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Acid, Protons and *Helicobacter pylori*

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The anti-ulcer drugs that act as covalent inhibitors of the gastric acid pump are targeted to the gastric H^+/K^+ ATPase by virtue of accumulation in acid and conversion to the active sulfenamide. This results in extremely effective inhibition of acid secretion. Appropriate dosage is able to optimize acid control therapy for reflux and peptic ulcer disease as compared to H_2 receptor antagonists. However, clinical data on recurrence show that *Helicobacter pylori* eradication should accompany treatment of the lesion. These drugs have been found to synergize with many antibiotics for eradication.

The survival of aerobes depends on their ability to maintain a driving force for protons across their inner membrane, the sum of a pH and potential difference gradient, the protonmotive force (pmf). The transmembrane flux of protons across the F_1F_0 ATPase, driven by the pmf, is coupled to the synthesis of ATP. The internal pH of *H. pylori* was measured using the fluorescent dye probe, BCECF, and the membrane potential defined by the uptake of the carbocyanine dye, $DiSC_3$ [5] at different pHs to mimic the gastric environment. The protonmotive force at pH 7.0 was composed of a ΔpH of 1.4 (-84mV) and a Δpotential difference of -131mV, to give a pmf of -215 mV. The effect of variations in external pH on survival of the bacteria in the absence of urea correlated with the effect of external pH on the ability of the bacteria to maintain a pmf. The effect of the addition of 5 mM urea on the pmf was measured at different medium pH values. Urea restored the pmf at pH 3.0 or 3.5, but abolished the pmf at pH 7.0 or higher, due the production of the alkalinizing cation, NH_3. Hence *H. pylori* is an acid-tolerant neutrophile due to urease activity, but urease activity also limits its survival to an acidic environment. These data help explain the occupation of the stomach by the organism and its distribution between fundus and antrum. This distribution and its alteration by proton pump inhibitors also explains the synergism of proton pump inhibition and antibiotics such as amoxicillin and clarithromycin in *H. pylori* eradication.

INTRODUCTION

The last quarter of this century has seen a remarkable change in the way that all diseases are treated, and treatment of peptic ulcer disease is no exception. It was early in the century that acid secretion was blamed for the occurrence of peptic ulcer disease [1], but it was not until the last quarter of the century that medical treatment of this common ailment became feasible with the introduction of the histamine-2 receptor antagonists (H_2RAs)[b] [2]. It was recognized then that even more effective inhibition of acid secretion would improve clinical results, and this led to the introduction of proton pump inhibitors (PPIs) as first-line therapy certainly for reflux disease and probably, as safety concerns faded, for all acid related diseases [3]. With the improvement in acid control came the realization that the diseases recurred when the acid control therapy was discontinued,

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[b]*Abbreviations:* H_2RA, H_2 receptor antagonist; PMF, protonmotive force; PPI, proton pump inhibitor; BCECF, bis-carboxyethyl carboxyfluorescein.

emphasizing that acid was not the only cause of peptic ulcer disease. It was then discovered by a pathologist observing sections of clinical material that gastric damage was associated with infection with a spiral organism, *Campylobacter*, now *Helicobacter pylori* [4]. Upon eradication of the organism from the stomach, recurrence of peptic ulcer disease dropped to the same level as found in those patients complying with maintenance treatment [5, 6]. This has now resulted in a large body of research on the organism itself and particularly on methods of eradication that coincide with healing of the ulcer.

Beyond acid related disease of the stomach or duodenum, infection by *H. pylori* is now thought to result in atrophic gastritis, a situation apparently accelerated by the co-administration of inhibitors of acid secretion [7]. Atrophic gastritis, be it of fundus or of antrum, is known to increase the incidence of gastric cancer [8, 9]. Hence eradication of the organism is important not only in treatment of peptic ulcer disease but for prevention of atrophic gastritis.

This review will evaluate the role of proton pump inhibition in dealing with the acid secretory component of upper gastro-intestinal disease and will then consider the mechanisms of synergism between proton pump inhibition and eradication of *H. pylori*. We postulate that the reasons for survival of the organism in the stomach determine the change of localization of the bacterium upon treatment with PPIs and the synergism between proton pump inhibition with growth dependent antibiotics [10].

PROTON PUMP INHIBITION

Although H_2RAs are selective only for the histamine-$_2$ receptor, these drugs are effective medically due to the importance of this receptor for gastric acid secretion and to the distribution of H_2 receptors giving few side effects. Inhibition of acid secretion by these agents is insufficient for optimal inhibition of acid secretion for treatment of reflux disease [11], and their effects fall short of the calculated ideal for acid suppression in duodenal ulcer disease [12]. Various biological factors account for this shortfall, such as alternate muscarinic pathways able to stimulate acid secretion [13] and tolerance [14].

Inhibition of the final step of acid secretion, that catalyzed by the gastric H^+/K^+-ATPase (the secretion of HCl), is able to reach the goal of elevation of intragastric pH to above 4.0 for 18 hours per day for reflux disease treatment and to pH above 3.0 for treatment of duodenal ulcer [11, 12].

The chemical mechanism of the benzimidazole class of anti-ulcer drugs

The currently available clinically used PPIs all conform to the substituted pyridyl methlysulfinyl-2-benzimidazole class of drug. The formulae for the registered examples of these as well as one that is undergoing clinical evaluation are presented in Figure 1. Figure 1 also illustrates their mechanism of action as discussed below.

These benzimidazoles are all membrane permeable weak bases with a pK_a of around 4.0 on the pyridine nitrogen. Below pH 4.0 they are more protonated than unprotonated. Since the protonated form is less membrane permeable, these compounds will accumulate in acid spaces with a pH_{in} of less than 4.0. Only the secretory canaliculus of the active parietal cell has this level of pH_{in}, reaching about 1.0 with stimulation of acid secretion. Hence their accumulation in this space should be about 1000-fold. They are, therefore, chemically targeted to the secretory canaliculus of the active parietal cell, as has been demonstrated by electron microscopic autoradiography [15, 16].

Following accumulation, they undergo an acid catalyzed conversion to a tetracyclic cationic sulfenamide structure as shown in Figure 1. The formation of this sulfenamide depends on the protonation of the benzimidazole nitrogen without protonation of the pyridine nitrogen. This increases the electrophilic reactivity of the number 2 carbon of the benzimidazole allowing reaction with the unshared electron pair of the unprotonated pyridine

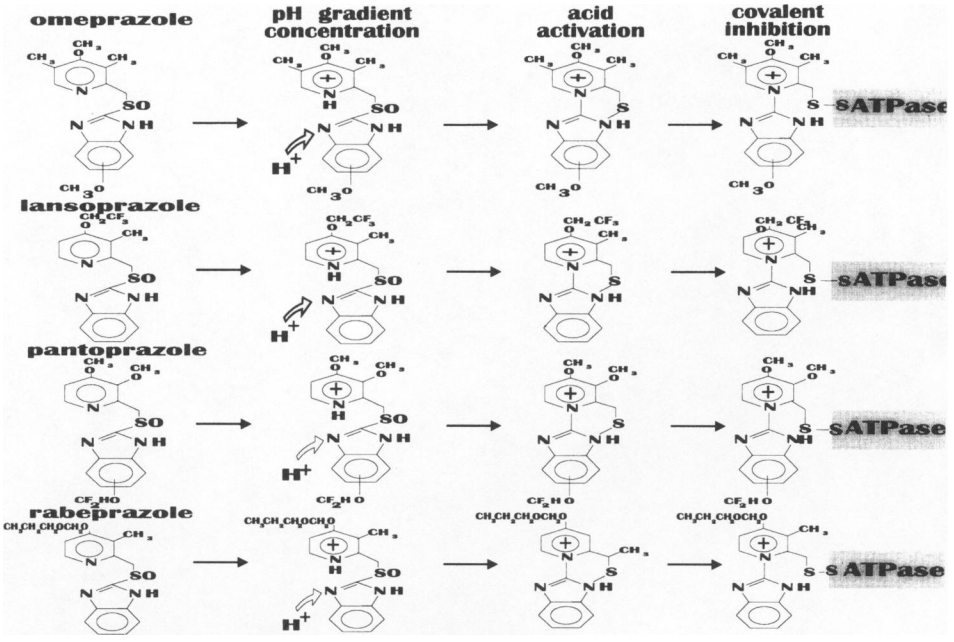

Figure 1. The three currently registered proton pump inhibitors (top), showing the chemistry involved in their action on acid secretion and the fourth that is in development (bottom).

nitrogen. The sulfenamide reacts covalently, in a stable fashion, with SH groups forming disulfides and perhaps in an unstable fashion with disulfides (Brandstrom, personal communication).

Since the cationic sulfenamide is formed within the secretory canaliculus and the membrane of the canaliculus contains large quantities of the gastric H^+/K^+-ATPase [17], the drug is targeted to those cysteines present in the H^+/K^+-ATPase, which are accessible from the outside surface shown in Figure 2. The compounds are, therefore, targeted to the secreting canaliculus by pH gradient dependent accumulation and to the acid pump by acid catalyzed activation to a membrane impermeable, SH-reactive sulfenamide.

The target amino acid on the gastric H^+/K^+-ATPase

An explanation for inhibition by the sulfenamide binding to the pump requires an understanding of the secondary structure of the H^+/K^+-ATPase, with consequent definition of those amino acids present in the membrane and extracytoplasmic domains. This has resulted in the two-dimensional model of Figure 2. This structure has been obtained by various means including (a) tryptic digestion of intact gastric H^+/K^+-ATPase vesicles and sequencing fragments [18] along with definition of labeling sites by the benzimidazoles [18, 19, 20]; (b) definition of the location of antibody epitopes [21]; and (c) by *in vitro* translation scanning [22].

Inspection of this model reveals several cysteines that are present in the extracytoplasmic and membrane domain and that are potential targets of the sulfenamides generated from the benzimidazole prodrugs, for example cysteines at position 321, 813, 822, 892 and 974. The presence of a large number of hydrophilic amino acids particularly in transmembrane segments 5 and 6 suggests that this region of the membrane domain might well be involved in ion translocation across the membrane. The significance of this region in the closely allied Na^+/K^+ ATPase and the sr Ca^{+2} ATPase has been emphasized by results

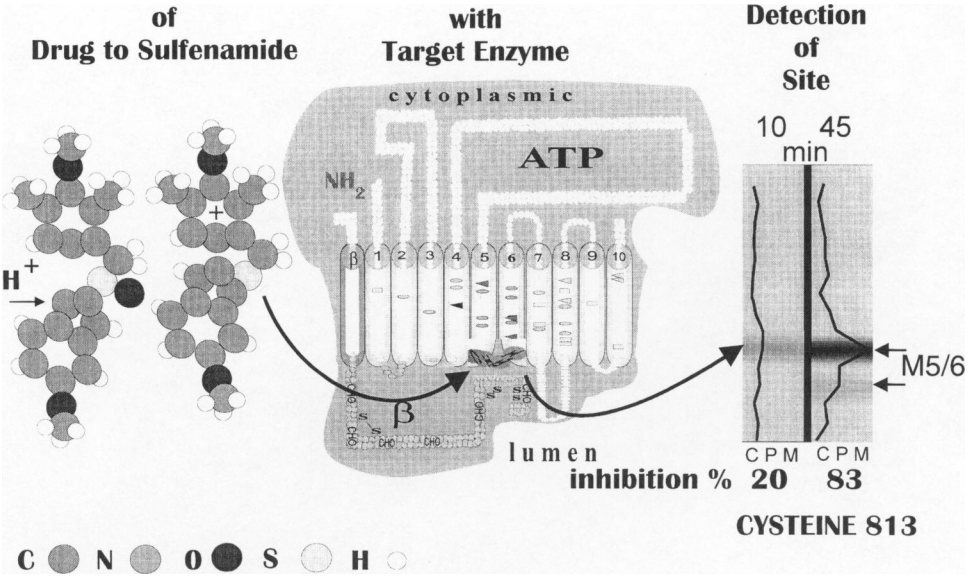

Figure 2. A summary of the critical chemical conversion of the drug into a sulfenamide, the target region on the two-dimensional model of the H^+/K^+-ATPase and the binding on the enzyme as revealed by radiolabelling followed by tryptic digestion, SDS gel separation and autoradiography with microsequencing.

found using site directed mutagenesis where mutations alter Na or Ca transport properties [23, 24, 25, 26].

The cysteines in the region enclosed by 5th and 6th transmembrane segments are cysteines 813 and 822, the former present in the extracytoplasmic loop between these segments, the latter predicted to be more buried within the membrane domain. Labeling studies by the various benzimidazoles (omeprazole, lansoprazole and pantoprazole [18, 19, 20] and the compound, E3810 currently undergoing clinical trials) has shown that they bind covalently to at least one of the cysteines in the 5th and 6th transmembrane region. From modeling studies predicting a surface location, it is likely to be cysteine 813. The results of a labeling reaction are also shown in Figure 2. Here, hog gastric vesicles containing the pump were reacted under acid transporting conditions with pantoprazole, digested with trypsin and the resulting fragments separated by tricine gradient SDS gels. Autoradiography and sequencing showed that the major fraction of labeling was in the 5th and 6th transmembrane segments and the connecting loop. Reactivity with the SH reagent, fluorescein maleimide, showed that only a single cysteine was labeled in the major peak, whereas lack of reactivity with fluorescein maleimide showed that both cysteines were labeled in the minor peak [20]. Inhibition correlated with the major labeling of cysteine 813. This was also true of the other benzimidazoles, although they were able to label other cysteines as well (e.g., cys 321 and 892 for lansoprazole [19] and cys 892 in addition for omeprazole [20]).

The finding that there is selectivity in terms of cysteines that are labeled argues that the chemistry is not the only determinant of reaction with the enzyme. There could be steric factors within the different sulfenamides that are formed that result in different

cysteines being selected. On the other hand, the slowest activating compound, pantoprazole, reacts only with transmembrane segments 5 and 6, as if activation occurred on the enzyme surface and not in free solution. If surface activation does occur, then the cysteine selectivity would derive from the ratio between compound that is activated on the enzyme surface and in free solution.

With such inhibitors, there is a marked improvement in control of acid secretion as compared to the H_2RAs, as well as improvement in clinical outcome in all acid-related diseases [28, 29, 30]. However, recurrence after stopping treatment with PPIs is found to be at the same rate as following stopping treatment with H_2RAs (Figure 3).

Eradication of *H. pylori* is necessary to prevent recurrence. Single antibiotics that are effective *in vitro* do not eradicate. However, it has been shown in several studies that the co-administration of a PPI with two antibiotics such as clarithromycin, amoxicillin or metronidazole improves eradication of *H. pylori* [31, 32]. Investigation of the bioenergetics of this organism has allowed us to offer an explanation for this synergism.

HELICOBACTER PYLORI AND pH

Bioenergetics

Aerobic bacteria, and presumably micro-aerophilic organisms such as *H. pylori*, generate an electrochemical gradient for protons across their inner membrane by virtue of oriented oxido-reductases. These oxido-reductases transport hydrogen ions electrogenically,

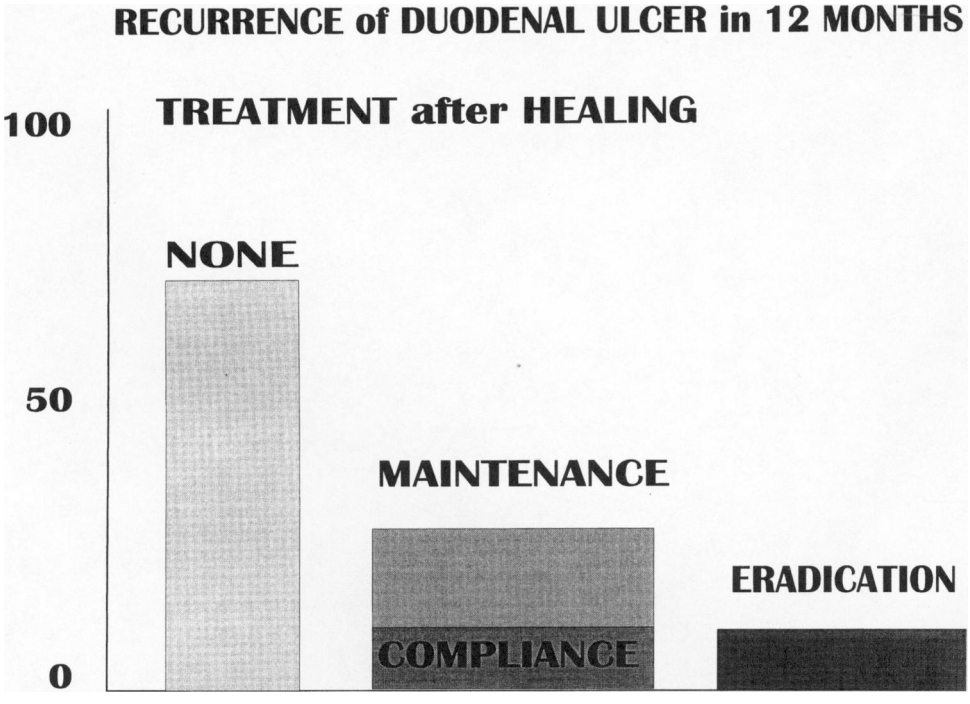

Figure 3. The recurrence of peptic ulcer disease after stopping treatment, with maintenance and maintenance where compliance is monitored and after eradication of *H. pylori*.

generating both an inward negative potential and an inward pH gradient to give what is called the protonmotive force (pmf) [33], the electrochemical gradient for H^+ ions namely:

$$pmf = \Delta \mu_{H^+} (mV) = -61\Delta pH + \Delta\Psi$$
where $\Delta\Psi$ is the transmembrane potential

The PMF is the means whereby the bacteria convert the energy of substrate metabolism into the synthesis of ATP. In these bacteria, where the pmf has been measured, it is about -200 mV. Figure 4 illustrates this concept, wherein the gradient for H^+ ions drives ATP synthesis.

The potential and pH gradient generated by substrate oxidation exerts a driving force on protons to flow inward across the ATP synthase. This is a multi-subunit protein composed of two domains: the F_0 domain, responsible for the transport of protons across the membrane and the F_1 domain, responsible for the generation of ATP from ADP and inorganic phosphate by a protonation-deprotonation reaction, which depends on the donation of protons from F_0. Thus, the coupling device between substrate oxidation and ATP synthesis is the electrochemical gradient of protons across the bacterial inner membrane.

Since *H. pylori* exists in an environment of varying acidity, the relationship of its PMF to external pH is of paramount importance in its ability to synthesize ATP and to survive. Both the internal pH and the transmembrane potential must be measured to evaluate the ability of the organism to react to varying environmental pH. The PMF must be kept at a level high enough to allow continuing ATP synthesis over the wide range of external pH that exists within the stomach. The pH and potential gradients will vary reciprocally over a range of pH at which the organism remains viable. Thus, as external acidity increases increasing the inward chemical gradient for H^+ ions, the internal negative potential

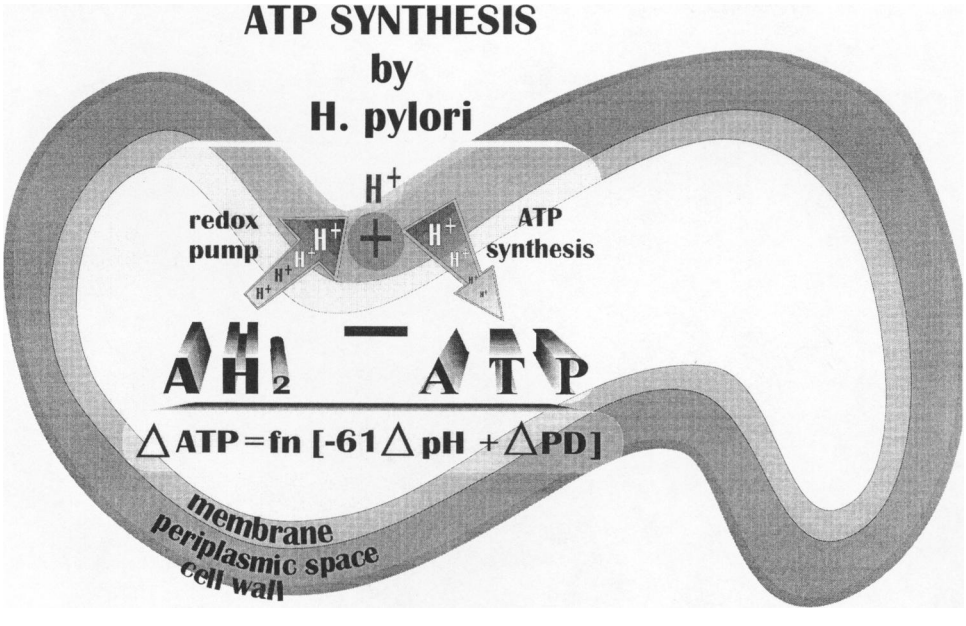

Figure 4. A diagram illustrating the protonmotive force concept. A redox pump is shown as a battery generating proton current across the inner membrane of the bacterium by substrate oxidation. The return limb of the proton current enters the cell across the F0F1 ATPase synthesizing ATP from ADP and Pi.

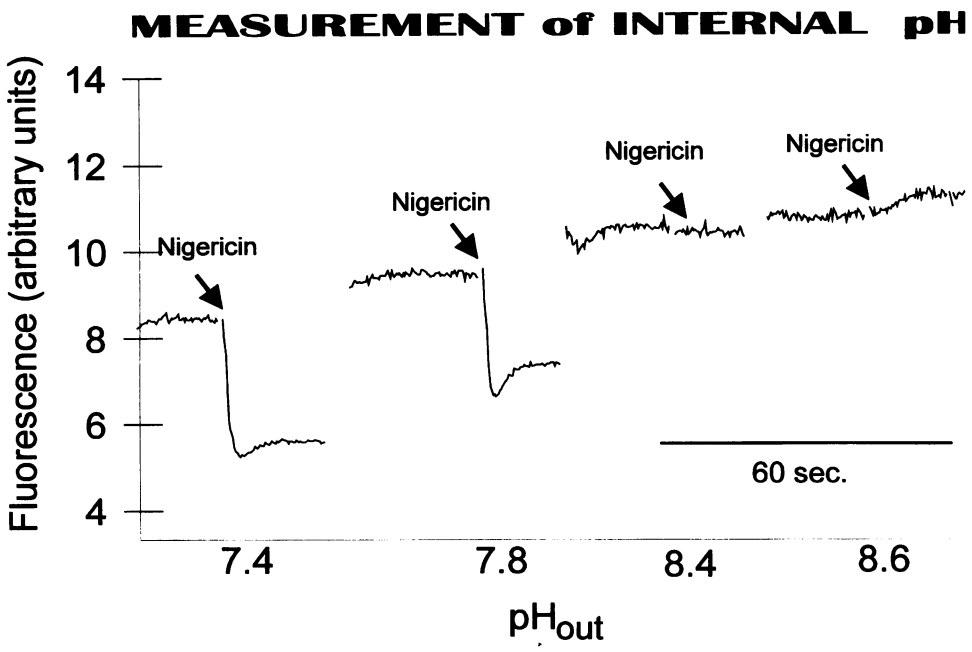

Figure 5. The calibration of internal pH using a null point method on BCECF loaded *H. pylori*.

decreases, and as external pH increases decreasing the inward chemical gradient for H^+ ions, the internal negative potential increases.

Internal pH of H. pylori

Measurement of the internal pH of the organism was achieved by using a fluorescent dye. The fluorescent pH sensitive dye, bis-carboxyethyl carboxyfluorescein (BCECF) was loaded into the bacteria by incubation with the permeant, esterified, non-fluorescent form, BCECF-AM. After hydrolysis of the ester the dye becomes fluorescent and is found within the cell or bound to the cell surface. Since the dye is not only intracellular, internal pH was measured by a null point method that measured only internal dye fluorescent changes [34]. This entails placing the organism loaded with BCECF into a well-buffered medium containing high K^+ such that no K^+ gradient is present across the bacterial membrane. This clamps dye fluorescence external to the plasma membrane. At different medium pH, the addition of the electroneutral H^+ for K^+ exchange ionophore, nigericin, allows equilibration of internal with external pH, and only a pH change inside the cell can result in a change of fluorescence. As can be seen from Figure 5, there is a decrease in fluorescence if the medium pH is between 7.4 and 8.0, an increase when the medium pH is 8.6, and no observable change when the medium pH is 8.4 [35]. This says that the internal pH of the organism is about pH 8.4. This value for pH_{int} depends on the accuracy of the assumption that the $[K^+]_{ext}$ in the solution is equal to $[K^+]_{int}$. This method when applied to *E. coli* gave a value of pH_{int} of 8.0, whereas other methods, such as flow dialysis, gave a value of pH_{int} of between 7.5 and 7.9 [36]. The internal $[K^+]$ was estimated from experiments measuring transmembrane potential as detailed below.

A second method for measurement of pH_{int} using BCECF is to add the electrogenic protonophore, tetrachlorsalicylanilide, to the organisms loaded with BCECF suspended in buffered media of different pH. Since tetrachlorsalicylanilide rapidly collapses the

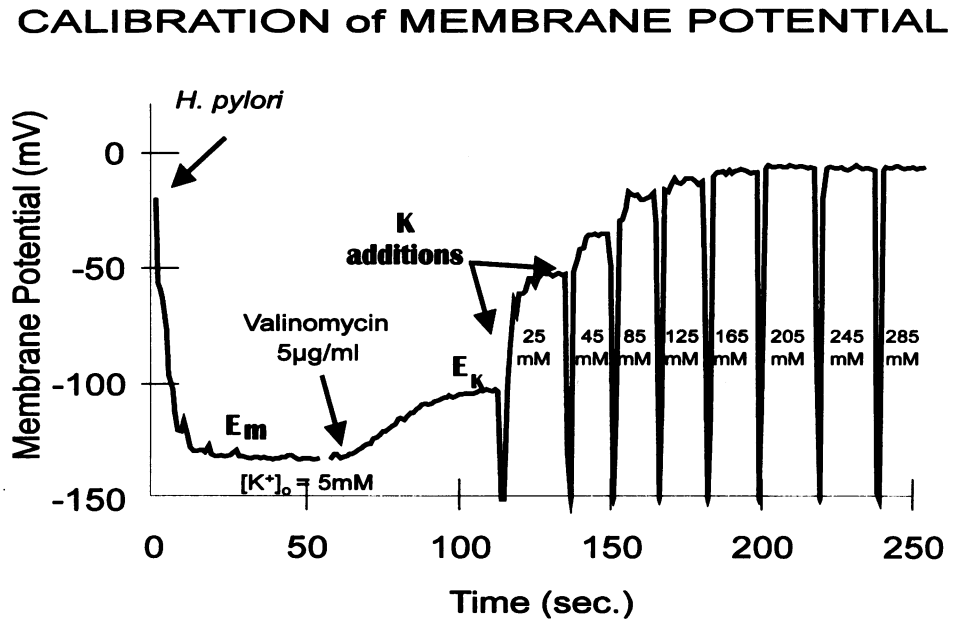

Figure 6. The calibration of transmembrane potential using $DiSC_3[5]$ and valinomycin with increasing $[K^+]_{ext}$ to obtain the null point.

potential rapidly to zero, abolishing the pmf, a change in BCECF fluorescence represents a pH difference between medium and the bacterial interior. Again, no change of BCECF fluorescence was found when the medium pH was 8.4.

Transmembrane potential

A lipid permeable cation was used to measure the transmembrane potential. The lipid permeable carbocyanine dye, $DiSC_3$ [5], accumulates in internal membrane-bound spaces due to uptake driven by internal negative potentials, and its fluorescence is quenched in proportion to the transmembrane potential [37]. The greater the quench, the greater the interior negative potential. When the K^+ selective conductive ionophore, valinomycin, is added, the transmembrane potential is set to the K^+ equilibrium potential, since the K^+ current overwhelms all other currents. As seen in Figure 6 (which was run at pH 7.0), there is an increase of fluorescence indicating that the K^+ equilibrium potential is less than the transmembrane potential present prior to the addition of valinomycin. There is now a progressive increase of fluorescence as the membrane is depolarized by the addition of increasing amounts of external K^+, until the external $[K^+]$ is between 200 and 240 mM, at which point there is no further change of fluorescence. This represents the situation when there is no K^+ gradient and the transmembrane potential is zero: the null point. Since the internal concentration of K^+ is 240 mM, the potential difference in the presence of valinomycin when $[K^+]_{ext}$ is 5 mM is calculated from the Nernst equation,

$$\Delta PD = RT/nF \ln K^+_{out}/K^+_{in} = -101 \text{ mV at pH } 7.0$$

This is the E_K. In the absence of valinomycin, the membrane potential can be calculated to be -131 mV. The PMF, using the above techniques, given a pH gradient of 1.4 units and a potential of -131 mV, approximates -216 mV [35]. Previous flow dialysis

measurement of the transmembrane potential using the distribution of a radioactive cation ($TPMP^+$) determined by flow dialysis gave a value of -132 mV [38], but measurement of the distribution of weak acids and weak bases (salicylate and benzylamine) to measure internal pH was probably inaccurate.

Transmembrane potential and external pH

The major contribution to the protonmotive force at neutral pH is from the transmembrane potential, hence measurement of this parameter was chosen to evaluate the effect of changes in medium pH on the ATP synthetic capacity of *H. pylori.*

Using $DiSC_3$ [5], it was possible to measure the effect of changes of external pH on the potential difference and, therefore, to calculate internal pH on the assumption that the PMF remains constant. Figure 7 illustrates the mean values of the potential difference in three experiments performed in the absence of urea over a pH range of 3.0 to 9.0.

Reversibility of pH effects on membrane potential

It can be seen that there is no measurable potential difference either at pH 8.4 or higher nor at pH 3.5 and lower. The latter would be expected as the theoretical pH gradient increases to about 5 units as the medium becomes more acidic. However, this would give a calculated protonmotive force of -300 mV assuming no change in internal pH. For the PMF to be constant, the actual pH gradient would have to decrease by about 1.5 units, bringing the internal pH to about 6.9 at pH 3.5 and the internal pH to 7.4 at pH 4.0 to maintain a PMF of about -216 mv. Is the absence of a membrane potential at pH 3.5 also indicative of a cytotoxic effect of external acidity? If so, the potential difference should not recover upon re-alkalinization.

At pH 8.4, the membrane potential should now represent the entire PMF of -215 mV, since the pH gradient is zero, but is found to be absent. Again, can the potential difference be restored by acidification?

The experiments of Figure 8 show that, over a short period of exposure (approximately 1 min), the potential difference can be restored following acidification or alkalinization

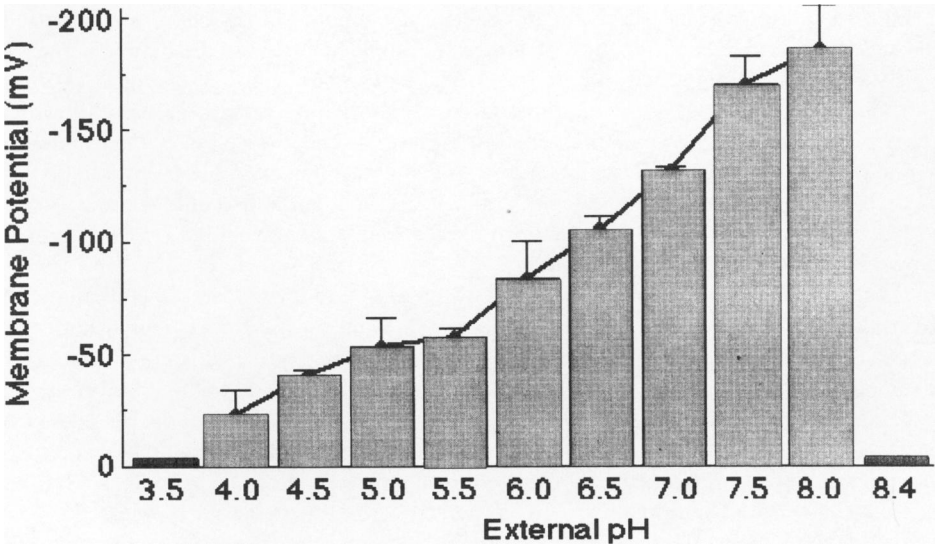

Figure 7. The transmembrane potential as a function of external pH (mean of three experiments +/- SEM)

by increasing or decreasing the pH respectively. In data not shown, longer exposure to acid (5 min) or alkali (15 min) results in irreversible loss of the potential difference component of the PMF. Given the varying pH of the gastric lumen and presumably the varying pH of the environment of *H. pylori*, these results would not be compatible with a lengthy survival of the organism in the stomach.

Measurement of *in vitro* survival of the organism in effective buffers in the absence of urea shows that it survives between a pH of 4.0 and 8.0 [35, 40], exactly the range determined for maintenance of a PMF by the organism. Growth occurs over a narrower range *in vitro*, not being found at a pH less than 5.0 [39, 40]. The organism, therefore, behaves as a neutrophile in standard buffers, namely it does not survive high levels of acidity nor does it survive excessive levels of alkalinity.

The effect of urease activity on membrane potential

The organism generates large quantities of urease much of which is found external to the bacterium. This enzyme hydrolyzes urea, $CO(NH_2)_2$, to $2NH_3 + CO_2$, thereby being able to neutralize significant acidity and to generate significant alkalinity. Urease negative mutants are unable to colonize the stomach [41, 42]. If urea is added to bacteria suspended at pH 3.0 or 3.5 early after exposure to the acid, there is a rapid increase of membrane potential as shown in Figure 9. This does not happen at a pH of 2.5. Hence urease activity is able to extend the pH range at which the organism can maintain a protonmotive force by about 1 unit, i.e. from pH 4.0 to 3.0, defining *H. pylori* as an acid-tolerant neutrophile due to this urease activity. On the other hand, when urea is added to weakly buffered medium at pH 7.0 or higher, the pH increases to well beyond 8.4 and the PMF collapses, as is also shown in Figure 9 [35]. Therefore, the urease produced by the organism allows it to extend its survival into the acid range but decreases its survival in the neutral range of pH.

These data can be offered as a partial explanation for the persistence of the bacterium in the stomach and not in the intestine and perhaps for the distribution of the organism in the stomach. Since the fundus secretes acid, acidity decreases in an outward direction, namely it is highest at the epithelial surface and decreases as neutralization occurs. Conversely, since the antrum secretes bicarbonate ion and no acid, acidity decreases with approach to the antral epithelium. In the fundic and antral regions under normal acid secretory conditions, some survival can occur in the fundus (in regions where the pH is 3.0 or greater), and the combination of acid secretion diffusing from the lumen, bicarbonate ion secretion coming from the antral glands, and urease activity sets up a favorable environment for the organism to inhabit and grow mainly in the antral region of the stomach in the pH range of 4 to 6 as illustrated in Figure 10. The interaction of environmental pH and *H. pylori* and its PMF (which allows ATP synthesis) is thus able to explain the distribution of the organism in the human stomach. Extrapolation also allows prediction of the environmental pH using the organism as a pH indicator, where survival cannot be below pH 3.0 or above pH 7.0.

The organism is found mostly in the antrum and there mostly on the epithelial cells and at the base of the antral glands, but some are found in the fundic region under the mucous layer [43]. Hence it can be deduced that whereas the pH at the surface of the antrum and in the lumen of the antral gland is compatible with life as defined by *H. pylori*, the pH at the fundic surface is much less inviting. At the fundic surface, the pH cannot be 6.0 as claimed by some investigators [44], otherwise there would be an abundance of *H. pylori* in this region even under control conditions. However, it can be as high as 4.0 in the immediate vicinity of the organism, given the activity of the bacterial urease. Therefore, survival in some of the fundic surface is possible, but growth is probably slight unless the pH can be elevated to pH 5.0 or above.

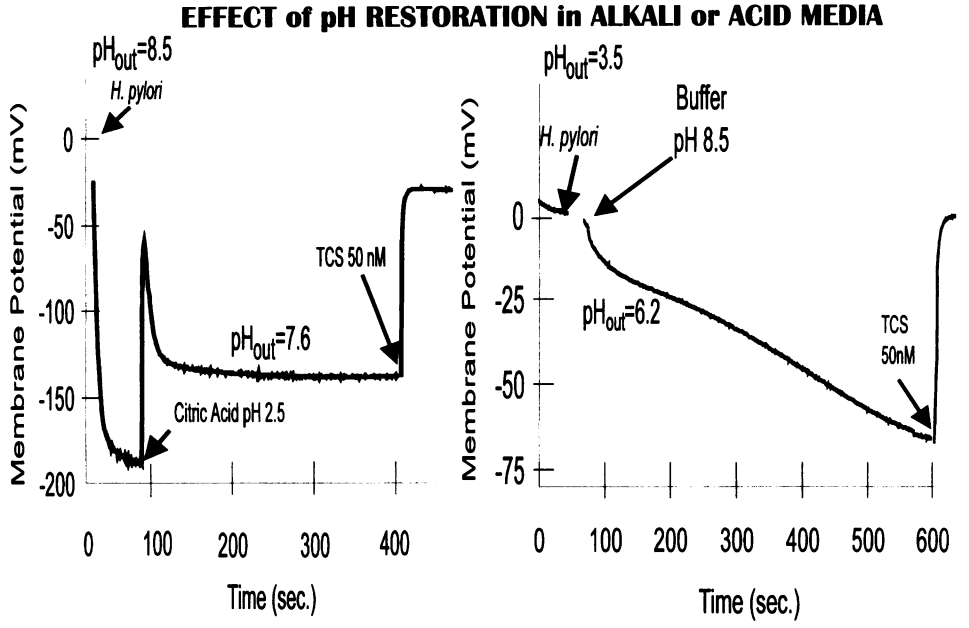

Figure 8. The effect of alkalinization or acidification and reversal of pH_{ext} on the transmembrane potential

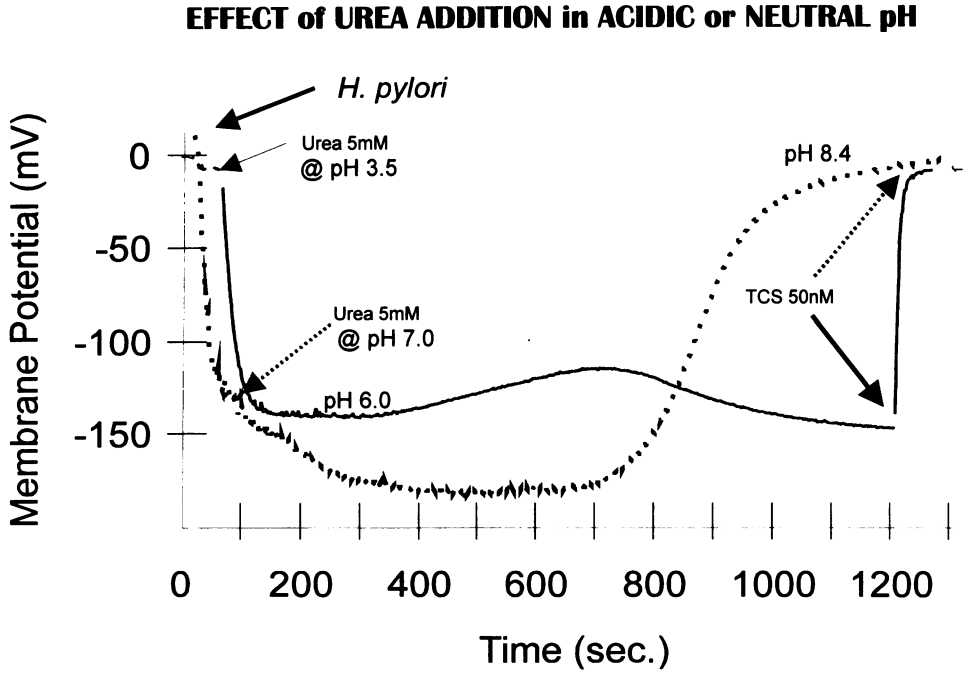

Figure 9. The effect of urea on the transmembrane potential following addition in acidic and neutral pH media showing protection against acid and generation of neutral pH intolerance.

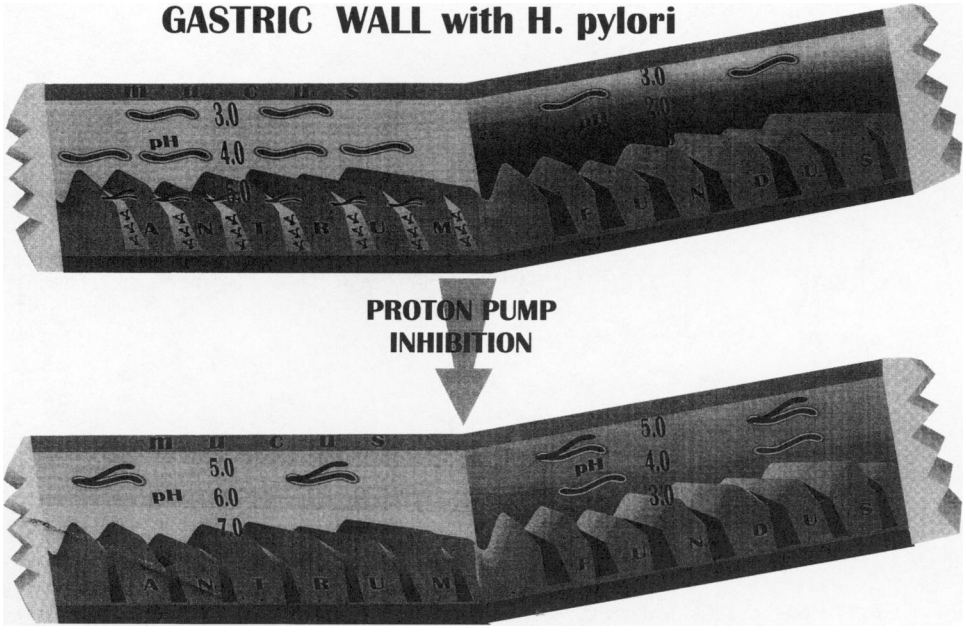

Figure 10. A schematic illustration of the population dynamics of *H. pylori* in fundus and antrum under control conditions and following treatment with proton pump inhibitors, illustrating pH dependence of the loss of organisms in the antrum and their increase in the fundus. The organisms are shown as being in stationary phase or dividing.

HELICOBACTER AND ANTIBIOTICS

Bacterial physiology can dictate the efficacy of different classes of antibiotics. The doubling time of *E. coli* is about 20 minutes, that of *H. pylori* in growth phase is about eight hours. Thus, over a short period of time, *H. pylori* is largely in stationary phase, *E. coli* and most pathogens largely in log phase. This is relevant to the efficacy of antibiotics *in vivo,* since access to the organism is determined by the dwell time of the antibiotic in the stomach and the plasma half-life of the drug. The stationary phase of *H. pylori* is in part determined by the prevailing pH. At pH 4.0, the organism survives but does not grow. At pH 5.0, the organism survives and grows. Hence, if urease activity is able to elevate the pH to 4.0 but not to 5.0, stationary phase organisms will be present.

The class of antibiotic represented by amoxicillin targets cell wall biosynthesis, therefore killing organisms in growth phase, without affecting stationary phase bacteria. Hence, amoxicillin can suppress organisms in the antrum where some are in log phase and will have little effect on the organisms in the fundus where they are mostly in the stationary state. Clarithromycin inhibits protein synthesis. Protein synthesis is required both prior to and during cell division, giving this antibiotic a broader activity against *H. pylori.* However, when the organism is in stationary phase, little if any protein synthesis is required, and, therefore, the activity of clarithromycin against the bacterium is blunted as well. Hence, neither antibiotic alone nor in combination is effective in eradication of *H. pylori* in the absence of additional drugs. Metronidazole targets DNA and, therefore, is independent of the stationary or growth phase distribution. On the other hand, this class

of antibiotic is a weak base. In acidic conditions, where the pH_{int} of the bacterium is much greater than the pH_{ext}, such weak bases will be excluded from the interior.

Helicobacter, bismuth and antibiotics

Two general triple-therapy regimens have been developed for eradication of the organism in man. One always contains bismuth. The mechanism of action of Bi^{3+} is unknown. It is usually administered as a water insoluble complex, such as Bi^{3+} subcitrate or subsalicylate, but more recent formulations contain ranitidine bismuth citrate, which is a water-soluble form [45]. There appears to be significant surface binding of the cation and a long dwell time on the gastric surface. Concentrations of Bi^{3+} up to 100 μM do not affect the transmembrane potential, therefore, the effects do not depend on inhibition of protonmotive force generation. Perhaps the cation inhibits surface binding of the organism to the gastric epithelium and this initiates cell division and hence, synergism with antibiotics.

Helicobacter, proton pump inhibition and antibiotics

Proton pump inhibition results in elevation of both luminal and wall pH. On once-a-day therapy, mean intragastric pH rises to about 3.0 [11, 12]. Hence, antral pH, especially that at the base of the antral glands is likely to rise to close to neutrality, given the HCO_3^- secretion of this region of the stomach. From the results discussed above, urease activity will likely raise the pH in the vicinity of the organism to toxic levels. PPIs will, therefore, suppress the organism in the antral glands. In some regions, the antral surface may be sufficiently acidic so as to neutralize the action of urease, and the organism will continue to thrive. In the fundus, the pH will remain acidic at the surface, but will rise regionally to levels, which, in combination with urease activity, may well exceed 5.0, allowing not only survival but growth. These considerations explain the observation that treatment with PPIs alone suppresses *H. pylori* and that the organism decreases in the antrum and increases its population in the fundus [47]. The pH elevation, apart from suppressing the organism in the fundus, moves more of the organism into growth phase in the fundus. Twice-a-day treatment with PPIs improves acid control and, therefore, changes the population dynamics of the bacterium due to the changing interaction between the alkali generating capacity of the bacterial urease and the acidic environment of the fundus or antrum (Figure 10).

The nature of the bacterial population is changed by PPI treatment. In the antrum, urease activity results in elevation of pH_{ext} to toxic levels, with consequent suppression of the organism in this region. In the fundus, where most of the bacteria are in stationary phase in the absence of PPIs, the elevation of pH resulting from inhibition of acid secretion moves most of the bacteria into growth phase. This allows the efficacy of both amoxicillin and clarithromycin to be enhanced, improving the results of eradication with these antibiotics when PPI triple therapy is used [31, 32, 4 6]. Metronidazole, which attacks DNA, might be expected to provide some advantage in triple therapy, and some clinical trials have indicated that this is so [46]. However, there is also resistance to this antibiotic, and for both clarithromycin and metronidazole there are alterations in taste sensation and other side-effects that could result in non-compliance. Thus, there are various forms of triple therapy, for example: twice a day PPI with twice a day 250 to 500mg clarithromycin plus 1000 mg amoxicillin or 400 mg metronidazole, for seven days. All have been shown to be effective in eradication to greater than the 85 percent level in a variety of clinical trials. The patient is required to take six tablets per day for seven days for eradication. This is many fewer than in regimens using various formulations of bismuth.

Compliance and bacterial resistance to clarithromycin or metronidazole are problems that will arise as this regimen becomes widely used. Recent data suggest that patients

undergoing chronic therapy for gastroesophageal reflux disease using antisecretory drugs also require eradication if they are *H. pylori* positive [7]. As knowledge of the organism progresses and rational therapy emerges, it may be anticipated that a monotherapeutic agent will be developed only to eradicate the organism in the absence of any obvious upper gastro-intestinal disease. It may be that generation of gastric wall achlorhydria by appropriate formulations of PPIs could act as monotherapy, if indeed these formulations would result in total suppression of acid secretion by the H^+/K^+-ATPase.

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