

Review

The Role of Gastric Acid in Preventing Foodborne Disease and How Bacteria Overcome Acid Conditions[†]

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ABSTRACT

The secretion of hydrochloric acid by the stomach plays an important role in protecting the body against pathogens ingested with food or water. A gastric fluid pH of 1 to 2 is deleterious to many microbial pathogens; however, the neutralization of gastric acid by antacids or the inhibition of acid secretion by various drugs may increase the risk of food- or waterborne illnesses. Peptic ulcer disease is often treated by decreasing or eliminating gastric acid secretion, and such treatment blocks the protective antibacterial action of gastric fluid. The majority of peptic ulcer disease cases originate from *Helicobacter pylori* infections. Treatment of *H. pylori*-induced peptic ulcers with antibiotics reduces the need for drugs that inhibit gastric acid secretion and thereby diminishes the risk of food- and waterborne illness for peptic ulcer disease patients. Many bacterial pathogens, such as *Escherichia coli*, *Salmonella* Typhimurium, and *H. pylori*, can circumvent the acid conditions of the stomach by developing adaptive mechanisms that allow these bacteria to survive in acid environments. As a consequence, these bacteria can survive acidic stomach conditions and pass into the intestinal tract, where they can induce gastroenteritis.

The stomach has a fundamental role in killing or inactivating pathogens present in food or water before they enter the intestinal tract. The stomach is a large-capacity organ located between the esophagus and the duodenum and functions as a reservoir for the storage, mixing, mincing, and partial digestion of food before it moves into the intestinal tract. In addition, the stomach is the primary site for hydrochloric acid (HCl) secretion (1). Gastric HCl enhances the absorption of dietary calcium and iron and activates pepsinogen to pepsin, which is involved in the partial hydrolysis of proteins (1). Most importantly, gastric HCl is a major defense mechanism against pathogens that may be ingested with food or water (48, 49). Gastric juice has a pH of ≤ 1 with HCl levels of 150 to 160 mEq/liter (5,475 to 5,840 mg/liter), and the stomach produces 1 to 2 liters of gastric juice per day (1, 49). In this review, the role of gastric acid as an antibacterial agent and the consequences of preventing the secretion of or neutralizing the acid in the stomach with regard to antibacterial activity and the mechanisms by which bacteria increase their resistance to acid environments, including that of the stomach, are discussed.

HCL SECRETION BY THE STOMACH

HCl is secreted from the parietal cells located in the gastric glands. These glands open into the gastric pits on

the gastric mucosal surfaces. The parietal cells deliver H⁺ and Cl⁻ into canaliculi, which crisscross the cellular cytoplasm. The canaliculi make up an interconnecting system of labyrinthine channels lined by microvilli. The canaliculi open onto the parietal cell luminal surface to deliver the acid secretions into the gastric gland lumen, and acid is eventually delivered to the stomach lumen (42, 49, 61).

The neurotransmitter acetylcholine, the hormone gastrin, and the paracrine (hormone-like) histamine are endogenous chemicals that act in concert to stimulate and control acid production by binding to parietal cell surface receptors (1, 42, 49, 104). Acid secretion is also dependent on oxygen, calcium, and cyclic AMP (cAMP) (87–89). The binding of acetylcholine, histamine, and gastrin to the parietal cell receptors results in an increase in intracellular calcium. Histamine activates adenylate cyclase, which catalyzes the synthesis of cAMP from ATP (42, 49, 89). Calcium and cAMP-dependent protein kinases are involved in the activation of the acid-secreting proton pump (42, 84, 87, 112).

The basic mechanisms involved in acid secretion by the parietal cell are presented in Figure 1. Carbon dioxide diffuses into the parietal cell from the blood side of the cell and combines with water to form HCO₃⁻ and H⁺ through the reaction catalyzed by carbonic anhydrase. The bicarbonate passes out of the parietal cells into the blood in exchange for chloride ions via the HCO₃⁻/Cl⁻ exchange protein. Chloride ions are eventually released from the parietal cells into the lumen of the gastric gland via a chloride ion channel. Hydrogen ions are released into the gastric gland lumen in exchange for K⁺ ions through the action of the H⁺/K⁺-ATPase (i.e., the proton pump). One hydrogen

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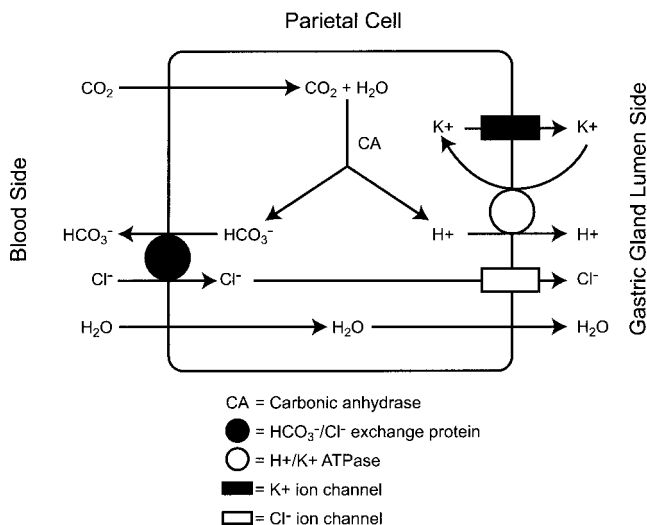


FIGURE 1. Mechanisms involved in the secretion of HCl by parietal cells.

ion is exchanged for a potassium ion for each ATP molecule hydrolyzed. The cytosolic K⁺ is transported back into the gastric gland lumen via K⁺ ion channels (42, 49) (Fig. 1).

ROLE OF SALIVARY NITRITE IN GASTRIC ACID ANTIBACTERIAL ACTION

A number of studies have indicated that acidic gastric juice is more effective in killing bacteria if nitrite is present. The major sources of nitrite in the human diet are fruits, vegetables, cereals, and water containing nitrate (29, 64). Ingested nitrate is absorbed from the gastrointestinal tract into the circulatory system, and up to 25% of this nitrate is eventually concentrated in the salivary glands. Nitrate is then secreted in the saliva, in which it is reduced to nitrite by mouth bacteria (28, 29, 64). Nitrite is mixed with gastric juice when saliva is swallowed. The major sources of nitrite in the human diet are not foods containing nitrite that was added during processing, but foods that naturally contain nitrate.

With the use of conditions simulating a normal stomach, Dykhuizen et al. (31) demonstrated that *Salmonella* Enteritidis, *Salmonella* Typhimurium, and *Yersinia enterocolitica* were killed after 30 min of exposure to nutrient broth acidified to pH 2.1 with HCl. Under the same conditions, *Shigella sonnei* and *Escherichia coli* O157 survived unless nitrite was present. The level of bacteria used was ca. 10⁷ CFU/ml. In nutrient broth acidified to pH 3.0, the addition of nitrite was necessary to kill all of the bacteria tested by Dykhuizen et al. (31). Other workers showed that nitrite present in nutrient broth acidified to a pH of 3.0 to 3.3 was microbicidal to *E. coli* and *Candida albicans*; however, acid conditions in the absence of nitrite did not lead to the killing of these pathogens (11, 120). These authors concluded that salivary nitrite present in gastric fluid may play an important role in a host's defense against ingested pathogens.

Data from an unpublished thesis cited by McKnight et al. (64) indicate that human gastric fluid obtained endo-

scopically exerted a killing effect on *Salmonella* Typhi and that the addition of artificial saliva containing nitrite to the gastric fluid enhanced the killing of the organism. The experiments reported by Benjamin et al. (11), Dykhuizen et al. (31), McKnight et al. (64), and Xu et al. (120) strongly suggest that salivary nitrite and the gastric acid of the stomach act synergistically to inactivate foodborne pathogens prior to the entry of these pathogens into the gastrointestinal tract. Archer (2) has recently reviewed the role of salivary nitrite potentiation of the antibacterial action of stomach acid.

NEUTRALIZING GASTRIC ACID OR INHIBITING GASTRIC ACID SECRETION

There are a number of ways to reduce gastric acid or inhibit its formation. HCl secreted by the stomach can be neutralized through the oral administration of absorbable antacids such as sodium bicarbonate or calcium carbonate. Nonabsorbable antacids such as aluminum hydroxide or magnesium hydroxide are also effective in neutralizing stomach acid (66). Acid secretion by the stomach can also be controlled surgically. Vagotomy is the surgical cutting of certain branches of the vagus nerve, thereby preventing the release of acetylcholine, which is a potent stimulator of gastric acid secretion. Another surgical procedure, gastrectomy, the removal of all or part of the stomach, results in a loss of the acid-secreting parietal cells (1, 49).

Cholinergic (muscarinic) receptor antagonists block gastric secretion by binding to the M₁ muscarinic receptors on postganglionic neurons to prevent the release of acetylcholine and its subsequent binding to parietal cells (99). Anticholinergic agents that inhibit acid secretion include atropine, pirenzepine, glycopyrrolate, and propantheline. Histamine H₂ receptor antagonists are competitive and reversible inhibitors of gastric acid secretion. These compounds inhibit the binding of histamine at the histamine H₂ receptor sites on the parietal cells (98, 99). Cimetidine, ranitidine, famotidine, and nizatidine are histamine H₂ antagonists that effectively inhibit gastric acid secretion. The substituted benzimidazoles, omeprazole, and lansoprazole (proton pump inhibitors) prevent gastric acid secretion by inhibiting the enzyme H⁺K⁺-ATPase (i.e., the proton pump). These compounds are acid-active prodrugs that noncompetitively bind and irreversibly inactivate the ATPase by binding covalently to two cysteine residues of the enzyme (88, 98).

Nalin et al. (70), in a study involving volunteers who ingested *Vibrio cholerae*, found that heavy smokers of cannabis had decreased levels of stomach acid and were more susceptible to the diarrhea induced by *V. cholerae* than were light smokers or nonsmokers. Gastrin- and histamine-induced gastric acid synthesis is inhibited by the binding of cannabinoids to CB₁ receptors (78). Fever has also been reported to inhibit gastric acid secretion (48). Cytokines stimulate the synthesis of prostaglandins, particularly prostaglandin E₂, which is considered the ultimate endogenous inducer of the febrile response (97). Prostaglandins prevent gastric HCl secretion by inhibiting adenylate cyclase synthesis of cAMP (42, 112).

DISEASE STATES THAT INDUCE HYPOCHLORHYDRIA OR ACHLORHYDRIA

A number of factors can lead to hypochlorhydria (i.e., a low level of HCl in the stomach) or achlorhydria (i.e., a lack of HCl in the stomach) with a concurrent decrease or loss of the gastric acid protective mechanism. For example, autoimmune and environmental atrophic gastritis can result in a loss of acid secretion. Autoimmune atrophic gastritis is a genetic disease in which there is an immune response against the parietal cells (121). The loss of the parietal cells leads to achlorhydria as well as to the loss of the parietal cell-derived intrinsic factor that facilitates vitamin B₁₂ absorption. Therefore, individuals with autoimmune atrophic gastritis will develop pernicious anemia (121). There is no treatment for the achlorhydria of autoimmune atrophic gastritis, but pernicious anemia can be treated with regular vitamin B₁₂ injections.

Environmental atrophic gastritis is a form of chronic gastritis associated with environmental factors, such as a diet containing a high level of pickled vegetables, salted fish, or smoked meat or *Helicobacter pylori* infection (121). Gastric acid secretion may be reduced in environmental atrophic gastritis, but it does not disappear completely; antiparietal cell antibodies are not present (121). More than 50% of patients with an acute *H. pylori* infection will have a transient hypochlorhydria for approximately 4 months (range, 2 to 8 months) (45, 118). Interleukin 1 β (IL-1 β) is upregulated by *H. pylori* infection; interleukin 1 β is a potent inhibitor of gastric acid secretion and may explain why hypochlorhydria is induced by *H. pylori* infection (33). Antibiotic treatment will eliminate *H. pylori*, with a resultant correction of the hypochlorhydric state. Individuals experiencing hypochlorhydria or achlorhydria are more susceptible to infections from food or waterborne agents (see "Effect of Gastric Acidity on Enteric Pathogens" section).

EFFECT OF FOOD ON GASTRIC EMPTYING AND GASTRIC ACIDITY

Gastric emptying time, that is, the length of time food remains in the stomach, is influenced by certain characteristics of food. Ingested liquids begin to empty into the duodenum almost immediately, whereas solid foods leave the stomach only after a lag period during which they are reduced to 1- to 2-mm particles (1, 49). The emptying rate for both solids and liquids depends on the chemical composition of these materials. A high level of fat, a low pH, a high viscosity, and/or a high caloric content delay gastric emptying of the ingested materials (49, 63).

Studies suggest that both gender and age influence gastric emptying. However, the effect of age on gastric emptying is not clear, since conflicting results have been obtained. Graff et al. (41) studied the time required for water or an omelet meal to clear the stomach by comparing the process for young men and women (i.e., 20 to 30 years old) with that for older men and women (i.e., 38 to 53 years old). Gastric emptying was significantly slower for women than for men (77.4 min versus 75.6 min, respectively, for liquid; 112.8 min versus 104.4 min, respectively, for solid

food). Emptying times for older individuals were accelerated compared with those for younger persons (50.4 min versus 96.0 min, respectively, for liquid; 104.4 min versus 124.8 min, respectively, for solid food) (41). In contrast, when Clarkston et al. (18) compared gastric emptying times for individuals aged 23 to 50 years (mean age, 30 years) with those for individuals aged 70 to 84 years of age (mean age, 76 years), they found that the elderly group had significantly longer emptying times for both liquids (47 min versus 35 min) and solids (182 min versus 127 min) than did the younger group. In total, 65 individuals were studied by Graff et al. (41) and Clarkston et al. (18), and the conflicting results obtained with respect to the effect of aging on gastric emptying may be due to the small number of individuals studied and to physiological differences between the two populations.

The entrance of food into the stomach leads to a transient elevation in gastric pH. The median fasting gastric pH for a group of elderly individuals ($n = 79$; mean age, 71 years; range, 65 to 83 years) was 1.3, compared with 1.7 for a group of young individuals ($n = 24$; mean age, 25 years; range, 21 to 35 years). After a standard meal comprising hamburger, bread, potatoes, and milk, the peak gastric pH for the elderly group reached 6.2, compared with 6.6 for the young group (26, 86). The times taken for the gastric pH to return to 2.0 were 100 min for the young group and 150 min for the elderly group. Thus, the fasting gastric pH and the peak pH reached after the ingestion of a meal were only slightly higher for the young group than for the elderly group; however, the time taken for the gastric pH to return to 2 was significantly longer for the elderly population. Both study groups contained men and women, and Dressman et al. (26) and Russell et al. (86) found no significant differences with respect to gender in their results.

The effect of an increase or decrease in gastric emptying time or of the temporary elevation of the gastric pH on the survival of bacteria in the stomach has received little attention. It is logical to assume that if the gastric emptying time is short, as it is for liquids, then pathogens have less contact with gastric acid and viable organisms may pass through the stomach into the intestine. While there are no data available concerning the effect of a temporary increase in stomach pH after eating on the survival of bacteria, it is possible that if a return to more acid conditions is slow, organisms may be better able to survive and pass into the intestinal tract.

EFFECT OF FOOD ON PATHOGEN SURVIVAL

In a study involving the inoculation of the acid-sensitive enteric pathogens *Salmonella* Typhimurium, *Salmonella* Typhi, *Campylobacter jejuni*, and *V. cholerae* (at ca. 10⁶/0.1 g) individually onto ground beef, Waterman and Small (117) found that the organisms survived for at least 2 h at 37°C in Luria-Bertani broth acidified to pH 2.5 with HCl; in the absence of ground beef, the organisms did not survive the 2-h acid treatment. When the acid-sensitive enteric pathogens were inoculated onto boiled rice or boiled egg white, only the pathogens inoculated onto boiled egg white

survived in pH 2.5 broth. Waterman and Small (117) postulated that a high protein content in a food may protect bacteria against the killing effects of gastric acid.

Fischer rats were used by Drouault et al. (27) to model the effect of food on the gastric acid killing of bacteria. When 10^9 CFU of *Lactococcus lactis* per ml was fed to rats in the absence of food, only 7% of the organisms survived in the stomach. However, when *L. lactis* was mixed with the rats' food, 100% of the organisms survived. Drouault et al. (27) postulated that the acid-buffering capacity of the rat diet protected the lactococci against the killing effect of gastric acid.

Gastric fluid obtained from fasting healthy volunteers and adjusted to pH 1.5 with HCl killed approximately 10^3 enterotoxigenic *E. coli*, *Shigella flexneri*, and *Salmonella* Typhimurium cells within 5 min at room temperature (79). However, there was an approximately two- to fourfold increase in survival after 5 min when the enteric pathogens were suspended in postfeeding gastric fluid adjusted to pH 1.5. The gastric fluid was collected from the volunteers 20 min after they had ingested a meal of steak and toast (79). Thus, Peterson et al. (79) demonstrated that postmeal gastric fluid was less effective in killing bacteria. Since the postmeal gastric fluid was adjusted to pH 1.5, the protective effect was not simply due to buffering of the gastric fluid acid by the food. Peterson et al. (79) did not speculate on how food protected the bacteria from gastric acid.

While the mechanism is not completely clear, it is apparent that certain foods do protect bacteria from acidic conditions and from gastric acidity in vitro. More research, particularly research involving in vivo experiments, is needed to assess how foods protect bacteria from the acid environment of the stomach.

PEPTIC ULCER DISEASE

The antibacterial action of gastric acid may fail during certain courses of treatment for peptic ulcer diseases such as gastric and duodenal ulcers. A peptic ulcer is characterized by an area of acid and pepsin damage to the mucous membrane that penetrates through the muscularis mucosa of the stomach or other parts of the digestive system. In the United States, the lifetime prevalence of peptic ulcer disease is about 10% (1); approximately 500,000 new cases and 4,000,000 recurrences of gastric and duodenal ulcers occur each year (113). Since peptic ulcer disease occurs only if the stomach secretes acid, it is generally treated with antacids, H_2 receptor antagonists, proton pump inhibitors, or other inhibitors of gastric acid secretion (113); such treatments increase the risk of food- or waterborne illness (see "Effect of Gastric Acidity on Enteric Pathogens" section).

H. pylori has been identified as the etiologic agent for >90% of duodenal ulcers and ca. 80% of gastric ulcers (39, 45). *H. pylori* infection can be effectively eliminated by treatment with antibiotics, which is usually followed by rapid healing of the ulcers. The use of a triple regimen consisting of a bismuth salt combined with metronidazole (or clarithromycin) and tetracycline (or amoxicillin) is generally successful in eradicating *H. pylori* from the stomach

(23, 45). A quadruple treatment incorporating a proton pump inhibitor (i.e., an inhibitor of the enzyme H^+K^+ -ATPase) along with bismuth and two antibiotics (see above) can be used if the triple therapy does not eradicate the organism (55). Therefore, only ulcers that are not caused by *H. pylori* infections or are refractory to antibiotic treatment should be treated with drugs that neutralize or prevent the secretion of gastric acid.

EFFECT OF GASTRIC ACIDITY ON ENTERIC PATHOGENS

Most ingested enteric pathogens are quite susceptible to acidic gastric juice, but this susceptibility decreases if the host has undergone a gastrectomy or is taking drugs that neutralize gastric acid or inhibit gastric acid secretion.

Bacteria. Individuals with hypochlorhydria or achlorhydria resulting from malnutrition or a prior gastrectomy were found to be more susceptible to infection with *V. cholerae* and often had a more severe disease (38, 90, 92). Volunteers who drank an inoculum of *V. cholerae* (10^8 CFU) with bicarbonate demonstrated a higher attack rate than volunteers who consumed the organisms without bicarbonate. In addition, bicarbonate decreased the number of bacteria necessary to cause disease from 10^8 to 10^4 organisms per individual (15, 46). The mean decimal reduction times for three strains of *V. vulnificus* exposed to simulated gastric juice at pH 4.0 and at pH 3.0 were 3.3 and 1.3 min, respectively. However, *V. vulnificus* strains did not survive if the pH of the simulated gastric juice was lowered to 2 (54).

For patients with acute salmonellosis, morbidity and mortality rates were highest for those individuals who had previously undergone a gastrectomy (44, 72, 74, 116). Recent treatment with histamine H_2 antagonists was associated with a twofold increase in the risk of *Salmonella* infections (72). With the use of aspirated gastric juice obtained from achlorhydric pernicious anemia patients or from normal individuals and adjusted to a pH of either 2.0 or 4.0, Giannela et al. (37) found that ca. 10^9 *Salmonella* Typhimurium, *Salmonella* Paratyphi, or *Salmonella* Enteritidis cells per ml were killed in about 30 min at pH 2.0 but survived for at least 120 min at pH 4.0. The killing of *Salmonella* species in pH 6.8 gastric juice collected from pernicious anemia patients and adjusted to pH 2.0 with HCl indicated that the major killing action of gastric juice resides in the HCl content (37). As another example, a 5.5-log_{10} decrease in the number of *Salmonella* Typhimurium DT104 cells was observed within 5 min in simulated gastric fluid adjusted to pH 1.5 (83).

The use of omeprazole, a proton pump inhibitor, predisposes individuals to *Campylobacter* infections (73). The use of this drug was associated with a 10-fold increase in the risk of infection. No risk of campylobacteriosis was noted with the use of histamine H_2 antagonists. Proton pump inhibitors reduce gastric acidity to a greater extent than histamine H_2 antagonists do (89).

In a foodborne outbreak of hospital-acquired listeriosis in Boston hospitals, patients receiving antacids or histamine H_2 antagonists were more likely than control patients to be

infected with *Listeria monocytogenes* (43). Cobb et al. (19) noted that there was an increased prevalence of *L. monocytogenes* fecal carriage for female patients receiving long-term treatment with histamine H₂ antagonists. The treated patients had a carriage rate of 20%, compared with a rate of 2.1% for control patients; however, listeriosis did not develop in any of the patients (19). Animal experiments conducted by Schlech et al. (93) indicated that the reduction of gastric acidity by the histamine H₂ antagonist cimetidine lowered the dose of *L. monocytogenes* necessary to induce an invasive infection in rats. The oral 50% infective dose for animals treated with cimetidine was 10² CFU, compared with an oral 50% infective dose of 10⁶ CFU of *L. monocytogenes* for untreated rats (93). Roering et al. (83) demonstrated that three strains of *L. monocytogenes* at 6 log₁₀ CFU/ml were killed by 30 min of exposure to simulated gastric juice at pH 1.5. These data suggest that gastric acid is bactericidal to *L. monocytogenes* and that drugs that inhibit or neutralize gastric acid increase the susceptibility of individuals to infection by this organism.

In a nursing home outbreak caused by *E. coli* O157:H7, elderly patients who had previously undergone a gastrectomy had an increased risk of infection (14). An *E. coli* O157:H7 outbreak in central Scotland revealed that one of the risk factors for hemolytic uremic syndrome was hypochlorhydria due to a gastrectomy or the use of proton pump inhibitors and/or histamine H₂ antagonists (30). Hypochlorhydria was significantly associated with death for these hemolytic uremic syndrome patients. Arnold and Kaspar (3) demonstrated that four of five strains of *E. coli* O157:H7 survived for 3 h in synthetic gastric juice at pH 1.5. In simulated gastric juice at pH 1.5, three strains of *E. coli* O157:H7 survived for at least 2 h, in contrast to survival times of 5 min for strains of *Salmonella* Typhimurium DT 104 and 30 min for strains of *L. monocytogenes* (83). With the use of an in vitro system to model the gastric acid inactivation of an *E. coli* O157:H7 strain, Takumi et al. (108) estimated that 20 to 80% of the ingested organisms would reach the small intestine in a viable state. Thus, the in vitro data obtained by Arnold and Kaspar (3), Roering et al. (83), and Takumi et al. (108) indicate that *E. coli* O157:H7 can survive the low pH of the stomach and pass into the intestine.

The influence of surgery or drugs that induce hypochlorhydria or achlorhydria on enteric infections has been well studied; however, the influence of malnutrition on gastric acid secretion has received little attention. Nalin et al. (71) and Cook (20) suggested that malnutrition-induced hypochlorhydria is common in developing countries and accounts for the prevalence of enteric diseases in those countries. Elderly individuals have a number of risk factors, e.g., type of medication, changes in mental status, and digestive disorders, that lead them to reduce their nutrient intake sufficiently to cause malnutrition (102) with possible hypochlorhydria, which may make them more susceptible to enteric illness.

Parasites. Trophozoites of *Giardia lamblia*, which are not resistant to gastric acid, can colonize the stomach mu-

cosa during hypochlorhydria. Results obtained by Doglioni et al. (25) for gastric biopsy specimens isolated from Italian patients undergoing upper gastrointestinal endoscopy indicated that 0.27% (41 of 15,023) of the patients involved in the study had gastric giardiasis. All 41 patients were suffering from chronic atrophic gastritis, and 37 of these patients were infected with *H. pylori*. Hypochlorhydria is a feature of atrophic gastritis due to a decrease in the number of parietal cells, and *H. pylori* infections can induce hypochlorhydria. In addition, 5 of 41 patients were being treated with antacids (25). The decreased gastric acidity allowed the colonization of the stomach by *G. lamblia*. Sanad et al. (91) also reported that under conditions of reduced gastric acidity, gastric giardiasis may occur in conjunction with intestinal giardiasis.

It is probable that the environmentally resistant forms (i.e., oocysts, cysts, and eggs) of foodborne parasites such as *Cryptosporidium*, *Cyclospora*, *Giardia*, *Taenia*, and *Toxoplasma* survive passage through the stomach. Most *Toxoplasma* infections are acquired through the ingestion of meat from infected animals in which the parasite had encysted in the tissues as walled bradyzoites. Asymptomatic *Toxoplasma* infections (evidenced by seropositivity) are common in meat-eating populations including that of the United States (101), and Mead et al. (65) stated that approximately 40% of individuals in the United States \geq 60 years of age are seropositive for *Toxoplasma*. Thus, the high prevalence of *Toxoplasma* seropositivity in humans indicates that bradyzoites of *Toxoplasma* are resistant to gastric acid even though they lack the extreme resistance of *Toxoplasma* oocysts (101).

Viruses. The half-life infectivity of several rotavirus strains suspended in pH 1.8 human gastric fluid was \leq 1 min (119). Nonetheless, human rotaviruses have been detected in the stools of 35 to 52% of infants and young children hospitalized with acute gastroenteritis but less often in older children and adults with gastroenteritis (50). More than 90% of children have rotavirus antibodies by age 3 (7). Gastric acid production in response to food and hormonal stimulation is normal in healthy infants (100). However, the buffering action of milk and the short gastric emptying time for infants (81) may permit the rapid passage of a rotavirus through the infant stomach.

Hepatitis A virus was found to retain infectivity in tissue culture after exposure for 5 h to pH 1.0 KCl-HCl buffer at room temperature and for up to 90 min after exposure at 38°C (94). The data obtained by Scholz et al. (94) suggest that hepatitis A virus may survive passage through the acid stomach. However, more research is needed to determine whether food- and waterborne viruses are effectively inactivated by gastric acid in vivo.

INDUCTION OF ACID RESISTANCE IN FOODBORNE PATHOGENS

While hypochlorhydria and achlorhydria are conducive to foodborne illness, not all cases of illness are due to decreased gastric acidity in the affected individuals. Healthy people with no gastric problems may develop foodborne

diseases because many pathogens have mechanisms that allow these pathogens to adapt to acidic conditions (34). For example, the neutrophilic *Salmonella* Typhimurium and *E. coli* have a preference for living and growing at a neutral pH but also have a variety of adaptive mechanisms that allow them to respond to potentially lethal challenges posed by acid stresses present in the external environment or in the animal host. Studies on acid resistance indicate that growth phase, growth conditions, and prior exposure to an acidic pH all play important roles in the development of acid resistance in bacteria.

***Salmonella* Typhimurium: log-phase ATR in minimal medium.** The log-phase acid tolerance response (ATR) is induced by the growth of *Salmonella* Typhimurium at pH 4.5 to 5.8, which allows the cells to survive subsequent exposure to a pH 3.0 environment for several hours. Prior growth of *Salmonella* Typhimurium in a medium acidified with an inorganic acid such as HCl allows the cells to survive not only exposure to HCl but also exposure to volatile fatty acids such as acetic acid. Prior growth of the organism in the presence of volatile fatty acids also provides protection against HCl (4). In addition, the exposure of log-phase cells to an acid stress protects them against a number of other stresses, such as oxidative stress or heat stress. However, exposure to oxidative stress or other stresses does not lead to protection against acid stress (4, 57).

The log-phase ATR involves the induction of emergency pH homeostasis systems by mild acid treatment (with a pH of >4.5), which allows the cells to maintain an internal pH of >5.0 as the organisms encounter a more severe external pH. Several inducible amino acid decarboxylases contribute to emergency internal pH maintenance in *Salmonella* Typhimurium. Emergency pH homeostasis, in turn, allows the synthesis of acid shock proteins (ASPs) at pHs of ≤ 4.5 . Approximately 60 ASPs are formed, and these ASPs protect the cellular machinery against acid damage (4, 9, 62). Three regulatory proteins control the expression of distinct sets of ASPs in log-phase ATR: the alternative sigma S factor (σ^s) (encoded by *rpoS*), the two-component sensor regulatory system PhoP/Q (encoded by *phoP* and *phoQ*), and the ferric uptake regulator Fur (encoded by *fur*) (35). A rapid shift to acid shock conditions for *Salmonella* Typhimurium increases the transcription of σ^s , and at the same time the concentration of σ^s increases due to decreased proteolytic turnover. Increased levels of σ^s drive the expression of σ^s -dependent ASPs, thereby leading to an increase in acid stress tolerance (4, 10).

Fur normally acts as a repressor of gene expression when it is bound to intracellular iron, but under acid shock conditions, Fur controls the synthesis of a subset of ASPs in an iron-independent manner. It is not known how Fur mediates ASP synthesis (4, 34). The regulators σ^s , Fur, and Ada (the *ada* gene is involved in DNA repair but not in ASP synthesis; *ada* mutants are acid sensitive) play important roles in tolerance to organic acids but are of minor importance with regard to inorganic acid stress (4, 8). The two-component regulatory system PhoP/Q protects against inorganic but not organic acid stress (8). The PhoP protein

is required for the low-pH induction of a subset of protective ASPs and is an ASP itself; *phoP* mutants are acid sensitive (8).

***Salmonella* Typhimurium: stationary-phase ATR in minimal medium.** The ASPs and regulatory proteins involved in stationary-phase ATR are different from those involved in log-phase ATR. As the cells enter the stationary phase, a general stress response that is pH independent but σ^s dependent is induced. Non-acid-adapted *Salmonella* Typhimurium in the stationary phase are at least 1,000-fold more acid resistant than log-phase cells and can tolerate pH 3.0 for approximately 4 h (4). In addition to the σ^s -regulated general stress response, there is also an acid-induced ATR in stationary-phase cells. Stationary-phase cells that are acid shocked at pH 4.5 can tolerate longer exposures to pH 3.0 than non-acid-shocked cells can. This acid-inducible stationary-phase ATR is σ^s independent but does depend on the two-component response regulator OmpR. Acid shock induces the production of OmpR, which in its phosphorylated state activates the expression of genes necessary for acid-induced stationary-phase ATR (5, 6). Stationary-phase *Salmonella* Typhimurium cells with insertional mutants in the *ompR* gene are acid sensitive, and the acid-inducible stationary-phase ATR is almost completely eliminated (4). The acid-inducible ATR is complex, with ca. 50 ASPs (OmpR is also an ASP) being induced during stationary-phase acid shock. There is little overlap between ASPs involved in acid-induced log-phase ATR and those involved in acid-induced stationary-phase ATR (4–6).

***E. coli* and *S. flexneri*.** Similar to the case for *Salmonella* Typhimurium, log-phase ATR, stationary-phase ATR, and the stationary-phase general stress responses are present in *E. coli*. *S. flexneri* does not have a detectible log-phase or stationary-phase ATR but does demonstrate the general stationary-phase stress response (34, 59, 62). Similar to the case for *Salmonella* Typhimurium, log-phase *E. coli* O157:H7 exposed to an acidic pH (pH 5.0 for 4 h) were protected against other stresses. The thermal and NaCl tolerances of acid-adapted cells were more extensive than those of non-acid-adapted cells (17).

***E. coli* and *S. flexneri*: log-phase acid habituation.** *E. coli* grown to the log phase in nutrient broth at pH 5.0 survived exposure to pHs of 3.0 to 3.5, whereas cells grown at pH 7.0 did not. Cells grown at pH 5.0 survived exposure to pH 3.5 for 25 min or to pH 3.0 for 7 min (9, 34, 40). Acid habituation can also be induced in cells grown at pH 7.0 in nutrient broth if glucose, glutamate, aspartate, FeCl₃, KCl, or L-proline is added to log-phase cells (34, 40, 85).

***E. coli* and *S. flexneri*: acid resistance.** Acid resistance (AR) systems, found in stationary-phase cells grown in complex media, permit the survival of *E. coli* and *S. flexneri* when they are exposed to pH 2. There are three AR systems: AR system 1 (oxidative, glucose repressed), AR system 2 (fermentative, glutamate dependent), and AR system 3 (fermentative, arginine dependent). The system induced depends on the type of medium and the growth conditions (4).

The σ^S -dependent, cAMP receptor protein-dependent, and cAMP-dependent glucose-repressed oxidative AR system (AR system 1) is induced when *E. coli* or *S. flexneri* cells are grown to the stationary phase in a glucose-free complex medium at pH 5.5. These cells survive acid challenge at pH 2.5 for several hours in minimal media (4, 16, 34). It is not clear how AR system 1 protects cells against acid challenge.

The glutamate-dependent AR system (AR system 2) is induced in cells of *E. coli* or *S. flexneri* grown to the stationary phase in a glucose-containing complex medium at pH 5.0. The cells can survive at pHs of 2.0 to 2.5 for several hours in minimal media containing glutamate. Of the three known AR systems, the glutamate-dependent AR system provides the highest level of protection against low pHs (4, 34).

The glutamate-dependent AR system depends on the induction of glutamate decarboxylase. During the decarboxylation of glutamate, protons that leak into bacterial cells during acid stress are taken up to form γ -aminobutyric acid (GABA). The GABA is transported out of the cell in exchange for glutamate via the glutamate:GABA antiporter. The GABA mechanism for sequestering protons is quite effective in preventing the internal pH of the cell from dropping to a lethal level (4, 16, 34).

There are three genes necessary for the glutamate decarboxylase system. The genes *gadA* and *gadB* encode for highly homologous glutamate decarboxylase isoforms, and the gene *gadC* encodes for the glutamate:GABA antiporter (16). Either *gadA* or *gadB* is sufficient for the survival of acid-stressed cells at pH 2.5, but both *gadA* and *gadB* are needed for survival at pH 2.0.

The arginine-dependent AR system (AR system 3) involves the induction of arginine decarboxylase when *E. coli* cells are grown to the stationary phase in a glucose-containing complex medium. Such cells are able to survive for several hours when challenged in an arginine-supplemented minimal medium at pH 2.5. For AR system 3, the structural gene for arginine decarboxylase, *adiA*, and the regulatory genes, *cysB* and *adiY*, must be present (4, 16, 34, 60). The expression of *adiA* is activated by the regulatory protein CysB, a global regulator for sulfur assimilation from inorganic sulfate and organic sulfur compounds in *E. coli* (114). It is not clear why *adiA* is regulated by CysB. The arginine-dependent AR system acts very much like the glutamate-dependent AR system. Intracellular arginine is decarboxylated to agmatine, and a proton is taken up during the reaction. The agmatine is transported out of the cell via an unknown mechanism (34). System 3 AR has not been demonstrated for *S. flexneri* (4).

Once the AR systems are induced in serotype O157:H7 strains of *E. coli* (and probably *S. flexneri*), the activity will persist at refrigeration temperatures for at least 1 month (60). The observation that AR is maintained for long periods during cold storage has obvious implications with respect to foodborne illness.

Lin et al. (60) have demonstrated that AR systems 2 and 3 protect *E. coli* O157:H7 against volatile organic acids such as acetic, butyric, and propionic acids, as well as HCl.

Thus, the AR systems probably protect cells against gastric acidity as well as against the volatile fatty acids produced by bacteria present in the intestine.

***H. pylori* and *Y. enterocolitica*.** At least two microorganisms, *Y. enterocolitica* and *H. pylori*, use urease for survival in the acid stomach. In humans, the metabolic breakdown of proteins leads to ammonia formation. The ammonia is detoxified in the liver via conversion to urea, which is secreted into the bloodstream and sequestered by the kidneys and is eventually excreted in urine (109). Individuals with normal blood urea nitrogen levels have blood urea and gastric juice urea levels of approximately 4.8 and 3.3 mM, respectively (53). The presence of urea in the stomach is due to its diffusion from the blood circulatory system through mucosal tissues into the stomach (105).

In the presence of urea, *H. pylori* survived in McIlvain's buffer at pH 4.0, but survival was poor when urea was omitted (77). Urease is an essential enzyme in *H. pylori*. The major portion of urease is located intracellularly, with <0.2% of the enzyme being located at the surface of the cell (95). Since urea is present in the stomach, it is the production of ammonia by urease that allows *H. pylori* to overcome the acidic conditions and to colonize the stomach (34, 105). Urease-negative mutants of *H. pylori* are unable to colonize the mouse stomach (32, 111).

The inner membrane protein UreI is essential for *H. pylori* survival at low pHs and for the gastric colonization of mice. The UreI protein is an acid-activated urea channel that allows rapid transit (at low pHs) of external urea to the intracellular urease of the organism (13). A low external pH stimulates the entry of urea via UreI, thereby leading to the production of ammonia by the urease present in the cytoplasm. The protons present in the gastric fluid diffuse into the *H. pylori* cytoplasm and are bound by ammonia (106, 107). The protonated ammonia (i.e., NH_4^+) exits the cell via an unidentified NH_4^+ exporter. Urease activity in *H. pylori* serves to maintain the cytoplasmic pH close to neutrality when the organism is exposed to the gastric acidity of the stomach (106, 107).

In addition to having the urease system to increase acid resistance, *H. pylori* has a urea-independent ATR system. Cells preexposed to pH 5 or pH 6 were found to survive exposure to pH 3 one hundred times as well as cells preexposed to pH 7 did (52). The iron uptake regulator Fur is also involved in acid resistance in *H. pylori*. Upon exposure to pH 3.5 or pH 4.8, wild-type *H. pylori* and *fur* mutants of *H. pylori* showed similar rates of survival; however, the growth rate for *fur* mutants at pH 5.0 was approximately 10-fold lower than that for the wild type (12). Thus, the presence of the *fur* gene does not affect the survival of *H. pylori* following acid shock but is necessary for growth under acid conditions. The involvement of *fur* in the growth of *H. pylori* at acid pHs is urease and iron independent (12).

Stationary-phase *Y. enterocolitica* suspended in pH 2.5 phosphate-buffered saline containing 0.1 to 10 mM urea survived the acid conditions for at least 2 h. None of the cells survived at pH 2.5 in the absence of urea (24). The rate of recovery of wild-type *Y. enterocolitica* from the ilea

of mice receiving an oral inoculum of the organism was 100%; however, the rate of recovery of a urease-negative mutant from mice ilea was only about 10% (24).

The *yut* gene present in *Yersinia* species encodes the urea-transporting protein Yut. The mechanism of Yut urea is transported via channel-mediated diffusion. Although they are structurally unrelated, *Yersinia* Yut and *H. pylori* Urel are functionally interchangeable (96). The acid sensitivity of *Yersinia* resulted when the *yut* gene was inactivated. The insertion of *urel* into *Yersinia* or the insertion of *yut* into *H. pylori* led to channel-mediated diffusion of urea into the bacterial cells (96). The urease of *Y. enterocolitica* has an optimum pH of 5.0, with little urease activity taking place at neutral pHs. In the acid stomach, the intracellular urease of *Y. enterocolitica* maintains the cytoplasmic pH at ca. 5.0 through the production of ammonia from urea (95). An internal pH of 5.0 is incompatible with growth, and therefore *Y. enterocolitica* cannot colonize the stomach but can resist the acid conditions and pass into the intestinal tract (95).

It is clear that urease action is necessary for the survival of *Y. enterocolitica* and *H. pylori* under acid conditions. It is urease activity that allows the passage of *Y. enterocolitica* through the stomach and into the intestinal tract. Urease activity is responsible for the survival of *H. pylori* survival in the stomach and for the subsequent colonization of that organ by the organisms.

Other bacteria. Other foodborne pathogens exhibit the induction of an ATR when they are exposed to mild acid conditions (ca. pH 5.0) for a short period, with resulting protection against acid stress at pHs of 3.0 to 3.5. Such pathogens include *Aeromonas hydrophilia* (51), *Clostridium perfringens* (115), *L. monocytogenes* (75, 76, 80), and *V. cholerae* (67–69). In addition to having an ATR system, *L. monocytogenes* cells also have AR system 2, the glutamate decarboxylase system. The glutamate decarboxylase system protects *L. monocytogenes* against the environment of gastric juice at pH 2.5 (21) and against the acid environment present in low-pH foods (22).

RELEVANCE OF BACTERIAL ACID ADAPTATION

It has been reported that the acid-adaptive mechanisms employed by bacteria are quite effective in “real-life” situations, which indicates that these mechanisms play a vital role in the survival of these organisms in acid foods or during passage through the acid stomach. For example, *Salmonella* Typhimurium induced for log-phase ATR by adaptation to HCl-containing medium (pH 5.8) survived at least 10 days longer than did unadapted cells in mozzarella, Cheddar, and Swiss cheeses stored at 5°C (56).

Overnight cultures of *E. coli* O157:H7 kept in nutrient broth at pH 5.0 (acidified with HCl) for 4 to 5 h showed survival rates that were ca. 100-fold higher than those for unadapted cells during lactic acid bacterial fermentation of meat to pH 4.4 (58). There was a ~10-fold increase in the survival rate for acid-adapted *E. coli* O157:H7 after 4 days in a hard salami (pH 5.0) stored at 5°C compared with the

rate for unadapted cells. In addition, acid-adapted *E. coli* O157:H7 survived four times as long as unadapted cells in apple cider at pH 3.5 stored at 6°C (58). The protection against these acidified foods observed for *E. coli* O157:H7 was probably due to the induction of log-phase ATR.

Hovde et al. (47) demonstrated that *E. coli* O157:H7 present in cattle feces were resistant to acid conditions (pH 2.0, 1 h, 37°C). *E. coli* O157:H7 residing in the ruminant gastrointestinal tract has AR systems 1 and 2 (4). In a study involving *rpoS* mutants of *E. coli* O157:H7, Price et al. (82) found that σ^s is required for the recovery of viable organisms following passage through the gastrointestinal tracts of mice and calves. The need for σ^s suggests the involvement of AR system 1. *E. coli* O157:H7 mutants lacking the *gadC* gene did not survive passage through the ruminant intestinal tract, indicating that AR system 2 is also necessary for the survival of *E. coli* O157:H7 in ruminants (4). However, only the σ^s -dependent AR system 1 was required for the survival of *E. coli* O157:H7 in apple cider at pH 3.5, since *gadC* and *adiA* mutants survived as well as wild-type *E. coli* O157:H7 did (4).

Log-phase *L. monocytogenes* adapted to lactic acid at pH 5.5 survived longer in yogurt at pH 3.9, in cottage cheese at pH 4.7, in orange juice at pH 3.8, and in salad dressing at pH 3.0 than unadapted cells did (36). There was no inactivation of acid-adapted *L. monocytogenes* during milk fermentation (with a final pH of 4.2) with the use of a lactic starter culture. The results obtained by Gahan et al. (36) indicated that the log-phase ATR system provided protection for *L. monocytogenes* present in acidic foods. Wild-type *L. monocytogenes* cells added to human gastric fluid (pH 2.5) containing 10 mM glutamate were not killed by the acid conditions during a 60-min treatment, whereas in the absence of glutamate, the numbers of wild-type cells were reduced >100-fold in 60 min (21). Mutants lacking *gadA* and *gadB* did not survive gastric acid exposure for 60 min whether or not glutamate was present, indicating that AR system 2 is necessary for the survival of *L. monocytogenes* in gastric fluid (21). Cotter et al. (22) found that *gadA* and *gadB* were required for the survival of *L. monocytogenes* in acidic foods such as apple juice, orange juice, tomato juice, salad dressing, mayonnaise, and yogurt. Thus, the glutamate-dependent AR system 2 is necessary to protect *L. monocytogenes* against acidic conditions found in food and in the stomach. These reports indicate that acid-adaptive mechanisms are important in the resistance of a number of enteric pathogens to acidic conditions found in the stomach and in foods.

CONCLUSIONS

The production of gastric acid by the stomach is an efficient mechanism for the destruction of pathogens ingested with food or water. However, a number of parameters can lead to hypochlorhydria or achlorhydria with a concurrent loss of the protective system afforded by gastric acid.

The manner in which many physicians treat peptic ulcer disease can cause the protective mechanism of gastric acid to fail. Since the majority of peptic ulcer disease cases

are caused by *H. pylori*, the use of antibiotics to eliminate this organism, rather than treatment with drugs that eliminate gastric acid secretion or activity, should be the treatment of choice. The use of antibiotics to eliminate the ulcer-causing organisms will put fewer individuals at risk for food- or waterborne illnesses due to drug treatments that induce hypochlorhydria.

Enteric pathogens do not always have to wait until the stomach is in an acid-deficient state to cause gastroenteritis. Bacteria have developed a number of adaptive mechanisms that permit them to survive the broad range of acid stresses they may encounter in the external environment, in food, or in an animal host. If bacteria first adapt to a moderate acidic condition, they will be able to survive more extreme acidic environments through the induction of adaptive mechanisms that lead to acid resistance. These inducible responses are of medical and applied significance because the ability of bacteria to resist extreme acid environments ensures that these acid-resistant pathogens can survive in nature, in acid foods, and in the acid stomach (85).

Since the gastric acid protective system can fail, it is necessary for individuals to practice a good water and food hygiene. Water used for drinking and food preparation should be properly purified and chlorinated. Raw foods should be obtained from a safe source and should then be properly handled and stored. Foods must be prepared and cooked in an appropriate manner and served properly (i.e., hot foods should be served promptly and cold foods should be kept refrigerated until they are served). Procedures for the safe handling of foods are given in Smith (103). Above all, a high degree of personal cleanliness must be observed. Hands should be washed with soap and hot water before foods are prepared and at intervals during the preparation, cooking, and serving of foods. Hand washing is the most effective means of preventing foodborne disease (110).

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