was stable over a 7-day period and that results were not influenced by degradation.

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Normal and Promoted GI Absorption of Water-Soluble Substances III: Absorption of Antibiotics from Stomach and Intestine of the Rat

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Abstract □ Absorption of soluble antibiotics was studied in the doubly ligated stomach of the rat, in the ligated small intestine, and in the intact GI tract. When surface-active absorption promoters are administered with the antibiotic in the ligated stomach, promoted absorption occurs but the onset is slow. When the drug–promoter combination is put in the ligated intestine, blood levels of the drug are elevated to severalfold the normal levels and the rise is extremely rapid. Absorption promoters exert a smaller influence in the intact GI tract. Their effectiveness is confined to the first 30 min., after which approximately normal levels of drug are seen. Emptying of the liquid dose from the stomach at 6–8 min. was followed by an immediate rapid rise of blood levels characteristic of the rapid response of the intestine to promoters. Accordingly, the blood level *versus* time curves obtained when promoters are employed in the intact rat are interpreted to be a result of a rapid but transient promoted absorption in the duodenum–small intestine, with little contribution from promoted gastric absorption.

Keyphrases □ Surfactants—role in enhancing antibiotic absorption, stomach, small intestine, and GI tract compared, rats, toxicity □ Antibiotics, soluble—comparison of doubly ligated stomach, ligated small intestine, and intact GI tract absorption, rats, effect of surfactants □ Absorption, soluble antibiotics—role of surfactants in doubly ligated stomach, ligated small intestine, intact GI tract, rats □ Surface-active absorption promoters—influence on water-soluble antibiotics in stomach and intestine, rats

Absorption of normally nonabsorbed or poorly absorbed water-soluble drugs from a Thomas gastric fundic pouch of the dog is greatly increased by certain surface-active agents (1). Nonionic, anionic, cationic, and zwitterionic surface-active agents were shown to promote the absorption of several types of antibiotics. Further work demonstrated similar promoted absorption in other segments and in the intact GI tract of the dog.

Another study demonstrated a greatly increased absorption of vitamin B_{12} from the stomach and the intact GI tract of the rat when an absorption promoter was added (2). The present study was initiated to characterize the response of the rat to absorption promoters and, particularly, to distinguish the response of the stomach and the intestine.

EXPERIMENTAL

Handling of Animals—Male Wistar and CFE strain rats, weighing 150–300 g., were fasted in individual cages with wide-mesh screen floors for approximately 15–20 hr. prior to operation and dosing. Blood samples were obtained by amputating the tip of the tail. Enough blood was collected to fill the capillarity capacity of from three to six 0.63-cm. (0.25-in.) diameter filter pads. The filter pads were placed directly on agar plates and were assayed for microbiological activity of the drug by standard disk-plate procedures[1].

Surgical Procedure—The animal was anesthetized with ether, and the stomach was exposed through a midline incision in the abdominal area. A ligation was placed at the pyloric sphincter. Aqueous drug solution was administered by stomach tube, after which an esophageal ligation was made at the cardiac sphincter, care being taken not to occlude blood vessels. The abdominal incision was then closed with wound clamps. After the rat recovered from the anesthesia in a restraining cage, it was conscious throughout the remainder of the experiment. Blood samples were periodi-

[1] Cephalothin and penicillin V were assayed against *Bacillus subtilis* (ATCC 6633). Cephaloridine was assayed against *Sarcina lutea* (PC1-1001-FDA).

Table I—Effect of Various Promoters on Absorption of Cephaloridine from the Doubly Ligated Rat Stomach

Promoter	Number of Animals	Time of Peak Blood Level, min.	Peak Blood Level and Standard Error, mcg./ml.[a]
Control			
No promoter	9	120	0.38 ± 0.17
Nonionic			
Polyoxyethylene-20-cetyl ether[b,c]	3	70	2.47 ± 0.28
Polyoxyethylene-20-stearyl ether[b,d]	3	120	2.39 ± 0.13
Polyoxyethylene-20-oleyl ether	9	120	1.88 ± 0.09
Polyoxyethylene-24-cholesterol ether[e]	3	70	4.87 ± 1.75
Surfonic LF-16[f]	3	60	2.61 ± 0.30
Surfonic LF-17[f]	3	70	1.84 ± 0.52
Anionic			
Sodium lauryl sulfate	4	60	3.80 ± 0.79
Cationic			
Cetyltrimethylammonium bromide[g]	2	120	3.20 ± 0.40
Cetylpyridinium bromide	2	120	1.60 ± 0.65

[a] Values are the mean of highest antibiotic value observed during 120 min. following administration to a rat of 1.5% antibiotic at 125 mg./kg. with and without 0.75% promoter at 62.5 mg./kg. in pH 7.0 isosmolar sodium phosphate buffer, except as noted. [b] Atlas Chemical Industries, Inc., Wilmington, Del. [c] Brij-58. [d] Brij-78. [e] Solulan C-24, American Cholesterol Products, Inc., Edison, N. J. [f] Modified primary alcohol–ethylene oxide adducts, Jefferson Chemical Co., Inc., Houston, Tex. [g] Concentration 0.91%.

cally taken from the tail. Absorption was followed for approximately 2 hr., after which time the rat was sacrificed, the stomach was excised, and the contents were examined. If any coprophagy occurred, the data from such animals were rejected.

For the intestinal ligation, the rat was anesthetized with ether. The intestinal area was exposed with the aid of a metal hook through an abdominal incision. Ligations were made at the pyloric sphincter and at the ileal end of the small intestine immediately adjacent to the cecum. The aqueous test solution was introduced into the small intestine using a 27-gauge needle inserted into the duodenum distal to the pyloric ligation. A third ligation was then made around the duodenum distal to the point of entry of the needle so as to prevent any leakage of the test solution from the puncture. The operation was then completed by closure of the abdominal cavity with wound clamps as previously described. The same holding and bleeding procedures were employed as for stomach ligation experiments.

To dose the intact rat, the animal was lightly anesthetized with ether, and the drug solution was introduced into the stomach through a stomach tube. The animal was placed in a restraining cage and was held there for the duration of the experiment while periodic blood samples were taken.

Preparation of Oral Dose—Drugs were dissolved in pH 7.0 isosmolar sodium phosphate buffer or in pH 3.7 isosmolar sodium citrate–sodium phosphate buffer. The administered dose was usually 125 mg./kg. antibiotic, with or without 62.5 mg./kg. surface-active agent. The volume of the administered oral dose varied in proportion to the weight of the animal. The concentrations of the two components in the dose were 1.5 and 0.75%, respectively. If other compositions were employed, they are noted.

RESULTS

Absorption from Ligated Stomach—Initial experiments were conducted in which a single (pyloric) ligation only was performed. In these experiments, little or no absorption of antibiotic was seen even when a promoter was added. The accumulation of large volumes of acidic fluid was observed. Excessive gastric acid secretion is known to be detrimental to promoted absorption in the dog. When the rats were predosed orally 1 hr. before the experiment with an anticholinergic compound[2], the gastric fluid volume remained low, and the pH remained within the range of 5–7 during the subsequent 2-hr. study. High antibiotic blood levels were achieved following this procedure, with promoters included with the antibiotic.

In 1966, Brodie and Knapp (4) showed that ligating the esophagus and the pylorus of a rat markedly decreased the volume and the titratable acidity of the gastric contents. With this procedure, the absorption promoter, polyoxyethylene-20-oleyl ether[3], enhanced the absorption of cephaloridine[4]. Good volume and pH control were also observed without employing an anticholinergic predose. This technique, without an anticholinergic predose, was thereafter employed for studying the gastric absorption.

The promoted absorption of cephaloridine under these conditions is dose responsive. Absorption is also promoted by some other, but not all, nonionic, anionic, and cationic surface-active agents (Table I). The absorption of cephaloridine is dose responsive also to its own concentration when a constant concentration of absorption promoter is employed.

A 2% solution of sodium cephalothin[5] in pH 7.0 isosmolar phosphate buffer, with and without polyoxyethylene-20-oleyl ether, was administered in the doubly ligated stomach. The peak blood levels of this antibiotic were increased by a factor of approximately two by the inclusion of this promoter.

The gastric absorption of potassium penicillin V[6] was examined in a pH 3.7 isosmolar sodium citrate–phosphate buffer and a pH 7.0 isosmolar sodium phosphate buffer. The efficiency of normal absorption at the lower pH value was much greater than at pH 7.0, approximately 3.5-fold. The resulting blood levels at pH 3.7 were approximately proportional to its concentration. The addition of the promoter with potassium penicillin V at pH 3.7 caused only a small increase in absorption.

Absorption from Ligated Duodenum and Small Intestine—Cephaloridine or cephalothin in pH 7.0 isosmolar sodium phosphate buffer, with and without promoter, was introduced into the doubly ligated duodenum and small intestine of fasted rats. The data in Table II clearly demonstrate that this agent effectively increased the absorption of these antibiotics from this segment. The rapidity with which blood levels rose when mixtures of antibiotic and promoter were placed in the segment may be contrasted with the much slower rate of rise of blood levels from the same dose placed in the ligated stomach. When the duodenum and small intestine were ligated only at the cecum, the resulting blood levels from either normal or promoted absorption were lower than when the segment was doubly ligated.

Absorption from Intact GI Tract—For correlation with absorption experiments in which promoters are employed in the intact animal, a series of experiments on stomach emptying was conducted. To determine the approximate time of emptying of a liquid dose from a fasted intact stomach, a solution containing 0.15% phenol red, 1.5% cephaloridine, and 0.75% polyoxyethylene-20-oleyl ether was given orally through a stomach tube to lightly anesthetized rats. This was the same dosing procedure later used in intact animal experiments. Phenol red reportedly is not absorbed from the rat stomach (7) or from the intestine (8). At various times the animals were reanesthetized, an abdominal incision was made, and

[2] α- and β-DL-(1-Methyl-3-pyrrolidinyl)-α-phenyl-α-(2-thienyl)glycolate methyl bromide (3), administered at 20 mg./kg.

[3] Brij-98, Atlas Chemical Industries, Inc., Wilmington, Del.

[4] 7-[α-(2-Thiophene)acetamido]-(1-pyridylmethyl)-3-cephem-4-carboxylic acid betaine, Loridine, Lilly (5).

[5] Sodium salt of 7-[α-(2-thiophene)acetamido]-cephalosporanic acid, Keflin, Lilly (6).

[6] Potassium salt of phenoxymethyl penicillin V-cillin K, Lilly.

Table II—Normal and Promoted Absorption from Doubly Ligated Duodenum–Small Intestine

Drug Combination	Number of Animals	Minimum Detectable Levels, mcg./ml.	Whole Blood Levels (mcg./ml.) and Standard Errors (Time Postdosing, min.) 20	40	60	80	120	180
Cephaloridine only	5	0.25	0.41 ±0.13	0.95 ±0.19	1.90 ±0.35	2.03 ±0.37	1.66 ±0.46	1.54 ±0.38
Cephaloridine with polyoxyethylene-20-oleyl ether	5	0.25	3.44 ±0.72	9.36 ±1.14	10.52 ±0.62	8.36 ±1.71	3.58 ±0.64	0.95 ±0.18
Cephalothin only	4	0.10	0.17 ±0.11	0.34 ±0.04	0.40 ±0.05	0.35 ±0.04	0.18 ±0.01	—
Cephalothin with polyoxyethylene-20-oleyl ether	5	0.10	1.39 ±0.10	5.90 ±0.69	5.96 ±0.97	2.25 ±0.37	0.76 ±0.20	—

Table III—Normal and Promoted Absorption from Intact Rat GI Tract

Drug Combination	Number of Animals	Minimum Detectable Levels, mcg./ml.	Whole Blood Levels (mcg./ml.) and Standard Errors (Time Postdosing, min.) 20	40	60	90	120	180
Cephaloridin only	15	0.25	0.33 ±0.09	0.82 ±0.23	1.12 ±0.28	1.35 ±0.30	0.79 ±0.17	0.45 ±0.12
Cephaloridine with polyoxyethylene-20-oleyl ether	11	0.25	1.16 ±0.37	1.10 ±0.28	1.04 ±0.21	0.93 ±0.13	0.79 ±0.17	0.57 ±0.12
Cephalothin only	4	0.10	0.21 ±0.02	0.33 ±0.06	0.33 ±0.05	0.34 ±0.10	—	—
Cephalothin with polyoxyethylene-20-oleyl ether	5	0.10	0.50 ±0.06	0.30 ±0.04	0.25 ±0.06	0.17 ±0.05	—	—

a clamp was quickly placed at the pyloric sphincter. Simultaneously with these ligations, a blood sample was taken. Additional clamps were also placed at various locations throughout the GI tract. The stomach and small intestine were then excised and separated. The fluid contents of the segments were emptied and flushed thoroughly with neutral buffer. These solutions were appropriately diluted with more buffer and centrifuged to remove any particulate matter. The optical density of each phenol red solution was determined at 550 nm. employing a spectrophotometer[7]. During the interval between 8 and 9 min. after dosing, approximately 80% of the administered dose passed into the small intestine[8]. This experiment and the corresponding blood levels of antibiotic are shown in Fig. 1. The appearance of the antibiotic in the blood correlated remarkably well with the time of emptying into the duodenum.

To follow the normal and promoted absorption of antibiotics from the intact GI tract of the rat, cephaloridine or cephalothin in pH 7.0 isosmolar sodium phosphate buffer, with and without promoter, was given *via* a stomach tube. In the blood samples taken at 20 min., antibiotic levels with absorption promoters were increased as much as three times over those observed without promoter. By 40–60 min., this difference disappeared. Averaged data are shown in Table III.

A second series of intact animals were orally dosed with antibiotic alone or with the promoter–drug combination as in the foregoing experiment, but they were additionally predosed with polyoxyethylene-20-oleyl ether 20 min. before the antibiotic administration. The early blood levels were then generally further elevated by factors averaging 1.6 times the levels without predosing. However, none of these levels was equal to the results seen in doubly ligated duodenum–small intestine animals.

Toxicity Effects of Absorption Promoters—Histological examinations were made of organs taken from the rat after treatment with this surface-active agent. The tissues were slightly damaged in all ligated specimens. However, identical effects were found in ligated tissues similarly exposed to saline solution or to drug solution without the surfactant. This observed slight damage is, therefore, attributed to the manipulation of the organ or to the ligation procedure.

Previous studies in these laboratories (1) indicated that no irreversible functional alteration of the absorbing tissues in the Thomas canine fundic pouch was attributable to promoters, since continued use in individual animals did not alter the absorption characteristic of the gastric tissue. In the present study, promoters were administered to rats over 2 weeks. Rats were placed in individual cages and given 0.75% solutions of sodium lauryl sulfate, polyoxyethylene-24-cholesterol ether, or polyoxyethylene-20-oleyl ether as their only source of drinking water for a 2-week period. The animals were given Purina Rat Chow and were free to eat and drink *ad libitum*. The food and water intake, weight loss or gain, and the general physical appearance of the animals were followed daily and compared to control animals given plain water and food. After 2 weeks, one-half of the animals in each category were returned to plain water and food and were observed for a 4-week period. One-half of the rats were fasted overnight and thereafter were employed for absorption studies.

These absorption experiments evidenced the ability of the animals to exhibit the typical promoted absorption after the continued administration of promoters. Daily water and food consumption and weight gain were greatly depressed when sodium lauryl sulfate solution was given (Table IV). These rats showed adverse physical changes during the test period. Among the animals placed on water and a normal diet after the 2-week test, the most notable change occurred among the sodium lauryl sulfate-treated rats. Their physical appearance improved immediately, and their food intake and growth rate far surpassed that of the others.

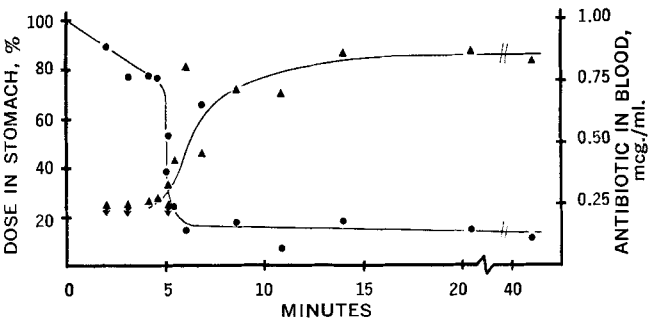

Figure 1—*Time course of emptying of a liquid dose of cephaloridine in the presence of polyoxyethylene-20-oleyl ether from the intact rat stomach (●) and of appearance of antibiotic in the blood (▲). Each point represents one animal from which one value for emptying and one for blood level were obtained.*

[7] Beckman model DU.

[8] Recovery of phenol red from the stomach and intestine averaged 103.6% in the 14 animals employed in this experiment. This value indicates that no great loss of dye from stomach or intestine occurred by promoted absorption.

Table IV—Food and Water Consumption and Weight Gain during 2-Week Administration of Promoters

Drinking Solution	Number of Animals	Initial Weight, g.	Intake, g.		Weight Gain, g.			
			Water	Food	Test Period	During Recovery 1 week	During Recovery 4 weeks	Overall
Control	2	165.5	349.0	245.0	80.0	—[a]	—[a]	—[a]
Sodium lauryl sulfate	2	164.5	267.2	191.2	14.7	—[a]	—[a]	—[a]
Polyoxyethylene-20-oleyl ether	2	159.0	341.6	236.7	74.2	—[a]	—[a]	—[a]
Polyoxyethylene-24-cholesterol ether	2	169.0	320.2	236.2	59.2	—[a]	—[a]	—[a]
Control	2	264.7	361.5	269.7	49.2	26.0	56.0	105.2
Sodium lauryl sulfate	2	257.5	262.0	182.5	2.0	72.0	133.0	135.0
Polyoxyethylene-20-oleyl ether	2	259.7	292.5	241.2	32.5	25.2	49.2	81.7
Polyoxyethylene-24-cholesterol ether	2	260.7	304.0	244.7	35.2	23.0	66.0	101.2

[a] Animals used for absorption experiments.

DISCUSSION

Previous investigators examined the absorption of acidic and basic organic compounds from the rat stomach and the rat duodenum and small intestine (7, 8). The stomach is often a significant site of absorption. Similar studies were done in other animals and in man, demonstrating the capabilities of the stomach in all species to absorb by passive diffusion a variety of acidic and weakly basic drugs (9). Such data are consistent with the hypothesis that the gastric or intestinal mucosa, acting as a lipoidal barrier, is selectively permeable to the more lipid-soluble unionized form of drugs, either free acid or free base.

In the present experiments in rats, the normal gastric absorption of potassium penicillin V was much more rapid when the drug was administered at pH 3.7 in sodium citrate–phosphate buffer than when administered at neutral pH in phosphate buffer. This finding is in agreement with expectations. At pH 3.7, approximately 10% of the antibiotic is in the nonionized free acid form, whereas a negligible fraction is in the free acid form at pH 7.0.

All other absorption studies reported here, either in the stomach or intestine, were done in a pH 7.0 isosmolar sodium phosphate buffer system, at which pH the carboxyl groups of the antibiotics, for all practical purposes, are totally dissociated. The normal absorption of cephaloridine (a zwitterion at pH 7.0), and of cephalothin (a fully ionized acid salt) is also extremely limited from the neutral stomach. These results are entirely expected, considering their negligible lipid solubility at pH 7.0.

The present results, concerned with the effect of absorption promoters on gastric absorption at pH 7.0, do not fall within the scope of the Schanker hypothesis which proposes correlation of absorption and lipid solubility. The hypothesis is useful in studying weak organic acids to allow understanding of relative absorbability of different drugs in the stomach or other GI segment as determined greatly by local pH. The dissociation constant determines the relative availability of the more lipid-soluble unionized form at the local pH. The use of buffer at neutral pH explicitly avoids the occurrence of the lipid-soluble form of the drugs.

It has been suggested that the mechanism of influence of surface-active absorption promoters, effective as they are under conditions of negligible lipid solubility of the drug, is to render the membrane a less effective lipid barrier to the passive diffusion of such ionized drugs. An earlier report from these laboratories showed that the surface-active absorption promoters act by a colloidal influence upon the absorptive organ and that the effect is rapidly reversible (1). The present work presents no additional information regarding mechanism or reversibility but appears consistent with the earlier conclusions.

In routine procedures employed here, the first blood samples were taken approximately 20 min. after dosing. Before this time, as seen from the experiment on stomach emptying in intact rats, most of the dose had emptied into the small intestine and a rapid rise in blood levels had already begun. Accordingly, the initial levels seen in these experiments on normal and promoted absorption in the intact animal should be attributed to intestinal rather than to gastric absorption. This initial rise in blood levels is rapid, as it is in ligated duodenum–small intestine, even though blood levels never rise to the high values seen in the isolated ligated duodenum–small intestine experiments. This rapid rise in blood levels contrasts with the slow rise when the drug–promoter mixture is administered in the ligated stomach, lending further evidence that the duodenum–small intestine is the responsible segment in the intact animal experiments.

The promoter appears to impose a very fleeting hyperabsorptive state on the duodenum–small intestine and then loses its effectiveness to elevate absorption. This may be due to rapid emptying of the stomach followed by dilution of the dose in the duodenum, or to the subsequent rapid passage of a liquid dose out of the more absorptive upper part of the small intestine. The comparatively poor results in the intact animals also raise the interesting question of possible specific incompatibilities of polyoxyethylene-20-oleyl ether with intestinal secretions in the intact GI tract.

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