Normal and Promoted Gastrointestinal Absorption of Water-Soluble Substances I: Induced Rapidly Reversible Hyperabsorptive State in the Canine Fundic Stomach Pouch

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Abstract □ Certain surface-active agents placed in a Thomas canine fundic pouch in buffered solutions influence the absorption of soluble drugs (antibiotics). Such agents induce a rapidly reversible hyperabsorptive state of the organ, resulting in blood levels of absorbed drug many times greater than control values. Effective surface-active agents may be nonionic, anionic, or zwitterionic. The influence of the surface-active agent is upon the organ and not upon the drug, as evidenced by the efficacy of the absorption promoter when it is employed and removed before the drug is introduced.

Keyphrases □ Absorption, antibiotics—surfactant effect □ Surfactant effect—antibiotic absorption □ Gastric fundus pouch, dog—absorption, antibiotics □ Plasma levels—antibiotics □ Microbiological analysis—plasma, antibiotic concentration

Detergents and bile salts interfere with the normal barrier to the movements of Na^+, K^+, and H^+ in the gastric mucosa (1). Many other reports in the literature describe examples of surface-active agents having a marked effect, either positive or negative, on passage of molecules through physiologic barriers (2–8).

Most of these observations, however, deal with translocation of substances under conditions complicated by insolubility of the compound (6), involvement of active transport processes (7, 8), or interaction of the surface-active agent with the penetrating species (4, 5). These complications prevent formulation of conclusions concerning the general nature of the influence of surfactants on the absorption of drugs from the gastrointestinal tract.

This and subsequent reports present observations regarding the general nature of the effects of surfactants on the absorption of drugs from the gastrointestinal tract. By restricting these studies to soluble drugs, the authors avoid variability due to differences in wetting, solubilization, and dispersion. In this initial study, the gastric fundic mucosa was used as a test membrane to confine the observations to a relatively simple system. By sometimes exposing the tissue first to surfactant and subsequently to the drug, the authors were able to separate the effects of surfactant–membrane interactions from those of surfactant–drug interactions.

Parenteral antibiotics were chosen for specific study because this class of drugs is readily detected in the blood by known quantitative methods. These compounds, furthermore, are ordinarily not well absorbed from the gastrointestinal tract. Their absorption in significant amounts would therefore be easier to demonstrate and be of greater practical interest than if the enhanced absorption of an already well-absorbed drug was the subject of study.

EXPERIMENTAL

Dogs were surgically prepared with Thomas-type pouches of the gastric fundus several months prior to the experimental period. Catheter access to the pouch permitted ready introduction and removal of solutions containing conditioning agents and drugs, either together or separately.

Dogs were fasted overnight before use so that observed gastric acid secretion was minimal. The secretory state, in addition to causing undesirable fluctuations in the pouch contents, was observed to oppose absorption where such absorption had been previously observed in the absence of secretion. In the early experiments, whenever the pouch contents were observed to be acidic, the animal was made nonsecreting by giving it 3 mg. of an anticholinergic drug[1] orally 30 min. before the experiment. This treatment had no apparent effect on either control or experimental results. To prevent the occasional inconsistencies attributable to the secretory state and to reduce the number of experimental variables, the treatment was subsequently made routine.

Solutions of sodium cephalothin[2] in pH 7.0 sodium phosphate buffer, with varying amounts of POE-24-cholesterol ether,[3] were placed in the pouch of a dog and replaced at approximately 40-min. intervals with a fresh solution. Heparinized samples of peripheral venous blood, removed periodically by venipuncture, were centrifuged; the plasma was assayed for microbiological activity of cephalothin by standard disk-plate tests.[4]

RESULTS AND DISCUSSION

Absorption of cephalothin from the gastric pouch was promoted by the presence of POE-24-cholesteryl ether. The plasma antibiotic level achieved was dose responsive to the pouch concentration of this nonionic surface-active agent (Fig. 1*a*) at constant antibiotic concentration.

Absorption of cephalothin from the gastric pouch under the same conditions was also promoted by many, but not all, other surface-active agents (Table I). The absorption of cephalothin was dose responsive to the concentration of coadministered sodium lauryl sulfate (Fig. 1*b*), but with the limitations to be discussed.

The absorption of cephaloridine,[5] an antibiotic related to cephalothin but amphoteric in nature, was also promoted by the presence of POE-20-oleyl ether[6]; the extent of absorption was again dependent on surfactant concentration (Fig. 1*c*).

The relationships between surfactant concentration and rate of drug absorption in these experiments illustrate an important aspect of the action of the surface-active agents. The critical micelle concentration (CMC) of sodium lauryl sulfate under comparable conditions is about 0.035% (12, 13), corresponding to the concentration at which the absorption response to increased surfactant concentra-

[1] α- and β-DL(1-Methyl-3-pyrrolidinyl)-α-phenyl-α-(2-thienyl)glycolate methyl bromide. Compound X in *Reference 9*.

[2] 2-(Thiophene acetamido)cephalosporanic acid (10).

[3] Solulan C-24, American Cholesterol Products, Inc., Edison, N. J. POE is used as an abbreviation for "polyoxyethylene."

[4] Cephalothin, methicillin, streptomycin, and penicillin G were assayed against *Bacillus subtilis* ATCC 6633. Cephaloridine and tylosin were assayed against *Sarcina lutea* PC1-1001-FDA. Tetracycline was assayed against *Bacillus megatherium*.

[5] 7-[α-(2-Thiophene)acetamido]-3-(1-pyridylmethyl)-3-cephem-4-carboxylic acid betaine (11).

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

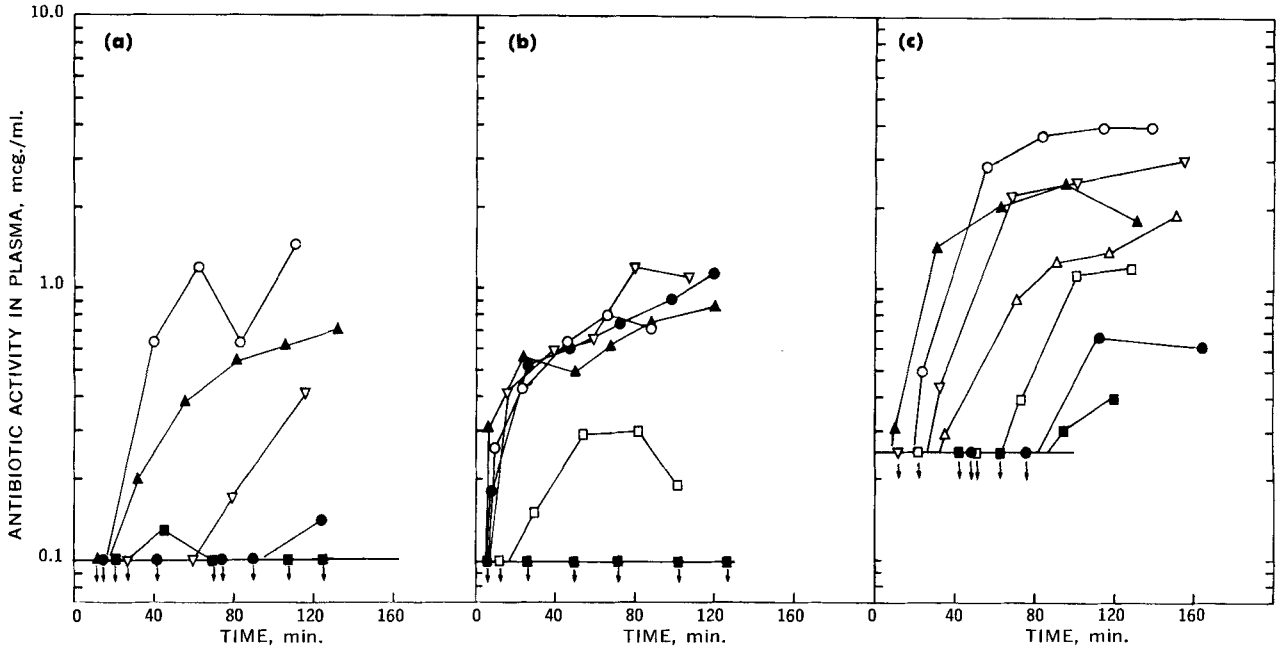

Figure 1—*Effect of surfactant concentration on the absorption of cephalosporin antibiotics from the dog fundic pouch. Pouch contents: 25 ml. 1% antibiotic and indicated concentration of surfactant, dissolved in isosmolar sodium phosphate buffer, pH 7.0. The horizontal lines are drawn at the minimum detection limit of the assay. Key: (a) Absorption of cephalothin promoted by POE-24-cholesteryl ether; surfactant concentration, w/v: ■, 0.0%; ●, 0.025%; ▽, 0.05%; ▲, 0.1%; and ○, 0.5%. (b) Absorption of cephalothin promoted by sodium lauryl sulfate; surfactant concentration, w/v: ■, 0.0%; □, 0.025%; ●, 0.05%; ▽, 0.2%; ▲, 0.4%; and ○, 0.8%. (c) Absorption of cephaloridine promoted by POE-20-oleyl ether; surfactant concentration, w/v: ■, 0.0%; ●, 0.15%; □, 0.031%; △, 0.062%; ▽, 0.125%; ▲, 0.25%; and ○, 0.5%.*

tion terminates. Therefore, the absorptive response to surfactant concentration develops only throughout the range in which the concentration of molecularly dispersed sodium lauryl sulfate is increasing. The absorptive response to nonionic surfactants (Figs. 1*a* and 1*c*) occurs over a wider range of surfactant concentration, in keeping with the continued increase in concentration of the molecularly dispersed species above the CMC which is a characteristic of nonionic surfactants. This dose response to molecularly dispersed surfactant is in contrast to the ability of surfactants to solubilize water-insoluble substances, which depends on and is proportional to the amount of surfactant in the micellar phase.

To characterize the onset of the hyperabsorptive state as a result specifically of contact of the stomach with the absorption promoter, POE-24-cholesteryl ether in buffered solution was placed in the pouch for periods from 5 to 60 min. and then removed. The pouch was rinsed several times with buffer (requiring about 5 min.), and then a solution of cephalothin (in buffer) without surfactant was introduced into the pouch. The resulting plasma levels of antibiotic activity rose more rapidly and to higher peak values as the duration of pretreatment with promoter was increased, demonstrating the gradual appearance of the hyperabsorptive state of the pouch with increasing time of exposure to the surfac-

Table I—Effect of Various Promoters on the Absorption of Several Antibiotics from the Dog Fundic Pouch

Antibiotic	Promoter	pH of Buffered Pouch Contents	Peak Plasma Level of Drug, mcg./ml.[a] Without Promoter[b]	With Promoter[c]
Methicillin	POE-24-cholesteryl ether	7.0	0.50	1.60
Streptomycin[d]	POE-24-cholesteryl ether	7.0	2.3	10.0
Streptomycin[d]	POE-24-cholesteryl ether	3.0	0.20	2.80
Tetracycline	POE-24-cholesteryl ether	2.3[e]	0.25	2.30
Penicillin G	POE-24-cholesteryl ether	7.0	0.39	2.45
Tylosin[d]	POE-24-cholesteryl ether	7.0	0.10	1.00
Cephaloridine	POE-24-cholesteryl ether	7.0	0.26	2.10
Cephaloridine	POE-32-cholesteryl ether[f]	7.0	0.26	3.00
Cephaloridine	POE-20-cetyl ether[g,h]	7.0	0.26	9.40
Cephaloridine	POE-10-stearyl ether[g,i]	7.0	0.26	3.85
Cephaloridine	POE-20-stearyl ether[g,j,k]	7.0	0.26	2.50
Cephaloridine	POE-20-oleyl ether	7.0	0.26	5.40
Cephaloridine	3-Carboxy-*N*-(*n*-tetradecyl)pyridinium·HCl[l]	7.0	0.26	2.75
Cephaloridine	Sodium lauryl sulfate[m]	7.0	0.26	1.65
Cephaloridine	Sodium lauryl sulfate[m]	4.0[n]	0.20	2.60
Cephalothin	POE-20-oleyl ether	7.0	0.10	2.40
Cephalothin	POE-2-stearyl ether[g,o]	7.0	0.10	0.14
Cephalothin	POE-X-stearyl ether[g,p]	7.0	0.10	0.20
Cephalothin	Polysorbate-40	7.0	0.10	0.22

[a] The results are the highest value observed during 120 min. following administration to a single dog with and without promoter. [b] Pouch contents: 25 ml. 1% antibiotic in isosmolar sodium phosphate, pH 7.0, except as noted. [c] Pouch contents: 25 ml. 1% antibiotic, 0.5% promoter, in isosmolar sodium phosphate, except as noted. [d] Concentration 2%. [e] Isosmolar citric acid–HCl. [f] Solulan C-32, American Cholesterol Products, Inc., Edison, N. J. [g] Atlas Chemical Industries, Wilmington, Del. [h] Brij-58. [i] Brii-76. [j] Brij-78. [k] Concentration 0.25%. [l] *Reference 16*. [m] Concentration 0.20%. [n] Isosmolar citrate–phosphate (sodium) [o] Brij-72. [p] Myrj 51.

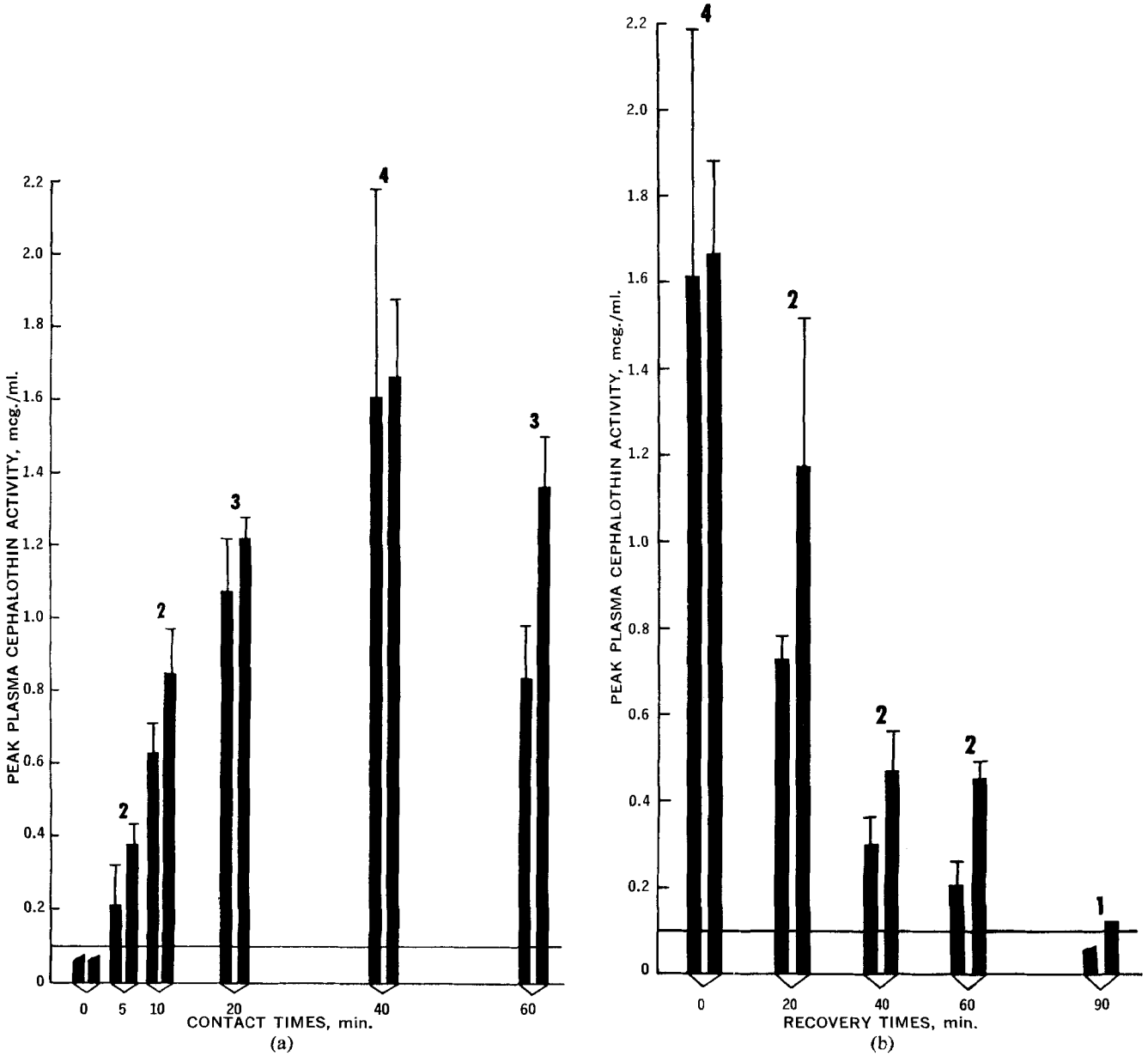

Figure 2—(*a*) *Effect of duration of contact between POE-24-cholesteryl ether solution and the dog fundic pouch upon the subsequent absorption of cephalothin. Sequence of pouch contents: (1) 25 ml. 0.5% POE-24-cholesteryl ether in isosmolar sodium phosphate buffer, pH 7.0, for the indicated time of contact; (2) three rinses with 25 ml. buffer; (3) 25 ml. 1% cephalothin in isosmolar sodium phosphate buffer, pH 7.0 (without POE-24-cholesteryl ether), for 120 min. Plasma activity was followed for 120 min. Each point is the mean ± SEM of the maximum levels from the number of experiments shown in bold numerals performed on each of two 21-kg. dogs. The horizontal line is the minimum detection limit of the assay.* (*b*) *Effect of recovery time on the hyperabsorptive state of the dog fundic pouch, the absorption of cephalothin after various time intervals between removal of POE-24-cholesteryl ether and introduction of cephalothin. Sequence of pouch contents: (1) 25 ml. 0.5% POE-24-cholesteryl ether in isosmolar sodium phosphate buffer, pH 7.0, for 40 min.; (2) three rinses with 25 ml. buffer; (3) pouch empty for indicated time; (4) 25 ml. 1% cephalothin in isosmolar sodium phosphate buffer, pH 7.0 (without POE-24-cholesteryl ether), for 120 min. Plasma activity was followed for 120 min. Each point is the mean ± SEM of the maximum levels from the number of experiments shown in bold numerals performed on each of two 21-kg. dogs. The horizontal line is the minimum detection limit of the assay.*

tant. The effect increased with time of exposure to this promoter at this concentration up to about 40 min., as seen in the plot of peak antibiotic plasma levels against time of contact (Fig. 2*a*). This graph also shows that as little as 5 min. of contact with 0.5% POE-24-cholesteryl ether solution resulted in some promoted absorption.

To quantitate the rate at which the fundic pouch loses the hyperabsorptive state after removal of the surface-active solution, 0.5% buffered POE-24-cholesteryl ether solutions were maintained in the pouch for 40 min. and then rinsed out with buffer. Then a time was permitted to elapse before the cephalothin solution without surfactant was introduced (Fig. 2*b*). As the elapsed time between removal of promoter and introduction of antibiotic increased, the maximum resulting blood levels declined rapidly. The peak plasma level obtained fell to one-half when the elapsed time was 25 min., and it fell below the detection limit when the elapsed time was 2 hr.

This rapid recovery of normal absorptiveness, suggesting the rapid restoration of a normal barrier to diffusion, is in contrast to the persistent hyperpermeability associated with damage to the gastric mucosa caused by fatty acids and acetylsalicylic acid at low pH, as reported by Davenport (14).

In addition to the observation that peak plasma levels of drug reach lower levels as the recovery time increases, other observations provide evidence of the rapid reversal of the hyperabsorptive state and the return to a normal absorptive state after removal of the promoter. When the drug is placed without promoter in the preconditioned pouch, blood levels decline rapidly after an initial

sharp rise, implying that absorption continues only briefly in the absence of promoter in the pouch contents.

The complete reversibility of this induced hyperabsorptive state and the absence of permanent damage in the Thomas pouch dogs used for almost daily experiments are indicated by the fact that they lost neither their characteristic control response nor their reactivity to promoters over the course of several years.

These experiments clearly show that the surface-active agent acts on the organ rather than on the drug. The effect of surface-active agents in rendering the stomach hyperabsorptive toward water-soluble drugs is thus distinguished from the reported effects of similar agents on the absorption of poorly soluble drugs, which is commonly attributed to improved physical dispersion or rate of dissolution.

All agents that the authors found to possess the ability to promote gastric absorption of drugs are surface-active agents, but not all surface-active agents are effective at the concentrations employed.

The phenomenon of promoted absorption is not limited by the specific molecular nature or charge of the soluble drug (Table I). It also can be observed at low pH and is seen in presence of citrate or phosphate buffers of a range of concentrations and with isotonic sucrose when these solutions are used to dissolve the drug and promoter.

The effectiveness of the surfactants in promoting gastric absorption of cephalothin is poorly correlated with the hydrophile–lipophile balance of the surface-active agent and is not proportionate to the ability of the agent to lower the surface tension at an air–water interface. A general correlation was observed, however, between the effectiveness of a surface-active agent in promoting gastric absorption and its ability to lyse erythrocytes *in vitro*. This lytic action, to which erythrocytes are notoriously sensitive, is generally regarded as due to the accumulation of the surfactant molecules in the lipid membrane of the cell wall in a manner that causes loss of functional or physical integrity of the membrane.

A high degree of order on a monomolecular or bimolecular leaflet level (15) is generally pictured as having an essential role in the maintenance of a barrier to passive diffusion across cell walls. The reversible changes in gastric absorption reported in the present paper are, the authors believe, the consequences of reversible alterations of this highly ordered barrier that accompany the addition or removal of surfactant as described in these experiments.

The hyperabsorptive state of the stomach epithelium is thus regarded as one in which interference with the diffusion barrier permits increased absorption of a wide variety of water-soluble substances placed in the organ. However, the net absorption (or excretion) of a substance by active transport processes may simultaneously suffer from this reduction of the barrier to free diffusion, since the barrier is presumably operative in both directions and lowering it would lend advantage to the flux opposite to the active transport. Indeed, some compounds used here as promoters have elsewhere been found to inhibit active transport processes (8, 17).

In this study, convenience of measurement has prejudiced the choice of drugs to antibiotics, and the authors have dealt with absorption from the dog fundic pouch only. The observations of promoted absorption of other drugs in the intact gastrointestinal tract, as well as in ligated segments of the gastrointestinal tract, of the dog and other animal species have confirmed the more general implications of this work to drug absorption.[7] Such studies will be reported in following communications.

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[7] Anello and Levy (18) have drawn similar conclusions regarding the effect of polysorbate-80 on the permeability of the absorptive membranes of the goldfish.