The spatial orientation of *Helicobacter pylori* in the gastric mucus

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The highly motile human pathogen *Helicobacter pylori* lives deep in the gastric mucus layer. To identify which chemical gradient guides the bacteria within the mucus layer, combinations of luminal perfusion, dialysis, and ventilation were used to modify or invert transmucus gradients in anaesthetized *Helicobacter*-infected mice and Mongolian gerbils. Neither changes in lumen or arterial pH nor inversion of bicarbonate/CO_2 or urea/ammonium gradients disturbed *Helicobacter* orientation. However, elimination of the mucus pH gradient by simultaneous reduction of arterial pH and bicarbonate concentration perturbed orientation, causing the bacteria to spread over the entire mucus layer. *H. pylori* thus uses the gastric mucus pH gradient for chemotactic orientation.

Helicobacter pylori, a motile Gram-negative human pathogen that causes gastritis and duodenal and gastric ulcers and increases the risk of gastric cancer (1), lives within the gastric mucus layer. The majority of bacteria are located deep in the mucus, close to the surface of the epithelium. The mucus is continuously secreted in the glands and by surface epithelial cells and is degraded at the luminal surface (2). Because of this rapid mucus turnover, the bacteria need motility and spatial orientation to avoid being carried into the lumen, where the acidic pH inhibits growth and paralyzes motility (3, 4). Orientation therefore plays a central role in acute colonization and the chronic persistence of *H. pylori*.

Motile bacteria sense chemical gradients by means of chemoreceptor proteins and relay the information to the flagellar motor (5). In the case of *H. pylori*, it must be a chemical gradient in the gastric mucus layer that guides the bacteria. Within the mucus layer, diffusion is effectively restricted, so that the concentration of most substances entering the mucus from the epithelial surface will remain constant through the entire width of the layer. Despite this, at least three chemical gradients are known to exist here: a proton (pH) gradient (2, 6), a bicarbonate/CO_2 gradient (7, 8), and, in the *Helicobacter*-infected mucosa, a urea/ammonium gradient (9) created by bacterial urease. We hypothesized that *H. pylori* may use one or several of these transmucus gradients for orientation *in vivo*.

We have previously developed a method to precisely determine the distribution and colonization density of *Helicobacter felis* in the gastric mucus of mice *in vivo* (3). This method uses a micromanipulator-controlled sampling device (10) to extract nanoliter samples from the mucus and mucosa of anesthetized animals. After immediate immobilization of the bacteria by cooling, the density and distribution of the bacteria can be studied by digital image processing.

To identify gradients involved in the orientation of *Helicobacter* spp., we inverted or modified individual gradients in anesthetized *H. pylori*-infected Mongolian gerbils or *H. felis*-infected mice and studied the effect of these changes on the distribution of bacteria in the mucus layer. The data show that gastric *Helicobacter* species use the mucus pH gradient for precise spatial orientation.

Materials and Methods

Animals. For the *H. felis* experiments, female CD^{R1} mice (25 g), 56 in total, were infected with 10^7 colony-forming units of *H. felis* (ATCC 49179) as described by Mohammadi *et al.* (11). At least 5 weeks were allowed after infection before the mice were used for an experiment. For the *H. pylori* experiments, 38 Mongolian gerbils (Hsd:Mon) (45–75 g) were infected, each with 10^8 colony-forming units of *H. pylori* SS1 (12, 13). To ensure a comparable phase of infection, animals were used between weeks 5 and 15 after infection. Successful infection of the gerbils was verified by ELISA detection of *Helicobacter* antigens in feces (FemtoLab *H. pylori* CnX, Connex, Martinsried, Germany).

Anesthesia and Stomach Preparation. All experiments were performed under general inhalation anesthesia. The animals spontaneously breathed a mixture of Isoflurane in 55% N_2O, 40% O_2, and 5% CO_2 in a semiclosed system (control conditions). Anesthesia was performed with the goal of maintaining a stable blood supply to the stomach, which was monitored by observing the color of the gastric mucosa and the diameter of the gastric vessels; also monitored were heart rate, blood pressure, respiratory frequency, and arterial oxygen saturation as well as the acid/base status. The stomach was carefully lifted through an incision in the ventral body wall, and the dorsal side was positioned on a bent spatula that was mounted on a micromanipulator. A small incision was made in the ventral wall of the antrum region. The chymus was removed from the stomach without touching the dorsal wall of the antrum from within.

Sample Extraction and Quantitation of the Bacteria. The technique of removing nanoliter samples of mucus has been described (3, 10). The sampling device operates by heating up or cooling down a sealed pipette filled with silicon oil. Within seconds, the samples were collected and immediately cooled to 5°C, completely inhibiting *Helicobacter* motility (14). Using digital microscopic imaging, it was possible to reconstruct the bacterial position with respect to the tissue surface.

Changing Chemical Gradients Within the Mucus. In the anaesthetized animal, luminal conditions are defined by the composition of the luminal superfusion fluid, whose concentrations of bicarbonate, CO_2, urea, ammonium, and pH could be set as required. A peritoneal perfusion of a high flow worked as a dialysis. Through this process of dialysis, the arterial concentration of urea and ammonium could be varied, and, together with the minute ventilation and the inspiratory pCO_2, it was also possible to change the concentration of bicarbonate, CO_2, and pH in the

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blood. More details are available in the supporting information, which is published on the PNAS web site.

The Juxtamucosal Mucus pH in the Explanted Antrum Mucosa. A more detailed description of pH measurements in the gastric mucus is given elsewhere (2, 15). The gastric mucosae of 20 guinea pigs were explanted and fixed in a chamber. The lower serosal chamber was continuously perfused by a solution similar to blood plasma containing an oxygen-carrying perfluorochemical emulsion. The upper chamber was perfused by an isotonic NaCl/citrate-solution buffered to a pH of 4 or 6.

Ultrafine-tipped double-barreled microelectrodes were advanced with very high acceleration to a position 10 μm above the tissue surface (juxtamucosal mucus). The mucus pH at this position was recorded after the change in the serosal solution. The composition of this solution was changed from physiological conditions (pH 7.4, bicarbonate concentration 24 mM) to the test conditions with varied pH, bicarbonate concentration, and pCO_2.

Results

The *in Vivo* Distribution of *H. pylori* in the Mucus of the Gerbil. Until now, only the distribution pattern of *H. felis* in the murine mucus has been studied in detail. *H. felis* shows very little adherence to gastric epithelium, whereas *H. pylori* shows strong adherence, which is likely to have an impact on the distribution of bacteria in the mucus. We used the previously established approach to study the distribution of *H. pylori* in the mucus of anesthetized Mongolian gerbils (*Meriones unguiculatus*). As expected, the distribution of *H. pylori* in gerbils differed significantly from the distribution of *H. felis* in mice. Similar to *H. felis*, *H. pylori* colonizes a thin mucus layer located 0–25 μm above the tissue surface (mean distance 11 $\pm$ 5 μm, Fig. 1). The remaining part of the mucus layer (total thickness $\approx$100 μm) was virtually free of bacteria. However, whereas *H. felis* avoids close proximity (<5 μm) to the tissue surface, the majority of *H. pylori* was found within the first 15 μm, and one-third of the bacteria were either swimming in the layer immediately adjacent to the epithelial cells (0–5 μm, Fig. 1) or adhering to the cells. The mean swimming velocities of *H. pylori* and *H. felis in vivo* were 30 $\pm$ 6 μm/s and 33 $\pm$ 19 μm/s, respectively (see supporting information).

Urea/Ammonium Gradient. To identify the gradients the two *Helicobacter* sp. use for orientation, chemical transmucus gradients were inverted or modified by varying the conditions of luminal superfusion, peritoneal dialysis, and respiration. Each experiment started with a series of samples taken under control conditions, followed by a period of 45 min, during which the gradients were altered. A second series of samples were then taken under the changed conditions. We first investigated the urea gradient, which was of particular interest, because *H. pylori* has been reported to perform chemotaxis toward urea *in vitro* (16). In the uninfected stomach, urea diffuses into the mucus layer and is transported into the gastric lumen along with the mucus. In the chronically infected animal, *Helicobacter* urease converts most of the urea to ammonia, which immediately reacts into ammonium. Under these conditions, a gradient is built up: the urea concentration is similar to the plasma concentration (5–8 mM) at the tissue side and low at the luminal side, and the ammonium concentration is high at the luminal side (5–15 mM) and low at the tissue side (9). Under physiological conditions, the mean bacterial densities were 3,900 *H. felis* and 2,900 *H. pylori* per nanoliter of juxtamucosal mucus, whereas a negligible number of bacteria (<100 per nanoliter in both) were found in the central and luminal mucus layer. To change the urea/ammonia gradient, the lumen was superfused with a solution free of ammonium and high in urea. At the blood side, we applied ammonium (5 mM) and reduced the urea concentration through peritoneal dialysis. These changes did not affect the distribution of bacteria, making it highly unlikely that chemotaxis toward urea plays a role in the spatial orientation of *Helicobacter* spp. *in vivo*. A scheme of the urea/ammonium gradient and its elimination is shown in Fig. 2.

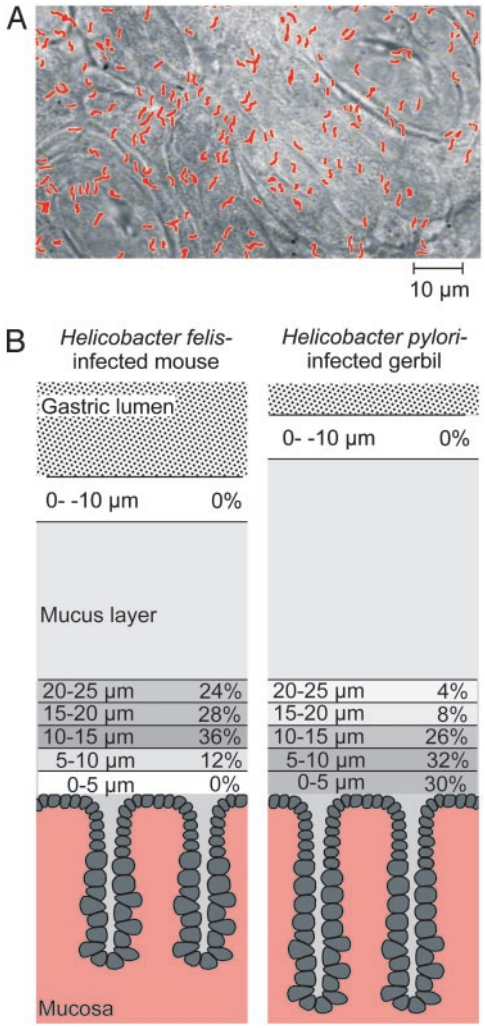

Fig. 1. Distribution of *H. pylori* and *H. felis* in the mucus layer of mice and Mongolian gerbils. (*A*) The tissue surface of the *H. pylori*-infected gerbil depicted from the luminal side of the antrum. Several focus planes have been digitally combined, the *H. pylori* in the mucus layer subsequently highlighted in red. (*B*) The gastric mucosa and mucus of the *H. felis*-infected mouse and the *H. pylori*-infected gerbil are shown as schematic cross sections. The first 25 μm of the mucus layer on the tissue side ("juxtamucosal" mucus) are subdivided into 5-μm sections. The numbers represent the percentage of bacteria present within each section. The first 10 μm from the luminal surface is referred to as "luminal mucus," the rest of the mucus layer as "central mucus." *H. felis* was found located between 5 and 25 μm from the tissue surface (3). *H. pylori*, however, colonizes the whole section 0–25 μm from the tissue surface. Some *H. pylori* were attached to cells.

Bicarbonate/CO_2 Gradient. We next addressed a possible role of bicarbonate, which was also reported to attract *H. pylori in vitro* (17). Within the mucus, the bicarbonate/CO_2 ratio and the surrounding pH are interdependent. Therefore, the pH gradient causes the build-up of a bicarbonate/CO_2 gradient, with equimolar amounts at the tissue side and low bicarbonate and high CO_2 at the luminal side (18, 19). Bicarbonate is secreted by the surface epithelial cells (20), the rate of secretion depending on sufficiently high plasma bicarbonate concentrations (21). At a

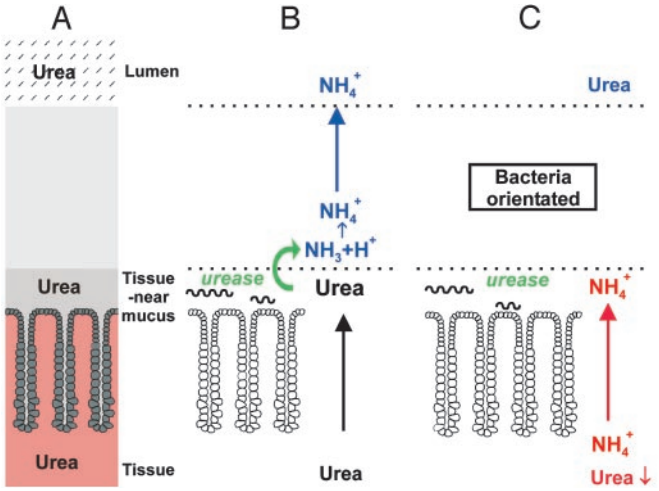

Fig. 2. Elimination of the urea/ammonium gradient. (*A*) A schematic cross section of the uninfected gastric mucosa. Under normal conditions urea diffuses from the arterial plasma into the mucus and is transported with it into the lumen. Thus the concentration of urea is constant throughout the mucus layer. (*B*) The build-up of a gradient in the *Helicobacter*-infected mucosa. The bacterial urease converts the urea into ammonia. The ammonia immediately reacts to ammonium, neutralizing the bacterial intracellular pH. Most of the urea entering the mucus layer is converted to ammonium, thus creating a gradient, urea at the tissue side and ammonium at the luminal side. (*C*) To invert these concentrations, we used dialysis to apply ammonium and reduce the plasma urea concentration. High flow of a luminal solution containing urea washed away the luminal ammonium. This inversion of urea and ammonium concentrations should eliminate the gradient, but it failed to affect bacterial orientation. Urea/ammonium is therefore not the critical gradient that *Helicobacter* uses to orient itself.

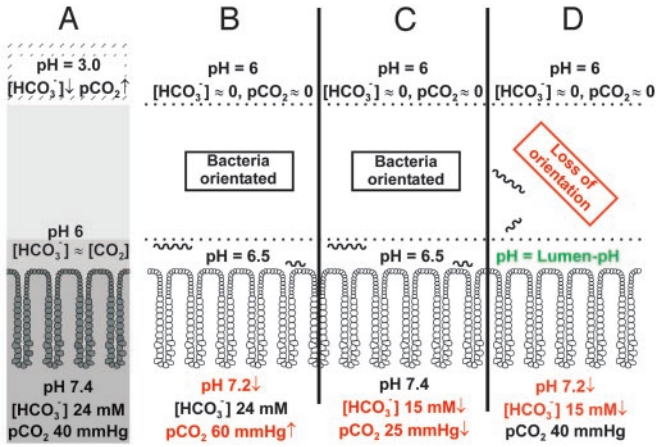

Fig. 3. pH and bicarbonate/CO_2 gradients. Shown are the alterations made in the interdependent pH, bicarbonate, and CO_2 concentrations in the mucus layer. (*A*) The normal conditions with a low luminal pH of 3 in either the infected or the uninfected mucosa. This luminal pH induces a pH of 6 in the juxtamucosal mucus. Under these conditions, secreted bicarbonate and diffused CO_2 have the same concentration in the juxtamucosal mucus, whereas at the luminal side of the mucus layer, the bicarbonate concentration is low and the pCO_2 high. The neutralization of the juxtamucosal mucus to pH 6 is caused by active bicarbonate secretion and a neutral pH in the newly secreted mucus. (*B–D*) To eliminate the pH bicarbonate/CO_2 gradient, the luminal pH was neutralized to 6, and three different constellations of arterial pH, bicarbonate and CO_2 were tested. (*B*) The first alteration of the gradient was achieved by doubling the inspiratory CO_2 fraction, bicarbonate concentrations maintained at normal values through dialysis. This caused a low arterial pH with a normal bicarbonate concentration. (*C*) In the second constellation, a reduced arterial bicarbonate concentration was combined with a normal pH. This was achieved by reducing the arterial pCO_2 through hyperventilation and lowering the bicarbonate concentration through dialysis. Neither the first nor the second alteration affected bacterial orientation. (*D*) However, a combined reduction of arterial pH and bicarbonate concentration through dialysis caused a loss of bacterial orientation, the bacteria spreading over the entire mucus layer.

normal arterial pH of 7.4, the bicarbonate concentration is 24 mM caused by an arterial pCO_2 of 40 mmHg (1 mmHg = 133 Pa) ($[CO_2]$ = 1.2 mM); the bicarbonate/CO_2 ratio is 20:1. According to the Henderson–Hasselbalch equation, it is possible to keep the arterial pH constant while reducing absolute concentrations of bicarbonate/CO_2, as long as the ratio between the two is maintained. The arterial pCO_2 was reduced to 20–30 mmHg through a moderate theophyllin-induced hyperventilation, whereas the bicarbonate concentration was decreased through dialysis to 14–18 mM. The arterial pH remained at 7.35–7.40. A reduction in the arterial bicarbonate along with high luminal bicarbonate concentrations would consequently invert the mucus bicarbonate/CO_2 gradient. This alteration of the bicarbonate/CO_2 gradient had no effect on the distribution of *Helicobacter*.

Luminal and Arterial pH. Another possible gradient helping *Helicobacter* to find its niche in the mucus is that of the pH, from a near-neutral pH at tissue surface to low pH values in the lumen. The pH at the antrum epithelial surface is determined by the pH of the secreted mucus and the amount of secreted neutralizing bicarbonate. We first asked whether raising the luminal pH affected the distribution of the bacteria. After 45, 90, and 120 min, at a lumen pH of 6.0, the mean density of the bacteria in the juxtamucosal mucus remained unaffected. Additional changes in luminal bicarbonate/CO_2 concentrations also had no effect on bacterial distributions.

Such a luminal solution of pH 6, free of CO_2 and bicarbonate (without effect), was used in the following experiments.

Because the reduction of the arterial bicarbonate concentration alone did not affect bacterial orientation, we next sought to reduce the pH of the secreted mucus. The pH of freshly secreted gastric mucus has never been measured directly but is believed to reflect the intracellular pH of the mucus-secreting cells (≈7) (15). It is known that gastric epithelial cells are sensitive to acidifications coming from the blood side (22). To decrease the intracellular pH in the mucus producing cells, the arterial pH was lowered to ≈7.15 through the increase of the inspiratory fraction of CO_2 to 8%. The arterial bicarbonate concentration was maintained at the normal value through dialysis. This acidosis did not change the distribution of *Helicobacter*.

Reduction of Arterial pH and Bicarbonate Concentration. It is possible that the effect of an acidification of the mucus-producing cells on mucus pH is counterbalanced by a regulated increase in bicarbonate secretion and vice versa, which may explain the failure of acidosis or bicarbonate reduction alone to affect the distribution of *Helicobacter* in the mucus. To achieve the desired elimination of the mucus pH gradient, we simultaneously reduced the arterial pH to ≈7.15 and the bicarbonate concentration to 12–13 mM. The arterial pCO_2 remained at ≈40 mmHg. Under these conditions, the density of bacteria in the epithelial mucus decreased remarkably both in the *H. pylori* and *H. felis* models, and about one-half of the bacteria left their mucus zone, spreading into the rest of the mucus layer and into the lumen. Fig. 3 summarizes the changes in bicarbonate/CO_2 and pH gradients.

Fig. 4 shows the different bacterial distributions following the changes of gradients. Details of measurements are shown in Table 1. Because of the small size of the mouse and gerbil stomachs, direct measurements of the pH in the gastric mucus

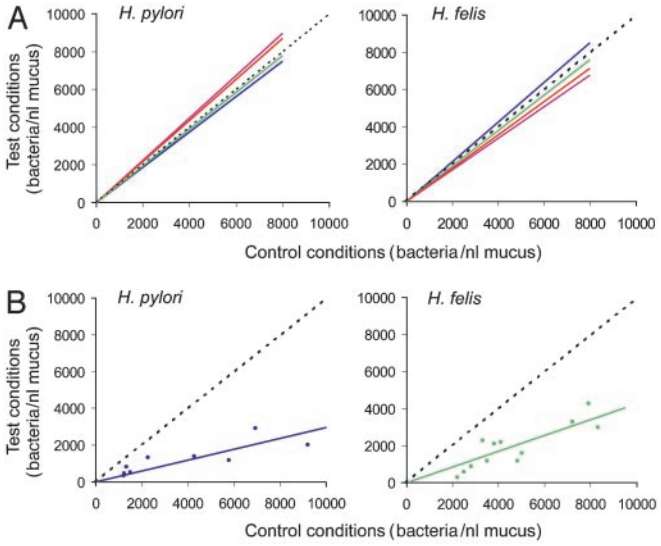

Fig. 4. Effects of gradient changes on the distribution of *H. pylori* and *H. felis* in the juxtamucosal mucus of gerbils and mice, respectively. Each animal is represented by one dot, which indicates the mean density of bacteria in the animal under control conditions plotted against the mean density of bacteria in the same animal under test conditions. The dotted line is the ideal curve showing where the density of bacteria under control conditions is identical to that under the test conditions. (*A*) Bacterial densities remained unaffected after changes in the urea/ammonium gradient (blue line), luminal pH (green line), arterial pH (red line), and arterial bicarbonate concentration (pink line). For these gradients, only the mean regression line of all values for one test series is shown. (*B*) The density of *H. felis* and *H. pylori* in the juxtamucosal mucus decreased significantly after the combined reduction of the arterial bicarbonate and pH, which eliminated the mucus pH gradient.

are not possible with the available technology. To demonstrate that the combined reduction of arterial pH and bicarbonate secretion does indeed lead to the predicted elimination of the mucus pH, we conducted an additional series of experiments in the explanted antrum mucosa of the guinea pig, a model system that permits direct pH measurements with ultrafine-tipped double barrel microelectrodes (2, 3). The conditions of low plasma pH and bicarbonate concentration used in the gerbil and mouse experiments were reproduced in this system, and the pH values in the gastric mucus were measured. Under these conditions, the pH of the juxtamucosal mucus fell to a value approaching that of the lumen pH, eliminating the mucus pH gradient as predicted (Fig. 5).

Discussion

The behavior of pathogenic bacteria within the host is poorly understood. In recent years, advances in animal model technology, such as the development of disease models using transgenic animals that carry defined human pathogen receptors, have led to major improvements in our understanding of host–pathogen interactions (23, 24). However, possibilities for direct *in vivo* observation of pathogens in their habitat are still very limited. In this study, we have used advanced *in vivo* sampling technology to study the behavior of the gastric mucosal pathogen, *H. pylori*, and the closely related species, *H. felis*, *in vivo*. We have individually eliminated the existing chemical gradients in the mucus to identify a gradient that the *Helicobacter* chemotaxis machinery uses for orientation *in vivo*. *H. pylori* has been reported to show chemotaxis toward urea and bicarbonate *in vitro* (16, 17). However, our data show that neither the urea/ammonium gradient nor the bicarbonate/CO_2 is essential for orientation of *H. pylori* or *H. felis in vivo*.

The Interdependence of Bicarbonate, CO_2, and the pH. The acid/base pair bicarbonate/CO_2 is involved in the acid/base equilibrium of the entire organism. The bicarbonate/CO_2 ratio is one of the most important factors that define the pH in organs and the blood. Due to this interdependence, it was difficult to determine which of the three parameters is detected by *Helicobacter* and used for orientation under *in vivo* conditions. Nevertheless, the reproduction of the three changes in arterial pH, bicarbonate

Table 1. Summary of all values

	H. pylori-infected gerbil		*H. felis*-infected mouse	
Conditions	Bacterial density, ratio test/control	Resulting conditions in the arterial plasma	Bacterial density, ratio test/control	Resulting conditions in the arterial plasma
Inverted urea/ammonium gradient	0.94 ± 0.26 (n = 7) No effect	5 mM ammonium Low urea	1.19 ± 0.29 (n = 9) No effect	5 mM ammonium Low urea
Neutralization of the lumen pH to 6	1.15 ± 0.30 (n = 7) No effect	Control conditions	1.01 ± 0.22 (n = 20) No effect	Control conditions
Reduction of arterial bicarbonate (low arterial bicarbonate, low CO_2, bicarbonate = norm)	1.18 ± 0.40 (n = 9) No effect	pH 7.38 ± 0.06 $[HCO_3^-]$: 13 ± 2 mM pCO_2: 23 ± 1 mmHg	0.95 ± 0.19 (n = 5) No effect	pH 7.30 ± 0.08 $[HCO_3^-]$:16 ± 3 mM pCO_2:33 ± 3 mmHg
Reduction of arterial pH (low arterial pH, high CO_2, bicarbonate = norm)	1.11 ± 0.14 (n = 6) No effect	pH 7.21 ± 0.04 $[HCO_3^-]$: 26 ± 2 mM pCO_2: 68 ± 7 mmHg	0.94 ± 0.24 (n = 10) No effect	pH 7.11 ± 0.12 $[HCO_3^-]$: 26 ± 3 mM pCO_2: 87 ± 36 mmHg
Reduction of arterial pH and bicarbonate (low arterial bicarbonate, low pH, CO_2 = norm)	0.39 ± 0.14 (n = 9) Significant effect $P < 0.05$	pH 7.14 ± 0.01 $[HCO_3^-]$: 12 ± 1 mM pCO_2: 38 ± 4 mmHg	0.40 ± 0.16 (n = 12) Significant effect $P < 0.05$	pH 7.17 ± 0.03 $[HCO_3^-]$: 13 ± 1 mM pCO_2: 38 ± 3 mmHg
Control	Control phase in each experiment (2,900 *H. pylori* per nanoliter of epithelial mucus)	pH 7.39 ± 0.05 pO_2: 136 ± 48 mmHg $[HCO_3^-]$: 26 ± 1 mM pCO_2: 41 ± 9 mmHg	Control phase in each experiment (3,900 *H. felis* per nanoliter of epithelial mucus)	pH 7.33 ± 0.03 pO_2: 104 ± 18 mmHg $[HCO_3^-]$: 23 ± 2 mM pCO_2: 46 ± 1 mmHg

Alterations in ventilation, luminal superfusion, and dialysis and the resulting conditions in the arterial blood. All values mean ± SD. *P*: Student's *t* test; all values vs. ''Low arterial bicarbonate − low pH − CO_2 = norm.'' norm., normal.

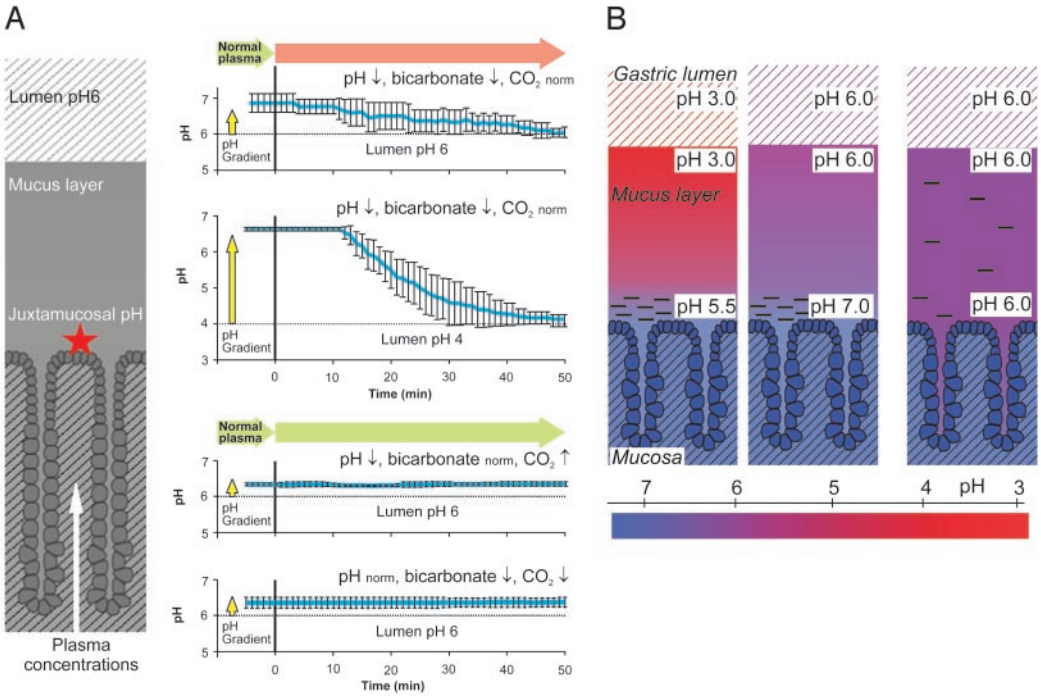

Fig. 5. The juxtamucosal mucus pH in the explanted antrum of the guinea pig. (*A Left*) By using pH microelectrodes, the mucus pH directly above the epithelial cells (0–10 μm between microelectrode tip and cell membrane = juxtamucosal pH) was measured over a time period of 1 hour. (*A Right*) The difference between the juxtamucosal pH and the lumen pH is the mucus pH gradient (yellow arrow). After 10 min, the pH/bicarbonate/CO_2 composition of the plasma solution was changed. (*A Right Upper*) After a combined reduction of plasma pH to 7.2 and bicarbonate concentration to 15 mM (pCO_2 normal) (red arrow), the juxtamucosal pH decreases from its normal value to the same value as the lumen pH, thus eliminating the mucus pH gradient. Curves for lumen pH values of 6 and 4 are shown. (*A Right Lower*) In contrast to the changes above, a reduction of the plasma pH with normal bicarbonate concentration (high pCO_2) or a reduction of the plasma bicarbonate concentration with normal pH (low pCO_2) did not change the juxtamucosal pH, thus preserving the mucus pH gradient (green arrow). (*B*) The pH values of the mucus layer are illustrated in a schematic cross section with a color continuum from deep red (pH 3) to deep blue (pH 7.4). At a lumen pH value of 3 (*B Left*), the mucus pH gradient ranges from 3 at the luminal side to 5.5 near the tissue surface. After a change of the lumen pH to 6 (*B Center*), we measured a mucus pH gradient from the luminal-side value of 6 to ≈7 approaching the tissue surface. This gradient was also found with the uneffective pH/bicarbonate/CO_2 changes in *A Right Lower*. Under the conditions shown in *B Left* and *Center*, *Helicobacter* is precisely oriented, guided by the pH gradients. *B Right* shows the transmucus pH profile after the critical reduction of the plasma pH to 7.2 and of the bicarbonate concentration to 15 mM at a luminal pH of 6. The mucus pH gradient is eliminated, and *Helicobacter* lose their orientation.

concentration, and pCO_2 in the explanted antrum mucosa showed that only the critical reduction in the arterial pH and bicarbonate concentration caused both the *in vitro* elimination of the mucus pH gradient and the *in vivo* disorientation of *Helicobacter*. It is therefore most likely the pH gradient, caused by the secretion of bicarbonate and a neutral mucus, that permits orientation of *Helicobacter*.

How Could *H. pylori* Sense pH? The sensing of pH or proton gradients in other bacteria is a part of "energy taxis," the directed movement toward an environment with optimal concentrations of both electron acceptors (e.g., oxygen) and proton/electron donors (e.g., redox equivalents), which maintains bacterial redox potential and proton motive force (PMF) at an optimum. The environmental pH has a profound effect on the bacterial PMF and the acquisition of cellular energy (25). Energy taxis can override all other responses of the bacterial chemotaxis system and, in the microaerophilic bacterium *Azospirillum brasilense*, the dominant behavior is indeed energy taxis (26). The mechanisms of energy sensing in bacteria are far from fully explained, although chemoreceptor proteins are known to play an important role. Energy taxis can involve PAS-domain sensor proteins (similar to *Escherichia coli* Aer) (27, 28) and can be mediated by other chemoreceptors responding to the cytoplasmic pH (*E. coli* Tsr) (25, 29) or by unusual heme-containing sensor proteins (27, 30). *H. pylori* has three classical membrane chemoreceptors and one putative cytoplasmic chemosensor, which has limited homologies to heme-like sensors but no PAS-domain chemoreceptor. Experiments are underway to analyze the role of the *H. pylori* chemoreceptors in pH-dependent orientation *in vitro* and *in vivo*. pH-dependent orientation of *H. pylori* could be aided by its increased motility and swimming velocity under moderately acidic conditions (pH 5) (ref. 31; T. Mizote and C.J., unpublished data).

The Ecological Niche of *H. pylori*. Our data show that *H. pylori* colonization differs significantly from colonization by *H. felis*. *H. pylori* are closer to the epithelial surface, which may facilitate exchange between the adherent and swimming populations, as predicted through mathematical modeling (32). The unexpectedly high bacterial densities ($10^6/\mu$l) achieved by the restriction of colonization to a thin segment of the mucus are likely to contribute to the uniquely high efficiency of genetic exchange between different strains of *Helicobacter* (33, 34). It has long been known that *H. pylori* colonization and gastritis are not evenly distributed over the stomach mucosa but are limited to the antrum and cardia, whereas the corpus is free of bacteria. This pattern was demonstrated experimentally in the *H. felis*-infected mouse by Danon *et al.* (35) and has been explained by differences of local acid production. We have confirmed these measurements in both our model systems (data not shown). It seems very likely that the same pH-sensing mechanism that *H. pylori* uses for orientation along the transmucus pH gradient is also involved in horizontal orientation. However, horizontal

orientation apparently involves other mechanisms in addition to pH sensing, such as adherence of the bacteria to carbohydrate epitopes that show a region-specific expression pattern (36).

Triple Therapy. The results may also help to elucidate the still unresolved role of proton pump inhibitors (PPI) in triple therapy of *Helicobacter* infection in human patients. PPI act on parietal cells in the corpus, leading to an increased luminal pH. In light of our results, it seems extremely improbable that this luminal neutralization could have any direct influence on the bacteria in the juxtamucosal mucus of the antrum, and it is also unlikely to affect the pharmacological activity of antibiotics in the juxtamucosal mucus zone. PPI inhibit *Helicobacter* motility, but this effect is weak for some of the most effective compounds (e.g., omeprazole) and unlikely to play a role (14). One possible explanation for the synergistic effect of PPI and antibiotics is that acid suppression in the gastric corpus may change the complex bicarbonate and pH regulation in the gastric antrum, which would disturb *Helicobacter* orientation, cause bacteria to be removed by mucus shedding, and thus support therapy by reducing bacterial loads. Finally, a more precise understanding of the ecological niche of *H. pylori* as provided by the present study will allow a more focused approach to the development of novel therapeutic substances, and the chemotactic system may be an attractive target. The approach used here of applying advanced physiological methodology to the study of the behavior of a pathogen within its host represents a previously undescribed paradigm that, once applied to other pathogens, could greatly refine our understanding of the interactions of bacteria with their hosts.

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