HCl causes less intracellular acidification in *Necturus* gastric mucosa surface epithelial cells than other acids

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Nylander-Koski, O., H. Mustonen, I. Vikholm, T. Kiviluoto, and E. Kivilaakso. HCl causes less intracellular acidification in *Necturus* gastric mucosa surface epithelial cells than other acids. *Am J Physiol Gastrointest Liver Physiol* 281: G675–G680, 2001.—Luminal acid causes intracellular acidification in the gastric epithelium, but the mechanism by which H+ enters surface cells remains obscure. This study addressed the problem by assessing how different acids affect intracellular pH in gastric surface cells. Isolated *Necturus maculosus* antral mucosa was exposed to HCl, HNO3, H2SO4, or H3PO4 at pH 2.30. Intracellular pH was measured with microelectrodes. The physicochemical interaction of a synthetic model of gastric phospholipids with the different acids was studied using Langmuir film balance. Exposure to luminal HNO3, H2SO4, or H3PO4 caused significantly larger intracellular acidification than exposure to HCl. The degree of acidification was not dependent on the valence or nature of the anionic counterion of the acid but significantly correlated with the amount of molecular acid. By Langmuir film balance, subphases acidified with HNO3, H2SO4, or H3PO4 caused more close packing of phospholipid molecules than those acidified with HCl, possibly allowing hydrogen bonding between head groups to facilitate H+ movement across the phospholipid membrane. HCl causes significantly less intracellular acidification in gastric epithelium than HNO3, H2SO4, or H3PO4. This may be caused by the lower amount of molecular HCl in solution and possible hydrogen bonding between the head groups of phospholipid molecules and the other acids.

phospholipid membrane; hydrogen ion; microelectrodes

THE GASTRIC MUCOSA IS CONTINUOUSLY exposed to relatively strong luminal acid, which implies that there are effective mechanisms to protect the mucosa against acid-induced damage and to maintain intracellular pH (pHi) within the physiological range in the surface epithelial cells. Direct measurements with microelectrodes in isolated *Necturus maculosus* antral mucosa indicate that exposure of the mucosa to physiological intragastric concentrations of HCl lowers pHi slightly but significantly in surface epithelial cells (10, 11), thus implying that some luminal H+ does penetrate inside the cells. These studies also showed that the surface cells are much more resistant against exposure to luminal acid than basolateral acid (10). One obvious reason for this disparity is the preepithelial mucus-HCO3 - buffer layer at the epithelial surface, which partly neutralizes luminal H+ “back diffusing” toward the mucosa (12). In addition, it has been proposed that the gastric epithelium is covered by a hydrophobic phospholipid layer, which likewise might impede the diffusion of luminal H+ inside the mucosa (9, 14). However, the exact mechanism by which luminal H+ enters the surface epithelial cell still remains to be delineated.

The apical membrane of gastric surface epithelial cells, like other cell membranes in eukaryotic cells, is composed of a phospholipid bilayer that, in principle, is virtually impermeable to electrically charged ions such as H+ (1). A cation (Na+) transport channel has been identified in the apical cell membrane of gastric surface epithelial cells (15). However, in the presence of luminal acid, these channels are blocked (17), thus precluding major transport of luminal H+ inside the cell via these channels.

Gutknecht and Walter (8) proposed that the entry of luminal H+ inside the gastric mucosa might occur in the form of molecular HCl. Phospholipid bilayers are, in general, highly permeable to small uncharged molecules (1), and direct calculations suggest that at normal intragastric pH, sufficient amounts of molecular HCl exist to account for “H+ back diffusion” in this condition (2). Gutknecht and Walter (8) proposed that monovalent acids are more permeant in phospholipid bilayers than divalent acids. On the other hand, Barreto and Lichtenberger (2) showed that it is the anionic counterion that dictates the permeation of H+ in phospholipid bilayers, Cl− permitting much higher H+ permeation than SO4 = or PO4 =.

In the present study, we investigated acid movement across the apical cell membrane of surface epithelial cells in isolated *Necturus* antral mucosa by exposing the mucosa to various inorganic acids with different valences and anionic counterions and by comparing their effects on pHi with that of HCl. Moreover, to...
explore the potential interaction of different acids with phospholipid membrane, a synthetic model of human gastric mucosal phospholipids was spread onto the air-water interface to form a monomolecular Langmuir film, which was used as a simple model system for studying the influence of the acids on the monolayer. According to calculated size and water-to-octanol partition ratios of HCl, HNO₃, H₃PO₄, and H₂SO₄, HCl should have the fastest diffusion rate through the plasma membrane.

MATERIALS AND METHODS

The experimental setup and microelectrode technique were described in more detail previously (10). Necturi (Necturus maculosus) were obtained from St. Croix Biological supplies (Stillwater, MN) and kept in filtered water at 15–18°C. Animals were anesthetized by immersion in 1% tricaine methanesulfonate. The antral portion of the stomach was resected, stripped of its seromuscular coat, and mounted mucosal side up in a perfusion chamber. The mucosal (volume 0.15 ml) and serosal (volume 0.15 ml) half-chambers were perfused individually at room temperature at a rate of 1.5 ml/min, keeping the pressure at the serosal side slightly negative to hold the tissue steadily against its wire mesh support. After the tissues were mounted, they were allowed to stabilize for 30–60 min before the experiment was started.

Solutions and Chemicals

The standard Ringer solution contained (in mM) 105 Na⁺, 2 Ca²⁺, 5 K⁺, 1 Mg²⁺, 93 Cl⁻, 18 HCO₃⁻, 1 SO₄²⁻, 1 PO₄³⁻, and 10 glucose. This solution, gassed with 95% O₂-5% CO₂ and having a steady state pH of 7.25 at 25°C, was used for perfusion of both sides of the mucosa at the beginning of the experiment. After stabilization of the tissues, the mucosal perfusate was changed to a Ringer solution buffered to pH 2.3. The pH was adjusted to 2.3 with HCl or one of the other anions in the Ringer solution. The mucosas were exposed to at least two different acids a random order, the HCl exposure step always serving as the control. Between the exposures to the different acids the mucosa was allowed to recover for 10–15 min in Ringer solution buffered to pH 6.0 with MES.

Measurement of pHi

pHi was measured with double-barreled proton-selective microelectrodes. In short, borosilicate tubing with fiber in both barrels (2 GC 150F; Clark Electromedical, Pangbourne, UK) was pulled into micropipettes with an outer tip diameter of <0.5 µm. The pH-selective barrel was silanized with N,N-dimethyltrimethylsilylamine (Fluka, Buchs, Switzerland) vapor at 140°C. The filling solution for the pH-selective barrel contained 150 mM NaCl and 200 mM HEPES at pH 7.5, and the liquid proton sensor was the Hydrogen Ion Ionophore II Cocktail A (Fluka). The electrodes were calibrated in a series of 10 mM Ringer-MES and Ringer-HEPES buffers before and after each experiment. The other barrel, filled with 0.6 M KCl and 0.8 M Na-acetate, was used for measurement of the apical transmembrane potential (Vᵦ). An Ag-AgCl reference electrode was connected to the mucosal half-chamber via an agar-KCl bridge.

Under microscopic control and with a micromanipulator (Prior), a surface epithelial cell was impaled by the double-barreled pH-sensitive electrode. The experiment was not started until stable readings were obtained for 10–15 min. The electrometer amplifier used with the pH-selective microelectrode had an input leakage current of <10⁻¹⁴ A and input impedance of ≥10¹⁴ Ω. The signals of the pH-selective barrel (H⁺ activity + Vₑ) and the voltage barrel (Vₑ) were subtracted to give the net H⁺ activity signal.

Measurement of Electric Potentials and Resistances

The transepithelial potential (Vₑ) was measured with an Ag-AgCl electrode connected to the serosal half-chamber via an agar-KCl bridge with a similar mucosal half-chamber electrode as reference. For resistance measurements, current pulses of 15–30 µA/cm² duration at 30-s intervals were applied from a current pulse generator through the epithelium by means of two Ag-AgCl electrodes located in the mucosal and serosal half-chambers.

Transmucosal current (I) was calculated from the voltage deflections generated by the current pulse across the apical (ΔVₑ) and basolateral (ΔVₑ) cell membranes (Rₑ/Rₑ = ΔVₑ/ΔVₑ). ΔVₑ was calculated from the taloltage deflection caused by the current pulse and ΔVₑ is the magnitude of a current pulse. The ratio of apical and basolateral cell membrane resistances (Rₑ/Rₑ, where Rₑ is resistance of apical membrane and Rₑ is resistance of basolateral membrane) was calculated from the voltage deflections generated by the current pulse across the apical (ΔVₑ) and basolateral (ΔVₑ) cell membranes (Rₑ/Rₑ = ΔVₑ/ΔVₑ). ΔVₑ was calculated from the voltage deflection caused by the current pulse across the apical (ΔVₑ) and basolateral (ΔVₑ) cell membranes (Rₑ/Rₑ = ΔVₑ/ΔVₑ). ΔVₑ was calculated from the voltage deflection caused by the current pulse across the apical (ΔVₑ) and basolateral (ΔVₑ) cell membranes (Rₑ/Rₑ = ΔVₑ/ΔVₑ).

Langmuir Film Balance

A KSV 2200 Langmuir trough (KSV Instruments) with a Pt-Wilhelmy balance was used for monolayer studies (13). A synthetic model of human gastric mucosal phospholipids was prepared with commercial phospholipids (Sigma). The composition of the phospholipid mixture was as follows: phosphatidylethanolamine 44.8%, phosphatidylycholine 31.5%, phosphatidylserine 10.8%, sphingomyelin 6.9%, cardiolipin/unknown 4.1%, and lysophosphatidylcholine 1.9% (18). The phospholipid mixture was dissolved in chloroform at a concentration of 1 mg/ml and spread onto a water subphase. The water was purified with a Millipore Milli-Q filtering system. The solvent was allowed to evaporate for a sufficiently long time, after which the surface pressure-area isotherms were recorded by compressing the spread phospholipids at a speed of 10 µm/min with the aid of a barrier. During compression, the monolayer undergoes a number of phase transformations. The different phases resemble two-dimensional analogs of gases, liquids, and solids. Several recordings were made to ensure the reproducibility of the isotherms. The isotherms were recorded at room temperature (21°C), and pH was reduced from 5.6 (pH of unbuffered water) to 3.0, 2.3, or 1.5 with one of the following acids: HCl, HNO₃, H₂SO₄, or H₃PO₄.

Molecular Modeling

Van der Waals volumes and octanol-to-water partition ratios (complete neglect of differential overlap method was used to calculate partial charges) were estimated using Molecular Modeling pro (ChemSW, Fairfield, CA).
Effect of HNO₃

Intracellular pH. pHi in mucosae exposed to luminal pH 6.0 was 7.24 ± 0.02. Luminal exposure to HCl (pH 2.3, pKₐ = 7), caused rapid intracellular acidification followed by a steady-state pHi of 7.15 ± 0.03 (n = 11; P < 0.001). During the recovery period after removal of luminal acid (10 min), pHi returned to the previous level (Fig. 1).

Resistances. Exposure to luminal HCl (pH 2.3) did not change transmucosal resistance (Rₑ) (from 766 ± 49 to 754 ± 50 Ω·cm²; not significant (NS)). At the same time, Rₑ/Rₐ increased significantly from 3.21 ± 0.63 to 6.19 ± 0.90 (P < 0.02) and returned to the previous level (3.18 ± 0.34) after the change back to luminal pH 6.0.

Electric potentials. Exposure to luminal HCl (pH 2.3) provoked a significant hyperpolarization of apical membrane potential (Vₐ; from −33.3 ± 5.2 to −44.3 ± 4.0 mV; P < 0.04). After removal of luminal acid (luminal pH 6.0 exposure), pHi recovered to 7.26 ± 0.02 in mucosae exposed to luminal pH 6.0 (n = 11). Exposure of the mucosae to HNO₃ (pH 2.3, pKₐ = 1.3) caused a rapid acidification of pHi, to 7.08 ± 0.03 in 4.5 min (n = 11; P < 0.01). After removal of luminal acid (luminal pH 6.0 exposure), pHi recovered to 7.09 ± 0.03 (n = 11; P < 0.01). When tissues were exposed to luminal pH 6.0 again, pHi recovered in 2 min to the baseline level (7.26 ± 0.04; Fig. 1). H₂SO₄ caused a significantly larger acidification than HCl, ΔpHi being 0.09 ± 0.01 and 0.17 ± 0.04 pH units for HCl- and H₂SO₄-treated tissues, respectively (n = 11, Fig. 2).

Resistances. Exposure to luminal H₂SO₄ (pH 2.3) did not induce a change in Rₑ (from 772 ± 58 to 718 ± 60 Ω·cm²; NS). Rₑ/Rₐ increased significantly (from 3.18 ± 0.34 to 7.34 ± 1.46; P = 0.02) and recovered to 4.37 ± 0.48 after return to luminal pH 6.0.

Electric potentials. Exposure to H₂SO₄ (pH 2.3) induced a significant hyperpolarization of Vₐ (from −37.6 ± 3.4 to −44.8 ± 5.3 mV; P = 0.03), which recovered to −33.7 ± 3.3 mV after return to luminal pH 6.0.

There were no significant differences in Rₑ, Vₑm, and Rₑ/Rₐ between the HCl and H₂SO₄ exposures in the same tissues.

Effect of H₃PO₄

Intracellular pH. Acidification of the luminal perfusate to pH 2.3 with H₃PO₄ (pKₐ = 3) caused a rapid decrease of pHi from 7.26 ± 0.02 to 7.09 ± 0.03 (n = 11; P < 0.01). When tissues were exposed to luminal pH 6.0 again, pHi recovered in 2 min to the baseline level (7.26 ± 0.04; Fig. 1). H₂PO₄ caused a significantly larger acidification than HCl, ΔpHi being 0.09 ± 0.01 and 0.17 ± 0.04 pH units for HCl- and H₂PO₄-treated tissues, respectively (n = 11, Fig. 2).

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There were no significant differences in Rₑ, Vₑm, and Rₑ/Rₐ between the HCl and H₃PO₄ exposures in the same tissues.

Effect of H₂PO₄

Intracellular pH. Exposure of the mucosae to luminal H₂PO₄ at pH 2.3 (pKₐ = 2.1) caused significant acidification of pHi from 7.27 ± 0.05 to 7.03 ± 0.05 (n = 11, P < 0.01; Fig. 1). Compared with the effects of HCl (pH 2.3) in the same tissues, H₂PO₄ (pH 2.3) caused a

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**RESULTS**

**Effect of HCl**

Intracellular pH. pHi was 7.26 ± 0.02 in mucosae exposed to luminal pH 6.0 (n = 11). Exposure of the mucosae to HCl (pH 2.3, pKₐ = 7) caused a rapid acidification of pHi, to 7.08 ± 0.03 in 4.5 min (n = 11; P < 0.01). After removal of luminal acid (luminal pH 6.0 exposure), pHi recovered to 7.28 ± 0.05. Compared with HCl at pH 2.3, HNO₃ at pH 2.3 caused a significantly (P < 0.05) larger acidification of pHi, ΔpHi being 0.09 ± 0.01 and 0.18 ± 0.04 pH units for HCl- and HNO₃-treated tissues, respectively (n = 11, Fig. 2).

Resistances. Exposure to HNO₃ (pH 2.3) induced no statistically significant change in Rₑ (from 650 ± 65 to 690 ± 77 Ω·cm²; NS) or in Rₑ/Rₐ (from 4.00 ± 0.55 to 3.45 ± 0.48; NS).

Electric potentials. HNO₃ at pH 2.3 caused a significant hyperpolarization in Vₐ (from −37.2 ± 3.4 to −56.7 ± 5.3 mV; P < 0.01), which recovered to −45.8 ± 2.7 mV after removal of luminal acid. There were no significant differences in the behavior of Rₑ, Vₑm, or Rₑ/Rₐ during HCl and HNO₃ exposures in the same tissues.

**Effect of H₂SO₄**

Intracellular pH. Acidification of the luminal perfusate to pH 2.3 with H₂SO₄ (pKₐ = 3) caused a rapid decrease of pHi from 7.26 ± 0.02 to 7.09 ± 0.03 (n = 11; P < 0.01). When tissues were exposed to luminal pH 6.0 again, pHi recovered in 2 min to the baseline level (7.26 ± 0.04; Fig. 1). H₂SO₄ caused a significantly larger acidification than HCl, ΔpHi being 0.09 ± 0.01 and 0.17 ± 0.04 pH units for HCl- and H₂SO₄-treated tissues, respectively (n = 11, Fig. 2).

Resistances. Exposure to luminal H₂SO₄ (pH 2.3) did not induce a change in Rₑ (from 772 ± 58 to 718 ± 60 Ω·cm²; NS). Rₑ/Rₐ increased significantly (from 3.18 ± 0.34 to 7.34 ± 1.46; P = 0.02) and recovered to 4.37 ± 0.48 after return to luminal pH 6.0.

Electric potentials. Exposure to H₂SO₄ (pH 2.3) induced a significant hyperpolarization of Vₐ (from −37.6 ± 3.4 to −44.8 ± 5.3 mV; P = 0.03), which recovered to −33.7 ± 3.3 mV after return to luminal pH 6.0.

There were no significant differences in Rₑ, Vₑm, and Rₑ/Rₐ between the HCl and H₂SO₄ exposures in the same tissues.

**Effect of H₃PO₄**

Intracellular pH. Exposure of the mucosae to luminal H₃PO₄ at pH 2.3 (pKₐ = 2.1) caused a significant acidification of pHi from 7.27 ± 0.05 to 7.03 ± 0.05 (n = 11, P < 0.01; Fig. 1). Compared with the effects of HCl (pH 2.3) in the same tissues, H₂PO₄ (pH 2.3) caused a

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**Statistics**

Results are given as means ± SE. Statistical analysis of the raw data was performed using Student’s t-test for paired variates. Pearson’s correlation coefficient R was used in correlation analysis.

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**Fig. 1.** Effect on intracellular pH (pHi) of HCl, HNO₃, H₂SO₄, and H₃PO₄ at luminal pH 2.3. *P < 0.05.

**Fig. 2.** Effect of luminal HCl at pH 2.3 on pHi (ΔpHi) compared with HNO₃, H₂SO₄, and H₃PO₄ at luminal pH 2.3. *P < 0.05.
statistically significantly larger acidification of pH$_i$, $\Delta$pH$_i$ being 0.09 $\pm$ 0.01 and 0.24 $\pm$ 0.05 pH units for HCl and H$_3$PO$_4$, respectively, ($P < 0.05$; Fig. 2). When changing back to luminal pH 6.0, the pH did not return to baseline level as with the other acids but tended to decrease to a very low level (from 7.03 $\pm$ 0.05 to 6.74 $\pm$ 0.23, NS; Fig. 1).

**Resistances.** Exposure to luminal H$_2$PO$_4$ (pH 2.3) decreased $R_t$ (from 712 $\pm$ 70 to 644 $\pm$ 54 $\Omega \cdot$ cm$^2$; $P < 0.01$). After return to luminal pH 6.0, $R_t$ did not recover but stayed at a low level (514 $\pm$ 42 $\Omega \cdot$ cm$^2$). $R_a/R_b$ did not change significantly (from 3.86 $\pm$ 0.67 to 6.57 $\pm$ 1.60; $P < 0.05$).

**Electric potentials.** Exposure to luminal H$_2$PO$_4$ (pH 2.3) did not significantly change $V_a$ (from $-45.9 \pm 2.9$ to $-27.6 \pm 4.2$ mV; NS). The decrease in $R_a$ and direction of change in $V_a$ were significantly different between HCl and H$_3$PO$_4$ exposures in the same tissues, whereas the changes in $R_a/R_b$ were not.

In experiments with lower H$_3$PO$_4$ concentration (Cl$^-$ substituted for phosphate), recovery of $pHi$, $R_a/R_b$, and $V_a$ was observed (data not shown).

### Surface Pressure-Area Isotherms

Figure 3 demonstrates the surface pressure-area isotherms of gastric mucosal phospholipids formed on HCl subphases at pH 1.0, 1.5, and 2.3. There is only a minor difference in the isotherms, but the extrapolated mean molecular area (MMA) of the phospholipid mixture increased slightly with increase in pH. This was also observed on the other acidic subphases (Table 1 and Fig. 4). The increase in MMA was most drastic on subphases of HNO$_3$, H$_2$SO$_4$, and H$_3$PO$_4$, with an increase between 11 and 28 Å$^2$. The increase in MMA on the HCl subphase was only ~7 Å$^2$. The phospholipids are more closely packed at pH 1.5 on all the acid subphases than on subphases with higher pH. The MMA on the HCl subphase was, moreover, much higher than that on the other acid subphases at pH 1.5, which showed no difference in MMA of the phospholipid mixture. There was no significant difference in collapse pressure (Table 1). The $pK_a$ values for each acid are given above in this section.

### DISCUSSION

The apical cell membrane of gastric surface epithelial cells, like other cell membranes in eukaryotic cells, is formed by a phospholipid bilayer, possibly uniquely covered by an additional hydrophobic phospholipid monolayer (9, 14). Phospholipid bilayers are highly impermeable to electrically charged ions but readily permeable to small uncharged molecules (1). The basal permeability of nonelectrolytes through the phospholipid bilayer is determined both by the octanol/water partition coefficient (which tells how easily the molecule enters the bilayer) and the diffusion coefficient of the molecule inside the membrane. For different nonelectrolytes the difference in the diffusion coefficient inside the same bilayer is mainly determined by the volume of the diffusing molecule, because the other factors (such as volume selectivity of the membrane and maximum diffusion speed) are independent of the diffusant (19). The estimated van der Waals volumes of the acids HCl, HNO$_3$, H$_2$SO$_4$, and H$_3$SO$_4$ are 24, 43, 64, and 63 Å$^3$, respectively. Different estimation methods of octanol-to-water partition ratios give slightly different values, but the order is always the same: HCl $>$ HNO$_3$ $>$ H$_2$SO$_4$ $>$ H$_3$SO$_4$. According to these results, the diffusion rate of HCl through the plasma membrane should theoretically be the fastest.

However, the cell membrane contains specific transport mechanisms, such as ion channels, ion exchangers, and pumps, that also permit ion movement across the phospholipid bilayer. An amiloride-sensitive Na$^+$ channel (15) has been identified in the apical membrane of gastric surface epithelial cells, and a Cl$^-$specific anion channel (16) and an Cl$^-$/HCO$_3^-$ exchanger (7) have been postulated. The amiloridesensitive Na$^+$ channel is probably also permeant to protons, but these channels are blocked in the presence of luminal acid (17).

It has been well documented that exposure of the gastric epithelium to acid leads to cytoplasmic acidifi-

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**Table 1. Extrapolated MMA and $R_a$ of human gastric mucosal phospholipid monolayers formed on various acidic subphases**

<table>
<thead>
<tr>
<th>Acid</th>
<th>pH 1.5 MMA, Å$^2$</th>
<th>pH 1.5 $R_a$, mN/m</th>
<th>pH 2.3 MMA, Å$^2$</th>
<th>pH 2.3 $R_a$, mN/m</th>
<th>pH 3.0 MMA, Å$^2$</th>
<th>pH 3.0 $R_a$, mN/m</th>
</tr>
</thead>
<tbody>
<tr>
<td>HCl</td>
<td>60.0</td>
<td>53.8</td>
<td>66.1</td>
<td>54.0</td>
<td>68.1</td>
<td>52.4</td>
</tr>
<tr>
<td>HNO$_3$</td>
<td>47.9</td>
<td>52.2</td>
<td>66.1</td>
<td>54.0</td>
<td>68.1</td>
<td>52.4</td>
</tr>
<tr>
<td>H$_2$SO$_4$</td>
<td>49.2</td>
<td>52.2</td>
<td>66.1</td>
<td>54.0</td>
<td>68.1</td>
<td>52.4</td>
</tr>
<tr>
<td>H$_3$PO$_4$</td>
<td>49.2</td>
<td>52.2</td>
<td>66.1</td>
<td>54.0</td>
<td>68.1</td>
<td>52.4</td>
</tr>
</tbody>
</table>

MMA, mean molecular area; $R_a$, collapse pressure.
cation in surface epithelial cells (10). However, the mechanism by which H\(^+\) gets its access to the cell interior remains to be delineated. Earlier studies on proton diffusion across artificial phospholipid vesicle membranes indicated that the permeability of phospholipid bilayers to H\(^+\) is unexpectedly high, being much greater than the permeability to other small monovalent ions (4). Gutknecht and Walter (8) proposed on the basis of their investigations of proton and gastric acid transport across artificial planar lipid membranes that in the presence of large pH gradients proton transport occurs primarily as diffusion of the molecular form of HCl across the membrane. They also showed that the monovalent acid HNO\(_3\) expressed transport through membranes similar to that HCl, whereas the divalent acid H\(_2\)SO\(_4\) showed no detectable H\(^+\) flux through artificial membranes (8).

To further investigate acid movement in gastric epithelium, we exposed isolated Necturus antral mucosa to four inorganic acids with different valence and molecular size, HCl, HNO\(_3\), H\(_2\)SO\(_4\), and H\(_3\)PO\(_4\), at pH 2.3. All acids provoked rapid intracellular acidification but, unexpectedly and deviating from the above studies, HCl caused less intracellular acidification in gastric epithelium than HNO\(_3\), H\(_2\)SO\(_4\), or H\(_3\)PO\(_4\). The reason for this discrepancy remains obscure, but our results are partially explicable in terms of the relative amounts of molecular acid. In contrast to monobasic acids, multibasic acids dissociate in several steps. Knowing the dissociation constants it is possible to calculate the amount of the acid, which is in the molecular form in an aqueous solution. At pH 2.3 in Ringer solution, the molecular concentrations for the acids used are as follows: 49 mM HCl, 25 \(\mu\)M HNO\(_3\), 161 nM H\(_2\)SO\(_4\), and 39 mM H\(_3\)PO\(_4\). Thus the molecular concentration of an acid (in logarithmic scale) in aqueous solution seems to correlate with the degree of intracellular acidification provoked by it [Pearson’s \(R = 0.48, P = 0.003, \log\) (molecular concentration) vs. \(\Delta\text{pH} \)], which is in agreement with the concept that gastric acid (like other acids) enters the surface cell, at least in part, in an ionized molecular form. The changes in osmolarity between different acid solutions used are small (compared with HCl pH 2.3 solution: 25 \(\mu\)mol/kgH\(_2\)O, 2.2 mmol/kgH\(_2\)O, and 3.4 mmol/kgH\(_2\)O for HNO\(_3\), H\(_2\)SO\(_4\), and H\(_3\)PO\(_4\), respectively) and most probably do not contribute to the results.

On the basis of studies with artificial phospholipid bilayer vesicles exposed to a high pH gradient Barreto and Lichtenberger (2) showed that the rate of intravesicular acidification is dependent on the nature of the anionic “counterion.” Exposure of the vesicles to ambient HCl provoked a significantly larger intravesicular acidification than H\(_2\)SO\(_4\) or H\(_3\)PO\(_4\) with the same pH gradient (2). Our results are not in accordance with these findings, because intravesicular acidification provoked by HCl was of lesser magnitude than that caused by H\(_2\)SO\(_4\) or H\(_3\)PO\(_4\). Further investigations with the vesicle model indicated that chloride is an important cofactor of acidification across artificial bilayers by forming a very membrane-permeant HCl molecule, and Barreto and Lichtenberger (3) proposed that this might be the mechanism for H\(^+\) back diffusion in the gastric mucosa.

The monolayer-forming properties of the synthetic model of human gastric mucosal phospholipids have been studied previously with the Langmuir film balance (pH 5.6 at room temperature and pH 2.1 at 37°C) (13). When different nonsteroidal anti-inflammatory drugs (in their aqueous solutions as sodium salts), which are weak acids, were used as a subphase, the MMA of the phospholipid mixture and their collapse pressure differed significantly (13).

In the present study the synthetic models of human mucosal phospholipids were spread onto an aqueous subphase acidified with HCl, HNO\(_3\), H\(_2\)SO\(_4\), or H\(_3\)PO\(_4\). There was a shift in MMA of the lipids depending on pH and acid. A clear distinction was observed at pH 1.5, where the phospholipids were more closely packed on subphases acidified with HNO\(_3\), H\(_2\)SO\(_4\), or H\(_3\)PO\(_4\) than at higher pH or on HCl subphases. The more compressed monolayers at low pH indicate a higher complexation between the phospholipid head group and the anion. Changes in the isotherm due to ionization will reflect on changes in the lateral attraction between the molecules and primary head group interactions. Apart from considering the degree of charge present, the specific nature of the head group must be taken into account. The phospholipid mixture was 31.5% phosphatidylethanolamine. Amine monolayers have been found to be more condensed on subphases containing divalent counterions such as sulfonate or hydrogen phosphate (5). Phosphatidylethanolamine, moreover, readily forms hydrogen bonds. The MMA of the dietary phospholipids at pH 5.6 was 84 Å\(^2\), which indicates that the phospholipids are getting more condensed as pH decreases (13). The MMA was higher on a subphase acidified with HCl than in those acidified with HNO\(_3\), H\(_2\)SO\(_4\), or H\(_3\)PO\(_4\) (pH < 3.0). Biegel and Gould (4) speculated that protons cross the phospholipid bilayer via a hydrogen-bond exchange mechanism along transmembrane H\(_2\)O bridges, which, in turn, are formed by H\(_2\)O molecules dissolved in the membrane hydrocarbon. Further studies demonstrated that such hydrogen-bonded chains of H\(_2\)O molecules can provide substantial discrimination between protons and other monovalent cations, thus specifically allowing H\(^+\) permeation across the phospholipid membrane (6). In our experiments, the orientational order of the hydrocarbon chains was increased on subphases of HNO\(_3\), H\(_2\)SO\(_4\), and H\(_3\)PO\(_4\) and the head groups are probably close enough for hydrogen bonding to occur, thus permitting H\(^+\) to permeate the lipid layer through hydrogen bonds. This is in accordance with our observation that HCl causes less intracellular acidification in gastric epithelium than HNO\(_3\), H\(_2\)SO\(_4\), and H\(_3\)PO\(_4\), and the difference in the degree of acidification may also be explicable, at least in part, in terms of this mechanism.

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