Luminal ammonia retards restitution of guinea pig injured gastric mucosa in vitro

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Suzuki, Hideo, Akinori Yanaka, and Hiroshi Muto. Luminal ammonia retards restitution of guinea pig injured gastric mucosa in vitro. Am J Physiol Gastrointest Liver Physiol 279: G107–G117, 2000.—The present study was conducted to elucidate the mechanisms by which Helicobacter pylori (HP)-derived ammonia causes gastric mucosal injury. Intact sheets of guinea pig gastric fundic mucosae were incubated in Ussing chambers. Both the luminal and the serosal pH were kept at 7.4. Transmucosal potential difference (PD) and electrical resistance (R) were monitored as indices of mucosal integrity. Restitution was evaluated by recovery of PD, R, and transmucosal [3H]mannitol flux after Triton X-100-induced mucosal injury. The effects of luminal or serosal NH4Cl on function and morphology of uninjured or injured mucosae were examined. In uninjured mucosae, serosal NH4Cl induced more profound decreases in PD and R and more prominent vacuolation in gastric epithelial cells than did luminal NH4Cl. In contrast, luminal NH4Cl markedly inhibited restitution in injured mucosae and caused an extensive vacuolation in gastric epithelial cells, as did serosal NH4Cl. Transmucosal ammonia flux was greater in the injured than in the uninjured mucosae. These results suggest that 1) basolateral membrane of gastric epithelial cells is more permeable to ammonia than apical membrane and 2) luminal ammonia, at concentrations detected in HP-infected gastric lumen, retards restitution in injured mucosae.

Helicobacter pylori; vacuolation; apical membrane; basolateral membrane; epithelial cells

It has been suggested that chronic gastric mucosal infection with Helicobacter pylori (HP) is closely associated with development of gastritis, peptic ulcers, and possibly with gastric carcinoma (2, 10, 14, 36). HP has been shown to possess strong urease activity, which may play an important role in the development of gastric mucosal injury (2, 10, 14, 36). For example, ammonia at concentrations produced by HP has been shown to cause injury in isolated human gastric epithelial cells in vitro (31) and in rat gastric mucosa in vivo (25, 35). Ammonia has been shown to increase H+ permeability across the gastric mucus gel layer (9). We have previously shown that luminal ammonia at neutral pH enhances H+ backdiffusion across intact sheets of bullfrog gastric mucosa in vitro (42). Ammonia has been shown to enhance formation of cytoplasmic vacuolation induced by HP-derived vacuolating cytotoxin (Vac A protein) in vitro (6). Ammonia in HP-infected stomach has also been shown to react with free radicals generated by intramucosal neutrophils, which results in production of an extremely toxic substance, mono-chloramine, thereby causing severe mucosal injury (7, 15, 17, 26, 34).

Some investigators, however, have pointed out that luminal ammonia alone, especially at concentrations detected in HP-infected subjects, does not cause injury in normal uninjured gastric mucosa (7, 17, 38). On the basis of these results, these investigators concluded that ammonia does not play a central role in the pathogenesis of gastric mucosal injury in HP-infected gastric mucosa in vivo. In contrast, we have previously shown that ammonia, when present at the serosal side of the mucosa, even at much lower concentrations than that detected in HP-infected stomachs, strongly inhibits both H+ secretion and Cl− transport across the gastric mucosa (42). On the basis of these findings, we hypothesized that luminal ammonia in HP-infected gastric mucosa may diffuse into the basolateral side through the injured apical surface and impair function of the gastric epithelial cells from the basolateral side, ultimately resulting in extensive mucosal injury.

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In the present study, we demonstrate that 1) the basolateral membrane of gastric epithelial cells is more permeable to ammonia than the apical membrane and 2) luminal ammonia, at concentrations detected in an HP-infected subject, does not cause injury in normal uninjured mucosa but does retard restitution of injured gastric mucosa.

**METHODS**

**Animals**

Female guinea pigs (250–300 g, Hartley strain) were killed under anesthesia induced by inhalation of diethyl ether. The stomachs were removed and divided into paired halves by incising the greater and lesser curvatures of the fundus. The muscularis propria was stripped from the mucosa with small scissors and fine forceps under microscopic control.

**Electrophysiology**

Intact sheets of resting guinea pig gastric fundic mucosae were mounted in Lucite Ussing chambers in vitro. The chambers were connected to water-jacketed gas-lift reservoirs, which were attached to keep the temperature of the mucosae constant at 37.0°C. The serosal side was bathed with a Ringer solution, buffered with 25 mM HEPES, pH 7.4 (see Solutions), and gassed with 100% O₂. The luminal side was initially bathed with 150 mM NaCl and gassed with 100% O₂ (pH −6.0–7.0). H⁺ secretion was inhibited by treatment of the serosal side with 0.1 mM omeprazole for 60 min. After removing omeprazole from the serosal solution, both sides of the tissue were bathed with a fresh HEPES-Ringer solution. Then the tissue was allowed to settle for another 60-min period. Transmucosal potential difference (PD) and electrical resistance (R) were monitored throughout the experiments. We confirmed that PD and R were maintained at reasonable values for 3–4 h, as long as the mucosae were kept at the initial control conditions. R was calculated from the change in PD induced by passing 100 μA of electrical current from the luminal to the serosal side for 0.5 s every 2 min. The mean initial values of PD and R in uninjured control mucosae were −24.5 ± 3.2 mV and 87 ± 7.2 Ω·cm², respectively (n = 10). PD and R decreased by 13.4% ± 3.2% and 7.5% ± 1.1%, respectively, during 4 h of incubation under control conditions.

**Morphology**

Gastric mucosal specimens for electron and light microscopy were fixed immediately after the electrophysiological experiments by immersion in 2.5% glutaraldehyde, 2% paraformaldehyde, and 0.02% trinitrophenol (picric acid) in 0.1 M cacodylate buffer for at least 2 h at room temperature. Tissues were then postfixed with 1% osmium tetroxide (OsO₄) in 0.1 M cacodylate buffer for 1 h, treated with 1% uranyl acetate in maleate buffer (pH 5.2), and embedded in an Epon-Araldite mixture. Embedded tissues were sectioned at ∼0.8–1.0 μm. The semithin sections were stained with toluidine blue and were examined by light microscopy. Some of the blocks were further sectioned at ∼0.07–0.09 μm. The ultrathin sections were stained with uranyl acetate and lead citrate for transmission electron microscopy. Five slides for light microscopy and five sections for electron microscopy were prepared from each group. Those samples were coded and were evaluated in a blind fashion by two investigators (H. Suzuki and A. Yanaka).

Cytoplasmic vacuolation was assessed by electron microscopy. The degree of vacuolation was classified according to the following criteria. When vacuolation was found in >90% of the gastric epithelial cells (i.e., the gastric gland cells and the surface mucous cells), the degree of vacuolation was referred to as severe. When vacuolation was noted in 50–90% of the cells, the degree was referred to as “moderate.” When vacuolation was noted in <50% of the cells, the degree was referred to as mild.

Restitution was evaluated by light microscopy. Degree of restitution was classified as either “complete” or “incomplete.” We referred to complete restitution only when the lamina propria was perfectly covered with epithelial cells (i.e., epithelial continuity is established) in all areas of the five samples we evaluated. In contrast, we referred to incomplete restitution when even a single focus of absence of epithelial continuity was found in any of the five slides we examined.

**[^H]Mannitol Flux Study**

To assess mucosal permeability, [³H]mannitol flux (MF) from the serosal to the luminal solution was measured under short-circuited conditions, as described by Moore et al. (24). In brief, [³H]mannitol, 5 μCi, was placed in the serosal solution. One-milliliter aliquots were collected from the luminal bath at 15-min intervals throughout the experiments. The entire luminal solution was replaced with reoxidized fresh luminal solutions at each 15-min interval immediately after the 1-ml aliquot was collected. Unlabeled mannitol (5 mM) was present in both the serosal and the luminal solutions throughout the experiment to eliminate a solute gradient between the two sides. MF at each 15-min period was evaluated from the radioactivity of [³H] detected in each sample.

**Experimental Protocol for Restitution Study**

To cause superficial mucosal injury, the luminal side was exposed to 2% Triton X-100 for 5 min, as described previously (24). This treatment induced rapid and marked decreases in PD and R, accompanied by a reciprocal increase in MF. Morphology of the Triton X-100-treated mucosae showed superficial injury of gastric mucosa (see RESULTS). After the luminal solution was replaced with fresh HEPES-buffered Ringer solution, the tissues were incubated for 2 h, during which PD, R, and MF showed gradual recovery. Restitution was quantitatively evaluated by the recovery of PD and R and/or the changes in MF after the injury, as described in our previous report (41) and in the report by Moore et al. (24). Restitution was also evaluated morphologically by light microscopy of the mucosae fixed at the end of the experiment.

**Measurement of Ammonia Flux From the Luminal to the Serosal Side**

To examine the mucosal permeability of ammonia, 10 mM NH₄Cl was added to the luminal solution of the uninjured or the injured mucosae. In injured mucosae, NH₄Cl was added immediately after removal of Triton X-100 from the luminal solution. One-milliliter aliquots of the serosal solution were collected every 30 min after addition of the luminal NH₄Cl. Concentrations of NH₃ in each sample were determined using a kit from Sigma (St. Louis, MO), which is based on reductive amination of 2-oxoglutarate in the presence of glutamate dehydrogenase, as described elsewhere (27). Total ammonia concentration in the serosal solution (NH₄⁺ + NH₃) was calculated from the serosal [NH₄⁺] taking into...
account that NH₃:NH₄⁺ is 1:40 at pH 7.4, since pKₐ of ammonia has been shown to be 9.0 (20). Transmucosal flux of ammonia from the luminal to the serosal side was estimated from the values of serosal [NH₄⁺ + NH₃]. Ammonia flux was expressed as the percentage of the total ammonia added to the luminal solution.

**Chemicals**

Omeprazole was kindly provided by Astra (Hässel, Mölndal, Sweden). All other chemicals were purchased from Wako Pure Chemical (Osaka, Japan).

**Solutions**

The serosal solution contained (in mM) 122 Na⁺, 5.0 K⁺, 1.8 Ca²⁺, 0.8 Mg²⁺, 130 Cl⁻, 0.8 HCO₃⁻, 0.8 H₂PO₄⁻, and 10.0 glucose (total calculated osmolarity was 300 mosM; pH in the solution was 7.4). The luminal solution contained (in mM) 150 Na⁺ and 150 Cl⁻ (total calculated osmolarity was 300 mosM).

**Statistical Analysis**

Student's paired or unpaired t-tests were used for the comparison of paired or unpaired values, respectively. P < 0.05 was considered significant. Results were expressed as means ± SE.

**RESULTS**

**Effects of Luminal or Serosal NH₄Cl on Uninjured Gastric Mucosae**

This series of experiments was conducted to determine whether the serosal side of the uninjured normal mucosa is more susceptible to the action of NH₄Cl than the luminal side. Both the luminal and the serosal side were bathed with the HEPES-Ringer solution (pH 7.4) throughout the experiment.

Changes in PD and R after addition of luminal or serosal NH₄Cl to uninjured control mucosae. Addition of 30 mM NH₄Cl either to the luminal or the serosal side for 2 h significantly decreased PD and R (Fig. 1). The magnitudes of the decreases in PD and R induced by the serosal NH₄Cl were significantly greater than those induced by the luminal NH₄Cl (Fig. 1)

In some tissues, 30 mM NaCl, instead of 30 mM NH₄Cl, was added to either the luminal or the serosal side to examine the effects of the increase in osmolarity on PD and R. Addition of 30 mM NaCl either to the luminal or the serosal side did not affect PD or R (Fig. 1).

Dose-response studies on the effect of luminal or serosal NH₄Cl/NaCl on PD and R. In this series of experiments, changes in PD and R were examined at 30 min after addition of graded doses of NH₄Cl or NaCl (1, 3, 10, 30, and 100 mM) to either the serosal or the luminal side. Addition of NaCl, at concentrations of 1–30 mM, to either the serosal or the luminal side did not affect PD or R. However, 100 mM NaCl in either the luminal or the serosal solution caused significant decreases in PD and R, although the magnitudes of the decreases in PD and R were significantly less than those induced by 100 mM NH₄Cl (Fig. 2).

Both the serosal and the luminal NH₄Cl, at concentrations approximately between 1 and 100 mM, dose-dependently decreased PD and R. The magnitudes of the decreases in PD and R caused by serosal NH₄Cl were significantly greater than those caused by luminal NH₄Cl (Fig. 2).

Effect of luminal or serosal NH₄Cl on morphology. Morphological examination by light and electron microscopy demonstrated that the mucosae treated with 30 mM NH₄Cl in the luminal solution for 2 h showed a mild degree of vacuolation, mainly in the gastric glands located toward the basolateral side of the mucosa (Fig. 3, A and B). In contrast, the mucosae treated with 30 mM NH₄Cl in the serosal solution showed severe degree of vacuolation, characterized by an extensive vacuolation of almost all the cells in both the gastric glands and the surface mucous cells (Fig. 3C). Low-
induced a rapid decrease in PD and an increase in R (Fig. 4, A and B). Serosal bumetanide also caused a rapid decrease in PD but did not alter R (Fig. 4, A and B). PD and R reached stable values during the 30-min period after addition of these agents. Subsequently, 30 mM NH₄Cl was added to the serosal side. Serosal BaCl₂, at concentrations approximately between 0.1 and 1 mM, dose-dependently attenuated the decreases in PD and induced by serosal NH₄Cl (Fig. 4, C and D). Serosal bumetanide, at concentrations approximately between 0.01 and 0.1 mM, also dose-dependently mitigated the NH₄Cl-induced decreases in PD and R (Fig. 4, E and F).

Effects of NH₄Cl on Restitution in Triton X-100-Treated Injured Mucosae

This series of experiments was conducted to determine whether luminal or serosal NH₄Cl, at physiological concentrations, could inhibit restitution. Therefore, restitution was evaluated in the presence or absence of 30 mM NH₄Cl in either the luminal or the serosal side. In other tissues, 30 mM NaCl, instead of 30 mM NH₄Cl, was added to either the luminal or the serosal side to exclude the possibility that increases in osmolarity affect restitution.

Effects of luminal or serosal NH₄Cl on electrophysiology and MF after injury. In the absence of 30 mM NH₄Cl, PD and R gradually increased, whereas MF rapidly decreased during a recovery period after the Triton X-100-induced injury (Fig. 5, A–C). In contrast, the recovery of PD, R, and MF was markedly inhibited by either the luminal or the serosal NH₄Cl (Fig. 5, A–C). There was no evidence of recovery of PD, R, or MF at 2 h after the injury in tissues exposed to NH₄Cl (Fig. 5, A–C).

Effects of luminal or serosal NaCl on electrophysiology and MF after injury. This series of experiments was conducted to examine the possibility that an increase in osmolarity affects restitution. Restitution was examined after addition of 30 mM NaCl, instead of 30 mM NH₄Cl, to either the luminal or the serosal side. Addition of 30 mM NaCl to either the luminal or the serosal side did not affect either the recovery of PD and R or the decrease in MF after injury (Fig. 5, D–F).

Effects of luminal or serosal NH₄Cl on morphology of injured mucosae. Exposure of the luminal surface to 2% Triton X-100 for 5 min induced superficial injury of the gastric mucosa, characterized by exfoliation of large amounts of the surface mucous cells without causing marked injury of the gastric glands (Fig. 6A). In the absence of NH₄Cl, the denuded surface of the injured mucosae was almost completely covered with the surface mucous cells at 2 h after the injury (Fig. 6B).

In contrast, in the presence of 30 mM NH₄Cl in the luminal solution, some denuded areas were still observed at 2 h after the injury (Fig. 6C). Prominent cytoplasmic vacuolation was noted in almost all the cells in the gastric glands (Fig. 6C). In the presence of 30 mM NH₄Cl in the serosal solution, morphology of

power electron micrographs showed numerous small vacuoles in the surface mucous cells and large vacuoles in oxyntic cells in gastric glands (Fig. 3D).

Effects of inhibition of the basolateral cation channels on the changes in PD and R induced by serosal NH₄Cl. This series of experiments was conducted to examine the possibility that the entry of NH₄⁺ into the gastric epithelial cells occurred via the basolateral K⁺ channels and the Na⁺₂K⁺₂Cl⁻ cotransporters, since this has been reported in renal epithelial cells (3, 20, 21) and in salivary acinar cells (12). The serosal side of the mucosa was exposed to various concentrations of either BaCl₂ (0.1, 0.3, or 1 mM) or bumetanide (0.01, 0.03, or 0.1 mM), which have been shown to block the K⁺ channel and the Na⁺⁻K⁺⁻2Cl⁻ cotransporter, respectively (21). Addition of BaCl₂ to the serosal side

Fig. 2. Dose-response studies on the effects of graded doses of luminal or serosal NH₄Cl/NaCl on PD and R in uninjured gastric mucosa. Omeprazole-pretreated resting fundic mucosae were incubated with HEPES-Ringer solution at pH 7.4 on both the luminal and the serosal side. Graded doses of NH₄Cl or NaCl, 1–100 mM, were added to either the serosal or the luminal side. The values of PD (A) and R (B) at 30 min after addition of NH₄Cl are shown. n, Number of experiments. Data are expressed as means ± SE. *P < 0.05 and ***P < 0.001, significant difference from the corresponding values in the mucosae treated with luminal NH₄Cl. \#P < 0.05, ###P < 0.01, and ####P < 0.001, significant difference from the corresponding values in the mucosae treated with luminal or serosal NaCl. *P < 0.05, significant difference from the corresponding values at 0 mM concentration.

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Effects of luminal or serosal NaCl on electrophysiology and MF after injury. This series of experiments was conducted to examine the possibility that an increase in osmolarity affects restitution. Restitution was examined after addition of 30 mM NaCl, instead of 30 mM NH₄Cl, to either the luminal or the serosal side. Addition of 30 mM NaCl to either the luminal or the serosal side did not affect either the recovery of PD and R or the decrease in MF after injury (Fig. 5, D–F).

Effects of luminal or serosal NH₄Cl on morphology of injured mucosae. Exposure of the luminal surface to 2% Triton X-100 for 5 min induced superficial injury of the gastric mucosa, characterized by exfoliation of large amounts of the surface mucous cells without causing marked injury of the gastric glands (Fig. 6A). In the absence of NH₄Cl, the denuded surface of the injured mucosae was almost completely covered with the surface mucous cells at 2 h after the injury (Fig. 6B).

In contrast, in the presence of 30 mM NH₄Cl in the luminal solution, some denuded areas were still observed at 2 h after the injury (Fig. 6C). Prominent cytoplasmic vacuolation was noted in almost all the cells in the gastric glands (Fig. 6C). In the presence of 30 mM NH₄Cl in the serosal solution, morphology of
the injured mucosae at 2 h after the injury also showed incomplete restitution, accompanied by prominent vacuolation in the gastric glands, which was quite similar to that demonstrated in the presence of luminal NH$_4$Cl (Fig. 6D).

Roles of basolateral K$^+$ channels in restitution. In this series of experiments, we tested the hypothesis that inhibition of restitution by NH$_4$Cl is caused by blockade of the basolateral K$^+$ channels by NH$_4^+$ in gastric epithelial cells, since recent studies have suggested the importance of the basolateral K$^+$ channels in cell migration (28, 29). We therefore examined the effects of inhibition of basolateral K$^+$ channels by serosal 1 mM BaCl$_2$ on restitution in Triton X-100-injured gastric mucosae. Serosal BaCl$_2$ markedly prevented recovery of R (Fig. 7A), and retarded the decrease in MF after injury (Fig. 7B), indicating that the basolateral K$^+$ channels play an important role in restitution of injured guinea pig gastric mucosae.

Ammonia Flux from the Luminal to the Serosal Side

In both the normal uninjured and the injured mucosae, [NH$_4^+$ + NH$_3$] in the serosal solution gradually increased over time (Fig. 8A). The [NH$_4^+$ + NH$_3$] detected in the serosal solution in the injured mucosa was significantly greater than that detected in the uninjured control mucosa (Fig. 8A). The ammonia flux from the luminal to the serosal side during the initial 30-min period was 0.08 ± 0.01% and 1.15 ± 0.11% in the uninjured and the injured mucosae, respectively (Fig. 8B).

DISCUSSION

The present study clearly demonstrates that the serosal surface of uninjured normal guinea pig gastric fundic mucosa is more sensitive to the action of NH$_4$Cl than the luminal surface. There are several possible explanations for the difference in NH$_4$Cl permeability between the luminal and the serosal membranes.

First, the difference could be attributable to asymmetrical distribution of the cation channels in highly polarized gastric epithelial cells (8, 13). Because ammonia has a pK$_a$ of 9.0 (20), calculated [NH$_4^+$]:[NH$_3$] at pH 7.4 is ∼40:1. The greater sensitivity of the serosal side of the mucosa to ammonia could be attributable to the large NH$_4^+$ permeability of the basolateral membrane of the gastric epithelial cells. As stated in the introduction, NH$_4^+$ does not diffuse through the phospholipid bilayer of the cell membrane but does pass through several kinds of cation channels. A number of recent studies in renal epithelial cells and in salivary acinar cells have clearly shown that NH$_4^+$ acts as a...
Fig. 4. Effects of serosal NH₄Cl on PD and \( R \) in the presence of various doses of BaCl₂ or bumetanide in the serosal solution. Omeprazole-pretreated resting fundic mucosae were incubated with HEPES-Ringer solution at pH 7.4 on both the luminal and the serosal side. Various doses of BaCl₂ (0.1, 0.3, and 1 mM) or bumetanide (10, 30, and 100 \( \mu \)M) were placed in the serosal solution. A and B: dose-response studies on the effects of graded doses of BaCl₂ or bumetanide on basal values of PD (A) and \( R \) (B). C and D: effects of NH₄Cl on PD (C) and \( R \) (D) in the presence of various doses of BaCl₂. E and F: effects of NH₄Cl on PD (E) and \( R \) (F) in the presence of various doses of bumetanide. n, Number of experiments. Data are expressed as means ± SE *\( P < 0.05 \), **\( P < 0.01 \), and ***\( P < 0.001 \), significant difference from the corresponding values in the absence of BaCl₂ or bumetanide.
Fig. 5. Effects of luminal or serosal NH₄Cl/NaCl on recovery of PD, R, and [³H]mannitol flux (MF) after injury. Omeprazole-pretreated resting fundic mucosae were incubated with HEPES-Ringer solution at pH 7.4 on both the luminal and the serosal side. Mucosal injury was induced by exposure of the luminal surface to 2% Triton X-100 for 5 min. After removal of the Triton X-100 from the luminal side, recovery of PD and R and changes in MF were monitored. Experiments were conducted in the presence or absence of 30 mM NH₄Cl or 30 mM NaCl in either the luminal or the serosal solution. A and B: effects of serosal or luminal NH₄Cl (30 mM) on changes in PD (A) and R (B) after the injury. C: effects of serosal or luminal NH₄Cl (30 mM) on changes in transmucosal MF after the injury. D and E: effects of serosal or luminal NaCl (30 mM) on changes in PD (D) and R (E) after the injury. F: effects of serosal or luminal NaCl (30 mM) on changes in transmucosal MF after the injury. n, Number of experiments. Data are expressed as means ± SE *P < 0.05, **P < 0.01, and ***P < 0.001, significant difference from the corresponding values in control mucosae. n.s., Not significant.
surrogate for $K^+$ and passes through both the $K^+$ channels (3, 21) and the $Na^+-K^+-2Cl^-$ cotransporters (12, 20, 21), although this has not been examined previously in gastric epithelial cells (8, 13). Since the existence of these cation channels has been demonstrated on the basolateral side of the gastric epithelial cells (8, 32, 33), it seems reasonable to propose that NH$_4^+$ passes through both the $K^+$ channel and the $Na^+-K^+-2Cl^-$ cotransporter on the basolateral surface of gastric epithelial cells, thereby rendering the basolateral membrane more permeable to NH$_4$Cl than the apical membrane. This possibility is strongly supported by the present results that the decrease in PD and $R$ induced by the serosal NH$_4$Cl was dose-dependently inhibited by serosal BaCl$_2$ or by serosal bumetanide (Fig. 4), agents known to block the $K^+$ channel and the $Na^+-K^+-2Cl^-$ cotransporters, respectively (21).

Furthermore, our morphological findings that serosal NH$_4$Cl caused more prominent cytoplasmic vacuolation in gastric glands than did luminal NH$_4$Cl also support this proposal. Thus the present results strongly suggest that the serosal membrane of well-polarized gastric epithelial cells is more permeable to NH$_4^+$ than the apical membrane. The significant difference in ammonia permeability of the serosal and the luminal membranes has also been demonstrated by other studies in isolated gastric glands (39) and in T84 cell monolayers (27).

Second, the difference in NH$_4$Cl permeability could be attributable to the difference in the permeability of NH$_3$ between the luminal and the serosal membranes of gastric epithelial cells, since recent studies have suggested the relatively impermeable nature of the apical membrane to NH$_3$ in well-polarized epithelial cells (18, 30).

Third, it is also conceivable that the basolateral Cl$^-$/HCO$_3^-$ exchangers in oxyntic cells raise ambient pH on the basolateral surface of the mucosa by extruding cytoplasmic HCO$_3^-$ (i.e., an alkaline tide) (1, 22), thereby enhancing conversion of NH$_4^+$ to NH$_3$. The NH$_3$ could then diffuse directly into the cells through the cell membrane. This possibility is supported by the present finding that luminal NH$_4$Cl induced more prominent vacuolation in gastric glands than in the surface mucous cells. It is also conceivable that oxyntic cells and/or peptic cells may be more susceptible to ammonia in terms of vacuolation than the surface mucous cells. The NH$_3$ caused more prominent cytoplasmic vacuolation in gastric glands than in the surface mucous cells. It is also conceivable that oxyntic cells and/or peptic cells may be more susceptible to ammonia in terms of vacuolation than the surface mucous cells, even though we do not have direct evidence for this possibility. The present results also suggest that vacuolation, per se, is not related to cell death, since we did not see necrotic changes in the vacuolated cells. Our findings are in concert with the studies on the effect of ammonia on morphology of isolated rat gastric glands in vitro (16).

The present study shows that the luminal surface of the Triton X-100-treated injured mucosa is more vu-
The present study shows that relatively low concentrations of ammonia, ~10–30 mM NH₄Cl at pH 7.4, which are almost equivalent to those detected in the gastric lumen in the HP-infected subjects (37), strongly inhibit restitution. We assume that the luminal surface of gastric epithelial cells in HP-infected subjects is exposed to higher concentrations of ammonia than that in gastric juice, since the majority of HP live within the adherent mucus gel layer. In contrast, in the present experimental system, [NH₄⁺ + NH₃] at the luminal surface of the mucosae is presumably not greater than that in the luminal solution, since we added NH₄Cl to the luminal solution and HP was not present within the mucus gel layer. Together, we believe that the

Fig. 7. Effects of serosal BaCl₂ on recovery of R and MF after injury. Omeprazole-pretreated resting fundic mucosae were incubated with HEPES-Ringer solution at pH 7.4 on both the luminal and the serosal side. Mucosal injury was induced by exposure of the luminal surface with 2% Triton X-100 for 5 min. After removal of the Triton X-100 from the luminal side, recovery of PD, R, and MF were examined in the presence or absence of 1 mM BaCl₂ in the serosal solution. A: changes in R after the injury. B: changes in transmucosal MF after the injury. n, Number of experiments. Data are expressed as means ± SE. *P < 0.05, **P < 0.01, and ***P < 0.001, significant difference from the corresponding values in the absence of BaCl₂.

Fig. 8. Difference in ammonia permeability between injured and uninjured gastric mucosae. Omeprazole-pretreated resting fundic mucosae were incubated with HEPES-Ringer solution (pH 7.4) on both the serosal and the luminal side. NH₄Cl concentration in the serosal solutions was measured at 30, 60, 90, and 120 min after addition of NH₄Cl to the luminal bath. Experiments were conducted in both the uninjured and the Triton X-100-treated injured mucosa. Total concentrations of ammonia ([NH₄⁺ + NH₃]) were calculated, based on the formula that [NH₄⁺]:[NH₃] = 40:1 at pH 7.4. A: time course of the ammonia flux across the uninjured and the injured mucosa. B: concentrations of ammonia detected in the serosal solution at 30 min after addition of the NH₄Cl to the luminal solution. The data are expressed as percentage of ammonia detected in the serosal solution to the total ammonia added to the luminal solution. n, Number of experiments. Data are expressed as means ± SE. *P < 0.05, **P < 0.01, and ***P < 0.001, significant difference from the corresponding values in uninjured mucosae.
[\text{NH}_4\text{Cl}], \sim 10–30 \text{ mM at pH 7.4}, used in the present study are clinically relevant concentrations.

The mechanisms by which \text{NH}_4\text{Cl} causes gastric mucosal injury and retards restitution have not been well clarified. It has been suggested that ammonia inhibits mitochondrial respiration and subsequent energy metabolism (38). We have previously shown that hypoxia inhibits restitution in bullfrog gastric mucosa in vitro (40). Thus it seems reasonable to assume that ammonia abolishes reorganization of actin filaments of the epithelial cells, which is an energy-dependent process, and is essential for cell migration on the denuded surface of the injured mucosae (4). Another possibility is that inhibition of the basolateral \text{K}^+ channels of gastric epithelial cells by ammonia may contribute to retardation of restitution. This possibility is supported by the present results, which showed that restitution is markedly prevented by blockade of the basolateral \text{K}^+ channels by serosal \text{BaCl}_2 (\text{Fig. 7}). Recent studies in renal epithelial cells have suggested that basolateral \text{K}^+ channels play an important role in migration of the cells (28, 29). Furthermore, we have previously shown that \text{NH}_3\text{Cl} inhibits electrogenic \text{Cl}^- transport across the bullfrog gastric mucosa by blocking the basolateral \text{K}^+ channels (42). Inhibition of the basolateral \text{K}^+ channels by ammonia has also been reported by studies in T84 cell monolayers (27). Thus it seems likely that inhibition of restitution by ammonia is, at least in part, related to inhibition of the basolateral \text{K}^+ channels by ammonia.

In the present study, we evaluated restitution by the recovery of both the transmucosal electrical resistance and the transmucosal flux of \text{H}^3\text{H}-labeled mannitol, since both of these parameters have been shown to correlate with morphological restitution (3, 20). Each of these methods, however, has some disadvantages. For example, the electrical resistance is affected by various factors, such as changes in \text{H}^+ secretion of the mucosae or by changes in concentration of ambient \text{Na}^+, \text{K}^+, or \text{Cl}^- . The value of the MF is not affected by those factors, although this technique takes more time and is more expensive than measurement of the electrical resistance. For these reasons, we have used both measurements to enhance the accuracy of assessing restitution.

With regard to changes in osmolarity on electrophysiological parameters, we have found that an increase in osmolarity from 300 to 500 mosM by the addition of 100 \text{ mM NaCl} decreases the value of \text{PD} and \text{R} in uninjured mucosae. However, we have also confirmed that an increase in osmolarity by 60 mosM by the addition of 30 \text{ mM NaCl} did not affect either the electrophysiological parameters in uninjured mucosae or the restitution after injury. These results indicate that the decreases in \text{PD} and \text{R} and the retardation of restitution after addition of 30 \text{ mM NaCl} were not induced by increases in osmolarity but rather were caused by the effects of \text{NH}_4^+ or \text{NH}_3 on the physiology of gastric epithelial cells.

In summary, the present study suggests that the serosal surface of uninjured normal guinea pig gastric mucosa is more susceptible to the effects of ammonia than the apical surface and that low concentrations of luminal ammonia, at physiologic concentrations, prominently retard restitution in injured mucosae.

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