Absorption of Bile Salts from the Gastric Mucosa During Hemorrhagic Shock[1] (36581)

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Intragastric bile salts increase gastric mucosal permeability to H^+ in man (1) and the dog (2), and are associated with a high frequency of mucosal erosions during hemorrhagic shock (3). Davenport (4) first reported that bile salts are absorbed from the gastric mucosa. He demonstrated a mean absorption of 12 to 304 μmole/hr after instillation of labeled taurocholate or natural dog bile into denervated canine gastric pouches. Studies in humans by Ivey, DenBesten and Bell (5) have shown a mean absorption of $10.7 \pm 0.8\%$ of 5 m*M* taurine-conjugated bile salts 15 min after intragastric instillation. In recent experiments (3) we observed a correlation between the incidence of stress ulcers and the presence of intragastric bile salts in hypotensive animals. In subsequent studies we have confirmed that shock does not influence the rate of hydrogen ion loss (back diffusion) from the gastric lumen in normal or vagotomized animals when the gastric mucosal barrier is broken by intragastric bile salts, but vagotomy protects against stress ulcer formation; that is, no stress ulcers occurred in the absence of H^+, and bile salts caused ulceration only in the presence of H^+ (6).

The present study was designed to measure the effects of hypotension and vagotomy on bile salt absorption and to correlate these data with the frequency of bile acid-induced mucosal damage.

Materials and Methods. Chronic mongrel dogs weighing 18-22 kg were randomly assigned to one of four groups. In Group I animals a test solution of bile salts and hydrochloric acid was instilled into the distally occluded stomach for two 30-min periods under normotensive conditions. Group II animals were treated as in Group I except that hemorrhagic shock was induced (mean blood pressure 40 mm Hg) prior to the instillation of the test solution. Animals assigned to Groups III and IV had a vagotomy and pyloroplasty carried out 3 weeks prior to the definitive study. Group III animals were treated as in Group I, that is, bile salts in acid were instilled into the distally occluded vagotomized stomach for two 30-min periods during normotensive conditions. The 16 vagotomized animals in Group IV were studied during hemorrhagic shock. Bile salts in acid solution were instilled into the distally occluded stomachs of eight animals, whereas in the remaining eight animals bile salts in neutral solution were left in the stomach during two successive 30-min periods.

Test solutions. All solutions containing bile salts were 10 m*M* taurocholic acid (containing $< 2\%$ other conjugates and $< 1\%$ unconjugated bile acids). For experiments using bile salts plus acid, the taurocholic acid was dissolved in 100 mEq/HCl. All test solutions contained 2 mg/ml of polyethylene glycol (PEG; mol wt 4000) as a nonabsorbable dilution indicator. The rinse solutions were indicator-free and contained the HCl concentration of the test solution. In experiments using bile salts in neutral solution the bile salts were dissolved in 0.9% NaCl and the rinse solution was 0.9% NaCl.

Experimental technic. Animals were anesthetized with intravenous Nembutal after an overnight fast. Orogastric and cuffed endotracheal tubes were placed. The abdomen was opened and the pylorus was occluded

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with a noncrushing clamp. At the end of the procedure the stomach was emptied, the clamp was removed, and the incision was closed. In Groups I (normotensive, 6 animals), II (shock, 6 animals), III (normotensive, 8 vagotomized animals), and IV-A (shock, 8 vagotomized animals), the stomach was initially rinsed with indicator-free HCl, and 100 ml of the test solution (bile salt + HCl) was instilled via the orogastric tube. After thorough mixing an aliquot was removed for analysis. At the end of the 30-min period, the gastric contents were aspirated and saved for analysis. The entire procedure was repeated a second time after rinsing the stomach with wash solution. In Group IV-B (shock, 8 vagotomized animals) the procedure was identical except that the wash and test solutions contained no HCl.

Hemorrhagic shock (mean arterial pressure of 40 mm Hg) was induced by rapid exsanguination from a cannulated femoral artery. Mean arterial pressure was monitored during the experiment in the contralateral femoral artery. At the termination of the experiment shed blood was returned to the animal over 30–40 min.

Laboratory measurements. After centrifugation to obtain a clear supernatant solution, PEG, H^+ and bile salt concentrations were determined in control, 1- and 30-min aliquots. PEG was measured by a turbidimetric method (7). Bile salts were determined by the steroid dehydrogenase method of Iwata and Yamasaki as modified by Rhodes *et al.* (8) The ΔOD was determined at 340 mμ in a Gilford spectrophotometer (Model 240, Gilford Inst., Inc., Oberlin, OH) and compared to curves derived from serial dilutions of pure cholic acid. The H^+ concentrations were determined by titration with 0.1 *N* sodium hydroxide to pH 7.2 using a glass electrode.

Calculations. The residual volume in the stomach and volume secreted during each experiment were calculated from dilution of the nonabsorbable indicator. The percentage of bile salts absorbed was calculated from the difference of the initial and terminal bile salt mass divided by the initial bile salt mass multiplied by 100 [percent bile salt absorbed = (bile salt initial — bile salt terminal) ÷ bile salt initial × 100].

Statistics. The Student's *t* test was used to analyze paired observations. The correlation coefficient (*r*) was used in relating bile salt absorption to H^+ back diffusion.

Results. The initial gastric pH was < 2 in animals with normal stomachs and > 4 in vagotomized animals. The bile salt absorption data expressed as absolute and percentage absorption are given in Table I.

The mean percentage of absorption for Groups I and II (normotensive and shocked animals with vagally intact stomachs) were 24.83 ± 3.65 and 27.88 ± 7.05%, respectively. In Group III (normotensive animals with vagotomy) the percentage of absorption was 21.96 ± 1.66%, which was not significantly different from Groups I or II. In Groups IV-A and IV-B (shocked vagotomized dogs) greater absorption of bile salts occurred (23.74 ± 3.57%) in the presence of H^+ than that observed when a neutral solution of bile salts was instilled into the stomach (7.68 ± 4.05%, $p < .05$). Absorption occurred in all

TABLE I. Gastric Absorption of Bile Salts/30-min Period.

Group	No. of periods	Initial amount (μmole)	Amount absorbed	% Absorption
I. Normotensive	6	601.97 ± 24.47	147.25 ± 19.73	24.83 ± 3.65
II. Shock	6	604.31 ± 19.88	164.16 ± 38.26	27.88 ± 7.05
III. Vagotomy normotensive	8	739.75 ± 10.68	166.58 ± 15.88	21.96 ± 1.66
IV. Vagotomy + shock				
A. Acid pH	8	642.87 ± 20.47	153.43 ± 23.27	23.74 ± 3.57
B. Neutral pH	8	639.37 ± 12.40	46.63 ± 24.23	7.68 ± 4.05[a]

[a] Δ Group IV-A and IV-B 16.06%, $p < .05$.

TABLE II. Gastric Absorption of Bile Salts/30 min Period of Instillation.

Group	Period	No. of samples	% Absorption/ 30 min
I. Normotensive BA–HCl[a]	1	6	24.83 ± 3.65
	2	6	19.35 ± 4.88
II. Shock BA–HCl	1	6	27.88 ± 7.05
	2	6	21.27 ± 4.59
III. Normotensive Vagotomy BA–HCl	1	8	21.96 ± 1.66
	2	8	19.39 ± 2.65

[a] BA = bile acid.

animals and in all periods (Table II). In Groups I, II and III absorption in the first 30-min period, although greater than in the second period, was not statistically different.

The mean net H^+ fluxes and the bile salt absorption/30-min period for the various groups are detailed in Table III. There was no correlation between bile salt absorption and H^+ back diffusion ($r > .5$) in any of the groups. Although no significant difference in H^+ loss was noted between the various groups, back diffusion was numerically less in Group II (shocked animals with vagally intact stomach) than in Group I (normotensive animals with vagally intact stomach). Six of six animals in Group II, and eight of eight animals in Group IV-A had extensive gastric erosions when sacrificed 1 to 3 days later, whereas none of the animals in Groups I, III or IV-B had mucosal injury.

Discussion. Conjugated bile salts are primarily absorbed by active transport in the ileum (9). Hislop, Hoffman and Schoenfeld (10) studied bile salt absorption in the small intestine and found that free bile acids are efficiently absorbed from a jejunal segment (60 ± 5.1%) whereas glycine conjugates are absorbed less rapidly (24.5 ± 5.6%) and taurine conjugates least of all (3.3 ± 0.8%). They concluded that the ionic group of the bile acids is a major determinant of the site of absorption in man. Hogben *et al.* (11) showed that the absorption of drugs such as aspirin from the stomach is determined by the pK_a of the drug, acetylsalicylic acid being absorbed more readily when the pH in the stomach is less than the pK_a. Davenport (4) found that the rate of absorption of taurocholate from the stomach was proportional to the concentration instilled, and postulated that bile salt absorption in the stomach is the result of passive diffusion through large pores rather than an active process. Ivey, DenBesten and Bell (5) found a rate of absorption from the stomach (7.4%/15 min) of taurocholic acid at pH 1 which was three times greater than that reported from Davenport's laboratories (4) (2.3%/15 min) at pH 7. This difference was postulated to be due to the presence of TCA in its nonionized lipid-soluble form at the lower pH. This thesis is supported by our observation that absorption was greater ($p < .05$) in vagotomized animals in the presence of acid (23.74 ± 3.57) compared to a neutral solution of taurocholic acid (7.6 ± 4.05).

All the shocked animals with bile salt in acid solution had mucosal gastric erosions at sacrifice compared to a total absence of mucosal defects in vagotomized shocked animals treated with bile salts in neutral solution. From our present observations we conclude that: (a) shock does not affect the rate of absorption of bile salts; (b) no correlation exists between bile salt absorption and hydro-

TABLE III. Gastric Absorption of Bile Salts and H^+ Back Diffusion/30-min.[a]

	Group	No. of samples	Mean % absorption	Mean net mEq H^+ flux
I.	Normotensive	6	24.83 ± 3.65	−4.1 ± 0.5
II.	Shock	6	27.88 ± 7.05	−2.7 ± 0.3
III.	Normotensive[b]	8	21.96 ± 1.66	−2.91 ± 0.2
IVB.	Shock[b]	8	23.74 ± 3.57	−2.40 ± 0.2

[a] $r = > .05$.
[b] Vagotomized animals.

gen ion back diffusion, the one occurring independently of the other; and (c) the absorption of bile salts with a low pK_a (taurocholic acid) is greater in the presence of an increased H^+ concentration.

Summary. We compared the absorption (loss) of bile salts from the distally occluded canine stomach of normal and vagotomized animals before and during hemorrhagic shock (mean BP 40 mm Hg) after serial instillation of 10 m*M* taurine-conjugated bile salts in neutral and acid solution (100 mEq/liter HCl). In normotensive animals mean ± SE absorption of taurocholic acid in acid solution was 24.83 ± 3.65% in the first 30-min period and 19.35 ± 4.88% in the second period. Absorption during shock showed a similar decrease in the first and second 30-min periods (27.88 ± 7.05 and 21.27 ± 4.59%) which was not different from absorption in normotensive animals. Absorption of bile salts in an acid soluction from the vagotomized stomach was 21.96 ± 1.66% in normotensive animals and 23.74 ± 3.57% during hemorrhagic shock. Absorption dropped to 7.68 ± 4.05% ($p < .05$) when bile salts in neutral solution was left in the vagotomized stomach for 30 min during hemorrhagic shock. None of the eight vagotomized animals developed mucosal erosions when a neutral solution of bile salts was placed in the stomach during hemorrhagic shock. In contrast, eight of eight vagotomized animals had numerous gross and microscopic mucosal defects at sacrifice, after the instillation of bile salts in acid solution during shock. We conclude that taurocholic acid is more readily absorbed from an acid solution, and produces extensive gastric mucosal injury in the presence of H^+ and hemorrhagic shock.

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