Effects of Bile Salts and Prostaglandins on Sodium Transport in Isolated Rat Gastric Mucosa

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Abstract. To determine whether prostaglandins may protect against bile salt inhibition of ion transport in the stomach, gastric mucosal tissue was isolated from the rat and mounted in flux chambers. Transport of Na^+ was traced with radioisotopes in the absence of bile salts and then in the presence of conjugated taurocholate or unconjugated deoxycholate at low, intermediate and high mucosal concentrations (1, 5 and 15 mmol/1). At a high (7.40) or low (3.4) mucosal pH, only the unconjugated deoxycholate inhibited active Na^+ transport from mucosa to submucosa with respect to untreated controls. Inhibition of Na^+ transport was apparent at a low level of deoxycholate, which also inhibited the electrical potential difference. Intermediate and high levels of deoxycholate lowered the tissue resistance. When the tissues were exposed to mucosal prostaglandin E_2 or its 16,16-dimethyl analogue before and during acidified taurocholate administration, Na^+ transport was not changed significantly but the electrical resistance remained high. Thus, unconjugated bile salt is more potent than conjugated bile salt in inhibiting Na^+ transport and breaking the gastric mucosal barrier, and prostaglandins may afford some small protection.

Key words: Bile — Stomach — Transport — Sodium — Prostaglandins

Introduction

The detergent action of bile salts in the stomach can result in breakdown of the gastric mucosal barrier. Davenport (1968) showed that sodium taurocholate (NaTC) in concentrations as low as 10 mmol/l increased the net Na^+ flux into Heidenhain pouches of the dog stomach. This apparent increase in gastric mucosal permeability may have been preceded by an inhibition of active absorptive Na^+ transport, which Kuo and Shanbour (1976) found to be reduced in the isolated gastric mucosa of the dog by a bile extract containing NaTC. These *in vitro* studies, however, were not conducted with pure NaTC nor in an acidic solution such as prevails *in vivo*.

Although the solubility of unconjugated bile salt is low in acid, the conjugated NaTC is soluble in moderately acidic solutions. Similarly, active Na^+ transport persits, although diminished, even after moderate acidification (pH 3.40) of the luminal solution bathing the gastric mucosa of the rat (Sernka and Hogben 1969) if not that of the dog (Kitahara et al. 1969). The present studies attempt to characterize the inhibitory action of pure NaTC in such a moderately acidic medium on Na^+ transport through the gastric mucosa of the rat. Actions of conjugated NaTC and unconjugated sodium deoxycholate (NaDC) are compared.

The damaging action of NaTC at a high concentration of 80 mmol/l to produce lesions in the rat stomach was reduced by addition of a prostaglandin, 16,16-dimethyl prostaglandin E_2 ($dmPGE_2$; Chaudhury and Robert 1980). This cytoprotective effect of $dmPGE_2$ observed *in vivo* was also examined *in vitro* (Chaudhury and Jacobson 1978). In the isolated gastric mucosa of the dog, $dmPGE_2$ addition stimulated active absorptive transport of Na^+. These studies, however, did not include actions of bile salts, interactions of bile salts and prostaglandins, or effects in a moderately acidic mucosal solution. The present studies extend the characterization of prostaglandin cytoprotection of the gastric mucosa in these respects.

A preliminary report of these studies has been presented (Nguyen and Sernka 1984).

Materials and Methods

Male adult rats of the Sprague-Dawley strain were fasted overnight and anesthetized by an intraperitoneal injection of sodium pentobarbital (60 mg/kg). The stomach was exposed by a midline incision of the abdomen and excised by severing the lower esophageal and pyloric sphincters. The forestomach was discarded, and the glandular stomach was divided along the greater curvature into two equal portions for paired studies. The gastric mucosa was separated from the serosal muscle coat by scissors and forceps dissection. The dissected mucosae were mounted in two flux chambers having exposed areas of 1 cm^2.

The isosmotic Ringer solution bathing both sides of the gastric mucosa had the following composition (in mmol/l): 10 N-tris (hydroxymethyl) methyl-2-aminoethane sulfonic acid (TES, Sigma), 133 NaCl, 5 KCl, 1 $MgSO_4$, 1 Na_2HPO_4, 1 $CaCl_2$ and 25 dextrose. This Ringer solution containing TES and phosphate buffers adequately maintained the pH at 7.40 or 3.40. (From the titration curve and the measured value of H^+ transport by isolated rat gastric mucosa (Sernka and Caplan 1982), 2.75 μmol/cm^2 h, it can be shown that in the 15-min periods studied the pH value would have fallen only slightly, from 7.40 to 7.37 or from 3.40 to 3.35 in the absence of any H^+ back-diffusion). The solution was maintained at 37 °C and oxygenated with 100% O_2 throughout the experiments. The electrical potential difference (*PD*), short-circuit current (*Isc*) and electrical resistance ($R = PD/Isc$) were measured with a voltage clamp apparatus (Cabler Biomedical) described elsewhere (Sernka et al. 1982). Short-circuiting was maintained except for brief determinations of *PD* and *R*.

After the gastric mucosae were mounted in the flux chambers, 10 ml of prewarmed Ringer solution was added to mucosal and submucosal sides. The *PD* was followed until a steady state was

reached. Following this stabilization, ^{22}Na (New England Nuclear) was added to opposite sides of the paired mucosae and the tissues were short-circuited. After 30 min for a new steady state to be attained, a control period of 30 min was initiated. The solutions from the unlabeled half-chambers were collected and replaced with fresh Ringer solution. Aliquots of the collected solutions were taken for radioactivity determination in a crystal well or liquid scintillation spectrometer (Packard). Unidirectional and net Na^+ fluxes were calculated from the radioactivity determinations as described previously (Sernka et al. 1982). Back flux from the unlabeled to the labeled sides was deemed negligible since the final radioactivity on the labeled side was about 1000 times that on the unlabeled side. Unidirectional flux values obtained in these studies are tabulated elsewhere (Nguyen 1983).

Four series of experiments were conducted to test the effects of pure bile salts on Na^+ transport. NaTC or NaDC were added to the mucosal sides so as to achieve final concentrations of 1, 5 and 15 mmol/l in successive 30-min periods. In two series, these bile salts were tested at a mucosal pH of 7.40, and in the other two series, at a mucosal pH of 3.40. The final concentration of NaDC in the moderately acidic solution was inexact due to flocculent precipitation.

Another two series of experiments tested the effects of PGE_2 and $dmPGE_2$ on Na^+ transport before and after inhibition by graded levels of moderately acidified NaTC. The 30-min control period was followed by an equal period of exposure on the mucosal side to a cytoprotective concentration of 1.6×10^{-8} mol/l PGE_2 (Sigma) or $dmPGE_2$ (kindly supplied by Dr. John E. Pike, Upjohn). With the background of prostaglandins maintained at this level, Na^+ fluxes were determined during two additional 30-min period exposures to 5 and 15 mmol/l NaTC at mucosal pH 3.40.

Two control series of experiments were conducted to test the effects of time and mucosal osmolality on Na^+ transport and the electrical parameters. In one series, the muscosal pH was maintained at a value of 7.40 and in the other series at a value of 3.40. In both controls, mannitol was added to the mucosal sides starting at 30 min so as to achieve final concentrations of 2, 10 and 30 mmol/l in successive 30-min periods. These mannitol Ringer solutions had the same osmolalities (Osmette Precision) as 1, 5 and 15 mmol/l NaTC, respectively.

The results of all series were expressed as means $\pm$SE for $n = 6$ animals. Results of single experiments were expressed as such. Significance of treatments was determined by analysis of variance for $P < .05$ followed by multiple comparisons utilizing the Studentized range test. Significance between two series of experiments was determined by an unpaired t-test for $P < .05$.

Results

The time course of the control rates of active Na^+ transport from mucosa to submucosa through rat gastric mucosa are given in Table 1. There was a slow but steady decline in Na^+ transport, expecially during the second hour in a moderately acidified mucosal solution. The reduction in Na^+ transport from the initial two time periods to the final two time periods was significant for both the neutral and acidified mucosal media. Na^+ transport was significantly smaller with acidification in five of the eight time periods studied.

Electrical parameters of the rat gastric mucosa showed relatively little change over the two-hour time period (Table 2). The *Isc* did not significantly decline in either neutral or moderately acidified mucosal solution, and *Isc* was not significantly smaller with acidification. The *PD* did not decline significantly in a neutral mucosal solution, and the decline in *PD* with acidification was significant only in the final two times examined. Acidification did not significantly change the *PD* at

Table 1. Time course of Na^+ absorptive transport through rat gastric mucosa

Time Period[1] (min)	Na^+ Net Flux (μmol/cm^2 h)	
	Mucosal pH 7.40	pH 3.40
0—15	3.9 ± 0.5	2.0 ± 0.6*
15—30	3.9 ± 0.7	2.1 ± 0.5
30—45	3.0 ± 0.4	1.2 ± 0.4*
45—60	3.2 ± 0.5	2.3 ± 0.9
60—75	2.4 ± 0.3	1.6 ± 0.9
75—90	2.5 ± 0.4	0.6 ± 0.2*
90—105	1.4 ± 0.3$^+$	−0.2 ± 0.4*$^+$
105—120	1.8 ± 0.3$^+$	0.1 ± 0.3*$^+$

[1] Mucosal mannitol concentration was 0, 2, 10 and 30 mmol/l during time periods 0—30, 30—60, 60—90 and 90—120 min, respectively.
* $P < .05$, pH 7.40 vs. pH 3.40 values.
$^+$ $P < .05$ from values at 0—30 min.

any time studied. The *R* did not decline significantly in neutral mucosal solution, and the decline in *R* with acidification was significant only in the final two times examined. Acidification significantly increased *R* at the 30-min time mark.

The effects of NaTC on the active transport of Na^+ from mucosa to submucosa through the rat gastric mucosa are shown in Fig. 1. At a mucosal pH of 7.40, no significant inhibition of Na^+ transport was observed upon successive mucosal exposure to 1, 5 and 15 mmol/l NaTC with respect to the corresponding controls with mannitol substitute. The *Isc*, *PD* and *R* also were not significantly lower than the corresponding control values.

The apparent refractoriness of the rat gastric mucosa to neutral NaTC did not appear to result from a species difference, since NaTC in concentrations from 1 to 10 mmol/l also had no apparent effects on the unidirectional or net fluxes of Na^+ through gastric mucosae isolated from the bullfrog, rabbit or dog in single experiments.

At a mucosal pH of 3.4, Na^+ transport through rat gastric mucosa fell with successive mucosal exposure to 5 and 15 mmol/l NaTC (Fig. 1). These reductions, however, were not significantly different from those of the mannitol controls. Similarly, changes in *Isc*, *PD* and *R* after mucosal NaTC exposure were not significantly less than those of the corresponding acidified controls.

Parallel experiments were conducted to test the effects of a pure but unconjugated bile salt on active Na^+ transport through this tissue. The responses to 1, 5 and 15 mmol/l NaDC added to the mucosal side are listed in Table 3 and depicted in Fig. 2. At a mucosal pH of 7.40, 1 mmol/l NaDC significantly inhibited

Table 2. Time course of electrical parameters of rat gastric mucosa

Time[1] (min)	*Isc* (μmol/cm^2 hr)		*PD* (mV)		*R* (Ω-cm^2)	
Mucosal	pH 7.40	pH 3.40	pH 7.40	pH 3.40	pH 7.40	pH 3.40
0	—	—	−18±1	−20±1	180±6	190±5
15	4.0±0.2	4.2±0.4	−18±1	−20±2	184±5	193±4
30	4.0±0.2	4.0±0.4	−18±1	−20±1	175±8	204±6*
45	4.1±0.2	3.8±0.3	−18±1	−18±1	179±6	191±5
60	4.0±0.3	3.7±0.3	−18±2	−16±1	181±5	189±6
75	4.0±0.3	3.5±0.4	−18±2	−16±1	177±6	184±6
90	4.0±0.3	3.4±0.3	−17±2	−15±1	173±6	183±5
105	3.9±0.3	3.3±0.4	−15±1	−14±1$^+$	168±6	180±5$^+$
120	3.6±0.3	3.1±0.3	−15±1	−13±1$^+$	159±3	171±6$^+$

[1] Mucosal mannitol concentration was 0, 2, 10 and 30 mmol/l during time periods 0—30, 30—60, 60—90 and 90—120 min, respectively.

* $P<.05$, pH 7.40 vs pH 3.40 values.

$^+$ $P<.05$ from values at 30 min.

active Na^+ absorptive transport and 5 and 15 mmol/l NaDC produced a significant reversals or net secretory fluxes as compared with the mannitol control fluxes at time periods 60—75 and 105—120 min. Successive mucosal exposure to 1, 5 and 15 mmol/l NaDC significantly reduced the *Isc* as compared to the mannitol controls. The *PD* was significantly reduced after 15 min mucosal exposure to 1 mmol/l NaDC and fell further and significantly as NaDC was increased to 5 and 15 mmol/l on the mucosal side in comparison to mannitol controls. The *R* was not significantly lowered by 1 mmol/l NaDC, but 5 mmol/l NaDC significantly reduced

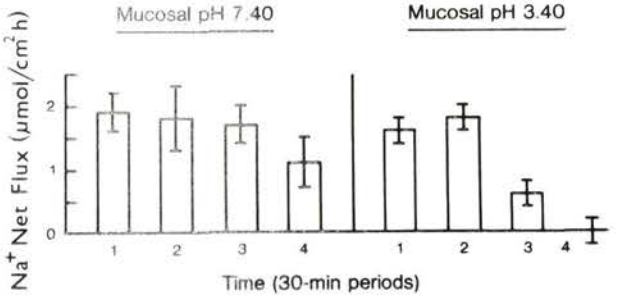

Fig. 1. Effects of increasing mucosal levels of sodium taurocholate (NaTC) on active transport of Na^+ from mucosa to submucosa through rat gastric mucosa in the absence (*left*) and presence (*right*) of a chemical gradient for H^+ back-diffusion. Periods.: 1 — control; 2 — 1 mmol/l mucosal NaTC; 3 — 5 mmol/l NaTC; 4 — 15 mmol/l NaTC. Mean values ± SE from 6 animals.

Table 3. Effects of sodium deoxycholate (NaDC) on Na^+ absorptive transport and electrical parameters of rat gastric mucosa at luminal pH 7.40

Time[1] (min)	NaDC (mmol/l)	Na^+ Net Flux (μmol/cm^2 h)	*Isc* (μmol/cm^2 h)	*PD* (mV)	*R* (Ω-cm^2)
0	0	—	—	24 ± 1	214 ± 9
15	0	1.8 ± 0.4	4.1 ± 0.1	23 ± 1	213 ± 9
30	0	1.4 ± 0.3	3.9 ± 0.2	22 ± 1	212 ± 8
45	1	0.7 ± 0.5*	2.9 ± 0.1*	13 ± 1*	195 ± 4
60	1	0.8 ± 0.3*	1.8 ± 0.1*	9 ± 2*	218 ± 10
75	5	−3.5 ± 0.7*	1.5 ± 0.1*	6 ± 1*	155 ± 6*
90	5	−0.4 ± 1.0*	1.5 ± 0.1*	6 ± 1*	149 ± 7*
105	15	−0.6 ± 0.9	1.6 ± 0.1*	5 ± 1*	144 ± 5*
120	15	−1.8 ± 0.7*	1.4 ± 0.1*	4 ± 1*	130 ± 5*

[1] 15 min time periods ending at these times for Na^+ net flux and *Isc* determinations.
* $P < .05$ from comparable mannitol control values.

R within 15 min as compared to mannitol controls. *R* fell further and significantly after mucosal exposure to 15 mmol/l NaDC as compared with mannitol controls.

At a mucosal pH of 3.4, similar responses to NaDC were noted (Fig. 2). Significant reduction in Na^+transport through rat gastric mucosa was effected by 1 mmol/l NaDC and significant reversal to net secretory flux was brought about by 5 mmol/l NaDC on the mucosal side with respect to mannitol controls. No further significant effect was evident with 15 mmol/l NaDC, since the control rate of Na^+

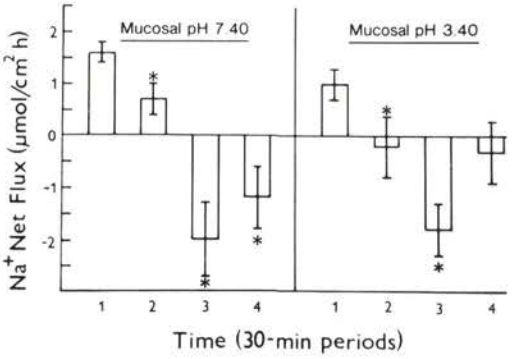

Fig. 2. Effects of increasing mucosal levels of sodium deoxycholate (NaDC) on active transport of Na^+ from mucosa to submucosa through rat gastric mucosa in the absence (*left*) and presence (*right*) of a chemical gradient for H^+ back-diffusion.
Periods: 1 — control; 2 — 1 mmol/l mucosal NaDC; 3 — 5 mmol/l NaDC; 4 — 15 mmol/NaDC. Mean values ± SE from 6 animals.
*Significant difference ($P < .05$) from value of corresponding control using mannitol as substitute.

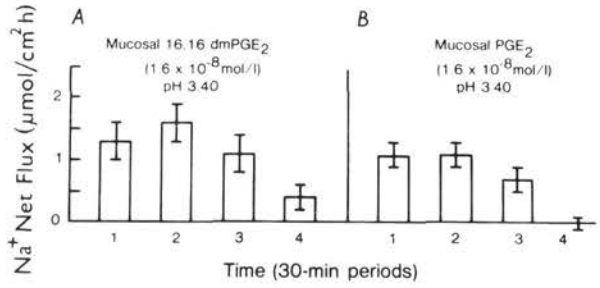

Fig. 3. Effects of cytoprotective, mucosal levels of 16,16-dmPGE$_2$ (*left*) and PGE$_2$ (*right*) on active absorptive transport of Na^+ through rat gastric mucosa exposed to intermediate and high mucosal levels of acidified sodium taurocholate (NaTC). Periods: *A*: 1 — control; 2 — 16,16 dmPGE$_2$; 3 — 16,16 dmPGE$_2$ and 5 mmol/l NaTC; 4 — 16,16 dmPGE$_2$ and 15 mmol/l NaTC; *B*: 1 — control; 2 — PGE$_2$; 3 — PGE$_2$ and 5 mmol/l NaTC; 4 — PGE$_2$ and 15 mmol/l NaTC. Mean values ± SE from 6 animals.

transport using mannitol was effectively zero at this time period (Table 1). *Isc* and *PD* was significantly lower than the mannitol controls at all levels of mucosal NaDC. *R* was significantly greater at the beginning and end of NaDC treatment than in the mannitol controls, but there was a significant decline from the value before NaDC, 248 ± 8 Ω-cm^2, to that after mucosal exposure to 5 mmol/l NaDC, 195 ± 4 Ω-cm^2.

In another series of experiments, possible protection afforded by prostaglandins against bile salt damage to the gastric mucosa was investigated. PGE$_2$ and its more stable, permeable analogue, dmPGE$_2$, were selected to test prostaglandin cytoprotection. Intermediate (5 mmol/l) and high (15 mmol/l) levels of moderately acidified NaTC were chosen to physiologically challenge the mucosa. The results of prostaglandins pretreatment followed by bile salt testing in the continued presence of prostaglandin are shown in Fig. 3. At a physiological, cytoprotective concentration of $1.6 = 10^{-8}$ mol/l on the mucosal side, neither PGE$_2$ nor dmPGE$_2$ altered significantly the active transport of Na^+ from mucosa to submucosa of rat gastric mucosa as compared with the untreated mannitol controls. With these prostaglandins remaining in the mucosal solution at this same level, neither 5 nor 15 mmol/l NaTC on the acidified mucosal side significantly changed Na^+ transport as compared with mannitol controls. However, *Isc* was significantly reduced by both levels of NaTC in the presence of dmPGE$_2$ or PGE$_2$ as compared with the values of the mannitol controls. *Isc* fell from 3.1 ± 0.1 μmol/cm^2 h prior to treatment to 2.3 ± 0.1 μmol/cm^2 h after mucosal exposure to dmPGE$_2$ and acidified 15 mmol/l NaTC. The *PD* did not show any significant changes from the mannitol control values, and the *R* was significantly elevated in the prostaglandin-NaTC treated mucosae over the mannitol controls except for the 15 mmol/l NaTC and PGE$_2$ treated tissues.

In all the experiments involving bile salts and prostaglandins at neutral or moderately acidic mucosal pH, the *Isc* exceeded net Na^+ flux from mucosa to submucosa. The difference, I_{sc} – net Na^+ flux, had a grand $\bar{x} \pm SE$ of 2.2 ± 0.1 μmol/cm^2 h. Thus, the rat gastric mucosa transported some other cation from mucosa to submucosa or, more likely, an anion like Cl^- from submucosa to mucosa (Sernka and Hogben 1969). Since I_{sc} – net Na^+ flux did not change much from the control value, 2.6 μmol/cm^2 h, to that with 15 mmol/l NaDC, 2.7 μmol /cm^2 h, the effects of unconjugated bile salt on probable Cl^- active transport appeared minimal. The comparable values of I_{sc} – net Na^+ flux for conjugated bile salt at neutral mucosal pH were control, 2.6 μmol/cm^2 h, and 15 mmol/l NaTC, 2.4 μmmol/cm^2 h. Pretreatment of the moderately acidified mucosa with $dmPGE_2$ and subsequent treatment with 15 mmol/l NaTC did not change the control I_{sc} – net Na^+ flux value of 1.9 μmol/cm^2 h. Thus, the effects of conjugated bile salt and prostaglandin on probable Cl^- active transport also appeared minimal.

Discussion

As a result of their detergent properties, bile salts that are regurgitated from the duodenum into the stomach may break down the gastric mucosal barrier. Conceivable mechanisms for such a gastric mucosal breakdown by bile salts include a reduction in mucosal blood flow, increased mucosal permeability and inhibition of active ion transport.

Mucosal ischemia is not a likely cause of gastric mucosal breakdown induced by bile salts, because bile salt elevates rather than reduces gastric mucosal blood flow. Ritchie (1975) showed that instillation of 5 mmol/l NaTC in a dog gastric flap preparation doubled gastric mucosal blood flow. Whittle (1977) found even larger increases in gastric mucosal blood flow after perfusion of the rat stomach with acidified NaTC.

Distinguishing between changes in gastric mucosal permeability and active ion transport is difficult with *in vivo* preparations. Increases in Na^+ fluxes found in the dog stomach after instillation of NaTC (Davenport 1968) very likely represented an increased mucosal permeability that permitted Na^+ to move down its electrochemical gradient from the blood to the lumen. However, the same secretory flux could have resulted from inhibition of active absorptive transport of Na^+. Using the isolated dog gastric mucosa, Kuo and Shanbour (1976) showed that a mixture of bile salts including NaTC first inhibited active Na^+ transport and then increased mucosal permeability. Our results on the isolated rat gastric mucosa are consistent with the hypothesis that damaging agents act initially on active ion transport and subsequently on permeability of the gastric mucosa (Shanbour et al. 1973). With pure NaDC in an acidified mucosal solution, we found that active Na^+ transport was inhibited at 1 mmol/l bile salt. Mucosal *R*, the inverse function of

tissue permeability, did not fall at this level of NaDC. Later, at 5 and 15 mmol/l, NaDC both inhibited Na^+ transport and increased mucosal permeability.

A somewhat more sensitive measure of instantaneous changes in active ion transport is the *PD* across the gastric mucosa. In neutral mucosal solutions, active Na^+ transport is a major source of the *PD* in the gastric mucosa of both the dog (Kitahara et al. 1969) and the rat (Sernka and Hogben 1969). In the present experiments, we found that the *PD* of rat gastric mucosa bathed by a neutral mucosal solution was reduced by a low concentration of NaDC. At this same concentration, 1 mmol/l, NaDC had no effect on mucosal *R*. These results also support the hypothesis of Shanbour that bile salts inhibit Na^+ transport prior to an increase in gastric mucosal permeability.

Our results differ from those of Kuo and Shanbour (1976) in the apparent potency of bile salts to effect breakdown of the gastric mucosal barrier. Whereas their results indicate total inhibition of active Na^+ transport by 1 mmol/l bile salt, we did not observe this degree of inhibition except at 5 and 15 mmol/l NaDC. (We did, nevertheless, observe the *PD* fall with 1 mmol/l NaDC). It is unlikely that there was a species difference between the dog that Kuo and Shanbour used and the rat that we used. When we treated dog gastric mucosa with a neutral solution of 1 to 10 mmol/l NaTC, Na^+ transport was not inhibited. More likely the source of discrepancy lies with the nature and purity of the bile salts utilized. Kuo and Shanbour used a mixture extracted from ox bile and containing 40% NaTC. We used pure NaTC or pure NaDC. Since NaDC was much more potent than NaTC in inhibiting Na^+ transport, unidentified biliary components like NaDC may have been the source of the high apparent potency observed by Kuo and Shanbour.

A major factor in rendering the gastric mucosa susceptible to bile salt damage is the presence of acid in the lumen. Russell et al. (1981) showed that none of the naturally occurring conjugated or unconjugated bile salts instilled in rat stomachs caused gastric erosions and bleeding unless administered in acidic solutions. They suggested that acid may make the conjugated bile salts more soluble and absorable. However, by the same token, acid makes unconjugated bile salts less soluble: the pKa of NaDC is 6.58. Also, the solubilized bile salt is more ionized and therefore less lipid-permeable and absorbable. Another explanation was suggested by the experiments of Rees et al. (1981). These investigators found that low concentrations of NaTC inhibited the alkaline secretion by isolated frog gastric mucosa. Since alkaline secretion provides chemical protection to the mucosa against luminal acid, the damaging actions of conjugated bile salts may only become apparent in acidic solutions. Rees et al. also found that NaTC could stimulate secretion of gastric acid, so that bile salt damage could occur even in the absence of instilled acid. This interpretation, however, fails to account adequately for the marked inhibition of Na^+ transport that we observed in the rat gastric mucosa exposed to a well-buffered, neutral luminal solution of NaDC. It may be that NaDC, unlike NaTC, does

not require acid for its barrier breaking. Studies by Hills et al. (1983) indicate that NaDC in neutral solution eliminates the hydrophobicity of the oxyntic mucosal surface. In these studies, acid did not affect the contact angle between fluid and surface that was used to measure hydrophobicity.

It is conceivable that conjugated bile salts that are regurgitated from the duodenum into the stomach act to impair the gastric mucosal barrier. With an increased mucosal permeability to ions brought about, H^+ back-diffusion from the lumen would increase. Chronic regurgitation of bile salt, as may occur in the process of gastric ulceration (Rhodes et al. 1969), may therefore lead to loss of gastric acid from the lumen. The resulting hypoacidity, in turn, may favor the survival and proliferation of micro-organisms regurgitated along with bile from the intestine. Some of these microorganisms can deconjugate bile salts (Hylemon and Glass 1983). Appreciable deconjugation would give rise to NaDC and other unconjugated bile salts that effectively break the gastric mucosal barrier at levels characteristic of gastric juice from gastric ulcer patients — about 5 mmol/l (Rhodes et al. 1969). Our studies also showed gastric mucosal barrier breakdown by this level of NaDC. Hence, permeability would increase further, leading to lower acidity, more bacterial growth and increased deconjugation. The result of such positive feedback, or vicious cycle, would be total breakdown of the gastric mucosal barrier in the area of the inner curvature of the stomach that is exposed to regurgitated bile, leading to gastric ulceration.

According to the studies of Robert et al. (1979), the gastric mucosa produces prostaglandins to protect itself against a variety of damaging agents, including NaTC (Chaudhury and Robert 1980). When these investigators inhibited prostaglandin synthesis by pretreatment of the rats with indomethacin, cytoprotection against damage by NaTC was lost. A possible mechanism for cytoprotection was suggested by the studied of Chaudhury and Jacobson (1978), who showed that active Na^+ transport in the dog gastric mucosa was stimulated by addition of $dmPGE_2$ and inhibited by addition of indomethacin. They also found that further addition of dibutyryl cyclic AMP or theophylline, a phosphodiesterase inhibitor, reversed the inhibition of Na^+ transport brought about by indomethacin. The postulated sequence of cytoprotective events appeared to be: prostaglandin synthesis, cyclic AMP formation, stimulation of Na^+ transport. The observations of Sernka and Caplan (1982) that $dmPGE_2$ increased oxygen consumption of rat gastric mucosal cells were consistent with this hypothesis, since stimulation of ion transport would require extra oxygen for metabolic energy.

One possible explanation for bile salt inhibition of Na^+ transport in the gastric mucosa would include depression of mucosal prostaglandin levels. This possibility was explored in the present experiments by testing the effects of exogenous $dmPGE_2$ or PGE_2 to restore prostaglandin levels and hence Na^+ transport to normal. Assuming a low rate of degradation and a high rate of permeation for the

dimethyl variety at least, one might expect addition of prostaglandin to reverse or lessen the inhibition of Na^+ transport produced by bile salt. There was little in our experiments to suggest that this happened, although the acidified NaTC used could not be shown to inhibit significantly Na^+ transport. The *PD* did not change significantly after NaTC treatment whether or not prostaglandin was present. Nevertheless, our experiments do not disprove that endogenous prostaglandin may protect the mucosa, since we only tested the effects of exogenous prostaglandin.

Several past studies have suggested that exogenous as well as endogenous prostaglandin protects the gastric mucosa against damage by bile salts. Mann (1976) utilized whole bile to cause acute erosive gastritis in rats and found that the number of lesions was significantly reduced when the animals were given PGE_2. Carmichael et al. (1977) showed that the incidence of gastric mucosal hemorrhage induced in rats by oral administration of acidified NaTC was reduced to about one-half by addition of the prostaglandin analogue, 15(R)15 methyl-PGE_2 methyl ester. Müller et al. (1981) reported that instillation of 4 mmol/l NaTC in the human stomach caused a transient, 35 % reduction in the *PD*; pretreatment with $dmPGE_2$ prevented this drop in gastric *PD*. Szelenyi et al. (1983) showed that aluminum hydroxide antacid gel increased PGE_2 content of the rat gastric mucosa and prevented the fall in PGE_2 content produced by NaTC. Erosions produced in rat stomachs by perfusion with NaTC were reduced in severity by simultaneous exposure to (15S)-15 methyl prostaglandin E_2 methyl ester (Whittle 1977). Except for this last study utilizing a less a common analogue, however, the concentrations of exogenous prostaglandins used to bring about cytoprotection against NaTC were some 3 to 10^5 times greater than that used by us. Robert et al. (1979) have distinguished the physiological and cytoprotective effects of prostaglandin at the level that we used, 10^{-8} mol/l, from the pharmacological and antisecretory effects at levels above 10^{-6} mol/l. Thus, prostaglandin cytoprotection against bile salt damage to the gastric mucosa may be more a pharmacological than a physiological phenomenon.

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