Intestinal Motor Effects of a Unique Analgesic Agent

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Abstract □ The effects of 2,3,5,6,11,11b-hexahydro-11,11b-dimethyl-14-indolizino(8,7,b)indole hydrochloride on intestinal motility were investigated. The indole derivative altered contractile activity in each preparation studied. Intestinal contractions *in situ* in anesthetized dogs were stimulated, but variable mild stimulatory or inhibitory effects occurred in cats and monkeys. Spontaneous contractions of monkey intestine were inhibited *in vitro*. Dose-related increases in motility were produced in dog intestine *in vitro*, although the experimental compound was considerably less potent than morphine as an intestinal stimulant. The stimulatory effects of the indole derivative in dog intestine were antagonized by atropine and by cyproheptadine. Indolazinoindole also inhibited propulsion of a test meal in mice *in vivo*.

Keyphrases □ 2,3,5,6,11,11b-Hexahydro-11,11b-dimethyl-14-indolizino(8,7,b)indole hydrochloride—intestinal motor effects studied in cats, dogs, and monkeys □ Analgesics, investigational indolazinoindole—effects on intestinal motility investigated □ Indole derivatives—intestinal motility effects of investigational indolazinoindole studied in cats, dogs, and monkeys □ Intestinal motility—effects of indolazinoindole analgesic in cats, dogs, and monkeys

The indole derivative 2,3,5,6,11,11b-hexahydro-11,11b-dimethyl-14-indolizino(8,7,b)indole hydrochloride[1], referred to in this article as indolazinoindole, has been investigated as an analgesic agent in rats and monkeys (1). Administered orally to rats, indolazinoindole was more potent than morphine as an analgesic. Administered intramuscularly to monkeys, it was again more potent than morphine. Animal studies thus indicate that this compound is a potent analgesic agent of potential value in the management of pain. A major side effect associated with virtually all potent analgesic agents is constipation. The constipating effects of the narcotic drugs appear to be secondary to stimulation of intestinal smooth muscle: an increase in nonpropulsive, phasic contractions which diminish the aboral pressure gradient required for effective propulsion of intestinal contents (2, 3).

A potent analgesic agent that, in therapeutic doses, does not stimulate intestinal smooth muscle would be desirable for clinical use. The intestinal effects of indolazinoindole were examined in four species, its intestinal stimulating potency was compared with that of morphine, and initial studies of its mechanism of intestinal actions in dog isolated intestine were performed.

EXPERIMENTAL

Propulsion of Test Meal in Mice—The effects of indolazinoindole on intestinal propulsion were tested in young adult male Swiss-Webster white mice weighing 24–28 g (4). The mice were divided into two groups of six and were administered 1 ml of a suspension of powdered charcoal in glycerin using a gastric tube. After 10 min, the mice were given intraperitoneal injections of saline (control group) or the test compound. The dose of indolazinoindole was 10 mg/kg; both it and saline were administered in volumes of 0.01 ml/g. The animals were sacrificed by cervical dislocation 20 min after drug or saline administration. The small bowel from the stomach to the cecum was removed and measured, and the percent of the bowel traveled by the charcoal marker was calculated.

Table I—Effects of Indolazinoindole on Passage of a Charcoal Meal in Mice

Treatment	Percent Passage	Confidence Limits (95%)
Saline	74.1	62.2–86.0
Indolazinoindole (10 mg/kg)	49.5	41.2–57.9

Intestinal Motility *In Situ*—Dogs, cats, and rhesus monkeys were anesthetized with sodium thiopental (15 mg/kg) and sodium barbital (250 mg/kg) administered intravenously. Femoral venous catheters were inserted for drug administration. Small and large bowel was exposed through a short midline abdominal incision, and the bowel was traced from the duodenal bulb to the cecum for accurate selection of recording sites. Latex balloons (undistended volume was 9 ml) attached to 8 Fr. nasogastric tubes were placed in ascending colon, ileum, jejunum, and duodenum. Each balloon was inserted through a 5-mm incision in the antimesenteric border of the bowel and threaded 8–12 cm from the site of the incision. A pursestring suture was employed to close the bowel incision and to secure the tube to the gut wall. Each balloon was filled with either 2 ml (in dogs) or 1 ml (in cats and monkeys) of water. The water-filled tubes were attached to pressure transducers[2], and intraluminal pressures were recorded on an oscillographic recorder[3]. The abdominal wound was closed with wound clips. All drugs were administered intravenously.

Intestinal Motility *In Vitro*—Dogs and monkeys were anesthetized as already described with thiopental and barbital. Cannulated mesenteric arteries of 8–10-cm sections of small intestine were perfused by means of a peristaltic perfusion pump[4]. The perfusion solution was warmed Krebs bicarbonate bubbled with 95% oxygen–5% carbon dioxide. Ligatures were placed around the intestine on both sides of the perfused arcade, and the segment was removed from the animal. Intraluminal pressure was measured from a latex balloon containing 2 ml (in dogs) or 1 ml (in monkeys) of water and was connected to a pressure transducer[2] by a water-filled 8 Fr. nasogastric tube. Perfusion pressure was measured by a pressure transducer[2] from a T-connection between the pump and the artery. Intraluminal pressure and perfusion pressure were recorded by an oscillographic recorder[3]. The rate of the constant-flow perfusion pump was set to produce a mean perfusion pressure of 60–70 mm Hg. Agonist drugs (indolazinoindole and morphine) were administered *via* the arterial cannula as boluses in volumes of 0.01–0.1 ml. Antagonist drugs (atropine and cyproheptadine) were added to the reservoir which contained the Krebs perfusion solution. Contractions of the isolated intestinal segments were measured as the increase in intraluminal pressure produced in response to the agonist drug.

Drugs and Chemicals—Drugs employed were indolazinoindole as the hydrochloride[5], morphine sulfate[6], atropine sulfate[7], and cyproheptadine hydrochloride[8]. Doses of all agents refer to the

[1] WY-12, 157.

[2] Statham P23Db.
[3] Beckman type RM dynograph.
[4] Sigmamotor type T-8.
[5] Wyeth.
[6] Merck.
[7] Baker.
[8] Merck, Sharp and Dohme.

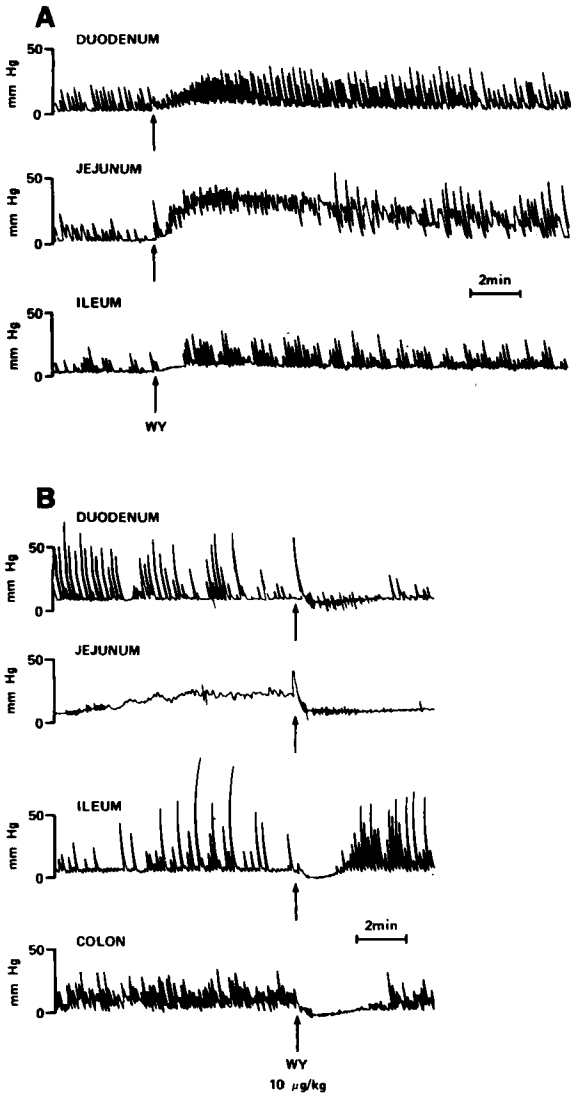

Figure 1—*Effects of indolazinoindole (WY) on intraluminal pressure of cat intestine* in situ. *Indolazinoindole was administered intravenously (10 μg/kg) at the arrows. In the cat illustrated in A, the analgesic agent stimulated motility in the duodenum, jejunum, and ileum. There was no effect on tone or contractions in the colon. In the cat illustrated in B, indolazinoindole produced inhibition of motility.*

salt forms. The composition of the Krebs bicarbonate solution was as follows (in millimoles): NaCl, 118.0; KCl, 4.7; $CaCl_2$, 2.5; KH_2PO_4, 1.1; $MgSO_4$, 0.5; $NaHCO_3$, 25.0; and glucose, 10.0. Due to apparent instability of indolazinoindole in solution, it was made up fresh daily in aqueous solution.

RESULTS

Propulsion of Test Meal in Mice—In a dose of 10 mg/kg, indolazinoindole reduced transit of a charcoal meal in mice to approximately 67% of control (Table I). With this dose, no gross behavioral effects in mice were observed.

Intestinal Motility *In Situ*—*Cat*—In doses of 10–100 μg/kg, the test compound produced either inhibition or mild stimulation of intestinal motility in different cats. Three of the five cats tested responded with increases in motility, two with primarily inhibitory responses. Examples of stimulatory and inhibitory motility responses are illustrated in Fig. 1. Initial doses of indolazinoindole also tended to block the intestinal effects of subsequent doses. Thus, when administered first, the lowest dose (10 μg) often produced larger responses than higher doses (30 or 100 μg). For this reason, meaningful dose–response curves could not be established for this compound in individual cats. Comparison of initial doses, however, indicated that the larger doses produced relatively greater effects than did smaller doses.

Monkey—In doses of 10 and 100 μg/kg, indolazinoindole often, but not always, produced brief tonic increases in intraluminal pressure (range of 5–56 mm Hg) in the ileum and colon, but usually produced no increases in pressure in the duodenum or jejunum. Stomach motility was measured in one monkey and was not affected by indolazinoindole. When spontaneous motility in the bowel was pronounced, the test compound often produced a mild inhibition of ongoing contractions. An excitatory response to indolazinoindole in ileum is illustrated in Fig. 2.

Dog—Indolazinoindole stimulated the upper regions of the bowel *in vivo*. In the doses employed (10, 20, and 100 μg/kg), it rarely caused significant contractions in the ileum or colon (range of 2–6 mm Hg). The duodenal and jejunal responses were characterized by transient, small decreases in intraluminal pressure followed immediately by small to pronounced phasic contractions (Fig. 3). The baseline intraluminal pressure was often elevated slightly during periods of maximal phasic contractions. The phasic contractions tended to occur in bursts lasting 2–4 min, with in-

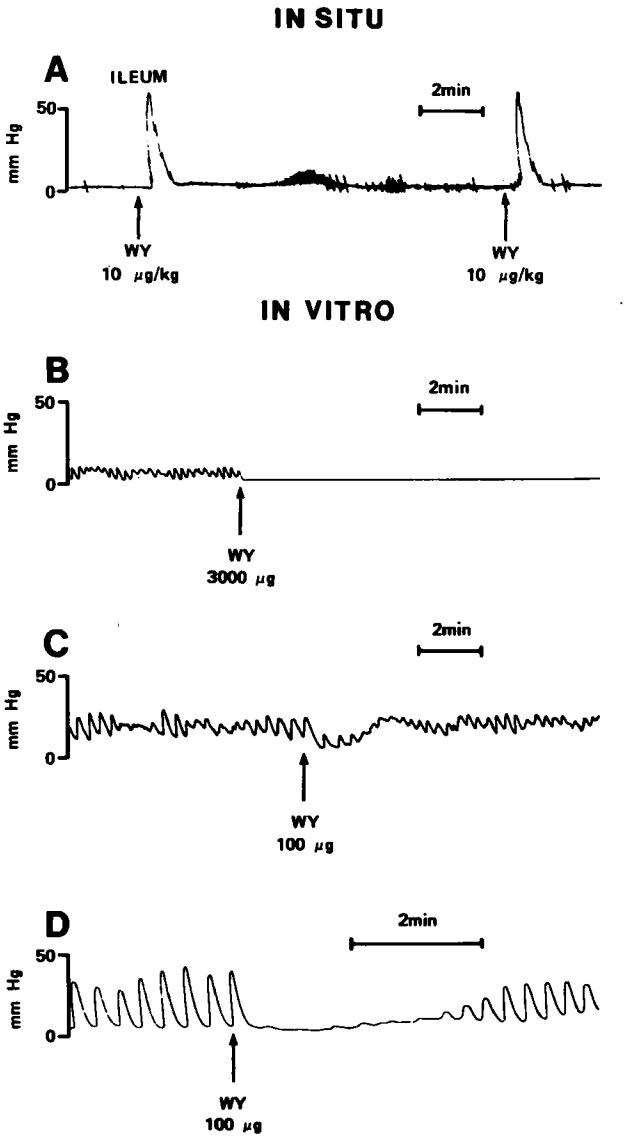

Figure 2—*Effects of indolazinoindole (WY) on intraluminal pressure of rhesus monkey small intestine* in situ *and* in vitro. *Record A illustrates the ability of indolazinoindole to produce brief tonic contractions of the lower small bowel. In this monkey, it had no effect on the duodenum or jejunum. Records B, C, and D illustrate responses of monkey isolated vascularly perfused small intestine to indolazinoindole.*

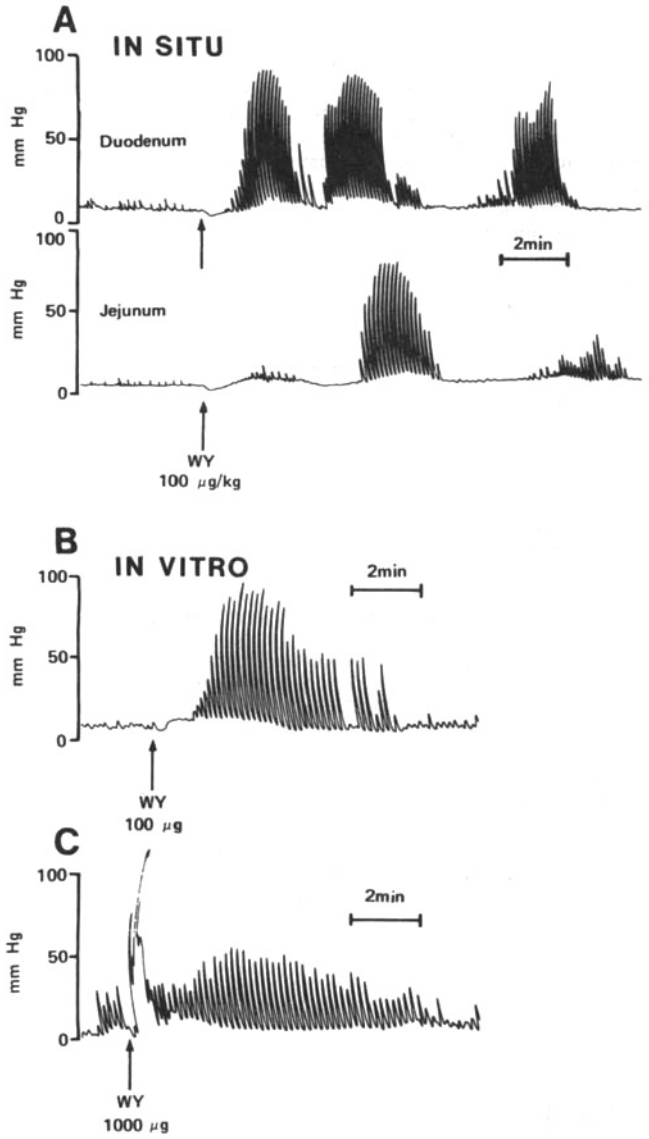

Figure 3—*Effects of indolazinoindole (WY) on intraluminal pressure of dog small intestine* in situ *and* in vitro. *Record A illustrates responses in the upper small bowel to indolazinoindole administered intravenously. In this dog, it had no effect on the ileum or colon. Records B and C illustrate responses of dog isolated vascularly perfused small intestine to two different doses of indolazinoindole.*

tervening periods of relative quiesence. These responses are similar to those produced in the dog by morphine.

Intestinal Motility *In Vitro*—*Monkey*—Indolazinoindole exerted primarily inhibitory effects on isolated gut segments; this effect was particularly prominent with doses of 100 μg or larger (Fig. 2). Except with doses of 1000 or 3000 μg, the period of inhibition was usually followed by recovery of intestinal tone and motility and was sometimes followed by enhanced rhythmic activity.

Dog—Indolazinoindole was studied most extensively in intestinal segments isolated from dogs. In bolus intraarterial doses of 0.1-3000 μg, the experimental agent produced dose-related increases in intraluminal pressure (Fig. 4). Responses to smaller doses (0.1-100 μg) consisted primarily of increases in the amplitude of rhythmic contractions. Larger doses (100–3000 μg) often produced tonic or sustained increases in intraluminal pressure, which were always followed by rhythmic contractions. These responses resembled those reported previously for morphine (5). On a weight basis, the test compound was found to be considerably less potent than morphine as an intestinal spasmogen, but the dose-response curves for the two drugs were of similar shape and the maximal responses were of equal magnitude (Fig. 4).

To obtain preliminary information about the mechanism of the intestinal stimulatory effect of indolazinoindole in dog intestine, studies were carried out with atropine and cyproheptadine. These were performed as paired experiments. Two intestinal segments were taken for each experiment; one was perfused with plain Krebs solution and the other with Krebs solution plus an antagonist. Dose-response curves for indolazinoindole were thus obtained in the absence and in the presence of atropine and cyproheptadine. Doses of indolazinoindole were administered in random order to rule out order effects. Intestinal stimulatory responses to the experimental analgesic were significantly reduced (Student's *t* test, paired comparison) in gut segments during perfusion with atropine or cyproheptadine (Fig. 4).

DISCUSSION

Effective separation of analgesic from smooth muscle actions of potent analgesic drugs is a highly desirable goal. The experimental indole derivative shows some promise in this regard. In both the monkey and the cat, the analgesic agent produced either mild stimulation or inhibition of intestinal motility. Its duration of intestinal action was quite limited in both species. The study in mice demonstrated that at fairly high dose levels (10 mg/kg), indolazinoindole can, however, inhibit intestinal propulsive activity. At this dose level, the analgesic could be expected to exert constipating effects. The intestinal stimulatory effects of indolaz-

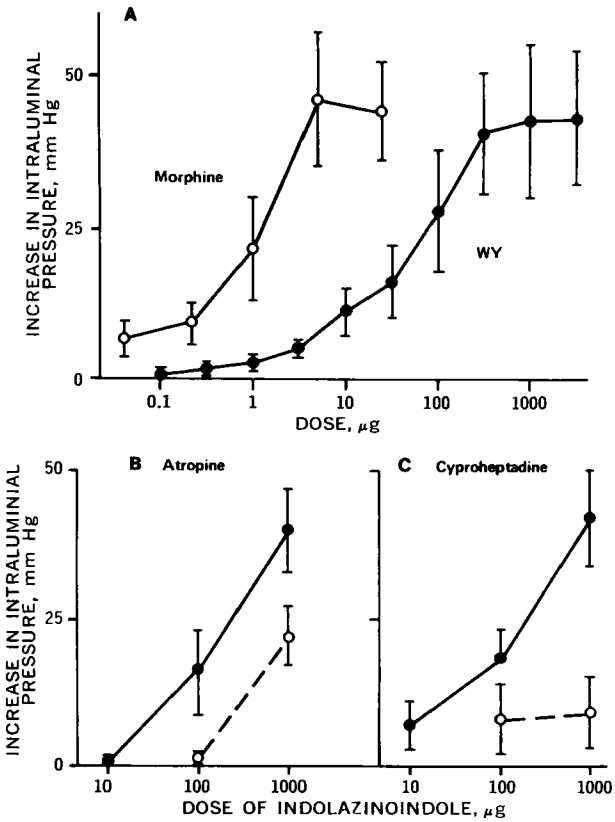

Figure 4—*Effects of indolazinoindole (WY) on intraluminal pressure of dog small intestine* in vitro. *Panel A shows dose–response curves for morphine and for indolazinoindole in dog isolated vascularly perfused intestinal segments. Each point is the mean (±SEM) of responses in preparations from six dogs. The potency ratio (with 95% confidence limits) of indolazinoindole in relation to morphine was 0.0195 (0.0136–0.1742). Panel B shows dose–response curves for indolazinoindole during perfusion with control Krebs solution (●) and with Krebs solution containing 0.1 μg/ml atropine (○). Panel C shows dose–response curves for indolazinoindole during perfusion with control Krebs solution (●) and with Krebs solution containing 1 μg/ml cyproheptadine (○).*

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