) and was used on occasion (Table 1). The fractions were used either on the day of preparation or following freezing and storage at $-84\,^{\circ}C$. The suspension was added to one side of the bilayer chamber at a final concentration of approximately 10–25 μg protein ml^{-1} after formation of the bilayer, with an activity of 60–90 μmole Pi mg^{-1} protein hr^{-1}.

The electrical properties of the bilayer were measured as described previously (Goodall, 1973). Open circuit potential difference or short circuit current were recorded under conditions of constant stirring from the time of film formation. Thus there is a 1/2 to 2-min period before the appearance of the black bilayer region and this is completed within 1–3 min. In view of the thickness dependence of the phenomena to be described, the time course in all records is measured from the initial formation. The outside chamber was connected to ground through the current amplifier and is referred to as "trans" with the sign conventions corresponding. The acceptable limit for bilayer background conductance was $2\text{–}3 \times 10^{-9}\ \Omega^{-1}\ cm^{-2}$. Thinning of the bilayer was monitored visually to the black stage (1–3 min) and thereafter by the current voltage ($I-V$) curve plotted on an $X-Y$ recorder (Esterline Angus 2411 TB) using a triangular wave form from a battery operated function generator (Krohn-Hite 5600). This showed a continuous and approximately linear increase of capacity to 50% at 20 min.

Bionic potentials were measured with 100 mM $SO_4^{=}$ salts of alkali metal cation pairs present on either side of the bilayer. For thallous ion 10 mM concentrations were used because of solubility limitations. Solutions were buffered with 2 mM tris acetate pH 7.4. For cation/anion selectivity a 10:1 concentration gradient of the Na salt was used.

For studies of ATP-dependent transport 10 mM Na_2SO_4 buffered with 2 mM tris acetate pH 7.4 was present on both sides of the bilayer and MgATP or other nucleotide buffered to the same pH was added at a final concentration of 0.75 mM to the "cis" side, i.e., the side containing the vesicles. Inhibitors such as *p*-chloromer curibenzoate (*p*CMBS) (10^{-4} M) were also added to this side. 50 mM K_2SO_4 was added to both

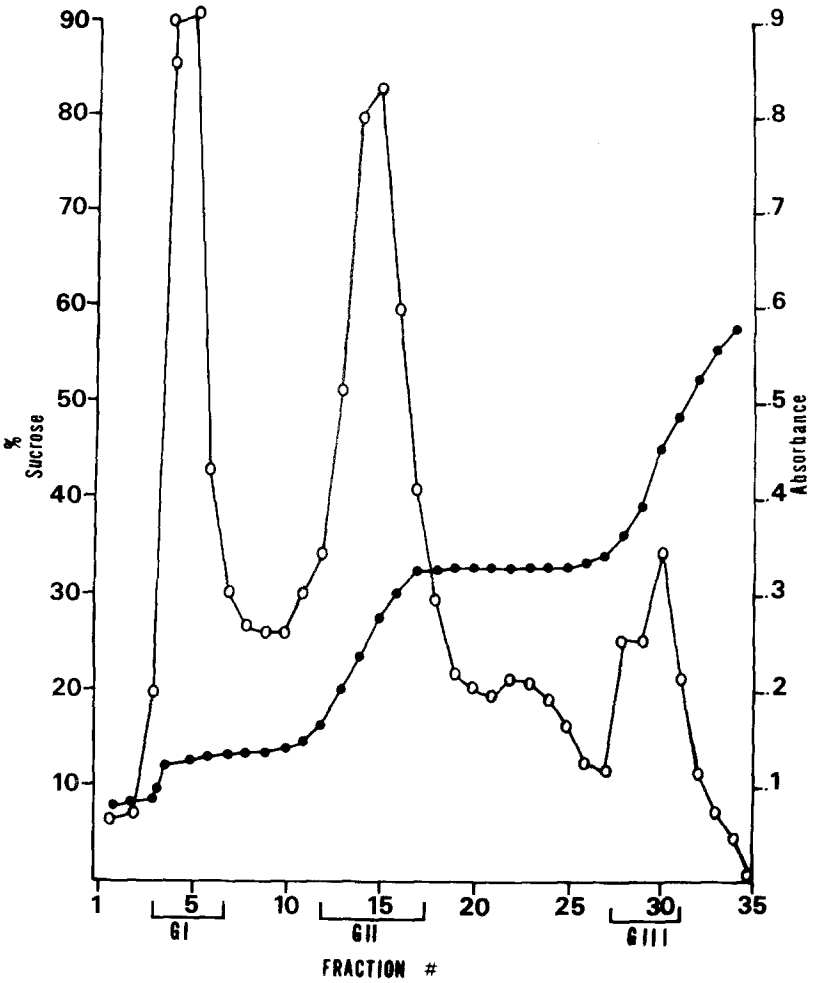

Fig. 1. The profile of protein obtained by fractionating the microsomal pellet of hog gastric mucosal homogenate on a step gradient in a Z60 zonal rotor. *G*1 corresponds to a step between 0.25 M sucrose and 7 % ficoll in 0.25 M sucrose, *G*11 is a step between 7 % ficoll in 0.25 M sucrose and 31 % sucrose and *G*111 between 31 and 60 % sucrose

solutions as necessary in the presence of ATP. The temperature of study was 34 °C and the lipid used was bovine phosphatidyl serine (chromatographically pure, Applied Science Labs) at 3–5 mg/ml in decane (Eastman Kodak).

Apart from the difficulty of incorporation, a major problem has been the sensitivity of the low conductance bilayer to artifacts of various types. The following pragmatic criteria of successful incorporation were used in this study:

(1) A low conductance membrane with only H^+ permeability and virtually no permeability to anions or cations was used throughout.

(2) With incorporation the increment in conductance was smooth or otherwise well characterized rather than the irregular noise which often occurs with contaminated bilayers prior to breakage.

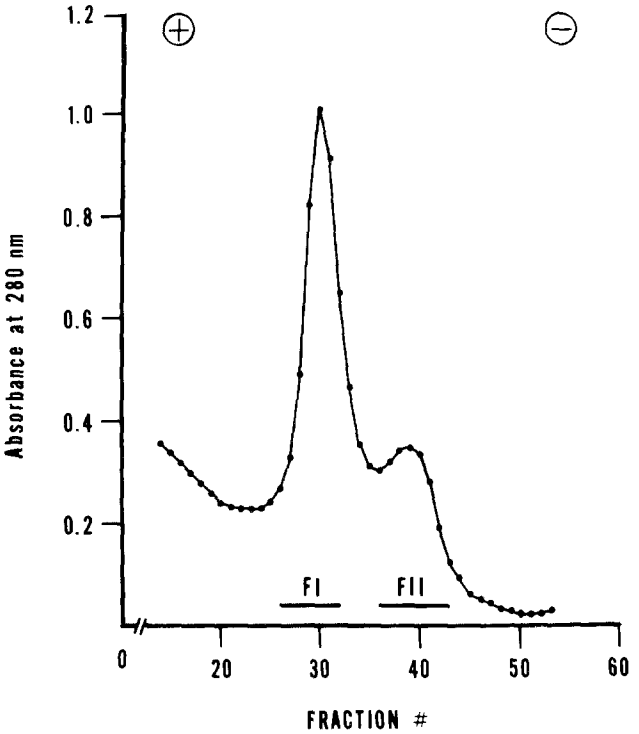

Fig. 2. The profile of protein obtained by fractionating the initial ($G1$) peak from the zonal gradient by free flow electrophoresis showing 2 peaks, $F1$ and $F11$, $F1$ containing the transport activity and the highest $H^+ + K^+$ ATPase activity as well as the bilayer activity of $G1$

Table 1. AMPase and ATPase activity of gastric membranes

	μmoles Pi mg^{-1} hr^{-1}		
	ATPase		AMPase
	Mg^{++}	K^+	
Total homogenate	6.2±0.6	2.0±1.0	0.5±0.3
Microsomal pellet	15.9±1.5	7.1±4.3	1.5±0.6
GI Fraction	6.8±1.1	35.8±2.2	8.8±0.2
FI Fraction	2.7±0.6	66.8±1.4	0.8±0.2

(3) The bilayer was generally stable for at least 30 min after or during incorporation.

(4) The increment in conductance was at least two or more orders of magnitude and was dependent on the concentration of material added.

(5) The denaturation of the protein resulted in an inactive preparation.

(6) The conductance modification corresponded in selectivity to the conductance selectivity of the natural membrane.

(7) The incorporation was reproducible qualitatively from batch to batch.

(8) Pump activity as determined by potential or current development had the same substrate, activator and inhibitor selectivity as the pump in the natural membrane.

(9) The material consisted of few components and was derived from a single structure of defined composition to allow further analysis. These criteria were met by fresh preparations of the gastric membrane fraction.

Results

Conductance

Addition of either the gradient or electrophoretic fraction produced a smooth, time-dependent and ion-selective conductance change in a lipid bilayer composed of a variety of lipids, the most activity occurring in phosphatidyl serine bilayers. The increase of conductance with time was exponential ($g_t = g_0\, e^{kt}$). The conductance obtained was dependent on protein concentration. The rate of increase of conductance, k, was largely independent of protein concentration (Fig. 3). Hence this time dependence, as subsequent results also show, does not appear to be due to rate limitation by incorporation. The actual value of the conductance at any given time is a function of the concentration of protein added, typically the conductance level reached before breakage was between 2 and 3 orders of magnitude above background, and the rate of change had a Q_{10} of about 2. Hence all experiments were performed at 34 °C. The incorporation and pump characteristics were obtained with $G1$ and $F1$ (Figs. 1 and 2). $F2$ did not show any transport characteristics in the bilayer or in the original preparation.

The selectivity sequence of the specific conductances (Table 2) was determined to be $H^+ > K^+ > Rb^+ > Cs^+ > Na$, $Li > Tl^+ > Cl^-$. This sequence corresponds to that found for H^+ uptake and for K^+-ATPase activity with the exception of Tl^+ which is the most active cation for the latter process. The relative intracationic activity for the different vesicular transport (i.e., H^+, K^+ and ANS) processes and bilayer conductance is also consistent with the supposition that the same groups are involved in the natural and artificial membrane.

Transport

Results using the intact vesicles (Sachs *et al.*, 1976*a*) indicated the possibility that the addition of ATP resulted in a K^+:H^+ exchange by the vesicle fraction. Of the different alkali cations tested Na^+ was only

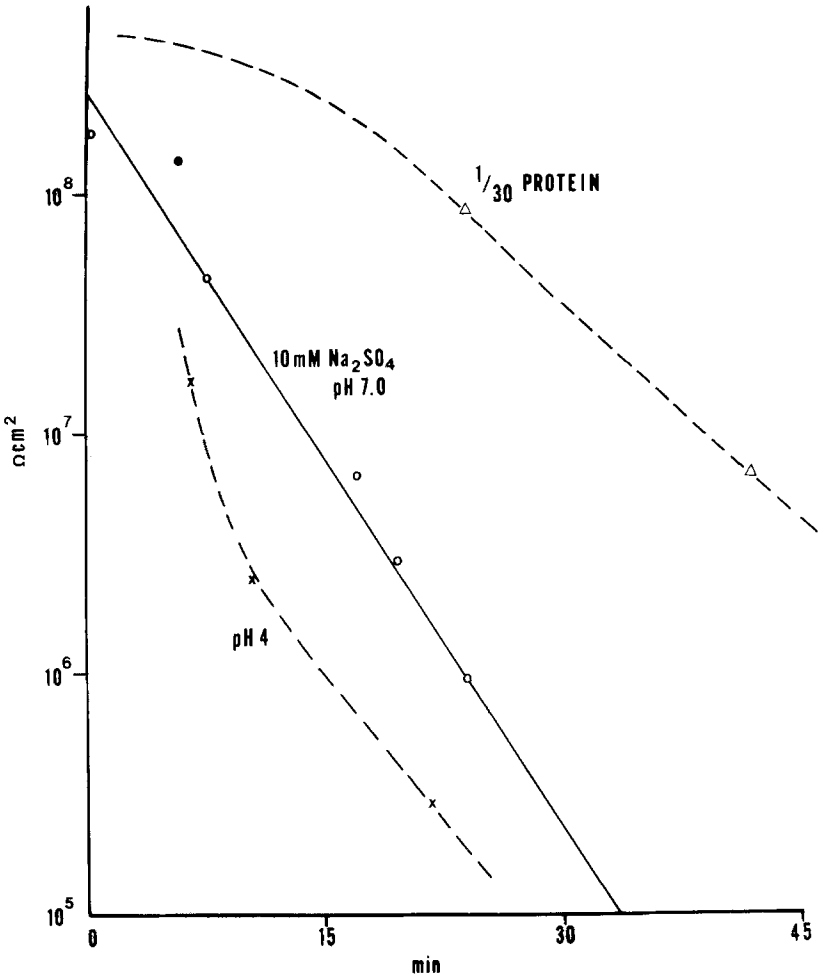

Fig. 3. The effect of addition of *G*1 fraction on the development of conductance in the bilayer. In the absence of addition no change in conductance was observed. At pH 7, in the presence of 10 mM Na_2SO_4 an exponential increase in conductance with time was observed ○——○. Altering the protein concentration △------△ altered the conductance level but had a much lesser effect on the time course of the conductance change. Lowering the pH ×------× increased the conductance but again had a relatively slight effect on the time course

Table 2

	H	NH_4	K	Rb	Cs	Na	Li	TI	Cl
Specific conductance (relative)	10^6	24	10	8	6	1	1	0.75	0.35
ATPase	–	14	14	11	2.3	1	0.56	>19	–

1% as effective as K^+ in the vesicles, hence Na_2SO_4 solutions were used for the bilayer experiments in the ATP studies to possibly unmask an electrogenic H^+ pump.

The addition of 0.25 mM ATP to the cis side of the phosphatidyl serine bilayer resulted in the development of a negative potential (Fig. 4). This orientation is compatible with a proton electrogenic pump directed away from the ATP and vesicle side. The dissipation of the potential by the addition of a protonophore such as tetrachlorsalicylanilide (TCS) would therefore be expected but the conductance increment induced by the ionophore would serve to shunt the potential whether due to H^+ transport or not. The magnitude of the potential was a function of the nature of the lipid. The mean potential obtained with phosphatidyl serine was 33.4 ± 4.7 mV ($n = 17$). The effect of ATP may be due to two factors, the effect of ATP on the pump and the effect of adsorption of ATP on the bilayer surface. $\beta-\gamma$ methylene ATP and ITP also adsorb on the bilayer and produce a transient potential corresponding to 20% of the ATP-dependent potential. The addition of ATP in addition to $\beta-\gamma$

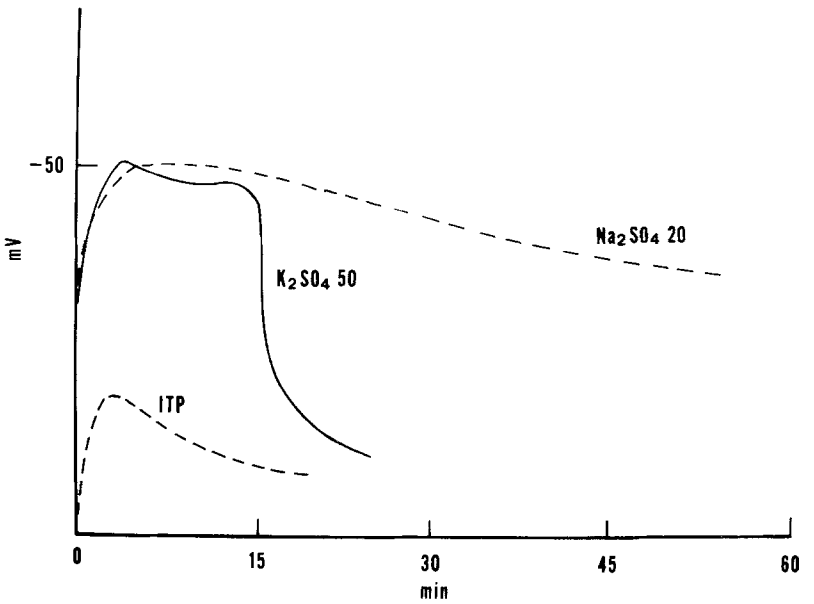

Fig. 4. The effect of ATP addition (upper 3 curves) and ITP addition (lower curve) on one side of the bilayer with incorporated *G*1. The addition of ATP at 0 time resulted in development of a potential of −50 mV which was well maintained in the presence of Na_2SO_4 (------). ITP produced only a fraction of the potential. The results of addition of 50 mM K_2SO_4 to both sides of the bilayer showed that ATP produced essentially the same potential, but after 15 min there was a sharp decline in the potential to about 10% of the peak value (——)

methylene ATP produces a normal ATP response. An inhibitor of the ATPase, *p*CMBS markedly reduces the potential obtained to about 20% of the initial value, but does not reduce the prevailing conductance (Fig. 6). Addition of ATP to the trans side produces a potential of opposite orientation, though of lesser magnitude (*see* Fig. 8 below), showing that although the orientation of the current flow is determined by the sidedness of ATP the incorporation of the protein is not completely

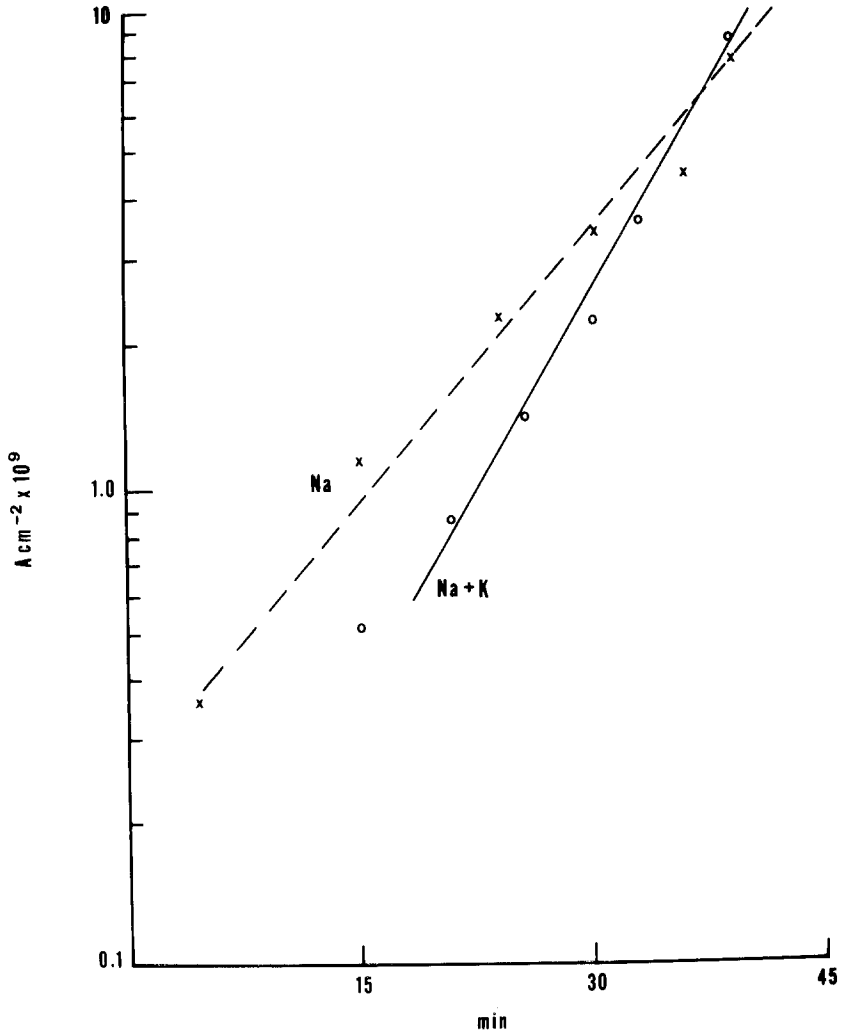

Fig. 5. The increase of short circuit current with time in the presence of Na (×------×) or $Na^+ + K^+$ (o——o). Zero time is the appearance of the initial black region. It should be noted that the presence of K^+ allows a similar exponential current increase which argues against an E_K^+ being present

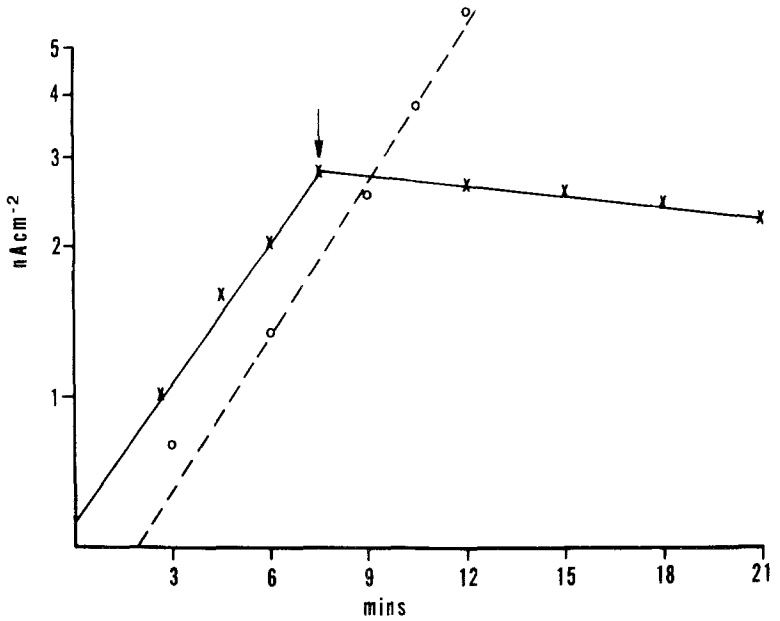

Fig. 6. The effect of 4×10^{-4} M *p*CMBS on short circuit current. The current in the absence of *p*CMBS is shown on the dashed line (o------o). The increase of current is blocked with the addition of *p*CMBS and decays. Higher concentrations of *p*CMBS result in a more rapid fall of current

symmetric, the inside of the vesicle presumably being incorporated preferentially away from the side of addition, i.e., the vesicle opens to the trans surface.

The short circuit current obtained under these conditions is what one would expect from a constant emf imposed on the exponentially increasing conductance (Fig. 5). The maximum (before breakage of the bilayer) corresponds to 12.1 ± 1.7 nA cm^{-2} and is about 1% of the maximal current calculated from the H^+ uptake rate of the native vesicles (Sachs *et al.*, 1976*a*).

The presence of a short circuit current appears to exclude the possibility of adsorption of the ATPase and the generation of a local H^+ concentration gradient.

Effect of K^+

The presence of K^+ symmetrically in both solutions characteristically alters the ATP response. Thus initially with the addition of ATP a potential is obtained similar in magnitude to that observed with Na^+. After some 15 min there is a fall in the potential to 7 ± 3 mV ($n = 10$) (Fig.

4), but with maintenance of the steady increase of the short circuit current (Fig. 5). Hence associated with the fall of potential there is a corresponding increase in conductance. With a further increase in K^+ concentration, the sign of the final potential does not change.

Effect of ATP on Conductance

It is an important question as to whether ATP affects the various conductances of the bilayer in addition to inducing an H^+ pump potential. The problem in answering this question is the finding that the conductance of the bilayer in the presence of vesicles continues to increase exponentially in the presence and absence of ATP. Hence, ATP could effect the conductance by altering the rate of change or the initial value. These two possibilities can be separated by the following analysis.

In a membrane containing two parallel limbs a proton pump with an emf, E_{H^+} and a conductance g_{H^+} and a K^+ pump with an emf E_{K^+} and a conductance g_{K^+} (Fig. 7) the transmembrane potential, E_M is given by

$$E_M = \frac{g_H E_H - g_K E_K}{g_H + g_K}. \tag{1}$$

This equation can be simplified based on the observation that varying the K^+ concentration does not change the final potential and that the short circuit current which would be given by the equation

$$I_{sc} = I_H - I_K$$

does not fall as would be predicted by the presence of an I_K. From these it would appear that E_K is very small, or that $\Delta I_H = \Delta I_K$, i.e., a neutral

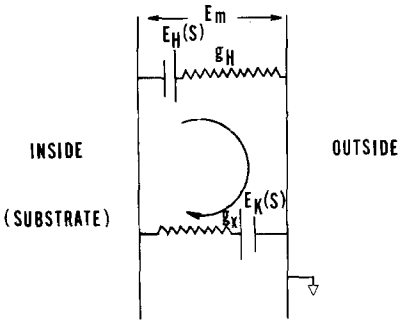

Fig. 7. An equivalent circuit illustrating a possible model for the pump which consists of 2 parallel patterns for K^+ and H^+ with their corresponding emfs and resistances

exchange occurs. Thus

$$E_M = \frac{g_H E_H}{g_H + g_K}. \tag{2}$$

Since the conductance changes exponentially with time, i.e.,

$$g_X(t) = g_X(o)\, e^{kt} \tag{3}$$

the potential at time t can be given by

$$E_M(t) = \frac{g_{H_0}\, e^{k_H t} \cdot E_H}{g_{H_0}\, e^{k_H t} + g_{K_0}\, e^{k_K t}}; \tag{4}$$

$$E_{M_{(t)}} = \frac{E_H}{1 + e^{k(t - t_0)}}, \tag{5}$$

where k, a rate constant is equal to the difference between the rate constants for the change in K^+ and H^+ conductances namely

$$k = k_K - k_H$$

and

$$kt_0 = -\ln g_{K_0}/g_{H_0}$$

where g_{K_0}, g_{H_0} are the initial conductances.

Fitting Eq. (5) to the data for open circuit potential in the presence of ATP we find $k = 0.002$ sec^{-1} and $g_{H_0}/g_{K_0} = 6.5$. Assuming a constant E_H with the appearance of a K^+ conductance, $k_H = 0.004$ sec^{-1} and thus $k_K = 0.006$ sec^{-1}. These data are obtained in the presence of ATP.

It is also possible to derive the rate constant for change of K^+ conductance from other experiments. Addition of ATP to symmetric solutions and on both sides of the bilayer results in only a small change of potential, i.e., there is only a small H^+ emf. Assuming that the H^+ emf can be neglected when ATP is added to both sides of a membrane in the presence of a $K^+ - Na^+$ trans-cis gradient, then current flow under these conditions is due to K^+ movement from trans to cis. The exponential rise of current without ATP gave $k_K = 0.0044$ sec^{-1} increasing to $k_K = 0.010$ sec^{-1} on the addition of ATP (Fig. 8) in fair agreement with the above value.

In the absence of ATP $k_K = 0.002$ sec^{-1} and $g_{H_0}/g_{K_0} \sim 10$ (pH 7.4, 50 mequiv K^+). Thus ATP increases the rate constant for changes of K^+ conductance but has little effect on the value of either K_H or g_{H_0}.

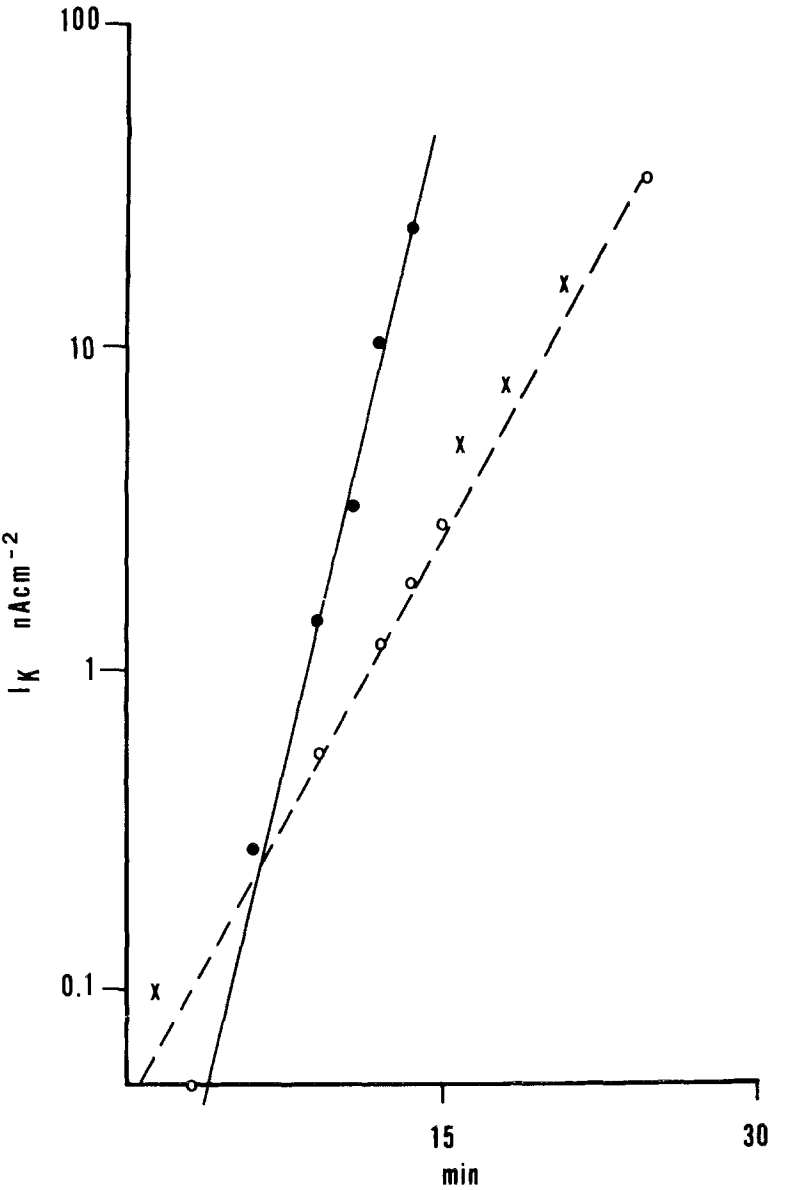

Fig. 8. The rate of change of K^+ current (due to a K^+/Na^+ gradient across the bilayer) as a function of the addition of ATP. This figure compares the K^+ current (inward) in the absence of ATP (o------o) to the current in the presence of ATP on both sides (●——●). It can be seen that ATP increases the magnitude of the current, and the rate of increase of the current

Effect of Bilayer Thickness

Fig. 9 shows that the rate-limiting factor for transport is not incorporation of vesicles but rather something which takes place in the

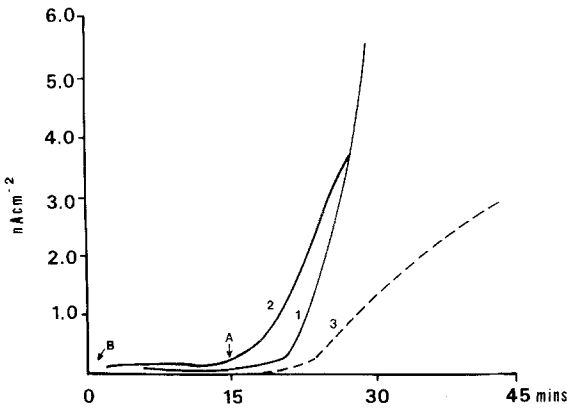

Fig. 9. The effect of bilayer status and sidedness of *G*1 and ATP on short circuit current. In curve *1*, in the presence of ATP, the bilayer was formed at zero time and *G*1 added at the arrow *A*. It can be seen that there is less than a 5-min lag before development of current. In curve *2* *G*1 and ATP are both present, arrow *B*, from the time of formation of the bilayer. It can be seen that there is a 15-min lag before development of current. Curve *3* shows the effect of adding *G*1 to the side opposite to that of ATP (arrow *B*) showing a lower current hence an asymmetry of the ATP site in the original preparation, as well as an even longer lag phase, indicating an additional restriction of incorporation of the ATP site in this configuration

bilayer prior to their addition. When ATP is present it is the time from the formation of the bilayer that is the determining factor in development of short circuit current. The analogous experiment with gramicidin is shown in Fig. 5 in Goodall, 1971. Also shown in Fig. 9 curve *3* is the SCC when the vesicles are added on the opposite side to ATP.

Discussion

Incorporation of membrane located pumps into synthetic membrane has been successfully achieved in many instances using liposomes (Kagawa, Kandrach & Racker, 1973; Hilden, Rhee & Hokin, 1974). In these instances the transport and electrical characteristics of the pump processes have been ascertained by the use of ionophores, lipid permeable ions, and ion flux measurements which, although informative, lack the sensitivity and resolution of direct electrical measurement. The suitability of the planar bilayer for electrical rather than chemical probing of transport events has led to much effort being expended in attempts to incorporate pumps into planar membranes. For various reasons connected with the criteria listed under *Materials and Methods*, success has been limited (Jain *et al.*, 1972).

Our studies on the gastric membrane material essentially fulfill the criteria as listed in the methods section. The initial conductance changes obtained in the absence of ATP show that if there are step changes these are less than $5 \times 10^{-13}\ \Omega^{-1}$. This suggests that the gastric membranes contain a carrier-like or shuttle mechanism. The selectivity of the conductance change corresponds closely to the measured selectivities of cation exchange efflux, H^+ uptake and K^+-ATPase activity in the original preparation (Sachs *et al.*, 1976*a*). The substrate selectivity and inhibitor sensitivity of the ATP dependent potential or current is the same as for ATPase activity. The incorporation, in contrast to the report on $Na^+ + K^+$ ATPase (Jain *et al.*, 1972) seems reproducible to the extent that in fresh preparations 80% of the experiments are successful. The most important factor found in this study is the freshness of the microsomes. The best results obtained here were with microsomes at 16 hr from slaughter. Aging at 0 °C resulted in loss of ability to modify bilayer conductance after about 48 hr. The material that is used for the incorporation experiments is usually density gradient purified fraction. This has been purified further by free flow electrophoresis and this material incorporates with the same characteristics as the density gradient fraction. The highly purified material has 75% of the Coomassie Blue staining material associated with the 100,000 dalton region of SDS polyacrylamide gels (Sachs *et al.*, 1976*a*).

Previous work on reconstitution of pumps in artificial membranes has been confined largely to reconstitution in liposomes. Reconstitution of the electrogenic ATPase of mitochondria results in retention of the electrogenic properties of that ATPase. The electrogenic properties of $Na^+ + K^+$ and Ca^{++} ATPases are more doubtful in the intact biological membrane. That a property change does occur on reconstitution of this type of ATPase is shown by the high anion permeability of the native *SR* vesicles and the low anion permeability of the reconstituted pump vesicles.

The evidence for nonelectrogenicity of the $H^+ + K^+$ ATPase in the isolated mature vesicles is convincing by the techniques used, namely effects of change of conductance of the membranes and redistribution of lipid permeable ions (Sachs *et al.*, 1976*a*). The most striking features of the bilayer reconstituted system on the other hand are (a) the presence of an electrogenic H^+ pump and the nonexistence of active K^+ transport, i.e., $E_K \sim 0$; (b) the exponential increase of all conductances with time; and (c) the increase of the exponential rate k_K in the presence of ATP.

It seems to us that the discrepancy as to active K^+ transport can be explained by considering the possibility that the pump exists as a dimer in the natural membrane, but may dissociate to a monomer in the artificial membrane. If the monomer has two configurations E_1 and E_2, which are H^+ and K^+ transporting, respectively, obligatory coupling in the form $E_1 E_2$ could give neutral exchange.

The evidence for existence of a dimer in the intact vesicles depends on (1) the demonstration of a Hill coefficient, n, of 1.8 when *p*-nitrophenyl phosphate is the substrate, (2) the doubling of the effectiveness of ATP as a competitive inhibitor of *p*-nitrophenylphosphatase with rupture of the vesicular structure (Sachs *et al.*, 1976*a*), (3) The twofold more rapid inactivation of ATPase and H^+ transport compared to inactivation of *p*NPPase (Hung *et al.*, 1976).

Evidence for E_1 and E_2, i.e., 2 sites or forms of the enzyme in the bilayer, comes from the different behavior of the exponential increases in g_H and g_K in presence and absence of ATP. Previous results obtained with gramicidin A (Goodall, 1971) show a similar exponential increase where it seems that the effect is due to a further thinning of the bilayer from 50 Å, at the initial collapse to the bilayer state, to 27 Å after a period of 10–20 min. The activation energy for gramicidin dimer formation, i.e., the conducting state, depends on thickness and is about kT/Å. In the present case one can suppose the ion transporting sites require an activation energy to reach the bilayer surface. Then the results for g_K show that the dependence of this activation energy on thickness (1/4 kT/Å) is increased (t_0 1/2 kT/Å) in the presence of ATP.

Our model assumes that only the $E_1 E_2$ form exists in the dimer. As pointed out elsewhere, (Glynn & Karlish, 1976) this implies that only half the sites are reactive with ATP and that H^+ and K^+ binding sites coexist. The transport sequence can then be written, where the asterisk identifies the same subunit:

$H_2O + E_1^* \cdot E_2$	$\leftrightarrow H^+ P \cdot E_1^* E_2 P + ADP$	H^+ binding
$H^+ P \cdot E_1^* E_2 P + K^+$	$\leftrightarrow H^+ P \cdot E_1^* E_2 PK^+$	K^+ binding
$H^+ P \cdot E_1^* E_2 PK^+$	$\leftrightarrow H^+ P E_2^* E_1 PK^+$	translocation
$H^+ P E_2^* E_1 PK^+$	$\leftrightarrow H^+ + P E_2^* \cdot E_1 + Pi + K^+$	dissociation

As written, this is an obligatory neutral exchange pump.

At the pH of 6.1 the actual ratio of H^+ or Rb^+ exchanged per mole ATP hydrolyzed seems to be 4 (Sachs *et al.*, 1976*a*). As the intracation stoichiometry varies so will the degree of electrogenicity. However, it may be simpler to develop a scheme for the transition between nonelec-

trogenic transport in the vesicle to electrogenic transport in the planar bilayer when we consider dissociation of the imer to the monomeric form. In this case the transport reactions may be written

$$\begin{array}{lll} H_2O + E_1 + ATP \leftrightarrow H^+E_1P & & \text{phosphorylation} \\ H^+E_1P \leftrightarrow H^+E_2P & & H^+\text{-translocation} \\ H^+E_2P \leftrightarrow H^+ + E_2P & & \text{dissociation} \\ E_2P + K^+ \leftrightarrow K^+E_2P & & \text{association} \\ K^+E_2P \leftrightarrow K^+E_1P & & K^+\text{-translocation} \\ K^+E_1P \leftrightarrow K^+ + E_1 + P & & \text{dissociation/dephosphorylation} \end{array}$$

From this scheme the movement of H^+ is now dissociated from the movement of K^+, provided an additional thickness dependent reaction

$$E_2P \rightleftharpoons E_1P \quad \text{(thickness increasing} \rightharpoonup\text{)}$$

is allowed; whereas in the dimer situation, this transformation can occur only during the H^+ and K^+ exchange. K^+ serves to accelerate this interconversion hence the K^+ conductance will increase as a function of phosphorylation. In the absence of K^+, in the presence of Na^+, for example, H^+ transport will result in a potential. K^+, due to the conductance of the reaction $K^+E_2P \rightharpoonup K^+E_1P$ will serve to shunt the H^+ emf but will not produce a K^+ emf. Hence there will not be a drop in the short circuit current associated with the presence of K^+. Also in the monomer, H^+ and K^+ sites sequentially, not simultaneously.

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References

Eisenman, G. 1961. Cation selective glass electrodes and their mode of operation. *Biophys. J.* **2(2):**259

Ganser, A., Forte, J.G. 1973. K^+-stimulated ATPase in purified microsomes. *Biochim. Biophys. Acta* **307:**169

Glynn, I.M., Karlish, S.J.D. 1975. Different approaches to the mechanism of the sodium pump. *In:* Energy Transformation in Biological Systems. Ciba Foundation Symposium. p.31. London

Goodall, M.C. 1971. Thickness dependence in the action of gramicidin A on lipid bilayers. *Arch. Biochem. Biophys.* **147:**129

Goodall, M.C. 1973. Action of two classes of channel forming synthetic peptides on lipid bilayers. *Arch. Biochem. Biophys.* **157:**814

Hilden, S., Rhee, H.M., Hokin, L.E. 1974. Na^+ transport by phospholipid vesicles containing purified $Na^+ + K^+$ ATPase. *J. Biol. Chem.* **249:**7342

Hung, H.H., Saccomani, G., Rabon, E., Schackmann, R., Sachs, G. 1977. H^+ transport by gastric membrane vesicles. *Biochim. Biophys. Acta* **464**:313

Jain, M.K., White, F.P., Strickholm, A., Williams, E., Cordes, E.H. 1972. Studies concerning the possible reconstitution of an active cation pump across an artificial membrane. *J. Membrane Biol.* **8**:363

Kagawa, Y., Kandrach, A., Racker, E. 1973. Partial resolution of the enzymes catalyzing phosphorylation. *J. Biol. Chem.* **248**:676

Lee, J., Simpson, G., Scholes, P. 1974. An ATPase from dog gastric mucosa. Change of pH in membrane vesicles accompanying ATP hydrolysis. *Biochem. Biophys. Res. Commun.* **60**:825

Saccomani, G., Stewart, H.B., Shaw, D., Lewin, M., Sachs, G. 1977. Fractionation of hog gastric mucosa. *Biochim. Biophys. Acta* **465**:311

Sachs, G., Hung, H., Rabon, E., Schackmann, R., Lewin, M., Saccomani, G. 1976a. A non-electrogenic H^+ pump in plasma membranes of hog stomach. *J. Biol. Chem.* **251**:7690

Sachs, G., Rabon, E., Saccomani, G., Sarau, H.M. 1976*b*. ATP and redox in acid secretion. *Ann. N.Y. Acad. Sci.* **264**:456