Physiological concentrations of bile salts inhibit recovery of ischemic-injured porcine ileum

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Bile is a normal constituent of the luminal contents of the mammalian small intestine, the main function of which is to aid fat digestion and absorption. The primary bile acids, cholic acid and chenodeoxycholic acid, are synthesized in the liver from cholesterol and are conjugated with taurine or glycine to form taurocholic and glycocholic acids. They can undergo further metabolism, by enteric bacteria for example, to form secondary bile acids such as deoxycholic acid and lithocholic acid (8).

The effect of bile salts on the intestinal epithelium has been studied by a number of investigators. For example, taurodeoxycholic acid caused an increase in the permeability of the gastric mucosa to varying sizes of polyethylene glycol molecules (21). In the rabbit small intestine, chenodeoxycholic acid and ursodeoxycholic acid (a tertiary bile acid derived from a secondary bile acid) increased the rate of transmural flux of lactulose in the jejunum and ileum (14), whereas the salt of deoxycholic acid, deoxycholate, increased the mucosal-to-serosal flux of mannitol in the rat and the rabbit small intestine (1). Electron microscopy revealed wider intercellular spaces with loss of contact points between the cellular membranes. Deoxycholate also caused increases in mucosal permeability in porcine colon (2, 19) and porcine ileum (18). Damage to the colonic mucosa ranged from slight (with loss of single cells) with 1.5 mM deoxycholate to extensive (with a barren mucosal surface and sloughed epithelial sheets) with 3–21 mM deoxycholate. Removal of the bile salts was followed by rapid recovery of mucosal barrier function in both the ileal and colonic studies.

Although this work has provided important insight into the mechanisms of injury and repair in intestinal mucosa subjected to bile salts, all of these studies used bile salt concentrations (0.1–21 mM) at the high end of the physiological range of bile salts documented in the intestinal lumen. For example, one of the most frequently used bile acids in these studies was unconjugated deoxycholic acid (1, 2, 19, 22), which we determined had a concentration of 3.0 ± 1.2 μM in porcine intestinal contents. Furthermore, most of these prior studies assessed the effects of bile acids on normal mucosa. However, one study (32) evaluated the effects of bile of rat jejunum subjected to 30 min of ischemia and showed that 1 mM taurodeoxycholic acid and taurochenodeoxycholic acid did not exacerbate gut permeability to sodium fluorescein. In another study (22), low dose deoxycholate (50 μM) added before the addition of 250 μM deoxycholate attenuated the injury produced by 250 μM deoxycholate alone on gastric mucosa. The mechanism of attenuation triggered by low-dose deoxycholate may have involved an effect on intracellular calcium. In addition, increasing calcium levels to 4 mM inhibited the damaging effect of 5 mM deoxycholate on colonic epithelium (35).

Previous studies (4, 5) of mucosal healing in porcine ischemic-injured ileum have shown that recovery of barrier function following ischemic injury occurs rapidly in studies performed in vitro, but these experiments did not take into account the effects of luminal contents. Because bile salts cause dose-dependent injury to intestinal epithelium and relatively high...
concentrations of bile may be present in mammalian small intestine, the objective of the present study was to evaluate the role of physiological doses of deoxycholic acid in recovery of mucosal barrier function in porcine ischemic-injured ileum. Deoxycholic acid was selected because it had been used extensively in previous studies on the effects of bile acids on intestinal mucosa (1, 2, 18, 19, 22).

MATERIALS AND METHODS

Experimental animal surgeries. All studies were approved by the North Carolina State University Institutional Animal Care and Use Committee. Six- to eight-week-old Yorkshire crossbred pigs of either sex were housed singularly, and maintained on a commercial pelleted feed. Pigs were held off of feed for 24 h before experimental surgery. General anesthesia was induced with xylazine (1.5 mg/kg im), ketamine (11 mg/kg im), and pentobarbital sodium (15 mg/kg iv) and was maintained with intermittent infusion of pentobarbital sodium (6–8 mg·kg⁻¹·h⁻¹). Pigs were placed on a heating pad and ventilated with 100% O₂ via a tracheotomy using a time-cycled ventilator.

To assess mucosal permeability after experimental treatments, 0.2 µCi/ml ³H-labeled mannitol or ¹⁴C-labeled LPS was placed on the mucosal side of Ussing chamber mounted tissues. A 15-min equilibration period, standards were taken from the mucosal side of each chamber and a 30-min flux period was established by taking 0.5-ml samples from the serosal compartment. The presence of ³H and ¹⁴C was established by measuring β-emission in a liquid-scintillation counter. Unidirectional ³H-labeled mannitol or ¹⁴C-labeled LPS fluxes from mucosa to serosa were determined using standard equations.

Morphometric measurements. Tissues were treated at 0 and 240 min for routine histological evaluation. Tissues were sectioned (5 μm) and stained with hematoxylin and eosin. For each tissue, three sections were evaluated. Four well-oriented villi were identified in each section. The length of the villus and the width at the midpoint of the villus were obtained using a micrometer in the eye piece of a light microscope. In addition, the height of the epithelial-covered portion of each villus was measured. The surface area of the villus was calculated using the formula for the surface area of a cylinder. The formula was modified by subtracting the area of the base of the villus and multiplying by a factor accounting for the variable position at which each villus was cross-sectioned. The percentage of the villous surface area that remained denuded was calculated from the total surface area of the villus and the surface area of the villus covered by epithelium. The percent denuded villous surface area was used as an index of epithelial restitution.

Electron microscopy. Tissues were removed from Ussing chambers after 240 min during three separate experiments (n = 3 for each treatment). Tissues were placed in Trump's 4F:1G fixative and prepared for transmission electron microscopy using standard techniques. For each tissue evaluated, five well-oriented interepithelial junctions were evaluated.

Data analysis. Data were reported as means ± SE. All data were analyzed using an ANOVA for repeated measures except where the peak response was analyzed using a standard one-way ANOVA or paired t-test (Sigmastat; Jandel Scientific, San Rafael, CA). A Tukey’s test was used to determine differences between treatments following ANOVA, and P < 0.05 was considered significant.
RESULTS

Bile acid assays. Ileal luminal samples from eight pigs were assayed for total bile acid concentration, using a commercial assay. The assay showed that the mean concentration of total 3α-hydroxy unconjugated bile acid in the porcine ileum was 6.0 ± 3.0 × 10⁻⁶ M (n = 8). The specific bile acids present in these samples were determined by mass spectrometry (Table 1). The average recovery of all unconjugated bile acid species in all samples was 101.8%. Unconjugated cholic acid had the lowest average recovery from porcine bile, with 92.1% of added cholic acid being recovered. Unconjugated ursodeoxycholic acid had the highest average recovery, averaging 112.8% of added unconjugated ursodeoxycholic acid being recovered. In general, the concentrations of unconjugated bile acids were in the same range as those determined using the commercial assay kit, with cholic acid having the highest concentration. However, conjugated bile acid concentrations reached levels in the millimolar range, with conjugated chenodeoxycholic acid having the highest concentration.

Deoxycholic acid inhibits recovery of ischemic-injured porcine ileum. After an initial 15-min equilibration period, incremental doses of deoxycholic acid were added to the mucosal surface of normal (control) tissues, resulting in dose-dependent reductions in TER (Fig. 1A). Ischemia for 45 min resulted in significant reductions in TER (34 ± 2.7 Ω·cm²) compared with controls (51 ± 4.8 Ω·cm²; P < 0.05), but ischemic-injured tissues recovered control levels of TER within 120 min (Fig. 1B). Mucosal treatment of ischemic-injured tissues with 10⁻⁶ M deoxycholic acid had no effect, whereas treatment with 10⁻⁵ M deoxycholic acid markedly inhibited recovery of TER. Similar experiments in which 10⁻⁵ or 10⁻⁶ M deoxycholic acid were placed on the serosal surface of ischemic-injured tissues revealed no significant effect on recovery of TER (data not shown), indicating that mucosal exposure was required for these physiological concentrations of deoxycholic acid to have an effect on TER. In further experiments assessing mucosal application of deoxycholic acid, higher doses above the physiological concentrations (10⁻³ and 10⁻⁴ M) completely inhibited recovery (Fig. 1B). \( I_{sc} \) was assessed as an index of mucosal transport function, because serosal addition of bile acids has been previously shown to stimulate Cl⁻ secretion (11). There were significant \( (P < 0.05) \) elevations in \( I_{sc} \) in control tissues following the addition of 10⁻³ to 10⁻⁶ M deoxycholic acid compared with untreated control tissues (Table 2), but there were no significant differences between differing concentrations of deoxycholic acid. Alternatively, in ischemic-injured tissues, there was no significant effect of deoxycholic acid at doses of 10⁻⁴ to 10⁻⁶ M, whereas the highest dose of deoxycholic acid (10⁻³ M) caused a significant \( (P < 0.05) \) drop in \( I_{sc} \) in ischemic-injured tissues vs. untreated ischemic-injured tissues immediately following the equilibration period (Table 2). There was no significant effect of 10⁻⁵ or 10⁻⁶ M deoxycholic acid on the serosal surface of ischemic-injured tissues.

Histological evaluation of tissues revealed denuding of villous tips immediately following the ischemic period (Fig. 2A), but denuded villi were fully restituted with flattened epithelium within 60 min (Fig. 2B). The completion of restitution (60 min)
before recovery of TER (120 min) in ischemic-injured tissues suggested increases in paracellular resistance were required following restitution to fully restore baseline levels of TER. Histological evaluation of control and ischemic-injured tissues treated with 10⁻⁵ or 10⁻⁶ M deoxycholic acid revealed no effect on epithelial morphology (Fig. 2, C and D), whereas higher doses of deoxycholic acid (10⁻³ and 10⁻⁴ M) caused disruption of epithelial restitution (data not shown). Morphometric analysis revealed significant reductions in villous height in control tissues treated with 10⁻⁵ and 10⁻⁶ M deoxycholic acid (Fig. 3). The villous height of ischemic-injured tissues was significantly lower than control tissues. Furthermore, 10⁻⁵ M deoxycholic acid addition to ischemic-injured tissues resulted in further significant reductions in villous height (Fig. 3).

Deoxycholic acid increases paracellular permeability. Because 10⁻⁵ M deoxycholic acid inhibited recovery of TER in ischemic-injured tissues without any histological changes in epithelial restitution, we considered the possibility that the deoxycholic acid inhibited recovery of TER via an action on the paracellular space. This paracellular premise was also supported by previous work (5–7) in which it was shown that in vitro recovery of ischemic-injured porcine ileal mucosa is associated with progressive reductions in paracellular permeability.

### Table 2. \( \Delta I_s \) in control (uninjured) and ischemic-injured tissues exposed to varying concentrations of deoxycholic acid

<table>
<thead>
<tr>
<th>Deoxycholic Acid Concentration</th>
<th>Tissue</th>
<th>Mucosal Application, ( \mu A/cm^2 )</th>
<th>Serosal Application, ( \mu A/cm^2 )</th>
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<tr>
<td>0</td>
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<tr>
<td>10⁻³ M</td>
<td>Control</td>
<td>74±6</td>
<td></td>
</tr>
<tr>
<td>10⁻⁴ M</td>
<td>Control</td>
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<td></td>
</tr>
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<td>−2±2</td>
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<td>Ischemic-injured</td>
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<td>Ischemic-injured</td>
<td>2±8</td>
<td>−7±12</td>
</tr>
</tbody>
</table>

Values are means ± SE for \( n = 6 \) samples. \( \Delta I_s \), change in short-circuit current.

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**Fig. 2.** Photomicrographs of porcine ileal postischemic mucosa. *A*: histological appearance of tissue immediately following 45 min of ischemia. Note sloughing of epithelium from the upper one-third of the villus, exposing subepithelial tissues. *B*: ischemic-injured tissue after 60 min of recovery in Ussing chambers. Villous contraction and epithelial restitution are evident, leading to complete epithelial coverage of the villus. Ischemic-injured tissues treated with deoxycholic acid had similar evidence of restitution at 60 min (not shown). Furthermore, ischemic-injured tissues treated for 240 min with 10⁻⁶ M deoxycholic acid (C) or 10⁻⁵ M deoxycholic acid (D) had continued evidence of complete restitution. Scale bar = 100 \( \mu m \).

**Fig. 3.** Morphometric analysis of normal and ischemic-injured porcine ileal mucosa. After the 240-min in vitro incubation period, significant reductions in villous height in control tissues treated with 10⁻⁵ M deoxycholic acid were noted (\( ^{\alpha}P < 0.05 \) vs. untreated control tissues). Ischemic-injured tissues had significantly reduced villous height compared with control tissues (\( ^{\beta}P < 0.05 \) vs. control tissues). Furthermore, 10⁻⁵ M deoxycholic acid treatment resulted in further significant reductions in villous height in ischemic-injured tissue (\( ^{\gamma}P < 0.05 \) vs. control and the other ischemic-injured treatment groups).
ability. However, the finding that $10^{-5}$ M deoxycholic acid stimulated significant villous contraction did not agree with this premise, because this action would tend to increase measurements of TER by reducing the surface area of the villus (31). Therefore, we chose to perform mucosal-to-serosal fluxes of the paracellular probe mannitol. Accordingly, these fluxes were significantly elevated in tissues treated with $10^{-5}$ M deoxycholic acid compared with tissues treated with $10^{-6}$ M deoxycholic acid or tissues that had no treatment (Fig. 4). We also performed mucosal-to-serosal fluxes of $^{14}$C-labeled LPS from Salmonella typhimurium, a bacterial toxin that has been shown to traverse epithelium via a transcytotic pathway (3) but that may gain access to the serosal surface of intestinal mucosa via the paracellular route following ischemia (14). The $^{14}$C-labeled LPS fluxes showed similar trends to $^3$H-labeled mannitol fluxes (Fig. 5).

**Effects of bile acid washout.** In further experiments, we sought to determine the effects of deoxycholic acid washout on recovery of TER, because the effects of treatments that have an action on paracellular structures are typically reversible (13, 15, 23, 28). Accordingly, tissues were treated with $10^{-5}$ M deoxycholic acid at the beginning of the recovery period. In select tissues, deoxycholic acid was washed out after either 60 or 120 min. This experiment revealed that the effect of $10^{-5}$ M deoxycholic acid was nullified following early washout but not after 120 min (Fig. 6). These experiments suggested either that the effects of deoxycholic acid are reversible after brief exposure (60 min) or that the effects of bile salts require up to 120 min to fully inhibit recovery of ischemic-injured tissue.

**Electron microscopic evidence of paracellular changes in the presence of deoxycholic acid.** As an additional method of assessing the role of deoxycholic acid in altering paracellular permeability, we performed electron microscopic analyses of ischemic-injured tissues during the 240-min recovery period. Compared with normal (control) mucosa (Fig. 7A), untreated ischemic-injured tissues had evidence of tight junction apposition by 120 min of the recovery period (Fig. 7B), and paracellular spaces appeared fully apposed by 240 min (Fig. 7C). Alternatively, ischemic-injured tissues exposed to deoxycholic acid ($10^{-5}$ M) for 240 min had evidence of widened tight junctions and dilated paracellular spaces (Fig. 7D) in tissues from the same experimental animals.

**Taurodeoxycholic acid has similar effects to deoxycholic acid.** Because deoxycholic acid is only one of a number of bile salts that may be present within the gastrointestinal lumen, we also chose to evaluate the effect of tauroconjugation of deoxycholic acid on mucosal recovery. The bile acid taurodeoxycholic acid was added to the mucosal surface of ischemic-injured tissue and had a similar effect to that of deoxycholic acid at $10^{-5}$ M (Fig. 8).

**Deoxycholic acid appears to mediate its effects via Ca$^{2+}$.** Because of the importance of Ca$^{2+}$ in regulation and reassembly of the tight junction (9) and in view of studies implicating
**Ca\(^{2+}\)** in bile salt-induced injury (22, 35), we wanted to investigate the effects of chelating **Ca\(^{2+}\)** and increasing intracellular **Ca\(^{2+}\)** in our ischemia model. Accordingly, EGTA (1 mM) was added to the mucosal and serosal sides of ischemic-injured tissue and significantly impaired recovery of TER (Fig. 9). Peak TER of tissue treated with EGTA was almost identical to that of ischemic-injured tissue treated with 10\(^{-5}\) M deoxycholic acid. In addition, we attempted to reverse the effects of 10\(^{-5}\) M deoxycholic acid with thapsigargin, an agent that has been shown to elevate intracellular calcium by inhibiting endoplasmic reticulum **Ca\(^{2+}\)**-ATPase without stimulating other second messenger signaling mechanisms (20). Application of thapsigargin (10\(^{-6}\) M) to the mucosal and serosal sides of ischemic-injured tissue resulted in recovery of TER to levels similar to those of untreated ischemic-injured tissue (Fig. 10).

**DISCUSSION**

Our studies on the intraluminal concentration of bile acid species indicated that the commercial bile assay kit detected levels of unconjugated bile acids in the micromolar range, similar to levels detected by mass spectrometry. We chose to study deoxycholic acid because this bile acid had been used in previous studies from our group (18), giving us a basis from which to initiate our studies. However, larger concentrations of conjugated bile acid species were noted, particularly chenodeoxycholic acid. There is little information on the concentration of individual bile acid species in intestinal contents in the literature, although millimolar concentrations of bile acids were detected in human meconium (38) and in rat intestinal contents (37). In the present study, combining both conjugated and unconjugated bile acids would have given similar millimolar levels. However, by applying physiological concentrations of a single bile acid species, we were able to show that doses as low as 10\(^{-5}\) M significantly inhibit mucosal recovery following ischemic injury, whereas doses in the millimolar range (10\(^{-3}\) M) completely nullify epithelial recovery. As far as specific bile acid species, the relative proportion of bile acid concentrations detected in porcine ileal contents was similar to that reported in human bile (26), including a preponderance of chenodeoxycholic acid (≈50% in human, ≈87% in pig), followed by cholic and deoxycholic acid (≈20–30% in human, ≈6% in pig) and far lesser concentrations of lithocholic acid and ursodeoxycholic acid in both species (<1%). The reason for the very large concentration of conjugated chenodeoxycholic acid in the present study is unknown, although this was strongly influenced by one pig that had >10,000 \(\mu\)M of this bile acid species in its ileal contents. Because chenodeoxycholic acid is a primary bile acid, these data suggest a wide variation in the degree of bile acid metabolic conversion by enteric bacteria such that the majority of chenodeoxycholic acid arrived at the ileum unmetabolized in some animals.
TER is a highly sensitive measure of the ionic permeability of intestinal mucosa. Recent studies (5) have shown close correlations between recovery of TER and reductions in mucosal permeability to inulin and mannitol in ischemic-injured tissues treated with prostaglandins. During the 240-min period of the present experiments, the TER of ischemic-injured tissue receiving \(10^{-6}\)M deoxycholic acid started at 34 \(\Omega \cdot \text{cm}^2\) and increased to 52 \(\Omega \cdot \text{cm}^2\) by the end of the experiment, which was very similar to control tissues (51 \(\Omega \cdot \text{cm}^2\)).

Histological analyses indicated that restitution was complete within 60 min, whereas TER required longer for full recovery. This discrepancy suggests that much of the recovery response was not associated with restitution, but rather that increases in paracellular resistance were required following restitution to fully restore barrier function.

The TER of ischemic-injured tissues treated with \(10^{-5}\) M deoxycholic acid was significantly less than that of control tissue at the end of the recovery period. Similar results were seen with the application of \(10^{-5}\) M taurodeoxycholic acid. The reduced recovery of TER in ischemic-injured tissues treated with \(10^{-5}\) M deoxycholic acid correlated with a significant increase in the mucosal-to-serosal flux of \(^3\)H-labeled mannitol and \(^{14}\)C-labeled LPS, indicating that these tissues failed to recover mucosal barrier function. Despite the increases in mucosal permeability in tissues treated with \(10^{-5}\) M deoxycholic acid, there was no appreciable difference in the histological appearance of ischemic-injured tissues treated with \(10^{-5}\) M deoxycholic acid. This suggests that changes in the paracellular space account for the decrease in TER and increase in mucosal permeability. \(^3\)H-labeled mannitol has been shown to traverse leaky epithelia via the paracellular space (24, 25), and \(^{14}\)C-labeled LPS may gain access to the serosal surface of intestinal mucosa via the paracellular route following ischemia (12), further supporting the possibility of disruption of paracellular pathways. In previous studies (16), taurochenodeoxycholic acid (4 mM) has been shown to alter the integrity of tight junctional complexes between the epithelial cells of rabbit colon. Furthermore, electron microscopy revealed wider intercellular spaces with loss of tight junctional integrity in rabbit small intestine treated with chenodeoxycholic acid (1 mM) (14). Similarly, in the present studies, the tight junctions and paracellular spaces appeared widened in repairing tissues in the presence of \(10^{-5}\) M deoxycholic acid compared with tissues in the absence of bile acid.

Whereas TER reached control levels in ischemic-injured tissues by the end of the recovery period, mannitol fluxes and LPS fluxes did not reach control levels. Because TER is essentially a measure of permeability of the mucosa to ions, which are much smaller than the macromolecules fluxed in this study, we expected macromolecular fluxes to return to control levels.
levels before normalization of TER. These findings suggest that there is more than one pathway by which ions and macromolecules can traverse tissue. For example, investigators studying the mucosal-to-serosal flux of a range of proteins and polysaccharides showed that transmucosal fluxes were not directly attributable to molecular weight or size. Their findings suggested instead that there were at least two distinct paracellular pores with distinct permeability properties (34). The recovery of TER in the absence of full recovery of macromolecules suggests that widening of small paracellular pores that would predominantly admit ions were not only recovered but possibly “over-tightened” to recover control levels of TER, whereas larger pores remained open to both ions and macromolecules. Previous studies (17) in porcine ileum subjected to bile acids have indicated by a series of mathematical models that much of the recovery of TER is attributable to changes in the crypt compartment.

Although neither mannitol nor LPS flux reached control levels in the present study, both had evidence of a similar reduction in flux over the 240-min recovery period. However, if both mannitol and LPS were traversing tissues via the same paracellular pathway, the LPS flux would have likely declined more rapidly and to a greater extent than mannitol fluxes. This may be explained by the fact that mannitol traverses epithelium via a paracellular pathway, whereas LPS has been shown to traverse epithelium via both paracellular and transcellular transcytotic pathways (3, 12, 24, 25). Thus continued transcellular LPS flux may partly explain the less than expected reductions in LPS flux over the recovery period as the paracellular spaces were progressively closed.

As far as the mechanism by which bile acids might have enhanced paracellular permeability, this is yet to be fully defined, but some clues were provided by experiments in which extracellular and intracellular Ca\(^{2+}\) levels were manipulated. For example, addition of thapsigargin, an agent that increases Ca\(^{2+}\); by inhibiting microsomal Ca\(^{2+}\)-ATPase reversed the effects of 10\(^{-5}\) M deoxycholic acid on ischemic-injured tissues, and the effects of 10\(^{-5}\) M deoxycholic acid on TER were simulated by addition of the calcium binding agent EGTA. The precise nature of the intracellular signaling pathways involving Ca\(^{2+}\) will require further work. However, we speculate that bile salts may impede restoration of paracellular function by reducing the availability of intracellular Ca\(^{2+}\), which may be required for recovery of tight junction integrity. For example, in Necturus gallbladder epithelium, the addition of the Ca\(^{2+}\) ionophore A23187 induced increasing numbers of tight junction strands associated with elevations in TER (33). This process could explain the recovery of TER induced by thapsigargin in the presence of deoxycholic acid.

Although the present study focused on changes in barrier function, changes were noted in \(I_{sc}\). In particular, significant elevations in \(I_{sc}\) were noted in control tissues following mucosal addition of deoxycholic acid (Table 2), possibly associated with the secretion of Cl\(^{-}\), as has been shown in previous studies (10, 27, 36) following the addition of bile acids to the serosal surface of tissues. On the other hand, there were notable changes in \(I_{sc}\) in ischemic-injured tissues until the highest dose of deoxycholic acid (10\(^{-3}\) M) was added to the mucosal surface. However, the deflections in \(I_{sc}\) were negative, rather than the positive deflections that would be expected with Cl\(^{-}\) secretion. The nature of these transport changes was not determined, but it is likely related to serosal-to-mucosal movement of Na\(^{+}\), as has been previously shown in porcine colon subjected to millimolar dosages of deoxycholic acid (2). Why these transport changes differed so dramatically from control tissues is not clear, but it may relate to the presence of intestinal epithelial injury in ischemic tissues, which resulted in early leakage of Na\(^{+}\) that may have overshadowed evidence of Cl\(^{-}\) secretion. These transport changes will require further experiments using unidirectional Na\(^{+}\) and Cl\(^{-}\) fluxes to isolate the source of changes in \(I_{sc}\).

In summary, this study shows that bile acids such as deoxycholic acid significantly impair mucosal recovery of epithelial barrier function at physiological concentrations. The effects of bile on barrier function appear to be due to changes in paracellular permeability. Thus bile acids result in increased permeability in tissues to the paracellular probe mannitol. Although the precise mechanism whereby bile acids disrupt the recovery of ischemic-injured mucosa is unknown, there appears to be a role for intracellular Ca\(^{2+}\). The importance of these findings is twofold. First, studies on in vitro recovery of injured native mucosa in the absence of normal luminal contents such as bile will tend to overestimate the recovery response that would be present in vivo in the presence of luminal contents. Second, the failure of recovery of TER in the presence of physiological concentrations of deoxycholic acid was accompanied by significant increases in transmucosal flux of LPS. Considering the importance of LPS in clinical syndromes such as sepsis (30), this suggests that treatments directed at minimizing the effects of luminal contents on injured intestine, such as agents that bind intraluminal bile, may be warranted.

**REFERENCES**


