Five milliliters of this solution was added to the above magnesium turnings-ether mixture. To induce the reaction to begin, a small crystal of iodine was added. The reaction proceeded with development of heat and decolorization of the iodine. The mixture was stirred by means of a magnetic stirrer, and the remainder of the benzyl chloride was added gradually over a period of 30 min., regulating the temperature of the reaction with the aid of an ice water bath. The reagent was used as described in the case of phenyl magnesium bromide.

All of the compounds listed in Table III were prepared by the following general method.

In a 200-ml. round-bottom flask, 0.02 mole of the appropriate aminoketone was dissolved in 75 ml. of anhydrous ether, and the solution was dried with anhydrous magnesium sulfate. Meanwhile, in a dry 300-ml. three-necked flask equipped with dropping funnel, magnetic stirrer, and condenser with drying tube on the upper end, 13.5 ml. of a 3 *M* solution (0.04 mole) of phenyl magnesium bromide and 30 ml. of anhydrous ether was placed. To this, a dry ether solution of the Mannich base as previously prepared, was dropped little by little with vigorous stirring. Stirring was continued for 2 hr., and the mixture was allowed to stand overnight. The reaction flask was cooled in an ice bath. A saturated solution of ammonium chloride was added to decompose the complex. The ether solution was decanted into a separator and washed with 10 ml. of amino-alcohol–ether solution, was filtered, cooled in an ice bath, and dry hydrogen chloride gas was passed through it. The hydrochloride was collected and recrystallized from the alcohol–acetone mixture.

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Keyphrases

Mannich reaction
β-Aminoketones—synthesis
γ-Aminoalcohols—synthesis
3-Azabicyclo[3.2.1]octane—amine component, Mannich reaction

Effect of Sodium Deoxycholate on Gastric Emptying in the Rat

By STUART FELDMAN*, RALPH J. WYNN, and MILO GIBALDI

Sodium deoxycholate delays considerably the gastric emptying of phenol red in rats. Gastric emptying of phenol red in control animals proceeds by apparent first-order kinetics, but a very different kinetic pattern emerges upon administration of the bile salt. Sodium deoxycholate also produces a large net secretion of fluids into the gastric pouch. It is proposed that the resulting increase in gastric volume is the immediate cause for the decreased rate of gastric emptying.

THE EFFECTS of orally administered bile salts on the gastrointestinal absorption of riboflavin (1) and other drugs are currently being investigated in this laboratory. These studies have prompted a consideration of the influence of bile salts on gastric emptying since the rate of passage of drug through the pylorus may have a profound influence on the overall rate and extent of drug absorption (2, 3).

Previous studies (4) have shown that the oral administration of sodium taurodeoxycholate and

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sodium deoxycholate markedly inhibits gastric emptying and proximal intestinal transit after gastric intubation in the rat. The present report concerns the influence of sodium deoxycholate on the pattern of gastric emptying in the rat and the pharmacologic basis of these effects.

EXPERIMENTAL

Male, Sprague-Dawley descent rats (Blue Spruce Farms, Altamont, N. Y.) weighing between 125 and 210 g. were used in all experiments. The animals were fasted for 20–24 hr. prior to gastric intubation. Water was permitted *ad libitum* until 1 hr. before the experiment. One and one-half milliliters of a 70-mg. % phenol red solution containing 0.026 *M* sodium deoxycholate (special enzyme grade, Mann Research Laboratories, New York, N. Y.), was introduced into the rat stomach by intubation. Control animals received 1.5 ml. of 70-mg.% phenol red solution in distilled water.

The rats were sacrificed by decapitation at various times after intubation. An abdominal incision was made immediately to expose the stomach. The stomach was then quickly ligated at the pylorus and cardia, excised, stripped of adhering fat, blotted, and weighed. The stomach and its contents were homogenized in an Eberbach homogenizer and assayed for phenol red as previously described (4). The analytical procedure is a modification of the method of Reynell and Spray (5) and involves alkalinization with 1 *N* NaOH, precipitation of proteins with 30% trichloroacetic acid, realkalinization with NaOH and colorimetric assay at 560 mμ. The stomach weights of a control group of six rats were obtained after sham intubation, immediate sacrifice, and removal of the stomach as described above.

The recovery of known amounts of phenol red added to stomach tissue homogenates is about 93% while recovery of phenol red after incubation in gastric pouches for 1 hr. before homogenization is 87% (6). The presence of bile salt has no effect on the recovery of phenol red added to tissue homogenates (4). In view of the high recovery from gastric preparations, the results are reported on a percent-of-dose basis.

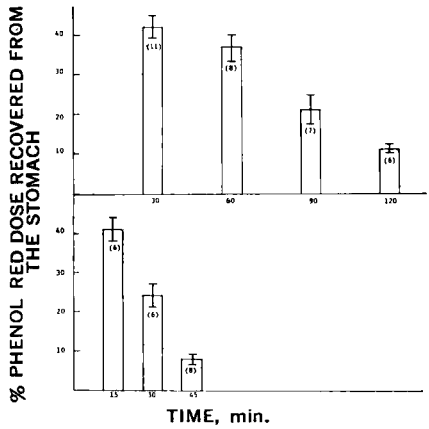

Fig. 1—Recovery of phenol red from rat stomach after gastric intubation. The lower half are data from control animals. The upper half are data from rats co-administered sodium deoxycholate. The vertical lines represent standard error of the mean. The numbers in parentheses indicate the number of animals used for each point.

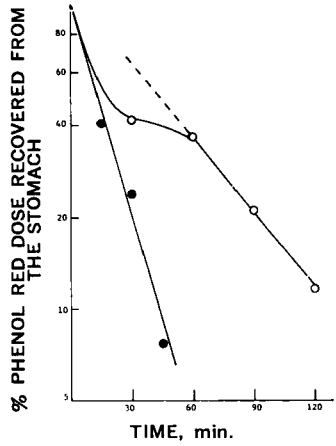

Fig. 2—Semilogarithmic plot of percent of phenol red dose in stomach versus *time. Key: ●, controls; ○, sodium deoxycholate.*

RESULTS

Figure 1 shows the recovery of phenol red from the rat stomach as a function of time after intubation. A considerable delay is observed in the gastric emptying of phenol red in the presence of sodium deoxycholate. This effect is illustrated further in Fig. 2, which shows a semilogarithmic plot of gastric retention *versus* time. The plot indicates that gastric emptying of phenol red in control animals proceeds by apparent first-order kinetics. This finding is consistent with the results of Hunt and MacDonald (7) who showed that the stomach content volume after a liquid test meal decreases in an exponential fashion with time. The "half-life" of gastric emptying in control rats was 13 min.

Figure 2 also shows that sodium deoxycholate modifies the pattern of gastric emptying. In the presence of the bile salt an initial rapid gastric emptying phase followed by a transition region, where little emptying occurs, is noted over the first hour. The second hour after intubation is characterized by an exponential decline in phenol red, with a "half-life" of 36 min.

The average stomach weight (expressed as mg./g. of body weight) of control rats after sham intubation was found to be 8.35 ± 0.23 (*SE*). The percent increases in stomach weight, relative to the control value, of rats receiving phenol red or phenol red and sodium deoxycholate are shown in Figs. 3 and 4, respectively. The greater increase in stomach weights of rats fed sodium deoxycholate is qualitatively consistent with the higher phenol red retention observed in these animals. However, the increase in stomach weight in bile salt-treated rats is significantly greater than predicted from phenol red recovery data.

The stomach weight after intubation reflects a number of factors which are summarized in the fol-

lowing equation:

$$(W_I - W_C) = (W_D - W_E) + (W_S - W_A) \quad \text{(Eq. 1)}$$

where W refers to weight and the subscripts denote stomach weight after intubation (I), stomach weight of controls after sham intubation (C), the weight of the dose (D), the amount of the dose emptied from the stomach (E), the amount of biologic fluids secreted into the gastric pouch (S), and the amount of intubated solution which is absorbed (A).

The term $(W_I - W_C)$ is the observed increase in stomach weight and the term $(W_D - W_E)$ is calculable from the phenol red recovery data. A positive value of the $(W_S - W_A)$ term indicates net secretion of fluids while a negative value indicates net gastric absorption of intubated solution. Values for the $(W_D - W_E)$ term are shown by the cross-hatched area in Fig. 3 at the 15-min. level and in Fig. 4 at the 30-, 60-, and 90-min. levels.

The data in Fig. 4 demonstrate a large net secretion of fluids, induced by the bile salt, which persists for at least 60 min. after intubation. In control rats, receiving only phenol red, there is an indication of net secretion 15 min. after intubation, but at 30 and 45 min. after intubation net absorption occurs (not shown on graph). Hence, sodium deoxycholate significantly enhances gastric secretion.

A semilogarithmic plot of the change in stomach weight after intubation *versus* time is shown in Fig. 5. Similar to the results noted previously with retention data in control animals, the stomach weight declines in an exponential fashion with time, *i.e.*, $(W_I - W_C) = W_D\, e^{-Kt}$. The half-life for this process was 10.5 min. The pattern of the bile salt data in Fig. 5 parallels that shown in Fig. 2. An exponential phase with a half-life of 18 min. is evident between 60 and 120 min. after administration.

DISCUSSION

The effect of sodium deoxycholate on gastric emptying may be explained by considering several

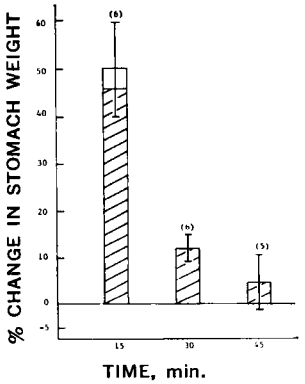

Fig. 3—Change in stomach weight as a function of time after administration of 1.5 ml. of a 70-mg.% phenol red solution. Hatched area at the 15-min. level denotes the value of ($W_D - W_E$). *See text for details. Vertical lines represent standard error of the mean. Numbers in parentheses indicate the number of animals used for each point.*

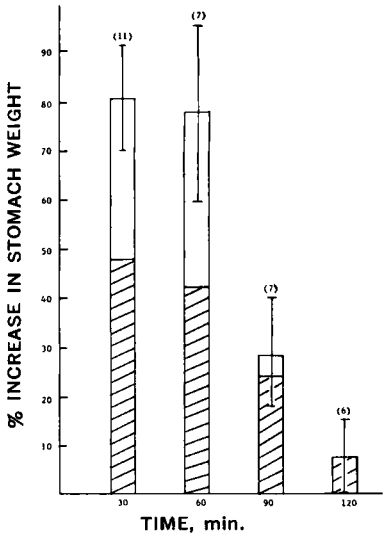

Fig. 4—Change in stomach weight as a function of time after administration of 1.5 ml. of a 70-mg.% phenol red solution in 0.026 M *sodium deoxycholate. Hatched area at 30-, 60-, and 90-min. levels denotes the value of* ($W_D - W_E$). *See text for details. Vertical lines represent standard error of the mean. Numbers in parentheses indicate the number of animals used for each point.*

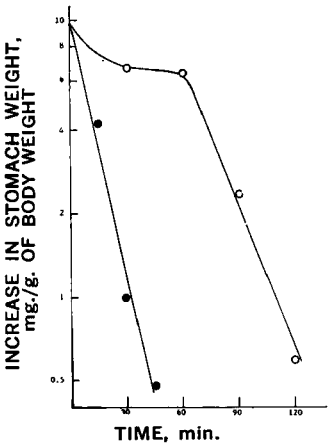

Fig. 5—Semilogarithmic plot of rat stomach weight after intubation minus stomach weight of sham-intubated rats versus *time. Key:* ●, *controls;* ○, *sodium deoxycholate.*

plausible mechanisms. A possibility exists that bile salts exert a direct effect on the musculature of the gastric pouch which results in a decrease in the gastric emptying rate. However, on the basis of literature reports, this possibility is unlikely. Sasaki (8) found that oral administration of bile salts to the rabbit resulted in a slight increase in gastric motility at low doses and in a small decrease in motility at higher levels. Anderson *et al.* (9) recently reported that bile placed in the duodenum had no effect on the contractility of the longitudinal and circular muscles of the canine gastric body and antrum. On the other hand, Davenport (10) ob-

served that natural bile in acid solution when placed in the canine gastric pouch results in an increase in gastric motility. This phenomenon would tend to promote gastric emptying. Hence, it would not appear that bile salts exert an effect on gastric emptying *via* an inhibitory mechanism on gastric motility.

The increase in gastric secretion and lumen volume in the presence of sodium deoxycholate offers an alternate explanation for the decrease in gastric emptying. Hunt and MacDonald (7) have shown that as the volume of a liquid test meal increases the percentage of gastric contents leaving the stomach per unit time decreases. Interestingly, these workers also observed curves similar to those presented in Figs. 2 and 5 for bile salt-treated animals, when large volumes of fluid were administered to the test subjects. The relationship of increased gastric secretions and decreased gastric emptying has also been considered by Sognen (11) who observed a concomitant increase in gastric volume and decrease in gastric emptying upon administration of calcium-binding salts to rats. The present results support the hypothesis that the increase in gastric secretions upon administration of sodium deoxycholate is the immediate cause for the depressed rate of gastric emptying.

The ability of sodium deoxycholate to elicit fluid secretions into the gastric pouch is related to the properties of the bile salts in modifying membrane permeability. Studies in this laboratory suggest that bile salts increase the permeability of the goldfish membrane to various drugs (12) and enhance the rate of translocation of salicylate in everted gut preparations (13). Faust and Wu (14) using the everted gut technique have shown that bile salts, when placed in the mucosal solution, produce a net flux of water from the serosal to mucosal side. Davenport (15) has reported that bile salts are capable of increasing the permeability of the gastric mucosa as judged by H^+ flux.

The alterations in membrane permeability produced by bile salts may or may not be specific. For example, the similar effects on gastric secretion observed with calcium binding substances (11) and sodium deoxycholate suggest a role for calcium depletion from the membrane. This possibility is particularly relevant since Webling *et al.* (16) have shown that bile salts are capable of complexing calcium ions. On the other hand, it appears that injury to the gastric mucosa may also result in changes in membrane permeability and net secretion of fluid. Davenport (17) has noted that the canine gastric mucosa, when damaged by salicylates or fatty acids, produces a large volume of fluid. It has been tentatively suggested that acid itself is the initiating stimulus for fluid production and that histamine liberation by damaging agents is an important mediator.

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Keyphrases

Deoxycholate, sodium—gastric emptying effect, rats
Phenol red—sodium deoxycholate delayed gastric emptying
Stomach weight—sodium deoxycholate effect
Colorimetric analysis—spectrophotometer