inoindole were more prominent in the dog. Large magnitude phasic contractions of the intestine are related to failure of intestinal propulsion, especially if the increase in nonpropulsive motility persists (2). However, indolazinoindole is less than one-fiftieth as potent as morphine as a stimulant of dog intestine. Since it may be approximately equipotent with morphine as an analgesic, it may offer significant advantages over morphine in terms of selectivity of action. It does possess the potential of causing constipation due to failure of propulsion, particularly at higher dose levels.

The relatively weak intestinal stimulatory property of indolazinoindole as compared to morphine may reflect its more pronounced inhibitory component of action. In cat and monkey intestine *in situ* and particularly in monkey intestinal preparations *in vitro*, inhibition of motility was often the primary response to indolazinoindole. Its actions in dog intestine may reflect the algebraic sum of morphine-like stimulatory effects and inhibitory effects as seen in monkey intestine. The inhibitory component of action could, therefore, account for the approximately 50-fold shift to the right in the dose–response curve in relation to morphine. In the cat intestine, the inhibitory effects of indolazinoindole sometimes outweighed its stimulatory effects. In the three species in which motility was measured, there was a spectrum of effects: primarily stimulatory in dogs, mixed in cats, and primarily inhibitory in monkeys. The decrease in meal propulsion in mice could possibly result either from stimulation of nonpropulsive contractions or from inhibition of propulsive intestinal contractions.

The ability of atropine and cyproheptadine to antagonize the intestinal stimulatory effects of indolazinoindole is significant. The narcotic analgesics are thought to produce their intestinal stimulatory effects by means of release of local 5-hydroxytryptamine from stores in the intestine (3, 5). The 5-hydroxytryptamine mobilized by the narcotics stimulates intestinal smooth muscle in two ways: by direct excitatory effects on smooth muscle 5-hydroxytryptamine receptors and indirectly by stimulation of cholinergic nerve elements in the wall of the intestine. The direct smooth muscle effect of 5-hydroxytryptamine can be reduced by 5-hydroxytryptamine receptor antagonists such as cyproheptadine (5). Since the indirect effect of 5-hydroxytryptamine is mediated in acetylcholine, the neural component of 5-hydroxytryptamine action can be antagonized by atropine (5). Cyproheptadine and atropine antagonized the intestinal stimulatory effects of indolazinoindole, and the intestinal motor effects of this compound may be mediated, at least in part, by 5-hydroxytryptamine.

Indolazinoindole thus appears to share with the narcotic analgesics the ability to affect intestinal smooth muscle, but its ratio of analgesic to intestinal stimulatory actions seems quite favorable. Potential contributions to constipation of the central components of indolazinoindole action remain to be determined.

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# Effect of Bile Salts on Partitioning and Oral Toxicity of the Bisquaternary Ammonium Drug Decamethonium Bromide


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**Abstract** □ The bisquaternary ammonium drug decamethonium bromide was studied in bile salt solutions in regard to partitioning *in vitro* and lethality when absorbed from duodenal and ileal segments of rats. Sodium glycocholate, when present below or above its CMC, had little effect on the partitioning of decamethonium from a phosphate buffer into *n*-octanol. *In vivo*, no significant change in lethality (absorption) could be attributed to either the absence of endogenous bile (ligated bile duct) or the presence of normal amounts of bile (unligated bile duct). Bile salts do not appear to alter lethality (absorption) of this bisquaternary drug when given to rats *via* the enteral route.



**Keyphrases** □ Decamethonium bromide—effect of bile salts on partitioning and oral toxicity, rats □ Bile salts—effect on partitioning and oral toxicity of decamethonium bromide, rats □ Partitioning—effect of bile salts on decamethonium bromide in water–*n*-octanol system □ Toxicity, oral—effect of bile salts on decamethonium bromide lethality in rats


Surfactants influence the GI absorption of various drugs and nutrients (1). Polysorbate 80, above its critical micelle concentration (CMC), inhibits salicylamide absorption from the small intestine of the rat (2). A similar inhibitory effect was shown for nitrobenzene in a micellar sodium lauryl sulfate system (3). Bile salts, being endogenous surfactants, also affect GI absorption of drugs, but the properties of bile salt–drug combinations vary, depending upon the state of aggregation of the bile salts. For example, the absorption of imipramine from the rat jejunum and ileum can be reduced in the presence of

micellar (aggregated) sodium taurocholate (4). Similar effects have been observed with a diethylaminoethylbenzamide compound (5) and with isopropamide iodide (6).

Some bile salts, below their CMC's, complex with various monoquaternary compounds to give products that partition into ethylene dichloride (7, 8) or *n*-octanol (6). In *in vivo* studies, cholic acid was shown to enhance the pharmacological response to a quaternary hypotensive agent when the two were administered concurrently (9).

Previously, micellar solutions were reported to inhibit partitioning of a monoquaternary compound (isopropamide iodide) from an aqueous to an organic phase (6). GI absorption of this drug is reduced, but not markedly, in the presence of micellar sodium glycocholate. The bisquaternary ammonium drug decamethonium bromide was chosen to be studied in relation to its tendency to interact in solution below and above bile salt CMC values. Thus, the ability of the doubly charged bisquaternary to interact like the monoquaternary can be evaluated. Oral (enteral) toxicity of the erratically absorbed (10) decamethonium was studied since the quantal response of death was readily measurable as a definite end-point of this drug's action. Water–octanol partitioning in the presence of various bile salts was also studied to test if an aggregate was present that could reduce transfer of decamethonium from an aqueous to a lipid phase.

## EXPERIMENTAL

**Materials**—The following bile salts were used as received: the sodium salts of glycocholic and taurocholic acids[1] and sodium cholate[2], deoxycholate[2], and dehydrocholate[2]. *n*-Octanol[2] (Grade I) was used as the organic phase in the partitioning studies because of its potential to model cellular membranes (11). Aqueous solutions for partitioning with or without bile salts were prepared in pH 7.4 phosphate buffer composed of 0.022 *M* $NaH_2PO_4 \cdot H_2O$ and 0.104 *M* $Na_2HPO_4$.

Partitioning of decamethonium bromide ($8 \times 10^{-4}$ *M*) between the aqueous phase and an equal volume of *n*-octanol was carried out for 24 hr at room temperature using $^{14}C$-labeled decamethonium[3]. A sample from both phases was counted by liquid scintillation[4]. Sampling at various times past 24 hr indicated equilibrium had been attained.

Solutions for the *in vivo* investigations were in the same buffer. Osmolarity was checked with an osmometer[5].

**Decamethonium Administration**—Male albino rats[6], weighing 200 ± 25 g and fasted overnight, were used. Following ether anesthesia, a midline incision was made. A segment of either duodenum or ileum was identified and ligated with two (00) silk sutures placed approximately 20 cm apart. These two areas were selected because bile salts are passively absorbed in all areas of the intestine and are actively transported in the ileum (12).

Drug solutions were coded and animals were assigned to treatment groups according to a completely random design. With a 27-gauge (0.5-in.) needle, 1.0 ml of the appropriate drug solution (with or without bile salt) was then injected into the segment. Gentle digital pressure over the injection site assured no solution leakage. All animals recovered from anesthesia within a few minutes. In some experiments, bile duct ligations were performed 48 hr prior to administration of the drug solutions to eliminate any possible influence of endogenous bile salts. The ligations were placed on the common bile duct at its exit from the liver, using (00) silk suture. Ligating at this level ensured that pancreatic secretions would not be interfered with. Sham operations were performed on animals with unligated common bile ducts.

Decamethonium was used at a standard dose of 25, 50, or 75 mg/animal. This amount is equivalent to 125, 250, and 375 mg/kg for a rat with an average weight of 200 g. Animals were observed for symptoms of toxicity; when death occurred, the time difference between drug injection and death was recorded as the "death time."

In one set of experiments, neomycin sulfate was used to disperse mixed micelles of bile salt, fatty acids, and decamethonium that may have been formed in the intestinal lumen.

[1] Mann Research Labs., New York, N.Y.
[2] Sigma Chemical Co., St. Louis, Mo.
[3] Amersham-Searle.
[4] Model 2002, Packard Instruments, Downers Grove, Ill.
[5] Osmette-S Automatic Osmometer, Precision Systems, Waltham, Mass.
[6] Rolfsmeyer Farms, Madison, Wis.

**Table I**—Partitioning of Decamethonium Bromide ($8 \times 10^{-4}$ *M*) into *n*-Octanol at pH 7.4 and 25°

| Bile Salt Concentration[a], m*M* | Decamethonium Partitioned, % |
|---|---|
| None (control) | 0.35 |
| 0.1 | 0.55 |
| 1.0 | 0.81 |
| 10.0 | 3.31 |
| 100.0 | 5.52 |

[a] Sodium glycocholate.

## RESULTS AND DISCUSSION

Essentially no partitioning (<0.35%) of the decamethonium bromide from the pH 7.4 buffer to the *n*-octanol phase occurred in the absence of bile salts (Table I). Concentrations of sodium glycocholate below its CMC (<10 m*M*) also had little effect. If lipid-soluble decamethonium–bile salt ion-pairs or complexes were present, they should have been extractable with some organic solvent system. Ethyl acetate, ethylene dichloride, ether, butanol, and triolein instead of octanol were used for this purpose, but no decamethonium partitioned. These results agree with those found for hexamethonium (13) with ethylene dichloride as the organic phase. Bile salt concentrations above the CMC allowed about 5.5% of the decamethonium to partition into the octanol. Slight emulsification may account for this small amount transferred. This is unlike the effects seen with the monoquaternary isopropamide iodide where the presence of sodium glycocholate and other bile salts enhanced partitioning from the aqueous phase into *n*-octanol (6).

Micellar sodium glycocholate had no significant effect on the toxicity of decamethonium (Table II). Likewise, other micellar bile salt solutions and neomycin had no significant effects (Table III).

One might expect that micellar bile salt solutions would reduce the degree of absorption of decamethonium *via* mixed aggregation or "micelloid" formation (14), as has been shown for a system of sodium taurocholate and a $C_{12}$ quaternary ammonium salt (15). These interactions would be extremely dynamic, however, and the *in situ* presence of mucin or residual food particles might also contribute to the variability of the response (16).

Neomycin sulfate has been shown to disrupt mixed micelles in solutions containing bile salts (17). If decamethonium interacts with aggregated bilt salts, then neomycin sulfate should destroy these aggregates and provide more free drug in solution to be absorbed by the intestinal epithelium. Pretreatment with neomycin sulfate had no effect on toxicity of decamethonium, suggesting little or no involvement of bile salt aggregates to retard absorption of this drug.

Decamethonium does not appear to form a lipid-soluble ion-pair or complex that will partition from an aqueous buffer *in vitro* or that will alter the oral toxicity of this drug in rats. The extraction of the monoquaternary isopropamide molecule was shown (6) to be enhanced by the presence of bile salts at concentrations below their CMC's but not at higher concentrations; however, bile salts at all concentrations appeared to diminish the absorp-

**Table II**—Death Times (Minutes) following Administration of Decamethonium Bromide to Rats with or without Common Bile Duct Ligation[a]

| Average Dose of Decamethonium Bromide, mg/kg | Ligated Bile Duct | | Unligated (Intact) Bile Duct | |
|---|---|---|---|---|
| | Duodenum | Ileum | Duodenum | Ileum |
| Control[b] | No death | No death | No death | No death |
| 125 | 29.5 ± 4.0 (6)[c] | 35.2 ± 9.4 (6) | 18.0 ± 4.5 (3)[d] | 32.5 ± 18.1 (6) |
| 250 | 18.8 ± 5.9 (6) | 28.0 ± 6.7 (6) | 9.6 ± 1.4 (5)[e] | 31.5 ± 13.8 (6) |
| 375 | 18.0 ± 4.0 (6) | 23.6 ± 6.7 (6) | 24.3 ± 8.8 (6)[f] | 17.0 ± 8.5 (6) |

[a] All comparisons between conditions at each dose level and over all doses were shown to be not significant at $p = 0.05$, using a Wilcoxon rank method for determining significance between two treatments with unpaired replicates (18). [b] Control = 1.0 ml of pH 7.4 sodium phosphate buffer; six animals were used. [c] Numbers in parentheses indicate the number of animals used to calculate the average death time listed (±*SEM*) and the number of animals that died out of the six given this treatment. [d] Average weight of the three animals that died was 222.3 g; average weight of the three that survived was 217.3 g. [e] Average weight of the five animals that died was 204.8 g. [f] These six animals had an average weight of 219.3 g. Correlations were attempted between body weight and death time, as well as between exact intestinal length or the presence of residual food particles and death time. No correlations existed between any given parameter and death time in any group.

**Table III**—Effects of Micellar Bile Salt Solutions[a] and Neomycin Sulfate on the Oral (Duodenal) Toxicity of Decamethonium Bromide[b] in Rats

| Micelle Forming or Dispersing Agent | Average Time of Death[c] (±*SEM*), min |
|---|---|
| None (control) | 12.5 ± 4.9 (6) |
| Neomycin sulfate, 30 mg[d] | 10.7 ± 3.7 (6) |
| Sodium cholate | 23.4 ± 11.4 (5) |
| Sodium deoxycholate | 12.5 ± 1.0 (6) |
| Sodium taurocholate | 15.6 ± 4.9 (5) |
| Sodium dehydrocholate[e] | 29.5 ± 7.9 (6) |

[a] Concentration = 10 m*M*. [b] Average dose = 150 mg/kg. [c] Numbers in parentheses represent the number of animals that died out of six used per experiment. [d] Volume of 1.0 ml of solution in pH 7.4 sodium phosphate buffer. Six nonfasted rats (200 ± 25 g) were given decamethonium 5 min after neomycin treatment. [e] Reported not to form micelles (19).

tion of the isopropamide from the small intestine of the rat. The results from the two investigations suggest that bile salts are not of physiological importance in the absorption mechanism of the highly water-soluble and protonated quaternary ammonium compounds.

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