J. Membrane Biol. 62, 19–27 (1981)

The Journal of
Membrane Biology

Thallium Interaction with the Gastric (K, H)-ATPase

E.C. Rabon and G. Sachs*
Laboratory of Membrane Biology, University of Alabama in Birmingham, Birmingham, Alabama 35294

Summary. The gastric (K,H)-ATPase has been shown to catalyze an electroneutral H^+ for K^+ exchange. Tl^+ is able to substitute for K^+ as an activating cation in the hydrolytic reaction with an apparent dissociation constant of 90 μM as compared to about 870 μM for K^+. The ability of Tl^+ to participate in transport is shown by the development of pH gradients in the presence of Tl^+ following addition of ATP to gastric vesicles and by the ATP-dependent efflux of Tl^+ from gastric vesicles. Inhibition of hydrolysis is observed at pH 7.4 with external Tl^+ concentrations above 3.0 mM. This inhibition of hydrolysis is correlated with inhibition of pH-gradient formation. The inhibition of transport activity is partially relieved by a decrease in medium pH. This inhibitory effect is attributed to Tl^+ binding at an external, low affinity cation site. In contrast to rubidium chloride, at high Tl^+ concentrations, following the initial Tl^+ efflux, there is reuptake of the cation. This rapid uptake is attributed to lipid-dependent Tl^+ entry pathways. The vesicles exhibit a high permeability to thallium nitrate demonstrating a half-time ($t_{\frac{1}{2}}$) for uptake of about 1.0 min in contrast to 46 min for rubidium chloride. In both gastric vesicles or liposomes, external Tl^+ concentrations in excess of 1 to 4 mM are able to dissipate intravesicular proton gradients by an electrically coupled H^+ for Tl^+ exchange. Thus, although Tl^+ is able to activate the gastric ATPase by mimicking K^+, the permeability of this cation in lipid bilayers tends to uncouple H^+ transport at concentrations high enough to generate detectable proton gradients.

Key words: Thallium transport, gastric (K,H)-ATPase, cation activation site, cation inhibitory site

Tl^+ is a group III cation with at least two types of interaction with biological membranes that have been examined in recent years. On one hand, it has been shown that Tl^+ can act as a lipid-permeable cation in mitochondria (Melnick, Monti & Motzkin, 1976; Skulskii, Savina, Glasunov & Saris, 1978) or starved, valinomycin-treated cells of streptococcus lactis 7962 (Kashket, 1979), distributing across the membrane at the same ratio as other lipid-permeable cations such as tetraphenylphosphonium. It also seems to behave as such a cation in red cells distributing in a ratio opposite to that of Cl^- (Skulskii, Manninen & Jarnefelt, 1973), which is in electrochemical equilibrium with the measured membrane potential. It has also been recognized that this cation interacts with the (Na,K)-ATPase of erythrocytes (Skulskii et al. 1973) and is transported by this enzyme in squid axon (Landowne, 1975). Presumably, therefore, in red cells the flux of Tl^+ via lipid pathways greatly exceeds the small pump capacity in this cell. Attempts to use this cation for measuring membrane potentials in bacteria were frustrated by the finding that bacteria contain K^+ transport systems which also transport Tl^+ (Bakker, 1978; Bakker-Grunwald, 1979; Kashket, 1979).

The gastric mucosal ATPase (Ganser & Forte, 1973*b*) catalyzes an electroneutral exchange of H^+ for K^+ with accumulation of H^+ in the vesicle lumen and transport of K^+ to vesicle exterior. The rate of transport is determined in part by K^+ binding to an internal high-affinity site which is required for activation of ATP hydrolysis and in part by K^+ binding to an external low-affinity site which slows hydrolysis and transport (Stewart, Wallmark & Sachs, 1980; Wallmark, Stewart, Rabon, Saccomani & Sachs, 1980). The relative affinity of Tl^+ (Ray & Forte, 1976) for participation in the K^+-dependent reactions of the (K,H)-ATPase may provide a clear distinction between K^+ activation and inhibitory sites.

The ability of Tl^+ to act as a lipid-permable cation may also prove useful in determining the electrogenic

* For reprint requests

0022-2631/81/0062-0019 $01.80

characteristics of the pump as well as helping to define the nature of the proton leak pathways in gastric vesicles.

Materials and Methods

Preparation of Gastric Membranes

The method used has been detailed previously. Briefly, the hog gastric mucosal epithelial surface was flooded with 3 M NaCl and incubated for 2 min. The NaCl solution and the majority of the surface epithelial cells were removed by wiping the surface with absorbant tissue. The remaining tissue was then scraped with a blunt spatula and the scrapings then homogenized in 0.25 M sucrose. Following differential centrifugations the microsomal pellet was centrifuged through an interface between 0.25 M sucrose and 7% Ficoll in 0.25 M sucrose. The material designated GI, that trapped at the interface, was used throughout these experiments (Sachs, Chang, Rabon, Schackmann, Lewin & Saccomani, 1976).

ATPase Activity

This was measured in a final volume of 1.0 ml containing 2 mM $MgNO_3$, 40 mM Tris(hydroxymethyl)aminomethane ($TrisNO_3$), pH 7.4, 10 μg enzyme protein and $TlNO_3$ between 0.001 and 50 mM and/or KNO_3 between 0.5 mM and 20 mM. On occasion, as detailed in the text, Tl^+ and K^+ were present simultaneously. The effect of ionophores on cation-activated ATPase activity was assessed with either gramicidin or nigericin added in 10 μl methanol to give a final concentration of 1 μg/ml of ionophore. Controls included in the assay indicate that a 10 μl addition of methanol did not appreciably stimulate the cation-stimulated component of ATP hydrolysis. The release of Pi was measured after 15 min at 37 °C by the method of Yoda and Hokin (1970) or by radiometric assay. Tl^+ above 3.0 mM interferes with the colorimetric assay of Pi release. γ^{32}P-ATP was used to measure enzyme activity when Tl^+ concentrations were higher than 3.0 mM. Here a 300 μl assay medium contained 40 mM (Piperazine-N, N′-*bis*{2-ethane sulfonic acid}) (PIPES/Tris) at pH 7.4, 2.0 mM $MgNO_3$, 2.0 mM γ^{32}P-ATP ($\simeq$1000 cpm/nmole), 5.0 μg protein and $TlNO_3$ within the concentration range 6.0 to 50 mM. After a 5-min incubation at 37 °C enzyme activity was stopped by the addition of 17 mM CDTA to the samples which were immediately placed on ice. The samples were diluted to 1.0 ml with ice-cold distilled water. A second volume of 1.0 ml which contained 5% ammonium molybdate in 4.0 N HNO_3 was then added and the resulting complex extracted into 2.5 ml of 1:1 isobutanol-benzene. ATPase activity was calculated from the ^{32}Pi partitioning into a 1.0 ml aliquot of the organic layer. Protein was measured by the Lowry method (Lowry, Rosebrough, Farr & Randall, 1951).

$^{204}Tl^+$ Trapping

Gastric vesicles at 0.5 mg/ml were added to an incubation solution containing (in mM): 150 KNO_3, 2.0 $MgNO_3$, 2.0 PIPES/Tris, pH 7.4, and 6.0 $^{204}Tl^+$ at a final activity of 0.011 μCi/μl. At different times following this addition, aliquots were taken from the mixture, filtered on HAWP Millipore filters and washed 4 times with ice-cold 276.0 mM mannitol, 2.0 mM $MgNO_2$ and 2.0 mM PIPES/Tris, pH 7.4. The filters were dried and counted in an LKB 81000 liquid scintillation counter.

ATP-Dependent Tl^+ Transport

The gastric vesicles at 0.5 mg ml^{-1} were preincubated for 2 hr at room temperature in a medium containing (in mM): 2.0 $MgNO_3$, 2.0 PIPES/Tris, pH 7.4, 150 KNO_3 and either 0.6 or 6.0 $^{204}Tl^+$ to give a radioactivity of 0.011 μCi/μl^{-1}. In addition to its contribution to pump activity K^+ contributed to the maintenance of lower Tl^+ blanks which can be problematic in this assay. Transport was initiated by adding to 100 μl of this suspension, 5 μl of a solution containing 42 mM ATP, 42 mM $MgNO_3$, 50 mM PIPES/Tris buffer, pH 7.4. At various times, the reaction mixture was filtered on a 0.45 μ Millipore HAWP filter and washed 4 times with a solution containing 276 mM mannitol, 2 mM $MgNO_3$ and 2 mM PIPES/Tris, pH 7.4. The dried filters were counted in 10 ml of ACS scintillation fluid in an LKB 81000 scintillation counter.

Incubation Procedure for Substrate-Dependent Transport and Experiments Involving pH Pulses

For studies of ATP-dependent proton transport measured by the acridine orange absorbance technique the gastric vesicles were preincubated for 2 hr at room temperature in a medium containing at final concentration 150 mM KNO_3, 2 mM $MgNO_3$, 2 mM PIPES/Tris, pH 7.4. For studies using the pH pulse technique 5.0 mM succinate/Tris, pH 5.5, was substituted for the PIPES/Tris buffer usually used. When a pH electrode was used to measure proton transport the pH was adjusted to 6.1 using 1.0 mM PIPES/Tris, pH 6.13 (Sachs et al., 1976).

Measurement of ATP-Dependent pH-Gradient Formation

Using the vesicles preincubated in KNO_3 media as above, the formation of a pH gradient was monitored following the addition of ATP by the time-dependent change in acridine orange absorbance. In previous experiments, the loss of the absorbance peak at 490 nm was correlated with acidification of the intravesicular space (Rabon, Chang & Sachs, 1978). This peak was measured in an Aminco DW-2 Spectrophotometer in the dual wavelength mode with the monochromators set at 490 and 446 nm. For this assay 0.8 mM MgATP was added to a 350-μl assay solution containing 150 mM KNO_3, 2.0 mM $MgNO_3$, 2.0 mM PIPES/Tris (pH 6.1 or pH 7.4), 15 μM acridine orange and 15 μg of membrane protein. When pH electrode measurements were used 0.3 mM MgATP was added to a 2.0-ml assay solution as described above buffered at pH 6.1 and containing 0.33 mg protein. Changes in external pH following substrate addition were measured using a Radiometer PHM 64 pH meter and an REA 12 high-sensitivity unit interfaced with the REC-61 Servorecorder. Signal calibration was as previously detailed (Rabon et al., 1978).

Formation of Liposomes

Thirty-five mg of L-α-phosphatidyl choline was sonicated for 40 min in 1.0 ml of a solution consisting of 276 mM mannitol, 2.0 mM $MgNO_3$, 5 mM succinate/Tris, pH 5.5, using a Laboratory Supplies Co., Inc. Sonicator at 80 W output. The liposomes were stored at 4 °C and used within 48 hr of preparation.

Generation of a pH Gradient

In the case of liposomes formed as above, 10 μl of liposomal suspension was added to a 340 μl solution containing 276 mM mannitol, 2.0 mM $MgNO_3$, 15 μM acridine orange and 1.0 mM succinate/Tris, pH 5.5. The pH gradient was induced by addition of 100 μl of 100 mM Tris/NO_3, pH 8.5, 176 mM mannitol, 15 μM acridine orange and 2.0 mM $MgNO_3$. For the gastric vesicles, 60 μl of the vesicles, equilibrated in KNO_3 medium as above, were diluted in 290 μl of medium containing 150 mM KNO_3, 2.0 mM $MgNO_3$,

15 μM acridine orange and 1.0 mM succinate/Tris, pH 5.5. The pH gradient was formed by addition of 100 μl of solution containing 150 mM Tris/NO_3, pH 8.5, 56 mM KNO_3, 2.0 mM $MgNO_3$ and 15 μM acridine orange. In the gastric vesicles 25-μl aliquots of Tl^+ at varying concentrations were added 35 sec after the pH pulse. Because of the leakiness of the liposomal vesicles to protons, a 25-μl aliquot of Tl^+ was added to the alkaline medium before its addition to the liposome suspension.

Materials

L-α-phosphatidylcholine and ATP were obtained from Sigma Chemical Co., $^{204}Tl^+$ from Amersham, acridine orange from Eastman Kodak and Ficoll from Pharmacia. All other chemicals were reagent grade or better.

Results

Tl^+-Dependent Activation of ATPase

The comparison of nonionophoric stimulation of hydrolysis by the (K,H)-ATPase by Tl^+ in intact gastric vesicles to the ionophoric stimulation is shown in Fig. 1. As shown, there is a significant gramicidin-stimulated component of hydrolysis up to 3.0 mM Tl^+. The insert of Fig. 1 is another experiment which uses a Lineweaver-Burk plot to compare the gramicidin-stimulated ATPase activity dependent upon Tl^+ (0.05 to 3.0 mM) to that of K^+ (0.5 to 20 mM). In this experiment, calculation of the apparent affinity of the cation activation site for these cations indicates that the affinity for Tl^+ is approximately 10-fold that of K^+. The apparent $K_{0.5}$ is 89 μM for Tl^+ and 870 μM for K^+. The V_{max} is approximately the same for each cation. Some experimental variability is noted in absolute activity between experiments. For example in 5 separate assays the $K_{0.5}$ for Tl^+ was determined to be 0.098 ± 0.013 mM while V_{max} was 129.8 ± 13.4 μmole mg^{-1} hr^{-1}. This variability does not affect the affinity difference noted between K^+ and Tl^+.

The addition of 20 mM K^+ and 1 μg of gramicidin per 10 μg protein is routinely used to produce maximal stimulation of hydrolysis in the gastric vesicles (Ganser & Forte, 1973*a*; Sachs, 1977). The addition of 1.0 mM Tl^+ to the assay medium containing 20 mM K^+ and gramicidin did not produce an additional stimulation. In Fig. 2, Tl^+ concentrations above 3.0 mM are shown to progressively inhibit maximal ATPase activity, as has been shown for K^+ under these conditions (Wallmark et al., 1980). At 50 mM Tl^+ only 15% of the maximal gramicidin-stimulated activity is present. At pH 7.4 and 37 °C the apparent affinity of this cation for the inhibitory site is at least 2 orders of magnitude less than that seen for activation. It must be noted that these assays are not directly comparable since conditions were not adjusted to maintain constant ionic strength or the time of incubation between these assays.

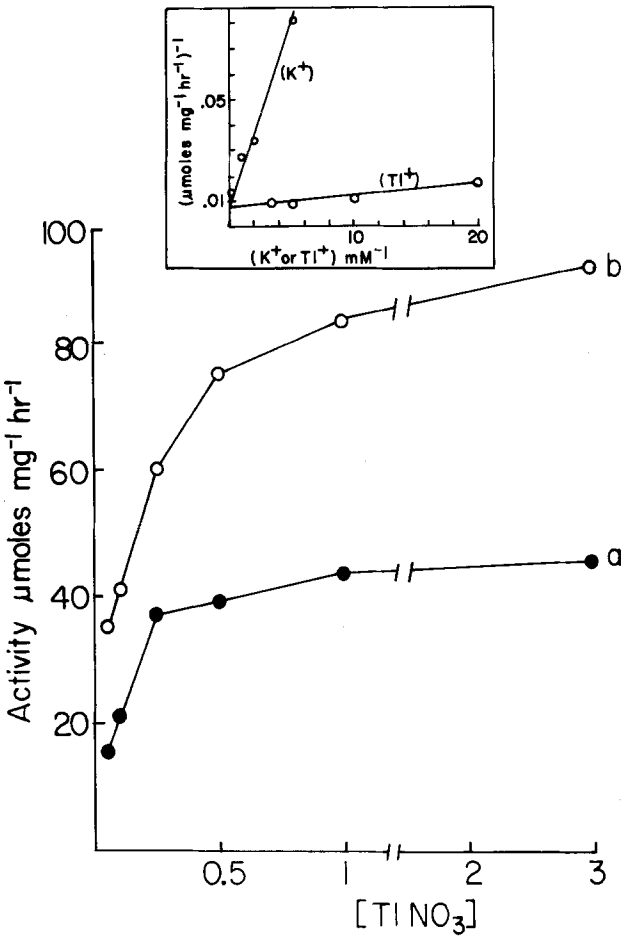

Fig. 1. Tl^+-dependent activation of the (K,H)-ATPase in gastric vesicles. Gastric vesicles containing 10 μg protein were incubated in Tl^+ concentrations from 50 μM to 3.0 mM for 15 min at 37 °C in the assay medium listed in Materials and Methods. Trace *a* indicates the Tl^+-activation in the absence of gramicidin. Trace *b* indicates the Tl^+-activation in the presence of gramicidin. Basal Mg^{++}-ATPase activity is subtracted in each case. The insert is a Lineweaver-Burk plot of the gramicidin-stimulated hydrolysis in nonidentical concentrations of either Tl^+ or K^+

$^{204}Tl^+$ Uptake by Gastric Vesicles

$^{204}Tl^+$ is rapidly accumulated by gastric vesicles as shown in Fig. 3. It can be seen that the $t_{\frac{1}{2}}$ at room temperature is approximately 1.0 min, which can be compared to the $t_{\frac{1}{2}}$ of 46 min observed for $^{86}Rb^+$ (Schackmann, Schwartz, Saccomani & Sachs, 1977). Since it has been shown in mitochondria (Skulskii et al., 1978) that Tl^+ distributes electrophoretically in accord with the membrane potential, the rapid uptake in gastric vesicles is presumably due to the lipid permeability of this cation. The equilibrium value for $^{205}Tl^+$ uptake gives a calculated vesicular volume of 3.4 μl mg^{-1} protein. This is about 1.5 times

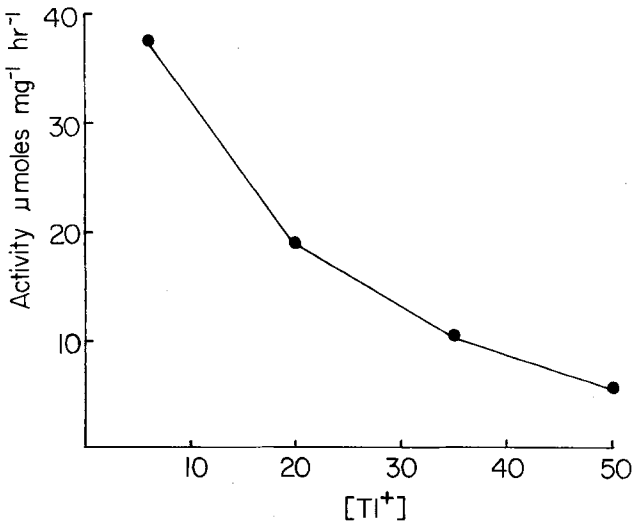

Fig. 2. Tl^+-dependent inhibition of the (K,H)-ATPase in gastric vesicles. ATP hydrolysis was measured as indicated in Materials and Methods using 2.0 mM γ^{32}P-ATP in a reaction medium containing from 6.0 to 50 mM Tl^+ buffered at pH 7.4 and thermostated at 37 °C. Activity was measured in the presence of gramicidin

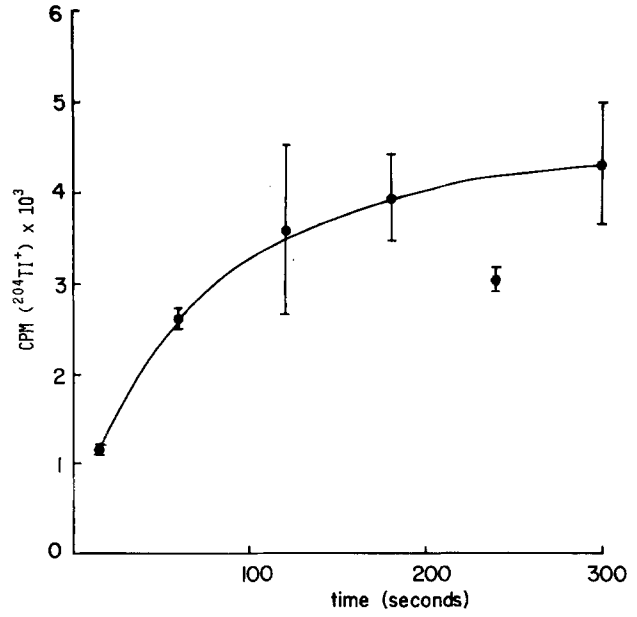

Fig. 3. Uptake of $^{204}Tl^+$ by gastric vesicles. The time-dependent uptake of $^{204}Tl^+$ was measured after the addition of the gastric vesicles to a medium containing (in mM): 150 KNO_3, 2.0 $MgNO_3$, 10 Tris NO_3, pH 7.4, and 6.0 $^{204}Tl^+$. At the indicated times a 100-μl aliquot containing 50 μg protein was removed from the medium, filtered and washed on a HAWP Millipore filter. Each point represents the average of duplicate aliquots

the volume found with other cations, such as rubidium. This might be taken to imply binding of Tl^+.

ATP-Dependent $^{204}Tl^+$ Distribution

As discussed below, progressive inhibition of substrate-dependent proton transport is produced by the

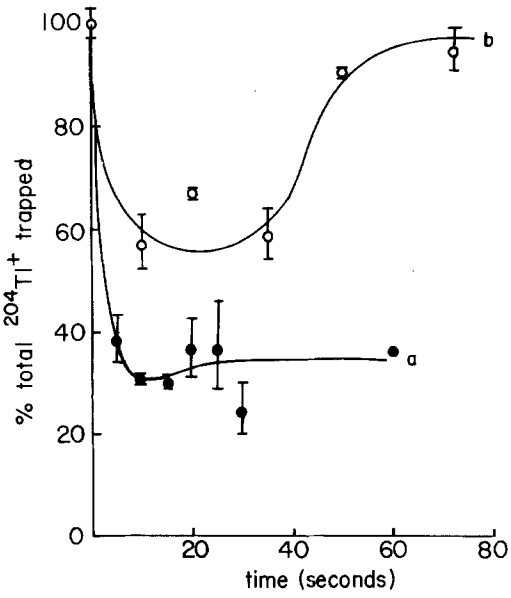

Fig. 4. ATP-dependent loss of equilibrated $^{204}Tl^+$ from gastric vesicles. Gastric vesicles were equilibrated in 150 mM KNO_3 and 0.6 or 6.0 mM $^{204}Tl^+$ medium as outlined in Materials and Methods. Following the addition of 2.0 mM MgATP, aliquots of the suspension were filtered on Millipore filters, washed and subsequently counted. In trace *a* the equilibrated $^{204}Tl^+$ concentration was 0.6 mM. In trace *b* the equilibrated $^{204}Tl^+$ concentration was 6.0 mM. Points are the average of duplicate assays

addition of Tl^+ (2.0 to 10 mM) to the equilibration medium containing 150 mM K^+. If this inhibition can be attributed to competition between a Tl^+_{in}/H^+_{out} leak and the proton pump this effect may be seen as rapid entry of pump-depleted intravesicular Tl^+. Hence the effect of 2.0 mM ATP on Tl^+ transport was assessed at 6.0 and 0.6 mM Tl^+ in the presence of 150 mM KNO_3. The higher Tl^+ concentration has been demonstrated to inhibit proton transport and the lower has no apparent effect under these conditions. In Fig. 4, trace *a*, the addition of ATP resulted in a rapid loss of intravesicular Tl^+ with a minimum achieved within 10 sec of substrate addition. Trapped Tl^+ at this point corresponded to 30% of the initial value. Similarly, ATP addition to the vesicles equilibrated in 6.0 mM Tl^+ plus 150 mM K^+ also produced a rapid loss of trapped Tl^+. In this case the trapped Tl^+ corresponded to 45% of initial levels. In 6.0 mM Tl^+ a rapid reuptake of ion occurred reaching 90% of initial levels within 1 min of ATP addition in contrast to that observed at the lower Tl^+ concentration. In both cases the quantity of ATP present at 1 min was sufficient to maintain transport; however, because of the requirement for intravesicular cation (either K^+ or Tl^+) the rate of the transport reaction is slowed following initial cation depletion.

Effect of Tl^+ on the ATP-Dependent Proton Transport

It has been shown previously (Sachs et al., 1976) that internal cation is required for ATP-dependent proton transport. This requirement may be met by the addition of valinomycin to nonequilibrated vesicles (in K^+ or Rb^+ media). In the case of Tl^+, in contrast to the equilibrium affinity of the valinomycin-Tl^+ complex which indicates some selectivity of valinomycin for Tl^+ (Pressman, 1976), we do not observe a valinomycin-dependent component of Tl^+-stimulated ATP hydrolysis (results not shown). Since nigericin or gramicidin also dissipate proton gradients, preincubation is the only simple means available for allowing Tl^+ entry. The activating effect of cations on ATPase activity is also complex. It has been suggested that whereas K^+ binding to an internal cation site activates the ATPase, K^+ binding to an external cation site inhibits the ATPase activity (Wallmark et al., 1980). At 2.0 mM ATP and 37 °C in medium at pH 7.4 this inhibition is seen at K^+ concentrations of 8 mM or higher, and also starts to become evident above 6.0 mM Tl^+ (Fig. 2).

When gastric vesicles were first pre-equilibrated with 50 mM $TlNO_3$ at pH 6.1 and diluted 17-fold into 50 mM KNO_3 or $NaNO_3$ (not shown because of similarity to experiment resulting from KNO_3 dilution) 30 sec prior to 0.5 mM ATP addition, the data of Fig. 5 were obtained. It was observed that under these conditions of high internal and low external Tl^+ (50 mM Tl^+_{in}/2.6 mM Tl^+_{out}) acidification of the vesicles' interior occurs upon ATP addition. The additional

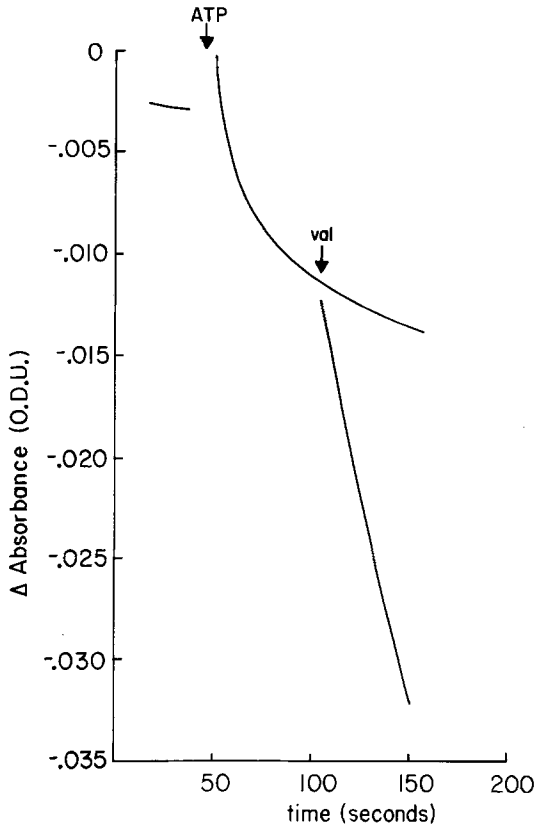

Fig. 5. Tl^+-dependent pH gradient formation. Thirty µl of Tl^+ (50 mM) equilibrated gastric vesicles were diluted into 320 µl of medium containing 50 mM KNO_3, 2.0 mM $MgNO_3$ and 2.0 mM PIPES/Tris, pH 6.1. Transport was initiated within 30 sec of this mixing by the addition of 10 µl of 30 mM MgATP. In a second experiment 5.0 µl containing 0.1 mg/ml^{-1} valinomycin was added approximately 50 sec after the addition of MgATP

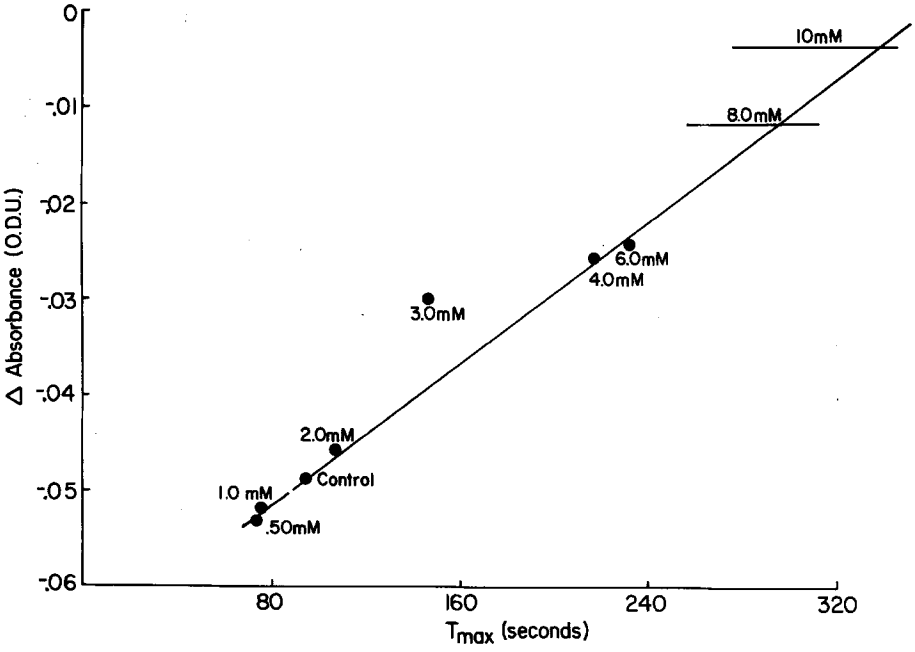

Fig. 6. Tl^+ inhibition of pH-gradient formation at pH 7.4. Gastric vesicles were equilibrated in a medium containing 150 mM KNO_3, 2.0 mM $MgNO_3$, 15 µM acridine orange, 2.0 mM PIPES/Tris, pH 7.4, and variable Tl^+ concentrations. The addition of 0.83 mM MgATP resulted in a decrease in the absorbance of acridine orange proportional to the magnitude of the pH gradient which is progressively inhibited by increasing Tl^+ concentrations. Both the time of development (T_{max}) of the minimum absorbance change and the absorbance change are plotted as a function of the equilibrated Tl^+ concentration. Each point is the average of two assays

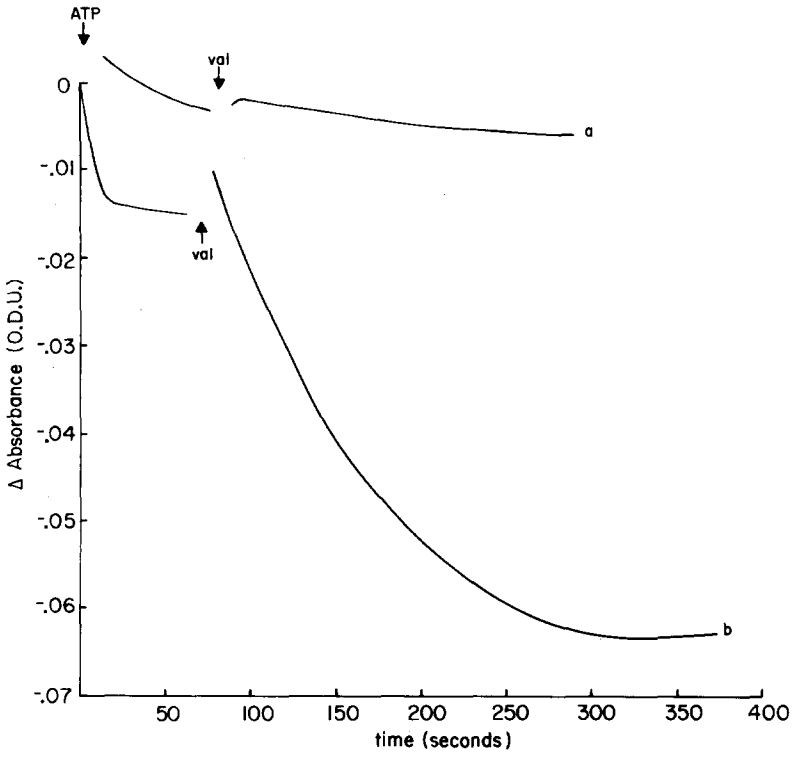

Fig. 7. Sidedness of Tl^+ inhibition of proton pump activity. Gastric vesicles were equilibrated in a medium containing 150 mM KNO_3, 10 mM $TlNO_3$, 2.0 mM $MgNO_3$ and 2.0 mM PIPES/Tris, pH 7.4. Immediately before the addition of 0.83 mM Mg^{++} ATP, 30 μl of equilibrated material was diluted into 320 μl of medium containing either 150 mM KNO_3, 2.0 mM $MgNO_3$, and 2.0 mM PIPES/Tris with or without 10 mM $TlNO_3$. Following the dilution MgATP was added and the absorbance change of acridine orange monitored. In each case, 5.0 μl of 0.1 mg/ml valinomycin was added approximately 1 min after the addition of MgATP. Trace *a:* ATP-dependent formation of a proton gradient in material diluted into equimolar Tl^+ (10 mM Tl^+_{inside}/10 mM $Tl^+_{outside}$). Trace *b:* ATP-dependent formation of a proton gradient of material diluted into Tl^+-free medium (10 mM Tl^+_{inside}/0.83 mM $Tl^+_{outside}$)

increase in acidification upon addition of valinomycin results from pump activity activated by the fresh supply of K^+ supplied through the valinomycin K^+ conductance pathway. If the vesicles are pre-equilibrated with 50 mM $TlNO_3$ and ATP added directly without dilution, no proton uptake is observed.

To extend the above findings, the effect of Tl^+ on K^+-dependent proton transport was measured in vesicles which were pre-equilibrated in a PIPES/Tris buffer, pH 7.4, containing 150 mM KNO_3 and Tl^+ at concentrations ranging from 0.5 to 10 mM as indicated in Fig. 6. In the vesicles equilibrated at 0.5 to 1 mM Tl^+($Tl^+_{in} = Tl^+_{out}$) little effect on the rate of formation or magnitude of the proton gradient as compared to the absence of Tl^+ is noted, other than a slight stimulation of the magnitude. In equilibrated medium containing higher Tl^+ concentrations there is a progressive reduction in the rate of formation and magnitude of the proton gradient so that at 8.0 mM and above virtually no pH gradient was established in spite of the prior equilibration of K^+ as well as Tl^+.

In order to establish the sidedness of the site responsible for Tl^+ inhibition, vesicles equilibrated in 150 mM KNO_3 and 10 mM $TlNO_3$ at pH 7.4 were either diluted into identical solutions or into Tl^+-free solutions containing 150 mM KNO_3. Fig. 7 shows that in the equilibrated Tl^+ medium (10 mM Tl^+_{in}/10 mM Tl^+_{out}) the addition of ATP does not induce the formation of a pH gradient. However, when the external Tl^+ concentration was decreased by dilution of the Tl^+- and K^+-equilibrated vesicles into Tl^+-free medium (10 mM Tl^+_{in}/0.83 mM Tl^+_{out}), the formation of an ATP-dependent pH gradient was stimulated. Valinomycin had no effect on the formation of the pH gradient in the former conditions, but markedly stimulated the formation of the pH gradient under the latter.

Since the ATP concentration used in these experiments is adequate to maintain effective substrate levels within the observed time limits, the valinomycin stimulation of pH-gradient formation can be ascribed to the ionophore-dependent supply of K^+ to the cation pump site located in the vesicle interior (Ganser & Forte, 1973*a*). The absence of this effect in the presence of 10 mM Tl^+_{ext} indicates external rather than internal Tl^+ inhibits the ATP-dependent proton pump.

It has previously been reported that the affinity of the external cation site is pH-sensitive (Stewart et al., 1980). Since the inhibition of the proton pump may in part be attributed to Tl^+ binding at an external site it is possible in the intact vesicle system to test for this by measurement of the pH-dependence of Tl^+-induced inhibition. Accordingly, the inhibition resulting from ATP addition to vesicles equilibrated in medium containing 150 mM K^+ and Tl^+ in a range from 3.0 to 30 mM was measured at pH 6.1 in contrast to a media pH of 7.4 used previously. In this case, the pH electrode was used to measure proton pump activity since it is a direct measure of the proton component of the cation/H^+ pump. In a control ex-

periment in which vesicles were equilibrated in medium containing 150 mM K^+ and 10 mM Tl^+ no ATP-dependent dye response was observed at pH 7.4 while only partial inhibition (approximately 25%) was seen at pH 6.1. In Fig. 8 progressive inhibition of net proton pump activity was again measured as a function of increasing Tl^+ concentration. However, the degree of Tl^+-induced inhibition is reduced at pH 6.1 in comparison to pH 7.4. At 10 mM Tl^+ only 40% inhibition of net proton uptake occurs in contrast to the almost total inhibition observed at pH 7.4. At 30 mM Tl^+, approximately 30% of the net transport measured in the absence of Tl^+ is present at pH 6.1. Although in accordance with the biphasic Tl^+ effect on the ATPase observed at pH 7.4 as well as the pH-sensitivity of the apparent affinity at the external cation site, the magnitude of this inhibition at pH 7.4 does not appear adequate to account for the complete loss of proton transport activity observed at concentrations of Tl^+ above 8.0 mM. The inhibition of the formation of the pump-dependent pH gradient has an additional explanation.

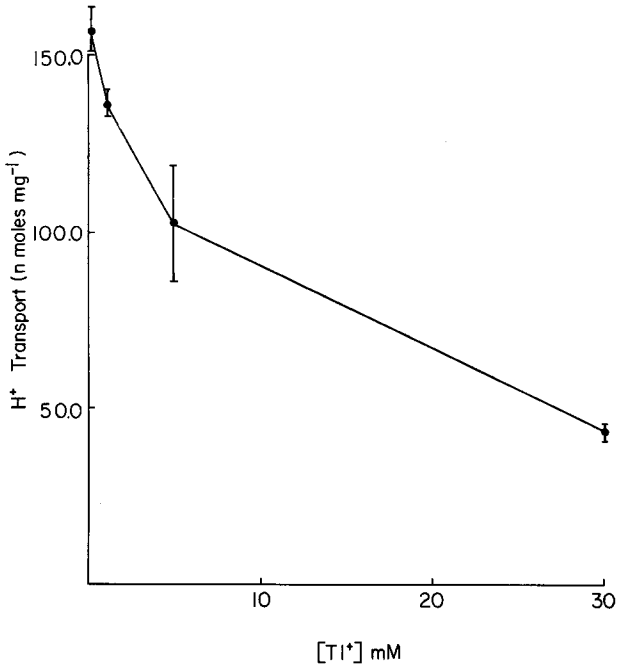

Fig. 8. Tl^+ inhibition of proton transport at pH 6.1. Gastric vesicles were equilibrated in a medium containing 150 mM KNO_3, 2.0 mM $MgNO_3$, 1.0 mM PIPES/Tris, pH 6.1, and $TlNO_3$ (1.0–30 mM). Each point is the average of duplicate assays which measure the maximum net levels of protons transported following the addition of 0.3 mM MgATP to each of the K^+- and Tl^+-equilibrated suspensions

Effect of External Tl^+ on Preformed pH Gradients

A pH gradient can be induced in gastric vesicles which are preloaded at pH 5.5 by the addition of external buffer at pH 8.5. The decay of the pH gradient in the absence of Tl^+ is shown in trace 1 of Fig. 9. At 350 sec 80% of the pH gradient-dependent absor-

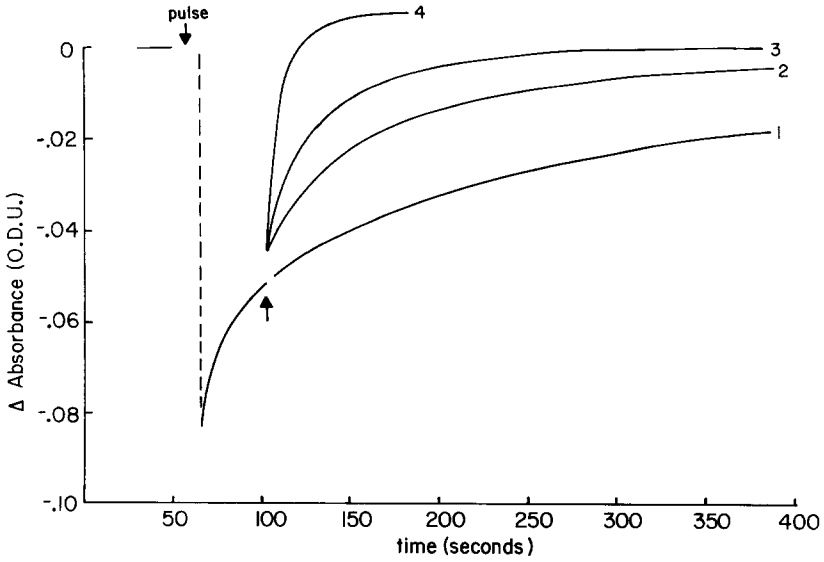

Fig. 9. Tl^+-induced dissipation of a pH gradient in gastric vesicles. Gastric vesicles were preloaded with 150 mM KNO_3, 2.0 mM $MgNO_3$ and 5.0 mM succinate/Tris, pH 5.5, as indicated in Materials and Methods. Thirty µl of the equilibrated material was diluted with 320 µl of 150 mM KNO_3, 2.0 mM $MgNO_3$, 15 µM acridine orange and 1.0 mM succinate/Tris, pH 5.5. At the indicated time 100 µl of a solution containing 56 mM KNO_3, 2.0 mM $MgNO_3$ and 15 µM acridine orange and 100 mM Tris NO_3, pH 8.5, was added to the equilibrated material. Trace 1 shows the decay of the pH gradient in the absence of Tl^+. Trace 2 shows the decay of the pH gradient induced by the presence of 3.8 mM Tl^+. Trace 3 shows the decay of the pH gradient induced by the presence of 8.0 mM Tl^+. Trace 4 shows the decay of the pH gradient induced by the addition of 0.5 µg nigericin

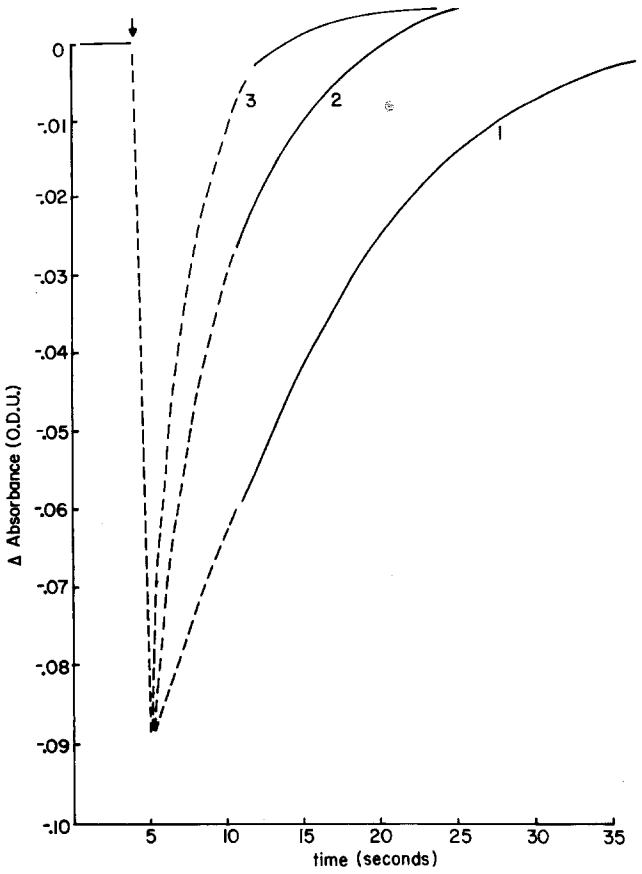

Fig. 10. Tl^+-induced dissipation of a pH gradient in liposomes. Liposomal vesicles were prepared as indicated in Materials and Methods. Ten μl of the equilibrated vesicles were then diluted into a 340-μl solution containing 276 mM mannitol, 2.0 mM $MgNO_3$, 15 μM acridine orange and 1.0 mM succinate/Tris, pH 5.5. At the indicated time a 100-μl solution containing 100 mM Tris NO_3, pH 8.5, 176 mM mannitol, 2.0 mM $MgNO_3$ and 15 μM acridine orange was added to the suspension containing liposomes. Trace 1 shows the pH-gradient decay in the absence of Tl^+. Trace 2 shows the decay of the pH gradient in the presence of 0.8 mM Tl^+. Trace 3 shows the decay of the pH gradient in the presence of 4.0 mM Tl^+

bance response has dissipated. In the following experiments shown in traces 2 and 3, 4.0 and 8.0 mM Tl^+ (final) were added to the external medium during the slow decay phase. These Tl^+ additions resulted in a concentration-dependent decay of the pH gradient. The addition of the H^+/K^+ exchange ionophore, nigericin, produced the rapid decay of the pH gradient shown in trace 4. The effect of external Tl^+ on the pulse-induced pH gradient in these gastric vesicles can be ascribed to the presence of a $Tl^+:H^+$ exchange (or $Tl^+:OH^-$ co-transport).

To distinguish between a Tl^+ interaction between membrane proteins or lipid, pH gradients were induced in a similar manner across protein-free pH 5.5 mannitol-loaded liposomes formed from phosphatidyl choline. In Fig. 10 the dissipation of the pulse-induced pH gradient is indicated in trace 1. In subsequent experiments 0.8 and 4.0 mM Tl^+ were introduced into the external medium with the pulse solution. As in the gastric vesicles the decay of the pH gradient was dependent on the concentration of externally added Tl^+. If the lipid permeable cation triphenylmethylphosphonium ($TPMP^+$) is substituted for external Tl^+ an equivalent dissipation of the pH gradient formed in gastric vesicles is obtained at 1 mM cation. This suggests a finite H^+ conductance is present in these vesicles and makes plausible the interpretation that the Tl^+/H^+ exchange is electrically coupled.

Discussion

The gastric ATPase which usually exchanges H^+ for K^+ is able to use Tl^+ as a substitute for K^+. Tl^+ activates the ATPase with a relatively high affinity in comparison to K^+. The gramicidin enhancement of Tl^+-stimulated hydrolysis is indicative of an intravesicular location of the Tl^+-activating site from which Tl^+ is depleted by the activity of the H^+/Tl^+ pump upon addition of substrate. Location of the Tl^+-stimulating site on the internal face of the membrane is also consistent with the protection from the inhibition of hydrolysis of the ATPase by the carboxyl group reagent ethoxycarbonyl ethoxydihydroquinoline by the presence of intravesicular K^+ (Saccomani, Barcellona, Rabon & Sachs, 1980).

The Tl^+ concentrations required for inhibition of gramicidin-stimulated ATP hydrolysis is at least two orders of magnitude greater than that required for activation. A biphasic effect has also been reported with K^+ with inhibition of maximal hydrolysis beginning at approximately the same range as observed with Tl^+ (Wallmark et al., 1980). With K^+ it was shown that inhibition resulted from inhibition of phosphorylation by bound external K^+. The inhibition by Tl^+ due to binding at an external site is strongly suggested by the antagonism between medium pH and Tl^+ inhibition observed in the measurements of proton transport, as well as by the Tl^+ dilution experiments.

As with Rb^+ (Schackmann et al., 1977) or K^+ (Lewin, Saccomani, Schackmann & Sachs, 1977) Tl^+ is transported by the ATPase. At low Tl^+ concentrations, ATP-induced Tl^+ efflux and the outward Tl^+ gradient is maintained. Tl^+ influx due to lipid permeation of the ion is considerably faster than that of the alkali metals K^+ and Rb^+ and at high Tl^+ concentrations this permeability presumably has the effect of causing Tl^+ efflux to be transient, efflux being followed by reuptake.

With pH gradients imposed across the membrane

in the absence of ATP, Tl^+ addition results in dissipation of the gradients. Tl^+ probably also dissipates the pH gradient due to the ATPase by a similar Tl^+/H^+ exchange. This interpretation is supported by the Tl^+-induced dissipation of pH gradients in protein-free liposome, and a similar effect of $TPMP^+$ in the gastric vesicles.

Thus the net effect of Tl^+ on the ATPase is determined by cation binding at the catalytic subunit with either the internal activating or external inhibitory cation-binding site. In parallel to the pump pathway for Tl^+ there is a Tl^+/H^+ exchange pathway which accounts for the uncoupling of ATPase activity from flux of cation and H^+, in the presence of external Tl^+.

This work was supported by NIH grants AM 15878 and AM 21588 and by NSF grants PCM 7809208 and PCM 7718951.

References

Bakker, E.P. 1978. Accumulation of thallous ions (Tl^+) as a measure of the electrical potential difference across the cytoplasmic membrane of bacteria. *Biochemistry* **17**:2899–2904

Bakker-Grunwald, T. 1979. Movement of thallous ion across the ascites cell membrane. *J. Membrane Biol.* **47**:171–183

Ganser, A.L., Forte, J.G. 1973*a*. Ionophoretic stimulation of K^+-ATPase of oxyntic cell microsomes. *Biochem. Biophys. Res. Commun.* **54**:690–696

Ganser, A.L., Forte, J.G. 1973*b*. K^+-stimulated ATPase in purified microsomes of bullfrog oxyntic cells. *Biochim. Biophys. Acta* **307**:169–180

Kashket, E.R. 1979. Active transport of thallous ions by Streptococcus lactis. *J. Biol. Chem.* **254**:8129–8131

Landowne, D. 1975. A comparison of radioactive thallium and potassium fluxes in the giant axon of the squid. *J. Physiol. (London)* **252**:79–96

Lewin, M., Saccomani, G., Schackmann, R., Sachs, F. 1977. Use of ANS as a probe of gastric vesicle transport. *J. Membrane Biol.* **32**:301–318

Lowry, O.H., Rosebrough, N.J., Farr, A., Randall, R.J. 1951. Protein measurement with the Folin phenol reagent. *J. Biol. Chem.* **193**:265–275

Melnick, R.L., Monti, L.G., Motzkin, S.M. 1976. Uncoupling of mitochondrial oxidative phosphorylation by thallium. *Biochem. Biophys. Res. Commun.* **69**:68–73

Pressman, B.C. 1976. Biological applications of ionophores. *Annu. Rev. Biochem.* **45**:501–530

Rabon, E., Chang, H., Sachs, G. 1978. Quantitation of hydrogen ion and potential gradients in gastric plasma membrane vesicles. *Biochemistry* **17**:3345–3353

Ray, T.K., Forte, J.G. 1976. Studies on the phosphorylated intermediates of a K^+ stimulated ATPase from rabbit gastric mucosa. *Biochim. Biophys. Acta* **443**:451–467

Saccomani, G., Barcellona, M.L., Rabon, E., Sachs, G. 1980. Site directed modifications of gastric ($H^+ + K^+$) ATPase: *In:* Hydrogen Ion Transport in Epithelia. I. Schulz, G. Sachs, J. Forte and K. Ullrich, editors. pp. 175–183. Elsevier/North-Holland Press, N.Y.

Sachs, G. 1977. H^+ transport by a non-electrogenic gastric ATPase as a model for acid secretion. *Rev. Physiol. Biochem. Pharmacol.* **79**:133–162

Sachs, G., Chang, H.H., Rabon, E., Schackmann, R., Lewin, M., Saccomani, G. 1976. A non-electrogenic H^+ pump in plasma membranes of hog stomach. *J. Biol. Chem.* **251**:7690–7698

Schackmann, R., Schwartz, A., Saccomani, G., Sachs, G. 1977. Cation transport by gastric $H^+:K^+$ATPase. *J. Membrane Biol.* **32**:361–381

Skulskii, I.A., Manninen, V., Jarnefelt, J. 1973. Interaction of thallous lons with the cation transport mechanism in erythrocytes. *Biochim. Biophys. Acta* **298**:702–709

Skulskii, I.A., Savina, M.V., Glasunov, V.V., Saris, N.E.L. 1978. Electrophoretic transport of Tl^+ in mitochondria. *J. Membrane Biol.* **44**:187–194

Stewart, H.B., Wallmark, B., Sachs, G. 1981. The interaction of H^+ and K^+ with the partial reactions of gastric ($H^+ + K^+$) ATPase. *J. Biol. Chem. (in press)*

Wallmark, B., Stewart, H.B., Rabon, E., Saccomani, G., Sachs, G. 1980. The catalytic cycle of gastric ($H^+ + K^+$) ATPase. *J. Biol. Chem.* **255**:5313–5319

Yoda, A., Hokin, L. 1970. On the reversibility of binding of cardiotonic steroids to a partially purified (Na+K) activated adenosinetriphosphatase from cat brain. *Biochem. Biophys. Res. Commun.* **40**:880–886

Received 9 September 1980; revised 13 January 1981;
revised again 24 February 1981