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PROTON SECRETION BY THE GASTRIC PARIETAL CELL

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SUMMARY

The parietal cell occupies a unique niche among eukaryotic cells in that it develops a proton gradient of more than 4 million-fold across the membrane of the secretory canaliculus. At rest, the cell is still able to develop a proton gradient across intracellular membranes, such that the acid compartment has a pH of less than 4. Acidification depends on the simultaneous presence of ATP, K^+ and Cl^- as demonstrated in permeabilized cells. With acidification of the luminal side of the proton pump, there is a corresponding alkalinization of the cytosolic face as revealed by carboxyfluorescein fluorescence enhancement. Disposal of the resultant alkali depends on carbonic anhydrase activity and the functioning of a coupled $Na^+:H^+$ and $Cl^-:OH^-$ antiport across the basal lateral membrane. Accordingly, with secretion there is an increased cellular Cl^- level, which is exported across the apical membrane in association with K^+. The Na^+ pump dependent secretion of KCl across this membrane is one of the major sites of regulation of acid secretion since K^+ is required at the luminal face of the gastric ATPase. Membranes isolated from secreting tissue contain a KCl permeation pathway largely absent from membranes isolated from resting tissue. The pump itself acts as an H^+ for K^+ exchange ATPase which is most probably composed of at least two peptides of 100 000 M_r. That catalytic cycle consists of formation and breakdown of a covalent aspartyl phosphate. Formation of the intermediate depends on loss of K^+ from cytosolic binding sites, and breakdown of the intermediate depends on K^+ binding to the luminal face of the enzyme. During breakdown, an acid labile E·P is formed, and, at high ATP concentrations, loss of this form of the enzyme is probably the rate limiting step.

INTRODUCTION

The parietal cell is dedicated to secretion of 160 mM-HCl across its apical membrane. In all species studied, this is a discontinuous process, subject to regulation by the secretagogues, histamine, acetylcholine and gastrin. Although much has been learned from studies *in vivo* and in the chambered mucosa *in vitro*, the discovery of a K^+-stimulated ATPase in parietal cells (Ganser & Forte, 1973*a*) and the recent development of simpler models such as isolated rabbit gastric glands (Berglindh & Obrink, 1976), dog parietal cell suspensions (Soll, 1978), permeable rabbit gastric

Key words: H^+ secretion, parietal cell, $(H^+ + K^+)$ ATPase.

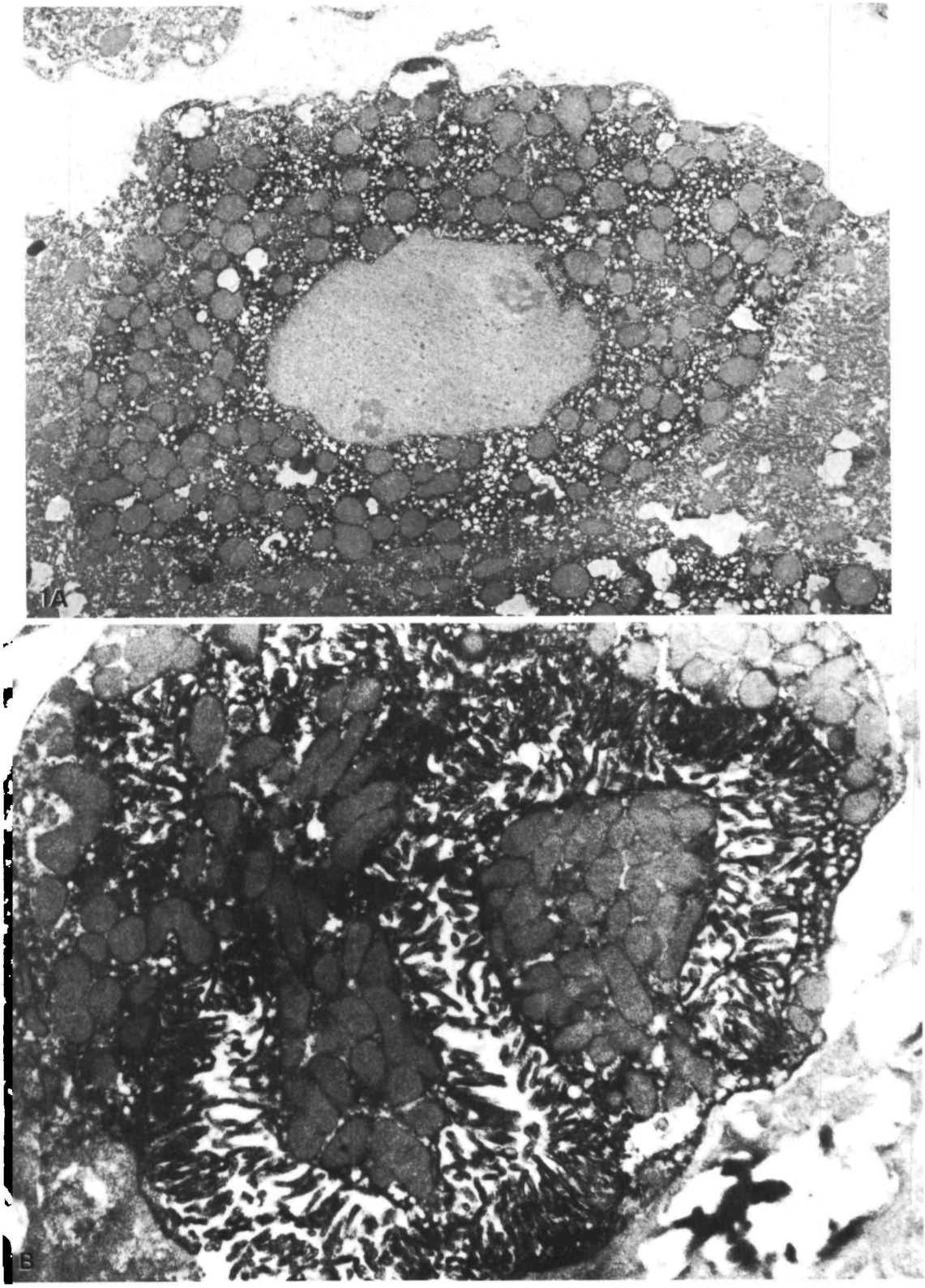
glands (Berglindh, Dibona, Pace & Sachs, 1980; Malinowska, Koelz, Hersey & Sachs, 1981) and vesicles isolated from resting (Sachs *et al.* 1976) or secreting parietal cells (Wolosin & Forte, 1981*a*) have provided new insights into the mechanism of acid secretion.

SITE OF ACID SECRETION

The unstimulated, resting parietal cell contains a large number of smooth-walled vesicular or tubular membranes known as tubulovesicles. There is also an intracellular membrane structure, the secretory canaliculus, which has a few stubby microvilli lining the extracellular face. Upon stimulation, the tubulovesicles disappear, and there is a massive expansion of the canalicular surface by the formation of long microvilli (Helander & Hirschowitz, 1972). This change possibly reflects a means of regulating the output of acid by translocation of membrane containing the proton pump from cytosol to secretory surface. This can be shown directly by staining resting and secreting parietal cells with antibody specific for a peptide(s) of the proton translocating ATPase. Fig. 1A shows staining of the resting cell where the stain is confined to the tubulovesicles; in Fig. 1B the tubulovesicles have disappeared, and the staining is confined to the microvilli of the expanded secretory canaliculus. The primary antibody used in these studies is a monoclonal antibody reacting specifically with an ATPase peptide of isoelectric point 5·8 which may also incorporate the terminal phosphate of ATP during enzyme turnover (Smolka, Helander & Sachs, 1983).

Given that the $(H^+ + K^+)$ATPase or the proton pump is located in the secreting cell at the membrane lining the canaliculus, it can be presumed that the site of acid secretion is into that space. This can be proved directly by the use of dyes such as fluorescein or carboxyfluorescein that respond to changes of pH, or acridine orange and 9-amino-acridine which accumulate as a function of a pH gradient across a membrane. The presence of the secretory canaliculus, essentially an intracellular compartment of high acidity, allows the use of these weak bases or the radioactive weak base, aminopyrine, for detection of the presence of acid in isolated glands or cell suspensions (Berglindh, Helander & Obrink, 1976). The acid space can be visualized by taking advantage of the hyperchromic shift of acridine orange from green to orange/red as it concentrates in the canalicular space. Fig. 2 shows the secretory canaliculus and glandular lumen as an orange/red stain in the secreting parietal cell. The use of weak bases has shown that the 'resting' cell is not totally devoid of accumulated acid. The rabbit gastric gland generates an aminopyrine ratio (gland/medium) of 200 or more when stimulated with histamine or dbcAMP, and in the presence of histaminergic or cholinergic receptor antagonists such as cimetidine or atropine, the ratio remains between 10 and 20. This ratio reflects continued functioning of the secretory proton pump, since the ratio is reduced to unity by terminal site inhibitors such as

Fig. 1. The staining of parietal cell membranes with monoclonal antibody against the gastric ATPase, using a peroxidase-linked second antibody. In (A) the resting cell is stained essentially only in the tubulovesicles, whereas in the secreting cell (B), the stain is now over the microvilli of the secretory canaliculus. Magnification: (A) ×6900; (B) ×10200. From Smolka, Helander & Sachs, 1983.


1A
B

SCN^-, picoprazole (Berglindh, 1977*a*; Wallmark, Sachs, Mardh & Fellenius, 1983) or by the removal of K^+ or Cl^- (Berglindh, 1977*b*, 1978).

Using acridine orange, it is seen that the cytosol contains small vesicular structures that continue to accumulate dye. Their size is considerably larger than vesicles of 0·2 μm diameter, but if the tubulovesicles are in fact tubular structures of about 1 × 0·2 μm, they would be visible by light microscopic techniques. On the other hand, the relative paucity of red acridine orange staining suggests vestigial secretion into the resting canaliculus. Since the proton secreted is ultimately derived from water, it could be anticipated that with secretion there would be alkalinization of the cytosol, or at least the local environment of the proton pump. This can be visualized using carboxyfluorescein. The diacetate derivative of this dye is membrane permeable (Thomas, Buchsbaum, Zimniak & Racker, 1979), but following entry into the cell, the diacetate is hydrolysed, and since the charged carboxyfluorescein is impermeable, it is trapped within the cell. Its fluorescence increases with increasing pH, and there is also a shift in the excitation wavelength of fluorescence. Staining of the unstimulated gastric gland with this technique is shown in Fig. 3A. Rather diffuse staining is seen, but peripheral fluorescence is more prominent. Upon stimulation, there is marked enhancement of the fluorescence with localization close to the cytosolic face of the secretory canaliculus (Fig. 3B). Prior studies in frog gastric mucosa using bromthymol blue (Hersey, 1978) have suggested that an alkali compartment is present during acid secretion.

The visualization of optically active substances responding to changes in pH can be extended further by adaptation of a photodiode array spectrophotometer to a microscope, either for absorbance studies or fluorescent measurements. Different configurations are possible, including a quartz fibre bundle leading the light to a holographic grating which is projected onto a regular or intensified photodiode array which is, in turn, coupled to a computer capable of signal averaging and other manipulation of the signal. Experiments using such a system are illustrated in Fig. 4. Fig. 4A shows the spectrum of a stimulated gastric gland, with the green emission of acridine orange at 530 nm (peak to the left) and the red emission at 630 nm (peak to the right) evident. Upon addition of nigericin, which catalyzes an H^+ for K^+ exchange, there is a loss of the red fluorescence as H^+ leaves the canalicular space and K^+ enters. In the cytosol, K^+ is lost in exchange for either H^+ or Na^+. The enhancement of green fluorescence, due to increased dye uptake, indicates significant acidification of the cytoplasm. Both of these changes can be seen in the difference spectrum (Fig. 4B). The parietal cell and its responses can therefore be studied using quantitative light microscopy.

MECHANISMS OF ACID SECRETION

The various arguments for and against ATP as an energy source for acid secretion were resolved with the development of permeable gland models. Differential permeabilization of parietal cell basal-lateral membranes, leaving the secretory or apical membrane intact was achieved either by electric shock treatment (Berglindh *et al.* 1980), which followed the work on the adrenal medullary cell (Knight & Baker, 1982), or by treating glands with the detergent digitonin (Malinowska *et al.* 1981). Using

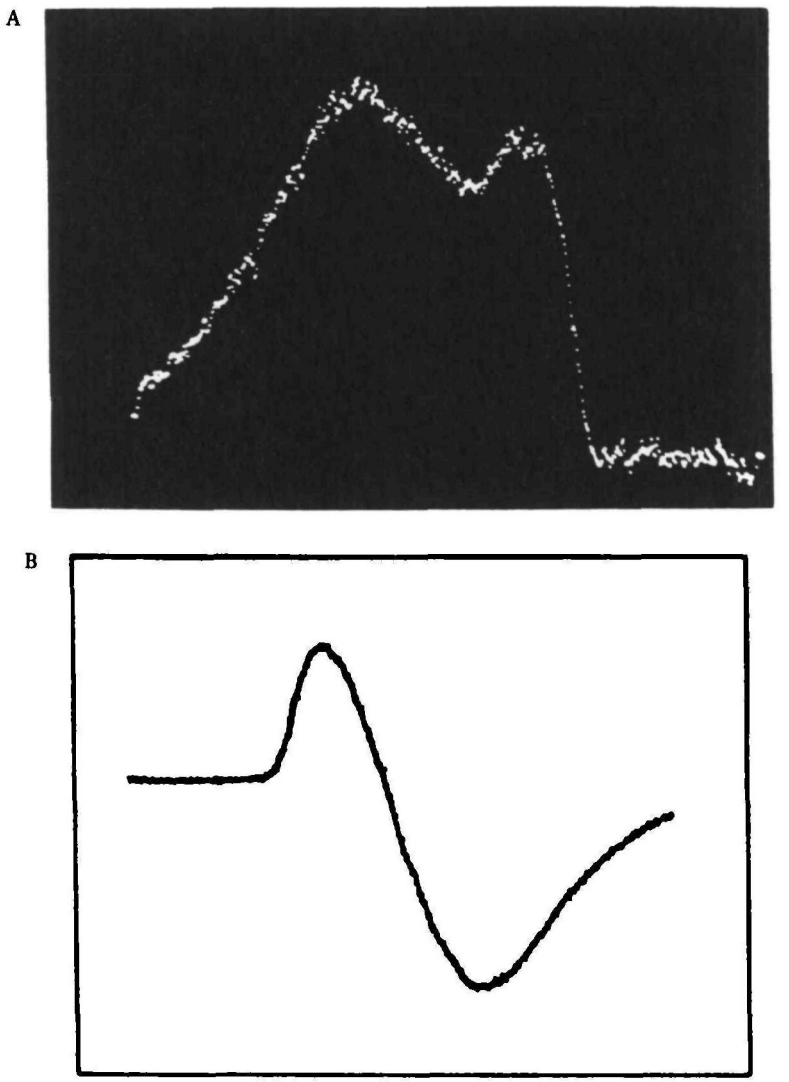
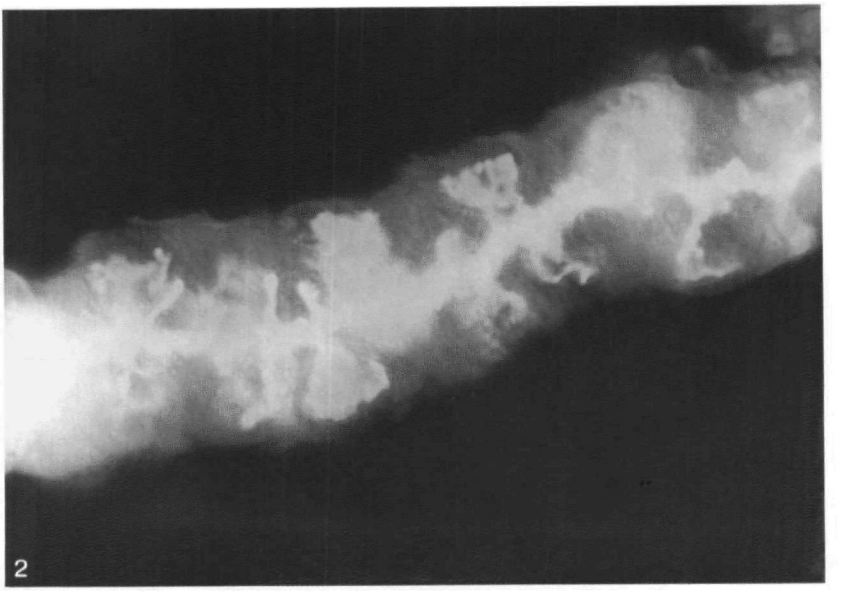


Fig. 4. The signal averaged spectrum of a stimulated gastric gland stained with acridine orange is shown in (A); (B) shows the difference spectrum obtained following the addition of nigericin, where there is a loss of the red peak (dissipation of acid from the canaliculus) and gain of the green peak (acidification of cytosol due to K^+ loss and H^+ entry).

digitonin-permeabilized gastric glands, it was shown that even with inhibition of mitochondrial metabolism by oligomycin and anoxia, ATP was able to drive the accumulation of aminopyrine. The response of this gland model to ATP was dependent on the presence of K^+ (Berglindh *et al.* 1980) and Cl^- (Malinowska, Cuppoletti

Fig. 2. The uptake of acridine orange by a secreting gastric gland, where the parietal cell canaliculi and glandular lumen are visualized as orange. Magnification: ~ ×850.

Fig. 3. The staining of rabbit gastric glands with carboxyfluorescein diacetate. In (A) the resting gland is shown with peripheral staining in the parietal cell and in (B) staining of a stimulated gland is seen intracellularly presumably at the border of the secretory canaliculus indicating alkalinization in that region. Magnification: (A) ~ ×570; (B) ~ ×700.



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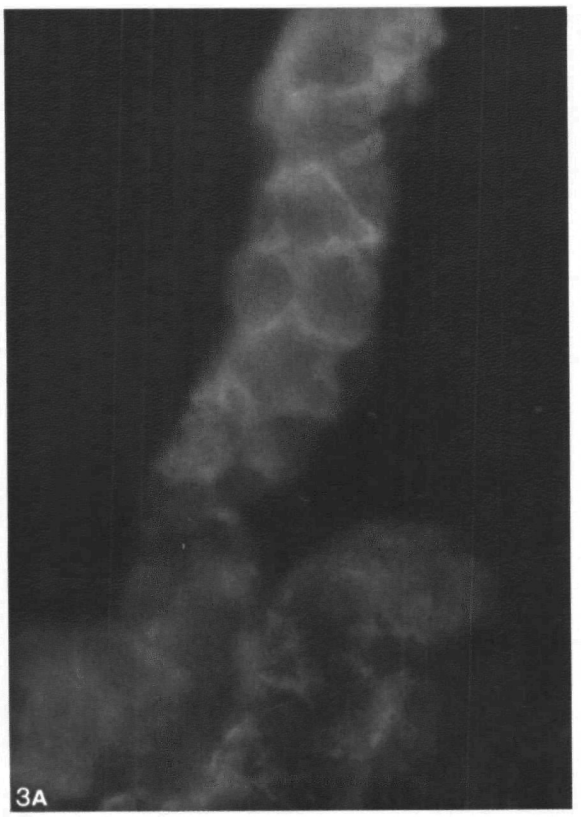
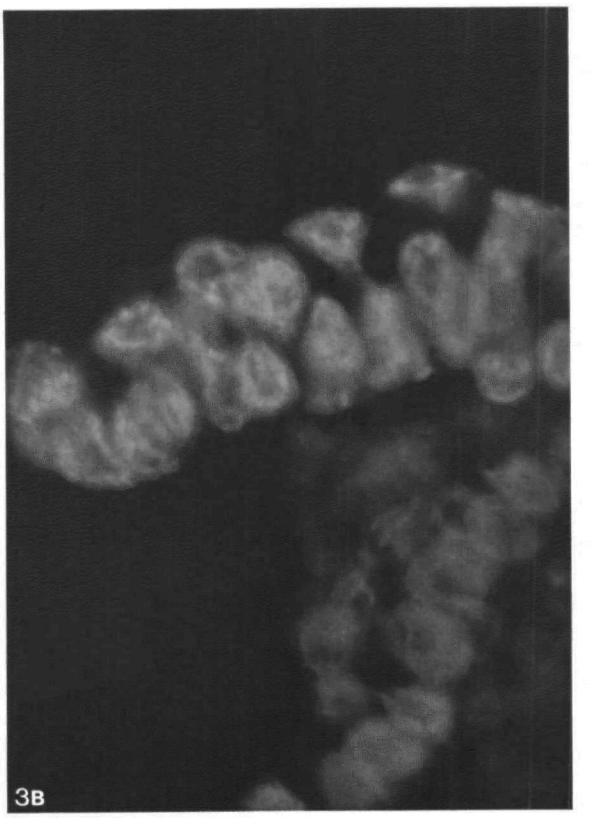


3A



3B

achs, 1983) as in the intact gland, and was inhibited by the ATPase inhibitor, picoprazole (a substituted benzimidazole) (Fig. 5). Thus, acid secretion appears to be driven in the intact system by the activity of an $(H^+ + K^+)$ATPase. This enzyme is present in isolated membrane fractions of gastric mucosa from several species, including man, and its properties have been described in several publications. The reaction pathway of this ATPase strongly resembles the $(Na^+ + K^+)$ and Ca^{2+} ATPases of plasma membranes. A conceptual diagram which is consistent with many of the kinetic properties of this enzyme is illustrated in Fig. 6. Here the reaction is perceived to commence with the displacement of K^+ from the cytosolic face of the enzyme by ATP. This is followed by a Mg^{2+}-dependent conformational change and phosphorylation of an aspartyl group on the enzyme (Walderhaug, Saccomani, Sachs & Post, 1983). As a consequence, the H^+ high affinity cytosolic site(s) is transformed first into an occluded form and eventually to a low affinity H^+ site on the luminal face of the enzyme. Luminal K^+ displaces these protons, and the enzyme phosphate now breaks down, initially into an acid labile form with phosphate still trapped on the enzyme, and then into a K^+ form of the enzyme with ATP being required to displace K^+, thus reinitiating the reaction cycle.

Three major conformations of the enzyme emerge from this scheme, as for the

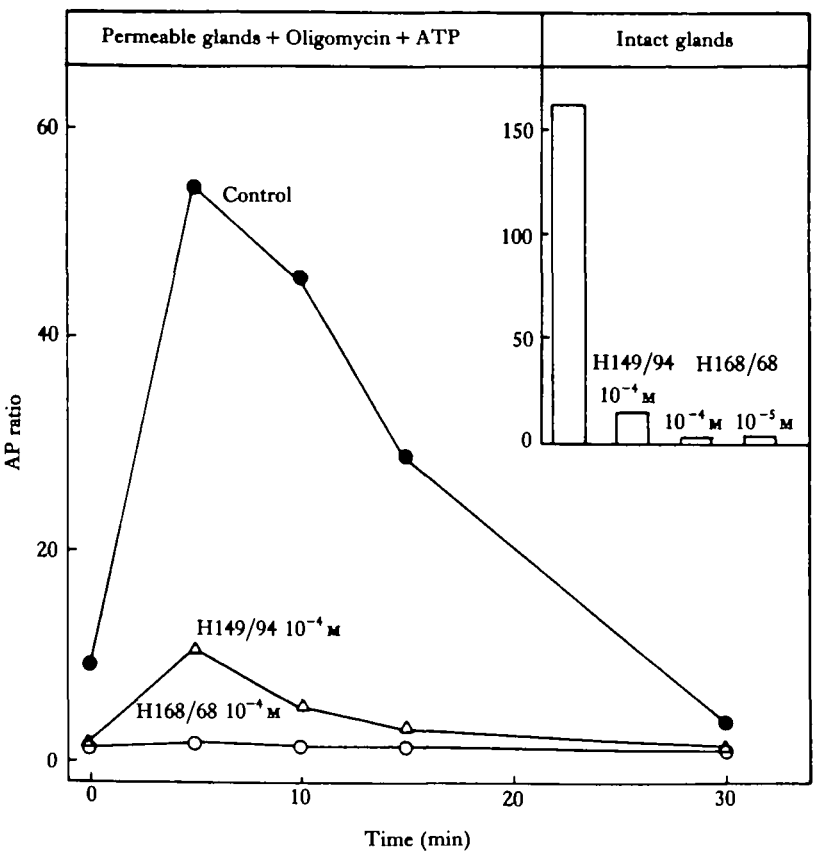


Fig. 5. The inhibition of the ATP-dependent aminopyrine uptake by substituted benzimidazoles picoprazole, H149/94; omeprazole, H168/68) in permeable rabbit gastric glands.

$(Na^+ + K^+)$ATPase: an E_1 form with ion sites exposed to cytosol, an occluded f[illegible] with ion binding sites buried within the enzyme and an E_2 form with the ion binding sites exposed to the lumen. The kinetic evidence for this model has been published in several papers (Wallmark & Mardh, 1979; Wallmark *et al.* 1980; Stewart, Wallmark & Sachs, 1981). This formulation indicates K^+ requirement at the luminal face of the enzyme for turnover, suggesting that for H^+ secretion to occur at a reasonable rate, a pathway for the entry of K^+ into the canalicular lumen must be provided. Indeed, the stimulation of ATPase activity by K^+ active ionophores (Ganser & Forte, 1973*b*), and the requirement either for valinomycin or preincubation in K^+ solutions for proton transport to occur, at least in hog gastric microsomes (Chang *et al.* 1977), was known before some of the kinetic features of the enzyme had been elucidated. As isolated in hog microsomes, therefore, the system appears deficient in terms of K^+ penetration to the luminal face of the enzyme.

STIMULATION OF ACID SECRETION

Isolated rabbit gastric glands show both K^+ and Cl^- dependence of acid secretion, as mentioned above. An example of this dependence on Cl^- is shown in Fig. 7. In unstimulated glands, there is no evidence for saturation of acid secretion as a function of Cl^- concentration. In contrast, with stimulated glands there is a hyperbolic dependence

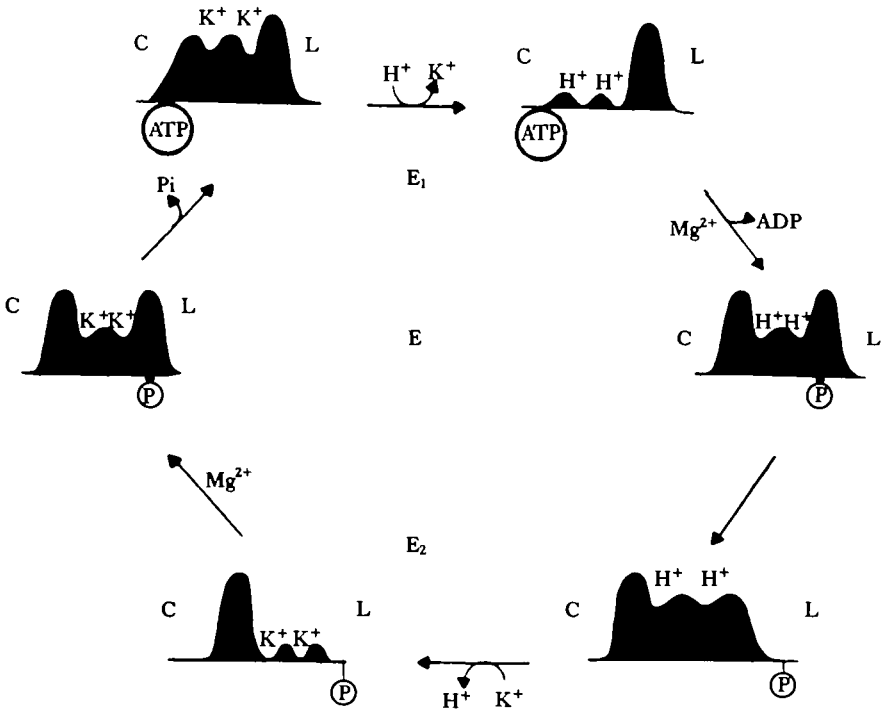


Fig. 6. A conceptual model illustrating the reaction pathway of the gastric $(H^+ + K^+)$ATPase. ATP and H^+ binding are followed by phosphorylation and change of E_1 conformation to E (occluded). This is followed by the formation of E_2-P, and with loss of H^+ and binding of K^+, breakdown of E_2-P to $E{\cdot}P.K^+$ (occluded) and then $E_1.K^+$. The latter conversion occurs by the binding of ATP and this also restarts the cycle. C, cytosol; L, lumen.

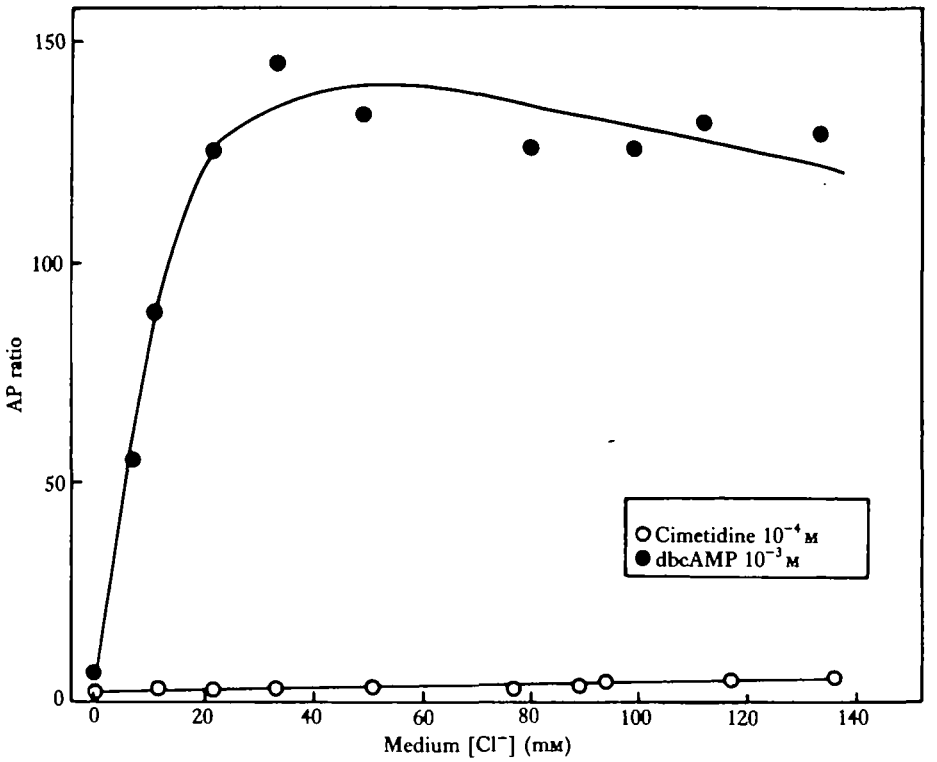


Fig. 7. The effect of medium [Cl^-] on acid secretion (aminopyrine uptake) in rabbit gastric glands. It can be seen that in unstimulated glands, no apparent saturation in terms of [Cl^-] is seen, whereas with stimulation the apparent K_m for Cl^- is about 10 mM. From Malinowska, Cuppoletti & Sachs, 1983.

of secretion on medium Cl^- concentration (Malinowska *et al.* 1983). This can be interpreted as evidence for the generation of a Cl^- site upon stimulation of acid secretion. Similar considerations apply to the relationship of K^+ to stimulation of secretion. Thus, from the intact cell it can be concluded that at least one site for activation of acid secretion is a KCl pathway in the secretory membrane either absent or inactive in the hog microsomal preparation discussed above. However, when membrane vesicles are isolated from stimulated rabbit mucosa, proton transport has been found to be independent of either valinomycin or KCl preincubation (Malinowska *et al.* 1983; Wolosin & Forte, 1981*b*). Similar data have been found in membranes isolated from stimulated rat mucosa (H. B. Im, unpublished observations). It is likely that activation of secretion in all mammalian species depends in part on activation of a KCl pathway.

PROPERTIES OF ACTIVATED MEMBRANES

In the case of hog gastric microsomes, a variety of tests for electrogenicity or electroneutrality suggested that proton transport was an electroneutral process. For example, as shown in Fig. 8, the response of the positively charged carbocyanine dye,

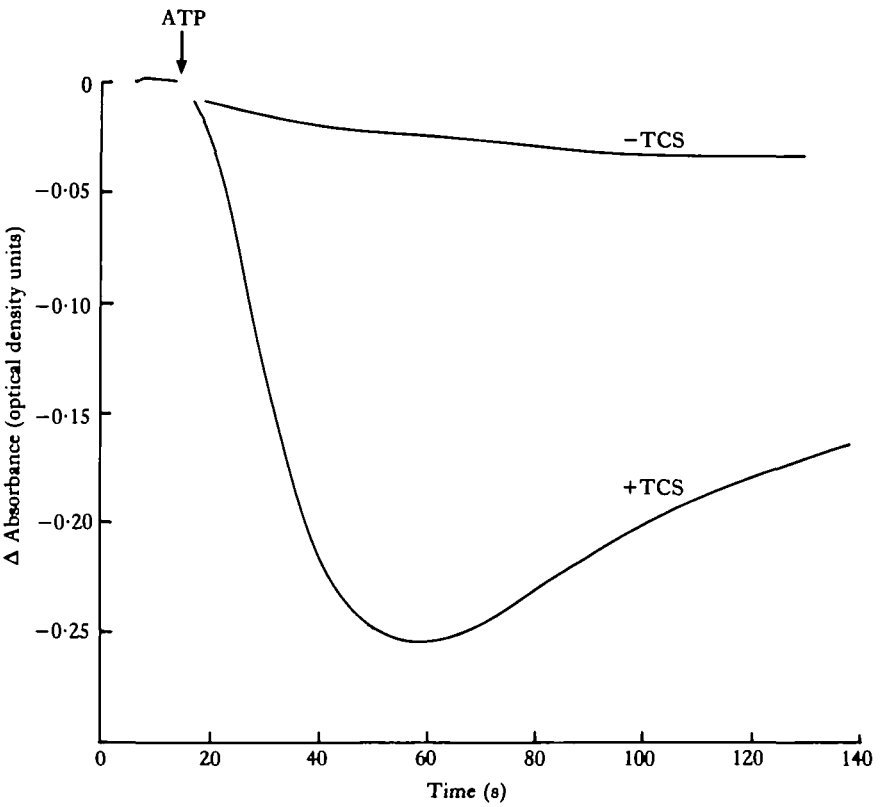


Fig. 8. The change in absorbance found with diethyl oxocarbodicyanine (DOCC) when ATP is added to hog gastric microsomes following equilibration in KCl. The upper curve shows the response in the absence of tetrachlorsalicylanilide (TCS), the lower curve shows the response in the presence of TCS.

diethyl oxocarbodicyanine (DOCC), was measured under a variety of conditions. A dye signal was obtained only when the protonophore, tetrachlorsalicylanilide (TCS), was present, with uptake of dye producing a change in absorbance or fluorescence. This signal was interpreted as being due to the protonophoric conductance allowing a diffusion potential to develop following generation of a proton gradient. If a Cl^- conductance were present in these vesicles, the protonophore would have dissipated the proton gradient and no DOCC signal would have been observed. In contrast, vesicles isolated from stimulated rabbit gastric mucosa show properties suggestive of a conductance in the membranes. Fig. 9 compares the fluorescent quenching of acridine orange in these vesicles in Cl^--containing solutions with the quenching of the negatively charged oxonol dye, $DiBAC_4(5)$, when Cl^- is replaced by sulphate, a less permeant anion in the system. The apparent uptake of the oxonol dye (curve 2) has a slightly slower time-course than the formation of the proton gradient detected by the acridine orange signal (curve 1). This suggests that a vesicle potential (positive interior) is developed during proton transport. Fig. 10 compares the signals as the Cl^-/SO_4^{2-} ratios are varied. It can be seen that whereas proton transport increases with increasing Cl^- concentration (Fig. 10A), potential development decreases (Fi

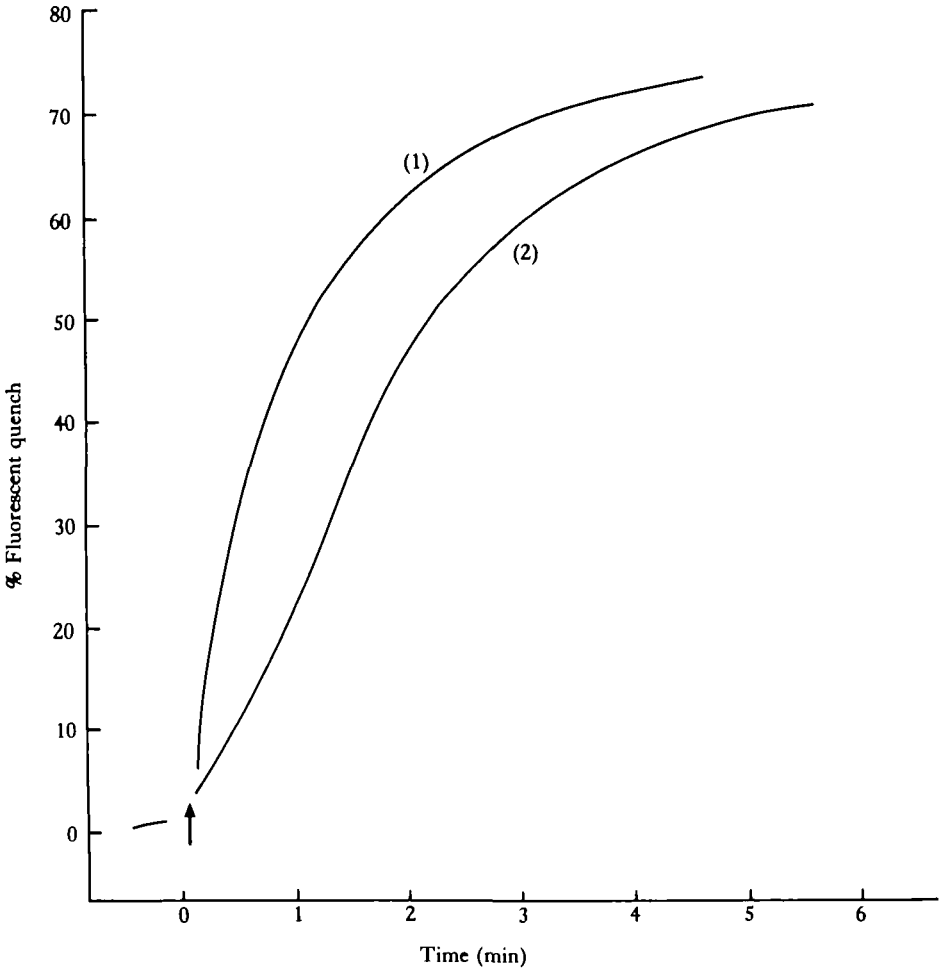


Fig. 9. A comparison of the rate of fluorescent quenching of (1) acridine orange (pH gradient) and (2) diBAC$_4$(5), a negatively-charged oxonol dye, following ATP addition to vesicles isolated from rabbit gastric mucosa in Cl^- (1) and SO_4^{2-} (2) solutions.

10B), suggesting the presence of a Cl^- conductance. Both processes are K^+ and ATP dependent, thus reflecting activity of the ATPase. Further, as shown in Fig. 11, TCS at concentrations above 1 μM inhibits the acridine orange and the oxonol dye signal. The inhibition by TCS of proton gradient formation in Cl^- solutions could result from either an H^+: K^+ exchange induced by the protonophore, or a Cl^- conductance coupled to the H^+ conductance induced by the protonophore. In the case of the oxonol dye, the effect of TCS is probably due to coupling of H^+ efflux to a K^+ conductance, since no Cl^- is present and only a small proton gradient is generated under these conditions.

Evidence for a K^+ conductance can be obtained by using the positively charged carbocyanine dye, DiSC$_3$(5), to examine diffusion potentials in K_2SO_4-loaded rabbit vesicles. Fig. 12 shows that upon dilution of these vesicles into K^+-free medium, dye uptake occurs, which is progressively decreased as K^+ is added externally.

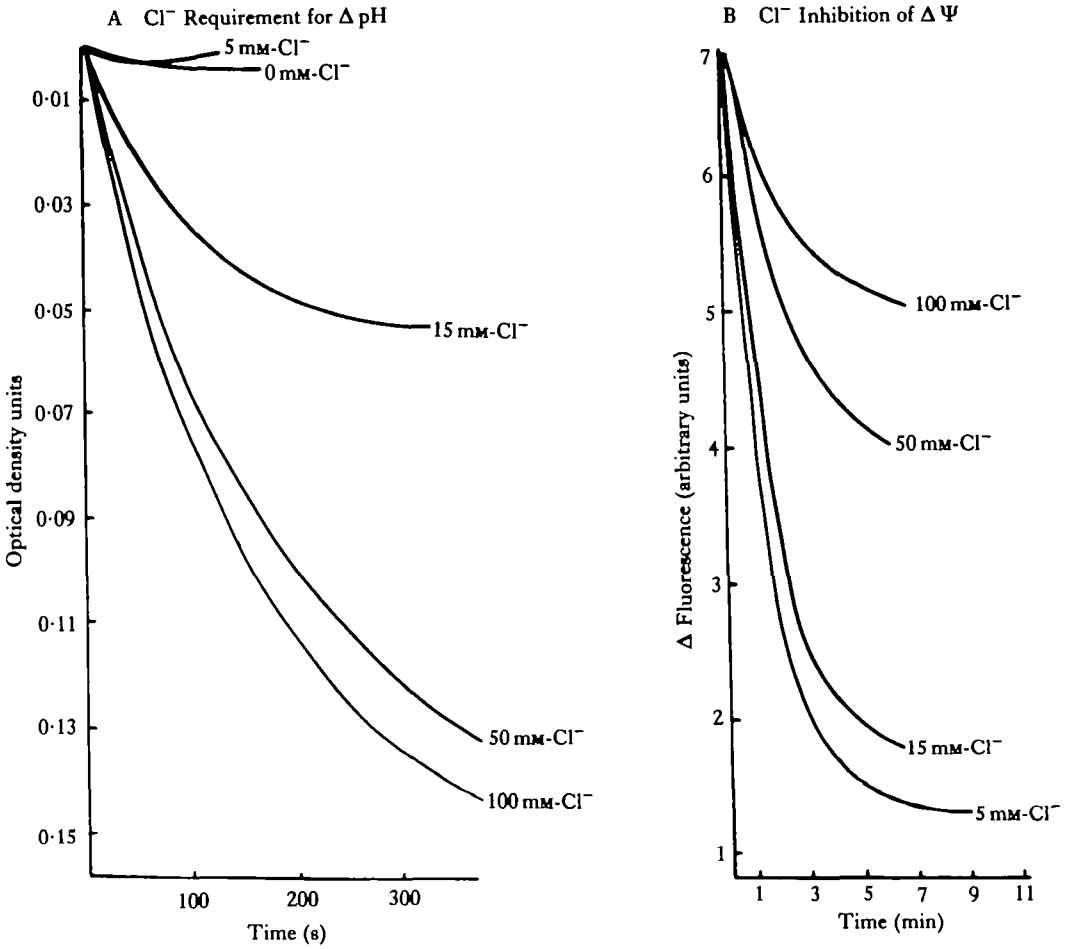


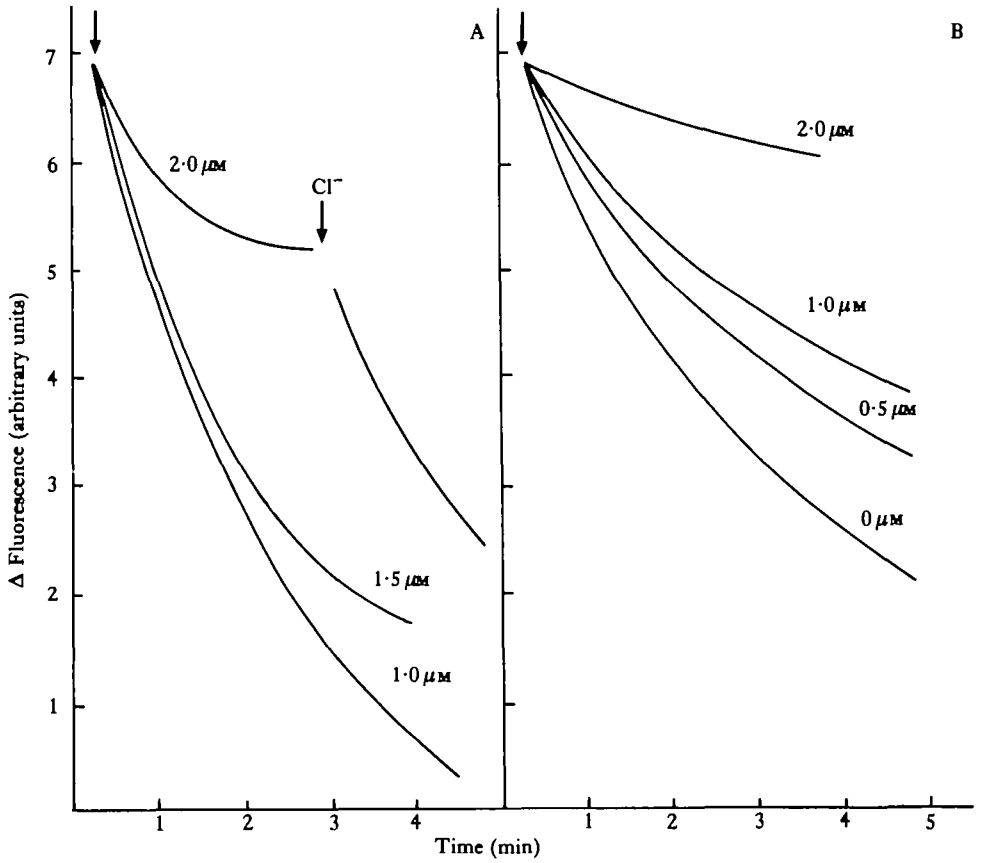
Fig. 10. (A) The uptake of acridine orange and (B) the uptake of the negatively charged $DiBAC_4(5)$ as a function of increasing Cl^-/SO_4^{2-} ratios in vesicles isolated from stimulated rabbit mucosa following addition of ATP.

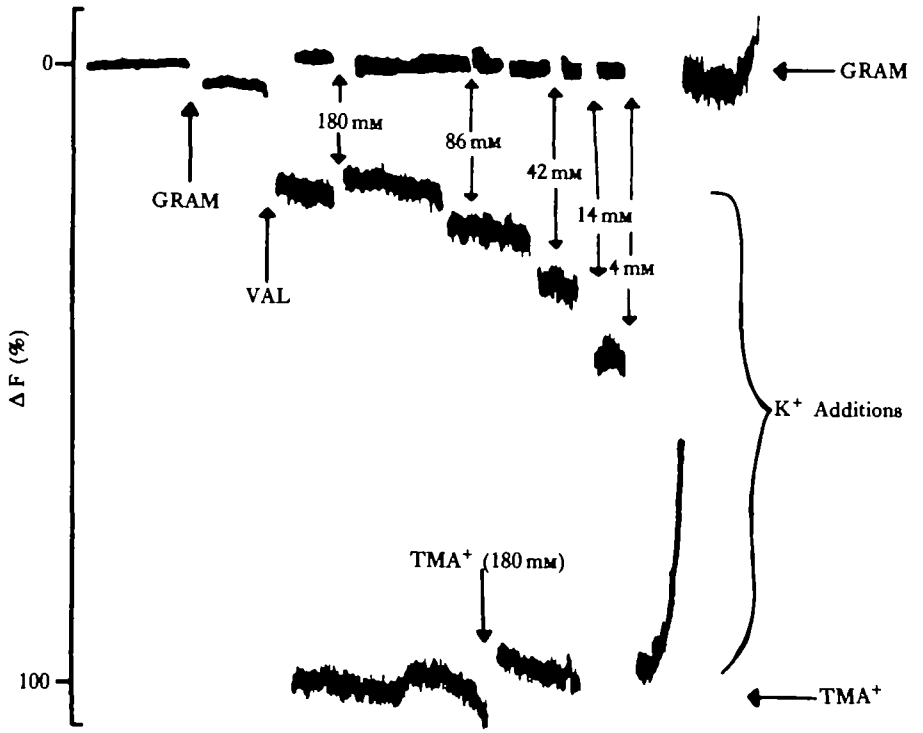
Gramicidin abolishes the dye signal, and no effect is seen when tetramethylammonium is added instead of K^+. When K_2SO_4-loaded hog gastric microsomal vesicles were diluted into K^+-free medium in the presence of high concentrations of TCS (7·5 μM), a proton gradient developed which was partially reversed by addition of K^+ to the external medium (Fig. 13). This was interpreted in terms of a K^+ diffusion potential-driven uptake of protons through the TCS pathway.

Fig. 14 shows an experiment also using hog gastric microsomes, where net Rb(K)Cl flux was measured and compared to the rate of $Rb^+ : Rb^+$ and $Cl^- : Cl^-$ exchange. Whereas the rate of net RbCl uptake and $Cl^- : Cl^-$ exchange had a slow time course,

Fig. 11. The effect of TCS on (A) the acridine orange response and (B) the lipophilic anion, $diBAC_4(5)$ signal in stimulated rabbit gastric membranes. It can be seen that both signals are dissipated, and that the addition of high Cl^- restores the pH gradient.

Fig. 12. The effect of dilution of K_2SO_4-loaded stimulated rabbit vesicles into K^+-free medium, followed by sequential addition of increasing K^+ to the medium, in presence of the lipophilic cationic dye, $diSC_3(5)$. This experiment shows that a K^+ diffusion potential can be detected with this dye. Abbreviations: GRAM, gramicidin; VAL, valinomycin; TMA^+, tetramethylammonium. Note: This figure should be read right to left.


A
B
2·0 μM
Cl⁻
1·5 μM
1·0 μM
2·0 μM
1·0 μM
0·5 μM
0 μM
Δ Fluorescence (arbitrary units)
Time (min)


GRAM
180 mM
86 mM
42 mM
14 mM
4 mM
GRAM
VAL
K⁺ Additions
TMA⁺ (180 mM)
TMA⁺
ΔF (%)

Figs 11 & 12

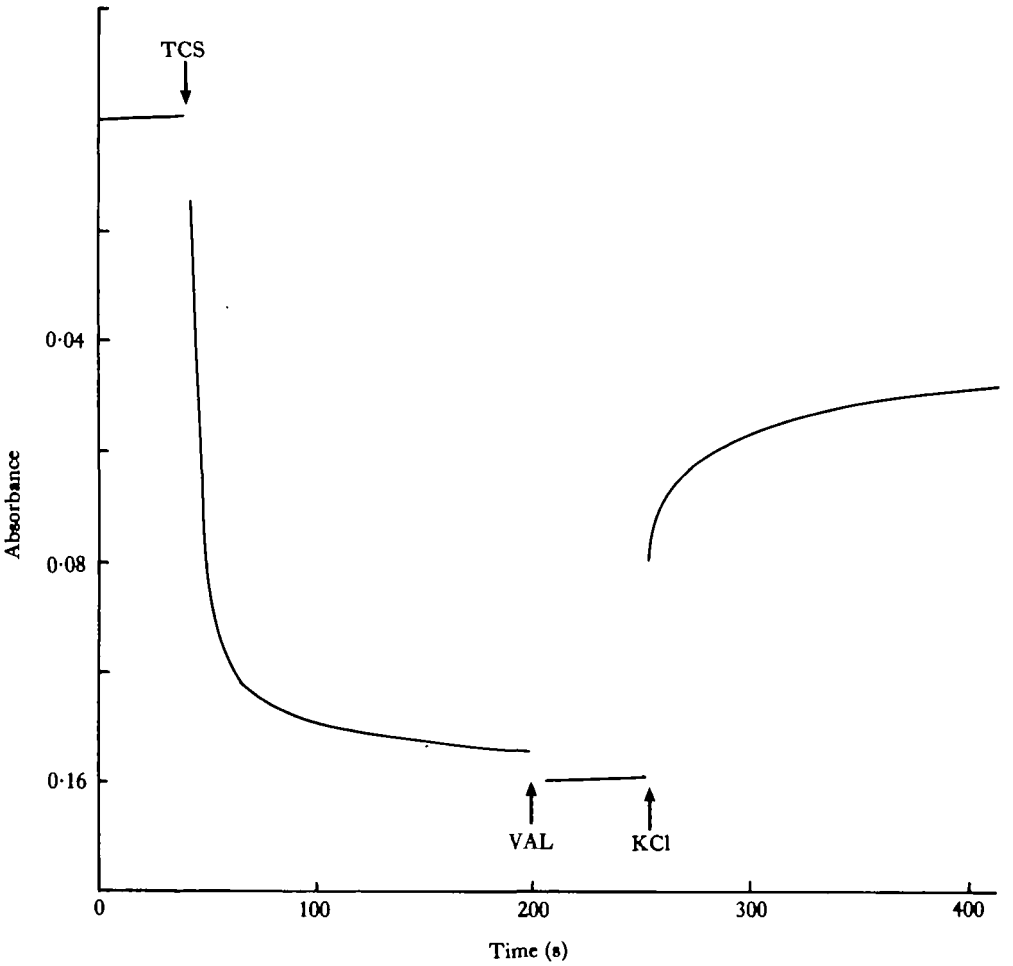


Fig. 13. The presence of a K^+ conductance in hog gastric microsomes is shown by the addition of TCS to K_2SO_4-loaded vesicles followed by dilution into K^+-free medium. A proton gradient is generated (detected by acridine orange uptake), due to coupling of the K^+ diffusion potential to H^+ uptake *via* the TCS pathway. No H^+ movement occurs in the absence of TCS. Abbreviations: VAL, valinomycin; TCS, tetrachlorsalicylanilide.

the rate of Rb^+: Rb^+ exchange was very rapid (Schackman, Schwartz, Saccomani & Sachs, 1977). In addition, the rate of Rb^+: Rb^+ exchange was blocked by vanadate in the presence of Mg^{2+}. Given that vanadate binds selectively to the ATPase complex (Faller *et al.* 1981), this would suggest that a K^+ pathway is intrinsic to the proton pump. Therefore, it is possible that the K^+ pathway of the stimulated system is present as an intrinsic property of the pump membrane, and activation of secretion involves the insertion or activation of a Cl^- component. In this regard, activation of acid secretion would be similar to activation of Cl^- secretion in the lower regions of the intestine which is thought to occur by activation or insertion of a Cl^- conductance into the apical membrane of the intestinal cell (Frizzell, Field & Schultz, 1979).

Although the above data may be interpreted as evidence for a K^+ conductance in vesicles derived from stimulated rabbit stomach, the ATP and K^+ dependence of the oxonol dye signal is a complicating factor. If a large K^+ conductance were prese

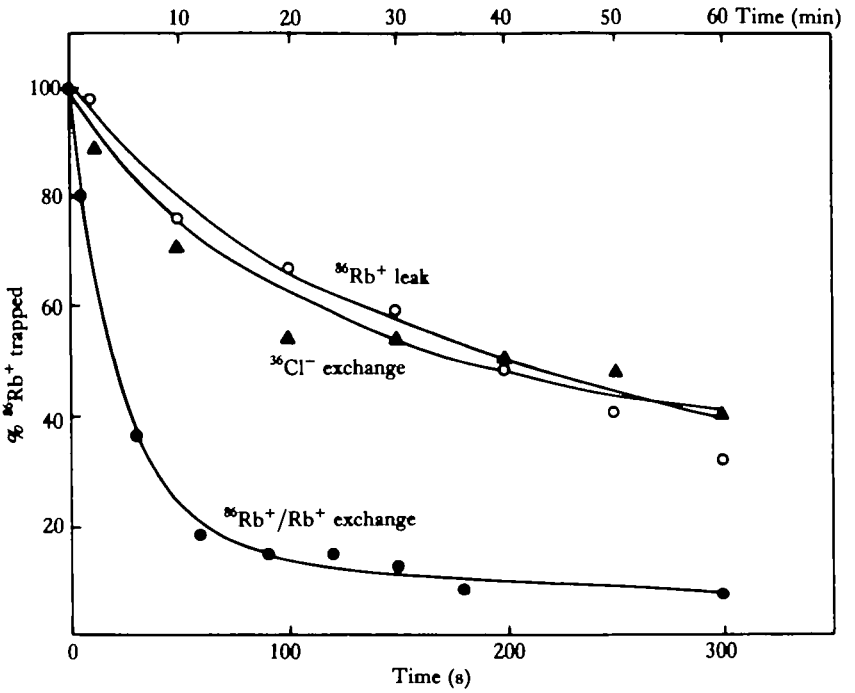


Fig. 14. The movement of isotopes across hog gastric microsomal membranes. The time-course of both net RbCl movement and Cl^-: Cl^- exchange is slow (upper time scale), but Rb^+: Rb^+ exchange is fast (lower time scale).

addition of K_2SO_4 in the absence of ATP would generate a dye signal limited only by the response time of the dye. Furthermore if the K^+ conductance were ATP dependent, then the addition of K_2SO_4 to ATP-pretreated membranes should also result in rapid dye uptake. Neither of these appears to occur. On the other hand, the slow time course of the response and its sensitivity to agents which either dissipate ion gradients by electroneutral mechanisms (nigericin), or act as lipid permeable buffer systems (NH_4^+, imidazole), argue for an ion gradient dependence of the dye signal, rather than for an electrogenic pump potential. Also the fact that dissipation of the proton gradient by TCS was reversed by increasing the external Cl^- concentration from 35 mM to 100 mM (Fig. 11A) suggests a possible explanation for the oxonol dye signal.

If a Cl^- site is closely associated with the pump, and if an intermediate pool of protons is trapped within the pump mechanism which reacts with TCS to provide dissipation, then the added Cl^- could perhaps compete with TCS for these protons, thereby antagonizing the action of TCS. Thus in the absence of Cl^-, a diffusion potential from this pool towards the vesicle interior could account for the generation of a positive potential, resulting in dye uptake.

Although aminopyrine uptake cannot be detected in isolated rabbit glands suspended in sulphate solutions, oxygen consumption is stimulated and further increased by secretagogues (Berglindh, 1977*b*). This apparent uncoupling action of sulphate suggests that in the absence of Cl^-, protons are held in an intermediate pool from which

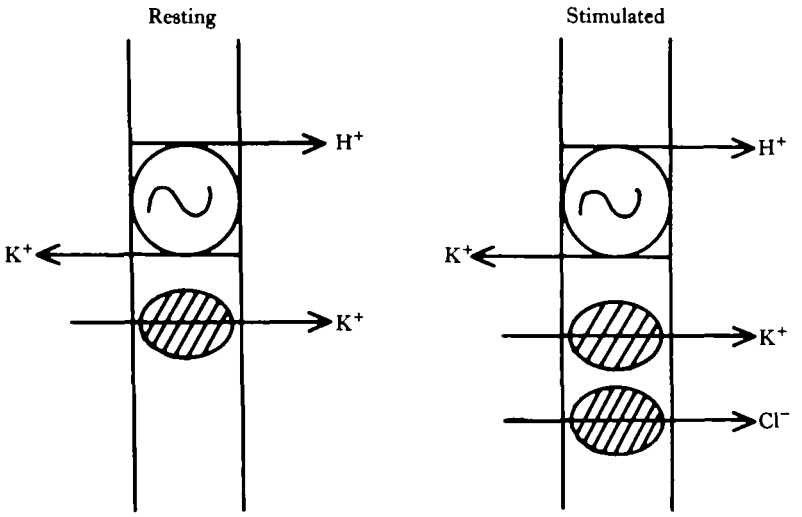


Fig. 15. A conceptual model of the resting gastric proton pump membrane showing the pump and the associated K^+ pathway (conductance?), but the absence of a Cl^- pathway (conductance?). Activation of secretion is due to activation of a Cl^- pathway (conductance?), or fusion of the tubulovesicle membrane with the apical membrane which contains the Cl^- pathway (conductance?), producing a system as shown on the right hand side of the figure.

they can be recycled through the pump. Thus the Cl^- pathway suggested above may be directly coupled to the pump mechanism, allowing the cation exchange to perform as a net cation: anion coupled pathway. Such a model is consistent with the observation that, under transporting conditions, ATPase activity is unaffected by substituting sulphate for chloride.

CONCLUSIONS

A working hypothesis for the mechanism of acid secretion across the apical (canalicular) membrane of the parietal cell is shown in Fig. 15. Here the membrane isolated from the resting cell contains the $(H^+ + K^+)$ATPase and a K^+pathway (conductance?), and a cAMP- and/or Ca^{2+}-dependent activation step results in a Cl^- pathway (conductance?) being added to the membrane. Whether this occurs as a result of fusion of tubulovesicular membranes with the secretory canaliculus, or as a result of activation of a peptide present in the pump membrane, remains to be shown. However, since the gastric mucosa secretes Cl^- by a rheogenic mechanism, even in the resting state, it is possible that the apical membrane of the parietal cell contains this Cl^- conductance at all times.

REFERENCES

BERGLINDH, T. (1977*a*). Effects of common inhibitors of gastric acid secretion on secretagogue-induced respiration and aminopyrine accumulation in isolated gastric glands. *Biochim. biophys. Acta* **464**, 217–233.

BERGLINDH, T. (1977*b*). Absolute dependence on chloride for acid secretion in isolated gastric glands. *Gastroenterology* **73**, 874–880.

GLINDH, T. (1978). The effects of K^+ and Na^+ on acid formation in isolated gastric glands. *Acta physiol. scand.* Special Suppl. 1978, pp. 55–68.

BERGLINDH, T., DIBONA, D. R., PACE, C. S. & SACHS, G. (1980). ATP dependence of H^+ secretion. *J. Cell Biol.* **85**, 392–401.

BERGLINDH, T., HELANDER, H. F. & OBRINK, K. J. (1976). Effects of secretagogues on oxygen consumption, aminopyrine accumulation and morphology in isolated gastric glands. *Acta physiol. scand.* **97**, 401–414.

BERGLINDH, T. & OBRINK, K. J. (1976). A method for preparing isolated glands from the rabbit gastric mucosa. *Acta physiol. scand.* **96**, 150–159.

CHANG, H., SACCOMANI, G., RABON, E., SCHACKMANN, R. & SACHS, G. (1977). Proton transport by gastric membrane vesicles. *Biochim. biophys. Acta* **464**, 313–327.

FALLER, L. D., MALINOWSKA, D. H., RABON, E., SMOLKA, A. & SACHS, G. (1981). Mechanistic studies of the gastric ($H^+ + K^+$) ATPase. In *Membrane Biophysics: Structure and Function in Epithelia*, pp. 153–174. New York: Alan R. Liss.

FRIZZELL, R. A., FIELD, M. & SCHULTZ, S. G. (1979). Sodium-coupled chloride transport by epithelial tissues. *Am. J. Physiol.* **236**, F1–F8.

GANSER, A. L. & FORTE, J. G. (1973*a*). K^+-stimulated ATPase in purified microsomes of bullfrog oxyntic cells. *Biochim. biophys. Acta* **307**, 169–180.

GANSER, A. L. & FORTE, J. G. (1973*b*). Ionophoretic stimulation of K^+-ATPase of oxyntic cell microsomes. *Biochem. biophys. Res. Commun.* **54**, 690–696.

HELANDER, H. F. & HIRSCHOWITZ, B. I. (1972). Quantitative ultrastructural studies on gastric parietal cells. *Gastroenterology* **63**, 951–961.

HERSEY, S. J. (1978). Intracellular pH and gastric acid secretion. *Acta physiol. scand.* Special Suppl. 1978, pp. 243–252.

KNIGHT, D. E. & BAKER, P. F. (1982). Calcium-dependence of catecholamine release from bovine adrenal medullary cells after exposure to intense electric fields. *J. Membr. Biol.* **68**, 107–140.

MALINOWSKA, D. H., CUPPOLETTI, J. & SACHS, G. (1983). The Cl^- requirement of acid secretion in isolated gastric glands. *Am. J. Physiol.* **245**, (in press).

MALINOWSKA, D. H., KOELZ, H. R., HERSEY, S. J. & SACHS, G. (1981). Properties of the gastric proton pump in unstimulated permeable gastric glands. *Proc. natl Acad. Sci. U.S.A.* **78**, 5908–5912.

SACHS, G., CHANG, H. H., RABON, E., SCHACKMAN, R., LEWIN, M. & SACCOMANI, G. (1976). A non-electrogenic H^+ pump in plasma membranes of hog stomach. *J. biol. Chem.* **251**, 7690–7698.

SCHACKMAN, R., SCHWARTZ, A., SACCOMANI, G. & SACHS, G. (1977). Cation transport by gastric H^+:K^+ ATPase. *J. Membr. Biol.* **32**, 361–381.

SMOLKA, A., HELANDER, H. F. & SACHS, G. (1983). Monoclonal antibodies against gastric $H^+ + K^+$ ATPase. *Am. J. Physiol.* **245** (in press).

SOLL, A. H. (1978). The actions of secretagogues on oxygen uptake by isolated mammalian parietal cells. *J. clin. Invest.* **61**, 370–380.

STEWART, B., WALLMARK, B. & SACHS, G. (1981). The interaction of H^+ and K^+ with the partial reactions of gastric ($H^+ + K^+$)ATPase. *J. biol. Chem.* **256**, 2682–2690.

THOMAS, J. A., BUCHSBAUM, R. N., ZIMNIAK, A. & RACKER, E. (1979). Intracellular pH measurements in Ehrlich ascites tumor cells utilizing spectroscopic probes generated *in situ*. *Biochemistry, N.Y.* **18**, 2210–2218.

WALDERHAUG, M., SACCOMANI, G., WILSON, T. H., BRISBIN, D., LEONARD, R. T., SACHS, G. & POST, R. L. (1983). Aspartyl residue may be phosphorylated in a variety of membrane-bound adenosine triphosphatases. *Fedn Proc. Fedn Am. Socs exp. Biol.* **42**, p. 1275, abstract 5762.

WALLMARK, B. & MARDH, S. (1979). Phosphorylation and dephosphorylation kinetics of potassium stimulated ATP phosphohytrolase from hog gastric mucosa. *J. biol. Chem.* **254**, 11899–11902.

WALLMARK, B., SACHS, G., MARDH, S. & FELLENIUS, E. (1983). Inhibition of gastric ($H^+ + K^+$)-ATPase by the substituted benzimidazole, picoprazole. *Biochim. biophys. Acta* **728**, 31–38.

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.

WOLOSIN, J. M. & FORTE, J. G. (1981). Changes in the membrane environment of the ($K^+ + H^+$)-ATPase following stimulation of the gastric oxyntic cell. *J. biol. Chem.* **256**, 3149–3152.

WOLOSIN, J. M. & FORTE, J. G. (1981). Functional differences between K^+-ATPase rich membranes isolated from resting or stimulated rabbit fundic mucosa. *FEBS Lett.* **125**, 208–212.