Aspirin-Induced Gastric Mucosal Injury: Lessons Learned From Animal Models

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This review of the mechanisms by which aspirin causes gastric mucosal damage points to the involvement of two potential mechanisms. Aspirin, which inhibits cyclooxygenase, is rapidly deacetylated to salicylate. Salicylate is toxic to cells and affects mucosal barrier function, reduces cytosolic adenosine triphosphate, stimulates sodium transport, and increases proton dissipation from surface epithelial cells. Cyclooxygenase inhibition makes the gastric mucosa more susceptible to injury, inhibits mucus and bicarbonate secretion, alters the physicochemical nature of mucus, stimulates fundic but not antral [3H]thymidine incorporation, and reduces epithelial surface hydrophobicity. No single mechanism seems to be involved, instead, that the toxic effects of salicylate and the effect of cyclooxygenase inhibition work in concert to render the mucosa more susceptible to injury, resulting in mucosal damage.

The acute or chronic use of aspirin is frequently associated with dyspepsia and often with mucosal erosions or ulceration. In vitro and in vivo techniques have been used to determine the mechanisms by which aspirin causes gastric mucosal injury. This review will focus on the published experimental work, addressing potential mechanisms of aspirin-induced mucosal injury. No one mechanism has emerged as the sole effector of aspirin-induced injury. In vitro effects of aspirin on transmembrane permeability, electrical activity, metabolism, transport, and proton handling appear to be primarily related to salicylate toxicity (Table 1). In contrast, the effect of aspirin on endogenous mucosal defense mechanisms such as endogenous prostaglandin activity, mucosal blood flow, bicarbonate and mucus secretion, epithelial cell proliferation, and epithelial surface hydrophobicity, appear to be related to cyclooxygenase inhibition (Table 2).

Pharmacokinetics of Aspirin

The pKa of aspirin is 3.5. This means that when aspirin is dissolved in a solution of pH > 3.5, more than half of the carboxyl groups are ionized; and at pH 6 virtually all of the acetyl salicylate molecules are negatively charged. This molecule is water-soluble and passage across plasma membranes is restricted to water-filled channels.

When aspirin is in a solution of pH < 3.5, more than half of it is undissociated and lipid-soluble. It is then able to move through plasma membranes. Ingested aspirin is therefore found in acidic gastric contents in the unionized form, and is therefore rapidly absorbed—after which it is hydrolyzed to salicylic acid by intestinal, hepatic, and plasma esterases (1,2). Salicylate does not inhibit cyclooxygenase activity in the gastric mucosa whereas aspirin (acetyl salicylate) does (3). The gastric mucosal toxicity of aspirin is therefore related both to salicylate-induced cellular or epithelial events and to aspirin-induced inhibition of cyclooxygenase.

Effect of Aspirin on Epithelial Integrity

Using light microscopy, electron microscopy, and freeze fracture techniques, the effect of acute and chronic aspirin administration on cellular epithelial integrity has been well described (4). The gastric mucosa of dogs showed dose-dependent epithelial damage and hemorrhage into the mucosal lamina propria within 4 h after intraluminal aspirin administration. Damage was most pronounced at 1 wk, with lesser injury present at 4 wk. This suggests that adaptation to chronic aspirin administration occurs. Cuboidal epithelial changes were present in the gastric mucosa of dogs given aspirin for 4 wk.
Endogenous prostaglandins
Epithelial surface hydrophobicity
Bicarbonate and mucus secretion
Blood flow

More recently, using freeze fracture techniques, aspirin has been shown to affect tight junctions of canine gastric mucosa (6) with focal discontinuity, variable strand number, and disorganization. Lanthanum, to which the normal canine gastric mucosa is normally impermeable, was able to pass through lateral intercellular spaces and tight junctions.

### Table 1. Effects of Aspirin on Cellular and Epithelial Events Related to Salicylate Toxicity

<table>
<thead>
<tr>
<th>Effect</th>
<th>References</th>
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<tbody>
<tr>
<td>Permeability changes</td>
<td>8, 9, 19</td>
</tr>
<tr>
<td>Electrical activity</td>
<td>8-10, 19</td>
</tr>
<tr>
<td>Metabolism</td>
<td>11, 12</td>
</tr>
<tr>
<td>Transport of ions</td>
<td>13-16</td>
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<td>Proton handling</td>
<td>17</td>
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### Salicylate-Induced Toxic Effects

Because of rapid deacetylation to salicylate, oral administration of 20 mg of aspirin to humans produces peak plasma aspirin concentration at 20 min, with a fall to nil by 60–70 min. In contrast, plasma concentrations and tissue levels of salicylate remain high for a prolonged period (7).

The studies to be described point to a number of toxic effects of salicylate on cellular and epithelial function quite independent of cyclooxygenase inhibition.

### The Gastric Mucosal Barrier

The gastric epithelium is normally able to maintain a large, 10^5, proton gradient under resting conditions. Because of this large concentration gradient, some protons diffuse into the mucosa, where they are efficiently handled by the surface cells and mucosa, allowing the tissue to remain at pH 7.4. After mucosal exposure to aspirin or salicylate, changes in this "mucosal barrier" function include increases in net cation flux, with large numbers of protons diffusing into the mucosa; sodium ions moving from mucosa into the lumen; and a reduction of transmucosal potential difference (8). Aspirin and salicylic acid had identical effects on these epithelial events, suggesting cyclooxygenase independence. In addition to aspirin and salicylic acid, several other compounds such as bile salts, detergents, and ethanol produced similar effects. These "barrier-breaking" agents cause intramucosal histamine release by mast cells, with resultant vascular congestion, edema, and transudation of plasma into the lumen.

Another report (9) of the effect of ASA and salicylate acid on net cation flux, transmucosal potential difference, and endogenous prostanoid generation further demonstrated that the "mucosal barrier" is independent of endogenous prostaglandin activity. In this study, aspirin or salicylic acid (0–40 mM in 150 mM hydrochloric acid) was administered to Heidenhain pouches of dogs. Change in net cation flux and transmucosal potential difference was identical for the two compounds; however, aspirin produced a dramatic reduction in endogenous generation of gastric mucosal prostacyclin, prostaglandin E2 (PGE_2), and prostaglandin F2α (PGF_2α), whereas salicylic acid did not.

Exposure of the Necturus gastric mucosa to 5 mM aspirin is associated with different effects on trans-epithelial and cellular potentials as a function of pH (10). At pH 4, aspirin produced a reduction in the apical membrane potential, from −37 to −22 mV, and the basolateral membrane potential, from −39 to −25 mM. After removal of the aspirin-containing

### Table 2. Effects of Aspirin on Mucosal Defense, Which in Large Part Are Related to Cyclooxygenase Inhibition

<table>
<thead>
<tr>
<th>Effects</th>
<th>References</th>
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<tbody>
<tr>
<td>Endogenous prostaglandins</td>
<td>20–25</td>
</tr>
<tr>
<td>Blood flow</td>
<td>26–33</td>
</tr>
<tr>
<td>Bicarbonate and mucus secretion</td>
<td>34–46</td>
</tr>
<tr>
<td>Epithelial surface hydrophobicity</td>
<td>47, 48</td>
</tr>
</tbody>
</table>
solution, the membrane hyperpolarized and returned to its original potential, which suggests that the epithelia were not irreparably damaged. In contrast, at pH 7 there was an increase in both apical and basolateral membrane potentials, from -40 to -47 mV and -42 to -50 mV, respectively. These observations are consistent with the hypothesis that aspirin-induced injury involves back-diffusion of protons, with resultant cellular acidification and influx of salicylate ions into cells, and with derangement of cellular metabolism.

In other studies of amphibian gastric mucosa, aspirin (10 or 20 mM) produced an initial rise in transmucosal potential and resistance from 0 to 15 min (11). From 15 to 30 min, the transmucosal potential declined toward zero. However, transmucosal resistance remained elevated for 30 min, at which time it, too, began to decline toward zero. Mucosal permeability to mannitol increased as transmucosal resistance fell. The initial rise in transmucosal difference was associated with inhibition of hydrochloric acid secretion, and the later decline was associated with inhibition of Cl- transport. Addition of a stable synthetic prostaglandin derivative to the serosal solutions did not prevent the production of these changes by aspirin.

In another study of the effect of ASA on amphibian gastric mucosa (12), it was shown that at concentrations of 10-20 mM, at pH 3, 4, or 6, mucosal concentrations of adenosine triphosphate (ATP) were reduced 21% and phosphocreatine 45%. Oxygen consumption initially increased after exposure to aspirin, but returned to control levels quite rapidly. There appeared to be no effect on mitochondrial respiration or respiratory control ratio. These observations suggest that aspirin-induced mucosal injury is related to its absorption from acidic luminal contents, intracellular hydrolyzation by an esterase to yield salicylate in sufficient concentrations to uncouple mitochondrial oxidative phosphorylation, reducing mucosal ATP and phosphocreatin content, resulting in inhibition of proton secretion and Cl- transport.

Other investigators have similarly shown that guinea pig gastric mucosa, perfused with 2 mM ASA at pH 4, was associated with a fall in transmucosal potential, reduction in ATP content and acid secretion, and an increase in proton back-diffusion (13). Changes in acid secretion and ATP content were correlated with reduction in transmucosal potential. These observations also suggest that one action of aspirin is to damage energy metabolism of the mucosal cells, causing cell death, with resultant back-diffusion of protons.

Membrane transport mechanisms are also affected by salicylate. Rabbit antral mucosa exposed to 5 mM salicylic acid at pH 4 was associated with a greater reduction in short-circuit current than resistance, reduced Cl- transport, and an initial increase in Na+ transport (14). These effects of salicylate may be related to inhibition of metabolic activity and occur as a result of the negative salicylate charge. Aspirin (5-20 mM) at pH 3 reduced transmucosal potential difference and increased transmucosal resistance within 30 min in a canine chambered mucosa model (15). Exposure of the mucosa to aspirin for more than 30 min was associated with increased unidirectional fluxes of Na+ and Cl-. Active transport of ions was inhibited by 1 mM aspirin at pH 3 or 20 mM aspirin at pH 7.4. Reduction in mucosal ATP content was also found by these investigators. High luminal concentrations of aspirin in acid have a greater effect on the mucosa than low concentrations of aspirin in acid or equal concentrations of aspirin in neutral solution. These observations suggest that aspirin inhibits active ion transport in the gastric mucosa, possibly through decreased ATP production as a result of enzyme inhibition or its uncoupling effect.

In isolated gastric mucosal cells from rat antrum and fundus, aspirin (1 mM) inhibited potassium and rubidium uptake, but had no effect on either Na+ uptake or K+, rubidium, or sodium efflux (16). Addition of prostaglandins to the aspirin-containing nutrient solution did not alter these effects. These isolated mammalian gastric cells showed active ion transport across plasma membranes by a mechanism independent of Na+-K+ pump or cyclooxygenase activity.

In a study of isolated gastric mucosal surface cells (17) loaded with H+ by exposure to NH4Cl and using acridine orange as a pH-dependent fluorescent probe, dissipation of intracellular protons was increased by exposure to salicylate (0-10 mM), which was associated with increased titratable H+ efflux. These effects were blocked by amiloride, indicating that salicylate increases Na+H+ exchange via an amiloride-sensitive pathway as well as via a leak of protons independent of extracellular sodium.

Studies comparing the effect of aspirin versus salicylate on mucosal injury in vitro have suggested that each compound is equally injurious. Using a pH-sensitive antimony microelectrode, the intramural pH of rabbit antral mucosa was measured after luminal exposure to sodium salicylate or aspirin at pH 7, 3.5, and 1 (18). Macroscopic and microscopic evaluation of the gastric mucosa under various conditions was used to assess mucosal integrity. Luminal perfusion with salicylic acid or acetylsalicylic acid (5 mM at pH 7) was associated with no change in intramucosal pH or ulceration. After luminal perfusion at pH 3.5, there was a fall in intramucosal...
pH to 6.75 and there were similar degrees of mucosal injury. Luminal perfusion of both compounds at pH 1.0 caused a prolonged reduction in intramucosal pH to 6.57 and severe mucosal injury.

When salicylic acid or aspirin was given parenterally with the luminal perfusate at pH 7 or 3.5, no change in intramucosal pH or ulceration was found. With luminal perfusates of both compounds at pH 1.0, there was no significant change in intramural pH, yet significant ulceration was present. It was concluded that reduction in intramural pH was not a prerequisite for ulceration, that the metabolic effects of salicylates make the mucosa more susceptible to proton injury, and that these effects do not appear to be related to cyclooxygenase inhibition.

Parenteral aspirin or sodium salicylate, given to anesthetized pylorus-ligated rats, produced similar extensive gross mucosal hemorrhagic lesions and similar microscopic damage when the luminal pH was 1.3 (19). Mucosal injury was not observed when the luminal fluid was 3.7. Either luminal or subcutaneous pretreatment with a synthetic PGE₂ analogue prevented the formation of macroscopic “red streaks” in aspirin-treated rats, but not in salicylate-treated rats.

It was concluded that intravenous salicylate is as damaging as intravenous aspirin, provided that the pH of the intraluminal fluid is low, and that the synthetic PGE₂ derivative protects the gastric mucosa against intravenous aspirin more than against salicylate. Therefore a mechanism of mucosal injury caused by intravenous acetyl salicylate is more complex than simply inhibition of endogenous cyclooxygenase.

Effect of Acetyl Salicylate on Mechanisms Contributing to Mucosal Defense

In contrast to the previously described studies, which indicate that salicylate is toxic to cells and epithelia by mechanisms independent of cyclooxygenase inhibition, there are a number of studies that implicate inhibition of cyclooxygenase as a factor contributing to the injurious effects of aspirin.

Endogenous Prostaglandins

Cyclooxygenase is the enzyme that is necessary for the conversion of arachidonic acid to endoperoxides. Aspirin, which inhibits cyclooxygenase, blocks the production of prostaglandins E₂ and I₂ in the gastric mucosa. Exogenous administration of prostanoids of the E, I, F, and D series inhibits the occurrence of gastric injury produced by a wide variety of experimental ulcerogens (20). Although these prostanoids have antisecretory activity when given exogenously, protection against ulcerogenesis occurs at doses that are nonantisecretory. These observations led to the hypothesis that aspirin-induced mucosal injury is directly related to cyclooxygenase inhibition. Several observations support this hypothesis.

Rats given intravenous or intragastric aspirin (60 mg/kg followed by a constant infusion or perfusion of 40 mg/kg·h) produced cyclooxygenase inhibition and macroscopic mucosal injury that were well correlated (21). Exogenous PGE₂ and prostacyclin completely prevented mucosal injury produced by both intravenous and intragastric aspirin. Additional dose-response studies using lower doses of aspirin (6 and 0.6, followed by 4 and 0.4 mg/kg·h) showed a significant correlation between cyclooxygenase reduction and mean ulcer area. It was concluded that aspirin injury may be related, at least in part, to cyclooxygenase inhibition. During histamine stimulation, parenteral administration of aspirin produced acute, deep penetrating ulcers in the gastric antra of cats (22). Comparison of the ulcerogenic actions of sodium salicylate and aspirin in this model suggests that injury produced by aspirin is associated with reduced cyclooxygenase activity in excess of 92%, compared with absence of macroscopic injury after sodium salicylate administration, which is not associated with cyclooxygenase inhibition.

Further support for the hypothesis that reduction of endogenous prostaglandins predisposes the mucosa to injury is found in recently reported studies in which rabbits were immunized with PGE₂ thyroglobulin conjugate (23,24), resulting in the production of circulating PGE₂ antibodies. Gastrointestinal ulcers occurred as early as 6 wk after the beginning of immunization. Passive immunization of rabbits with PGE₂ hyperimmune plasma also led to acute gastric ulceration. These observations support the notion that endogenous prostaglandins are important for maintenance of mucosal defense, and that inhibition of prostaglandin biosynthesis or neutralization of endogenous prostaglandins is an important mechanism of aspirin-induced mucosal injury.

Evidence that aspirin-induced gastric mucosal injury is related both to the direct effects of aspirin on mucosal cells and cyclooxygenase inhibition comes from a study in which rats were given either oral or parenteral aspirin (25, 50, or 100 mg/kg) and were evaluated for macroscopic mucosal injury and cyclooxygenase inhibition at 1, 3, 6, 12, and 24 h (25). Cyclooxygenase inhibition of 87%–95% was not associated with macroscopic injury when the lumen was acidified. Cyclooxygenase inhibition of 95%–98% was associated with macroscopic injury only when aspirin was given orally.
During pentagastrin stimulation, aspirin reduced mucosal blood flow 39% and increased acid output.

Mucosal blood flow measured by electromagnetic flow meters around the left gastric artery, whereas no effect on basal acid secretion was observed (28). Given intravenously, there was a 26% reduction of blood flow by virtue of intramucosal histamine release and vasodilatation. "Barrier-breaking" agents probably increase mucosal blood flow. These effects are not unique for aspirin, as both sodium taurocholate (40 mM) and ethanol (25%) also produced an increase in mucosal blood flow. These "barrier-breaking" agents probably increase mucosal blood flow by virtue of intramucosal histamine release and vasodilatation.

Gastric Mucosal Blood Flow

Gastric mucosal blood flow contributes to maintenance of gastric mucosal defense. Experimentally, reduction of mucosal blood flow is associated with potentiation of injury, whereas increased blood flow reduces injury in various experimental ulcer models. The effect of aspirin on mucosal blood flow is a function of route of administration. Intraluminal administration of acidified aspirin (20 mM) in Heidenhain-pouch canine preparations was associated with a 43% increase in aminopyrine clearance, reflecting an increase in gastric mucosal blood flow (26). Using both aminopyrine clearance and radiolabeled microspheres, aspirin (20 mM) placed in contact with preparations of canine fundic mucosa caused a doubling of mucosal blood flow (27). These effects are not unique for aspirin, as both sodium taurocholate (40 mM) and ethanol (25%) also produced an increase in mucosal blood flow. These "barrier-breaking" agents probably increase mucosal blood flow by virtue of intramucosal histamine release and vasodilatation.

In contrast, parenteral administration of aspirin has been shown to significantly reduce basal and pentagastrin-stimulated mucosal blood flow. In anesthetized dogs to which aspirin (100 mg/kg) was given intravenously, there was a 26% reduction of mucosal blood flow measured by electromagnetic flow meters around the left gastric artery, whereas no effect on basal acid secretion was observed (28). During pentagastrin stimulation, aspirin reduced mucosal blood flow 39% and increased acid output 104%.

In conscious dogs prepared with Heidenhain pouches with luminal acidification, aminopyrine clearance was used to measure the effect of parenteral aspirin on mucosal blood flow (29). Aspirin (100 mg/kg) reduced aminopyrine clearance 31%, and another cyclooxygenase inhibitor, indomethacin, reduced aminopyrine clearance 52%. These observations, considered in conjunction with others showing that prostacyclin and PGE2 are vasodilators in the canine gastric mucosal circuit (30,31), and that prostacyclin given exogenously increases mucosal blood flow and reduces acid secretion (32), suggest that endogenous gastric vasodilator prostanooids exert a tonic dilatory effect on the vasculature.

The initial effect of oral aspirin ingestion is an increase in mucosal blood flow that is independent of cyclooxygenase activity. Mucosal blood flow is likely to be reduced subsequently as a function of cyclooxygenase inhibition and loss of tonic vasodilator effects of endogenous prostanooids. This may be one mechanism by which aspirin compromises endogenous mucosal defense.

Another mechanism by which aspirin may reduce flow in the gastric mucosal microcirculation was studied with in vivo microscopy (33). Luminal application of 20 mM aspirin and 50 mM hydrochloric acid resulted in a significant reduction in red blood cell flow, in contrast to hydrochloric acid alone, which had minimal effects on red blood cell flow. White thrombi were observed flowing through the microcirculation, after which mucosal hemorrhages developed in the areas where the circulation had ceased. After application of acidified aspirin, direct measurement of arteriolar diameter showed constriction of small and large arterioles to 38% and 43% of their control diameters, respectively. These observations indicate that aspirin-induced back-diffusion of acid causes thrombus formation in mucosal microvessels and in submucosal arteriolar constriction. These changes may play a causative role in cessation of mucosal blood flow, mucosal injury, and focal hemorrhage.

Gastric Mucosal Proliferation

The gastric epithelium is one of the most rapidly replaced epithelia, the entire gastric surface being replaced every 2–3 days. The mucus neck cell is a pluripotent cell that usually differentiates to become a surface epithelial cell, and it is therefore the neck of the gland that has the highest metabolic activity. This rapid proliferative activity and epithelial replacement serves as another form of endogenous mucosal defense.

The effect of chronic aspirin ingestion on epithelial proliferation was studied in rats, using [3H]thymidine incorporation into the gastric fundus and antrum (34). Aspirin was added to the drinking
water of rats to deliver a dose of 120 mg/kg · day for 4 wk. Chronic aspirin ingestion stimulated epithelial proliferation in fundic mucosa, but had no effect in the antrum. All objective measurements—the number of labeled cells, proliferative zone thickness, mucosal thickness, labeling index, and proliferative zone to mucosal thickness ratio—were affected in fundic mucosa.

Fundic mucosal adaptation to chronic aspirin administration may be related to increased proliferative activity; however, this does not occur in the antrum. This effect may be mediated by cyclooxygenase inhibition, as exogenous prostaglandin administration has been shown to exert trophic effects on the gastric mucosa (35,36). This represents another mechanism by which aspirin may influence epithelial function and affect mucosal defense in the gastric antrum.

**Gastric Mucus and Bicarbonate Secretion**

Gastric surface epithelial cell secretion of bicarbonate, and synthesis and release of mucus, result in a layer equivalent to an unstirred water layer overlying the entire gastric epithelium. Protons are neutralized to some extent in mucus, producing a pH gradient from luminal to epithelial interface. When the gastric luminal fluid has a pH of >2.5, the apical membrane of the surface epithelial cell may be surrounded by fluid of pH >5 (37). This mucus layer is in dynamic equilibrium in that as mucus is released from surface epithelial cells, it is also degraded by acid and pepsin on the luminal surface.

Aspirin has been shown to inhibit, and exogenous prostaglandins to stimulate, bicarbonate secretion and mucus release, and to change the chemical composition of gel mucus. Basal bicarbonate output from guinea pig gastric mucosa fell from 40 to 20 mEq/L after administration of parenteral aspirin (5 mg/kg) (38). Several other nonsteroidal antiinflammatory compounds, fenclofenac (3 mM) and indomethacin (100 μM), significantly reduced basal bicarbonate secretion from amphibian gastric mucosa in an in vitro preparation (39). A synthetic PGE₂ analogue not only stimulated basal bicarbonate secretion but also blocked the inhibitory effect of indomethacin. The same synthetic PGE₂ analogue produced greater bicarbonate secretory rates when given luminally than when given intravenously in dogs with a Heidenhain pouch (40). The greatest bicarbonate rate when the synthetic analogue was given intravenously was 72 μmol/h compared with 144 μmol/h after intraluminal administration.

It seems reasonable to conclude, on the basis of these data, that the bicarbonate secretory mechanism of the surface epithelial cell is affected by cyclooxygenase inhibitors and exogenous prostaglandins. Inhibition of cyclooxygenase by aspirin or other nonsteroidal antiinflammatory compounds may compromise mucosal defense by reducing endogenous bicarbonate secretion.

Similar effects of aspirin on mucus production have also been implicated, as it reduces mucus synthesis and secretion and changes the biochemical structure of mucus. Parenteral administration of aspirin in rats (200 mg/kg over 72 h) produced a significant reduction in gastric mucosal content of mucus, as measured by concentration of hexosamines and 1-fucose in freeze-dried scrapings (41). The same authors subsequently found that the effect of oral administration of aspirin in dogs with denervated antral pouches was to reduce both the volume of mucus and the carbohydrate concentration in secreted gastric mucus. It was concluded that one mechanism of aspirin-induced injury is related to a decrease in the rate of renewal of mucus and to alteration in the quality of secreted mucus, reducing the ability of the gastric mucosa to resist injury. Aspirin (200 mg/kg), markedly inhibited the incorporation of 35SO₄, measured by column chromatography, into gastric glycoproteins (42) within a few hours. In contrast, salicylic acid required 24 h to produce similar decreases in 35SO₄ incorporation.

Several other nonsteroidal antiinflammatory compounds with high ulcerogenic status, such as aspirin, inhibit the incorporation of 35SO₄ into glycoproteins, whereas those with low to moderate ulcerogenic status do not. Luminal 16,16-dimethyl PGE₂ administration increases hexosamine output from canine gastric mucosa (43) and increases the measured thickness of the gel mucus layer in rats (30). It can be concluded from these data that control of gastric mucus synthesis and release is due in part to endogenous prostaglandin activity.

In addition to the effect of aspirin on gastric mucus synthesis and release, it also affects the biochemical and physicochemical characteristics of gastric mucus. The change in gastric mucus after administration of aspirin (300 mg/kg) in dogs, using column chromatography, identified three fractions (44). The first peak, corresponding to gastric mucus macromolecular, neutral, and acidic glycoprotein sulfate, was diminished at 3 h after aspirin administration. These data suggest that gastric ulceration induced by aspirin may be caused by a deficiency of gastric mucus macromolecular glycoproteins.

The proteolytic activity of pepsin on gastric gel mucus was enhanced by the addition of aspirin (1.0 μM) (45). The apparent Km value of pepsin toward mucus glycoprotein was 8.7 μM without aspirin and 6.9 μM with aspirin. In addition, after exposure to aspirin there was a 75% reduction in mucus viscos-
ity and an 18% increase in proton mobility in mucus. These results indicate that aspirin may weaken the integrity of gastric mucus by promoting peptic digestion, thereby reducing viscosity and increasing the mobility of protons within mucus. In contrast, preincubating mucus glycoprotein with the synthetic PGE₂ analogue, 16,16-dimethyl PGE₂ increased glycoprotein viscosity by 180% and improved the capacity to impede proton mobility (46). Taken together, these observations point toward the effects of aspirin on the physicochemical characteristics of gastric gel mucus—negatively influencing mucosal defense by promoting peptic degradation, reducing viscosity, and increasing proton mobility.

Surface Hydrophobicity

The hydrophobic nature of the surface epithelium represents a mechanism of endogenous mucosal defense. Surface hydrophobicity is estimated by measuring the contact angle between an applied drop of distilled water on the gastric mucosa and the tangent line at the point of contact between the mucosa and the drop of water, using a goniometer. Intraluminally administered aspirin (8.3 mM) reduced epithelial surface hydrophobicity when the luminal pH was <4.0, whereas it was not affected by acidic solutions to pH 2.6 without aspirin, or by aspirin-containing solutions when the pH was >4.0 (47). Reductions in hydrophobicity were correlated with the proportion of aspirin in its lipid-soluble, undissociated form, and were associated with decreased transmucosal potential differences that were also pH- and time-dependent. These data are consistent with the hypothesis that the undissociated form of aspirin negatively affects the mucosa, making it less hydrophobic.

Exogenous administration of 16,16-dimethyl PGE₂ not only increased basal surface hydrophobicity of canine gastric mucosa, but also prevented the decrease in surface hydrophobicity that occurs after luminal exposure to acidified aspirin (48). It is possible that these effects of aspirin and exogenous prostaglandins on surface hydrophobicity, too, play a role in the mechanism of aspirin-induced gastric mucosal injury.

Summary

This review of the effects of aspirin on gastric mucosal function, although not exhaustive, suggests that two modes of injury occur. Because of rapid deacetylation of aspirin to salicylate in cells, interstitial fluid, and plasma, serum concentrations of aspirin after oral administration are short-lived. However, salicylate is toxic to cells and affects epithelial function. Intraluminally, salicylates are associated with "barrier" injury, resulting in large net cation fluxes, hydrogen back-diffusion, and fall in transmucosal potential difference. Both apical and basolateral plasma membrane resistances are affected by aspirin, as a function of pH. Mucosal content of ATP is reduced by salicylate, affecting ion transport, stimulating sodium transport, and increasing proton dissipation from surface epithelial cells. These effects of aspirin do not appear to be related to cyclooxygenase inhibition, but rather are related to the product of aspirin deacetylation, salicylate.

The effect of aspirin on several mechanisms of endogenous mucosal defense seems to be related to cyclooxygenase inhibition. Dose-response studies indicate a significant correlation between mucosal injury and cyclooxygenase inhibition. Salicylic acid, which does not affect gastric mucosal prostaglandins, does not cause injury to the gastric mucosa as does aspirin.

Further support for the important role of endogenous prostanooids in mucosal defense comes from the observation that when rabbits are immunized against prostacyclin and PGE₂, gastric mucosal ulcers develop. Although cyclooxygenase inhibition alone, with intraluminal acidification, does not produce macroscopic mucosal injury, it is clear that the gastric mucosa becomes more vulnerable to other forms of injury as a result of cyclooxygenase inhibition.

Reduction in endogenous prostaglandin activity by aspirin is associated with compromised mucosal defense. Oral administration of aspirin may cause an initial increase in mucosal blood flow as a function of intramucosal histamine release. However, because of aspirin's effect on cyclooxygenase, reduction in endogenous vasodilator prostaglandins causes vasconstriction and reduced mucosal blood flow. This also reduces endogenous mucosal defense, as blood flow is important for delivery of oxygen and nutrients, for removal of protons that have diffused into the mucosa, and for prevention of the formation of oxygen-derived free radicals. Gastric mucus and bicarbonate are secreted from the surface epithelial cells and provide a mechanism by which protons can be neutralized within an unstirred layer overlaying the epithelium.

Aspirin reduces the synthesis of mucus glycoproteins, reduces basal bicarbonate secretion, and affects the physicochemical characteristics of mucus, making it less of a barrier to protons. Aspirin stimulates fundic mucosal epithelial proliferation as measured by [³H]thymidine incorporation. This may represent a form of fundic adaptation to chronic aspirin administration that does not occur in the antrum, making it more vulnerable to mucosal in-
jury. Finally, the hydrophobicity of the surface epithelium is negatively affected by aspirin and positively affected by exogenous 16,16-dimethyl PGE₂. The importance of this mechanism has yet to be delineated. However, it is reasonable to assume that in this way, too, aspirin may compromise mucosal defense.

After oral administration, aspirin passes through plasma membranes in the unionized form and is rapidly deacetylated, leaving salicylate to exert effects on surface cells and epithelia. Locally, aspirin inhibits cyclooxygenase, perhaps reducing surface hydrophobicity and mucus and bicarbonate secretion. Circulating aspirin inhibits endothelial cyclooxygenase, causing vasoconstriction. Although circulating levels of aspirin return to nil within an hour after oral administration, its effect on cyclooxygenase is present for 12–24 h. The effects of salicylate and cyclooxygenase inhibition together appear to be important contributors to this form of mucosal injury. These observations may also explain the lesser toxicity of enteric-coated or suppository forms of aspirin in comparison with oral ingestion of plain tablets. It is not known whether all nonsteroidal antiinflammatory agents have similar local and systemic toxicity to the gastric mucosa, but there is certainly a spectrum of toxic effects that may reflect differences in local toxicity, considering that all these agents are cyclooxygenase inhibitors [49].

References
32. Konturek SJ, Robert A, Hanchar AJ, Nezamis JE. Comparison of prostacyclin and prostaglandin E2 on gastric secretion,

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