Intestinal Permeability Enhancement: Efficacy, Acute Local Toxicity, and Reversibility

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The absorption of the polar drug phenol red was assessed in a rat intestinal perfusion model, in the presence of a variety of potential intestinal permeability enhancers. Both the absorption rate constant K_A and the plasma phenol red concentration were measured. Perfusates were also assayed for the presence of lactate dehydrogenase (LDH) and lipid phosphate, as biochemical markers of intestinal wall damage. Histological evaluation of surfactant-perfused intestines was also carried out. The potential permeability enhancers studied were the surfactants sodium dodecyl sulfate (SDS), sodium taurocholate (TC), sodium taurodeoxycholate (TDC), polysorbate-80 (PS-80), and nonylphenoxypolyoxyethylene (NP-POE) with an average polar group size of 10.5 POE units. Among these, SDS and NP-POE-10.5 were the most potent permeability enhancers. The bile salt TDC was a more effective enhancer than the more polar TC. The polar non-ionic surfactant PS-80 was an ineffective enhancer. Phenol red K_A and plasma level were generally correlated with biochemical and histological measures of intestinal damage. These observations indicate that permeability enhancement and local damage are closely related sequelae of the interaction of surfactants with the intestinal wall, and suggest that local wall damage may be involved in the mechanism of permeability enhancement. The reversibility of permeability enhancement and acute local damage was assessed for the surfactants TDC and NP-POE-10.5. Enhancement of phenol red permeability was reversed within 1–2 hr of the cessation of enhancer treatment. Biochemical markers of local damage also fell to control values within 1-2 hr of removal of enhancer from the perfusate. Histological evaluation of perfused intestines revealed that morphological damage was reversed within 3 hr. These results demonstrate that surfactant-induced acute intestinal wall damage is rapidly repaired.

KEY WORDS: permeability; absorption; intestinal toxicity; bile salts; non-ionic detergents; sodium dodecyl sulfate.

INTRODUCTION

The absorption of highly polar drugs is frequently limited by poor intestinal wall permeability. Surfactants have often been reported to be effective enhancers of intestinal wall permeability for polar drugs (reviewed in 1–4). This capability has been exhibited by surfactants with a broad range of structures, including anionic detergents, bile salts, glycerides, lysophospholipids, and others. A major limiting factor in the application of the permeability enhancer approach is the potential toxicity of the enhancers themselves (reviewed in 4, 5). For example, certain bile salts have been shown to cause acute local damage to the intestinal wall (6–9). Likewise, intestinal wall damage has been observed as a result of treatment with certain non-ionic surfactants (10), sodium dodecyl sulfate (SDS) (11–12), and lysolecithin (13).

In the present study, we have directly compared a variety of surfactant types in a single experimental system (single pass rat intestinal perfusion), in an effort to rank surfactants for their absorption enhancing ability. In addition, we have carried out biochemical and morphological evaluation of local intestinal damage, in order to ascertain whether surfactant-induced absorption enhancement and acute local toxicity are correlated. Finally, we have evaluated the reversibility of acute local intestinal damage, in order to gain insight into the potential long-term consequences of this damage.

MATERIALS AND METHODS

Materials. Sodium dodecyl sulfate was obtained from Stepan Co. (Northfield, IL). Sodium taurocholate, sodium taurodeoxycholate, and phenol red were from Sigma Chemical Co. (St. Louis, MO). Nonylphenoxy(polyoxyethylene)-10.5 (Igepal CO-710) was provided by Rhone-Poulenc (Princeton, NJ). Polysorbate 80 was obtained from Ruger Chem. Co., Irvington, NJ. All other chemicals and solvents were reagent grade or better.

Model Drug. Phenol red (phenolsulfonphthalein) was chosen as a model polar drug which is poorly orally absorbed (14,15). For the sodium salt, we measured an octanol/perfusion buffer partition coefficient of 0.4, and a saturation solubility of 357 mg/ml in perfusion buffer.

Intestinal Perfusions. Single pass intestinal perfusions were carried out using an experimental design similar to that described by Ho *et al.* (16). Fasted (16 hr) male CD rats (Charles River Laboratories, Mass.) of 275 to 350 gm body weight were anesthetized with urethane (1.5 gm/kg by intramuscular injection). The jejunum was exposed via a midline incision. Sutures were placed 5 cm distal to the ligament of Treitz in a position which minimally disrupted blood flow, and a small incision was made. Sutures were similarly placed at a position 22 cm distal to the first incision, and a second incision was made. The intestine was gently rinsed with 20 ml of 37°C saline to remove residual contents, then securely fastened to stainless steel cannulae at both the proximal and distal incisions. The perfused segment was moistened with saline and was covered with Parafilm. Body temperature was maintained by a heating pad. The 22 cm intestinal segment was perfused with solutions containing 0.25 mg/ml phenol red ± various surfactants in isotonic sodium phosphate/sodium sulfate buffer (pH 6.5), using a Sage Model 355 syringe pump (Orion Research Inc., Boston, MA). The perfusion buffer consisted of 66 mM sodium phosphate and 72 mM Na_2SO_4, adjusted to pH 6.5. The perfusion solution was maintained at 37°C using a water bath circulator. Perfusate samples were collected into tared vials over four 15 min

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intervals. An absorption rate constant was calculated for each 15-min interval, utilizing the relationship

$$K_A = \frac{Q(1 - C_0/C_i)}{V}$$

where Q is the measured flow rate (generally 0.2 ml/min), V is the volume of the perfused intestinal segment (2.7 cc), C_i is the ingoing phenol red concentration, C_0 is the outgoing phenol red concentration, and K_A is the absorption rate constant in min^{-1}. An average phenol red K_A was calculated for each perfusion experiment, using the C_0/C_i data gathered for the last 3 fifteen minute intervals. Plasma phenol red concentrations were determined in a separate group of rats at the end of a one hour perfusion, in which the starting perfusate phenol red concentration was 2 mg/ml. At the end of each 1 hr perfusion, blood was collected from the heart into a heparinized syringe, and plasma was stored frozen until the time of assay. In control experiments, the phenol red K_A was found to be independent of phenol red concentration over the range 0.25 to 2.0 mg/ml.

In reversibility experiments, perfusions were carried out for 4 hr, with perfusate collection in 15 minute increments. The phenol red K_A was calculated for each 15 minute interval, and each K_A value was averaged over 3 or 4 rats. At the end of each 4 hr perfusion, cardiac blood was collected.

Perfusate phenol red concentration. Ingoing and outgoing perfusate phenol red concentration was determined by HPLC using a mobile phase composed of 55% methanol and 45% 0.05 *M* KH_2PO_4 adjusted to pH 2.6. A Waters (Milford, MA) model 510 pump delivered the mobile phase at 1 ml/min. The stationary phase was a Beckman (San Ramon, CA) Ultrasphere C-18 25 cm column. The retention time of phenol red was 4.5 minutes. Phenol red was detected at 430 nm using a Kratos/ABI Spectroflow 783 detector (Applied Biosystems, Ramsey, NJ) and quantitated by external standardization using a Spectra-Physics ChromJet Integrator (Spectra-Physics Inc., San Jose, CA) and peak area quantitation.

Plasma phenol red concentration. HPLC-grade acetonitrile (0.5 ml) was added to 0.5 ml of plasma to precipitate protein and release phenol red from albumin. Following centrifugation at 11,000 rpm for 5 minutes (Eppendorf microcentrifuge, Hamburg, Germany), 0.5 ml of supernatant was diluted with 0.5 ml of perfusion buffer described above. The plasma phenol red concentration was determined by HPLC as described above for perfusate, except that the mobile phase was 48% methanol and 52% 0.05 *M* KH_2PO_4. Retention time of phenol red in this system was 9.5 minutes. Plasma concentrations were determined from linear regression of peak height of plasma standards of known phenol red concentration.

Lactate dehydrogenase. Lactate dehydrogenase (LDH) was assayed utilizing a kit (LD-L-20) supplied by Sigma Chemical Co. This assay involves spectrophotometric determination of the reduction of NAD in the presence of lactate and LDH, and was validated using LDH supplied by Sigma (Multienzyme Lin-Trol). The only tested surfactant which interfered with the determination of LDH activity was SDS; therefore LDH release could not be determined in the presence of SDS.

Lipid Phosphate. The lipid phosphate content of intestinal perfusates was determined by extraction into methylene chloride (a modification of the method of Bligh and Dyer (17)), followed by assay of inorganic phosphate according to Gomori (18). The presence of surfactant did not interfere with the extraction of phospholipid. Recovery of known amounts of lecithin from the various surfactant solutions ranged from 85 to 92%. No correction was made for recovery of phospholipid, as there was no trend of high or low recovery from a particular surfactant solution.

Thin Layer Chromatography (TLC). TLC was carried out using Merck silica gel-60 plates, with development in 65:25:4 $CHCl_3$:CH_3OH:H_2O. The following lipid standards were chromatographed: egg phosphatidylcholine (PC), egg lysophosphatidylcholine, egg phosphatidylethanolamine (PE), brain cerebrosides (monoglycosylceramides, MGC), cardiolipin, sphingomyelin, fatty acid (oleic acid), triglyceride (olive oil), cholesterol, and SDS. Lipids were identified using I_2 vapor. The presence of glycolipids was verified by spraying with 0.5% orcinol in 4:1 H_2O:CH_3OH containing 2N sulfuric acid, followed by heating the plate at 100°C until colored spots appeared. Spraying with 0.5% ninhydrin in CH_3OH, followed by heating at 100°C, verified the identify of amine-containing PE.

Histology. Single pass rat intestinal perfusions were carried out as described above. At the end of a 1 hr or 4 hr perfusion, the lumen was filled with 2.5% glutaraldehyde/2% paraformaldehyde in 0.1 M cacodylate buffer (pH 7), and was fixed *in situ*. The perfused intestinal segment was then removed and immersed in the same fixative. Cross sections were prepared and stained with hematoxylin-eosin, and were examined by light microscopy. Various measures of histological abnormality were quantitated on an arbitrary scale of 0–3, with 0 indicating no effect and 3 indicating an extensive effect. This evaluation was carried out by an experienced veterinary pathologist in a blinded fashion.

RESULTS

I. Efficacy and Acute Local Toxicity

Absorption enhancement. Table I presents phenol red absorption rate constants, obtained by rat intestinal perfusion of phenol red in the absence and presence of various surfactants. A surfactant concentration of 1% (w/v) was chosen for this comparison, because this concentration represents the approximate maximum surfactant concentration which could be achieved in the human intestinal lumen, using a practical dosage form which could contain no more than approximately 1 gm surfactant. The non-ionic surfactant polysorbate-80 (PS-80) exhibited little or no enhancement of the absorption of phenol red. In contrast, the non-ionic surfactant nonylphenoxypolyoxyethylene-10.5 (NP-POE-10.5) caused significant enhancement of the permeability of phenol red in this model. NP-POE-10.5 is significantly more non-polar than PS-80. The bile salts sodium taurocholate (TC) and sodium taurodeoxycholate (TDC) enhanced phenol red absorption. The more non-polar dihydroxy bile salt TDC was more effective than the more polar trihydroxy bile salt TC. Of the surfactants studied, the anionic surfactant sodium dodecylsulfate (SDS) was the most effective enhancer of the phenol red K_A. It should be

Table I. Effects of surfactants (1%, w/v) on the absorption of phenol red, in rat intestinal perfusions. Absorption rate constant K_A is averaged over 2nd, 3rd, and 4th 15 min intervals of a 1 hr perfusion. Plasma phenol red concentration was measured at the end of a 1 hr perfusion. (Na Salicylate is not a surfactant; see text.)

Treatment	n_A*	K_A ($\times 10^3$ min^{-1})	n_B†	Plasma Phenol Red (microgm/ml)
Control[a]	8	2.3 ± 0.4	8	0.35 ± 0.12
PS-80	4	4.0 ± 1.1	4	0.59 ± 0.18
NP-POE-10.5	6	9.6 ± 2.0	5	31.06 ± 9.50
TC	5	6.7 ± 1.0	4	1.22 ± 0.33
TDC	5	12.1 ± 1.8	4	11.75 ± 1.48
SDS	4	18.8 ± 1.3	5	37.04 ± 8.16
Na Salicylate	4	5.7 ± 1.3	4	0.3 ± 0.4

* Number of rats averaged for KA measurement.
† Number of rats averaged for plasma phenol red.
[a] Phenol red alone.

Table II. Biochemical markers of intestinal wall damage. Total units of lactate dehydrogenase (LDH) and total lipid phosphate released into the perfusate were measured during a 1 hr rat intestinal co-perfusion of phenol red (0.25 mg/ml) with various surfactants or sodium salicylate (1%, w/v).

Treatment	n*	LDH (units)	Lipid Phosphate (micromoles)
Control[a]	8	2.26 ± 1.04	0.17 ± 0.17
PS-80	4	1.65 ± 0.54	0.50 ± 0.18
NP-POE-10.5	6	30.72 ± 9.12	1.16 ± 0.33
TC	5	17.14 ± 5.97	0.21 ± 0.15
TDC	5	39.81 ± 10.19	1.75 ± 0.89
SDS	4	[b]	1.76 ± 0.60
Na Salicylate	4	2.61 ± 1.63	0.17 ± 0.03

* Number of rats averaged.
[a] Phenol red alone.
[b] SDS was found to denature LDH in control experiments.

noted that the phenol red K_A's observed in the presence of absorption enhancers, while greater than control, are still relatively low. Sodium salicylate, which is not a surfactant, has been reported to enhance the rectal permeability of a variety of polar drugs. Sodium salicylate was marginally effective in its ability to increase the phenol red K_A.

Table I also presents phenol red plasma levels at the end of a 1 hr perfusion. Large increases in phenol red plasma level are achieved in the presence of certain surfactants. For example, 1% (w/v) NP-POE-10.5 caused an 89-fold increase in phenol red plasma level, compared to the control level (in the absence of surfactant). The rank order of surfactants with respect to phenol red plasma level is similar to that observed for K_A, but the relative effectiveness of the surfactants exhibits a broader range in the plasma level data. For example, the phenol red plasma levels indicate that TDC is ~10-fold more effective than TC, while the K_A data suggest that TDC is 2× as effective. Sodium salicylate was ineffective. In absolute terms, we consider the plasma level data to be more reliable than the K_A data, because K_A measurements can be subject to artifacts related to water flux into and out of the intestinal lumen in the presence of surfactants (9,19,20). However, the K_A data were acceptably reproducible, as evidenced by the moderate observed standard deviations.

Biochemical markers of intestinal wall damage. Table II presents the release of lactate dehydrogenase (LDH), an intracellular enzyme, into the lumen of the small intestine during a 1 hr co-perfusion of phenol red with various surfactants. PS-80 perfusion resulted in LDH levels similar to control. The bile salts TC and TDC caused significant release of LDH, with the more non-polar TDC exhibiting a larger effect. The non-ionic surfactant NP-POE-10.5 also caused release of LDH into the lumen. The effect of SDS could not be determined, because SDS was shown to inhibit LDH in control experiments. Table II also presents the release of lipid phosphate into the lumen of the small intestine during a 1 hr co-perfusion of phenol red with various surfactants. PS-80 and TC caused minor release of lipid phosphate into the lumen, while the more effective absorption enhancers TDC, NP-POE-10.5, and SDS caused greater release of this marker. The non-surfactant sodium salicylate did not cause release of either damage marker into the intestinal lumen.

Membrane lipid identification. In order to verify the presence of membrane lipids in a typical perfusate, rat jejunum was perfused for 1 hr with 1% SDS in buffer or with buffer alone, and lipid extracts were prepared from the perfused fluids. Thin layer chromatographic analysis was carried out, with detection by orcinol and ninhydrin sprays, and by I_2 vapor. In the SDS perfusate, the following lipids were positively identified: phosphatidylcholine (PC), phosphatidylethanolamine (PE), cardiolipin, cholesterol, and monoglycosylceramide (MGC). No lipids were identified in the extract of perfused control buffer. The presence of cardiolipin, a mitochondrial lipid, suggests that cell lysis has occurred. The glycolipid MGC is a major component of the apical membrane of the intestinal epithelial cell, and is a very minor component of the other cell membranes (with the exception of kidney tubules and neural myelin). The presence of MGC in perfusates is positive evidence for the disruption of the brush border membrane. PC and PE are common components of plasma membranes and intracellular membranes. Their positive identification indicates that part or all of the measured "lipid phosphate" in perfusates is reflective of the presence of membrane phospholipids.

Histological evaluation. Figure 1A presents a cross section of rat intestinal villi after perfusion for 4 hr with phenol red. A normal intestinal epithelium is observed, with well aligned viable epithelial cells, and some normal sloughing of mature cells at the villus tips. Figure 1B presents a cross section of rat intestinal villi after co-perfusion for 1 hr with phenol red and 1% NP-POE-10.5. Large quantities of mucus and debris are observed, the villi are contracted and swollen, and the epithelial cells are rounded and poorly aligned. Histological sections were scored for a variety of abnormalities, using a scoring range of 0–3, where 0 represents no effect and 3 represents a severe effect. Scoring was carried out by a single pathologist who was unaware of the identity of the sections.

In Table III, scores are presented for various measures of damage, for intestines perfused for 1 hr with a variety of surfactants and phenol red. Mucus/debris refers to the pres-

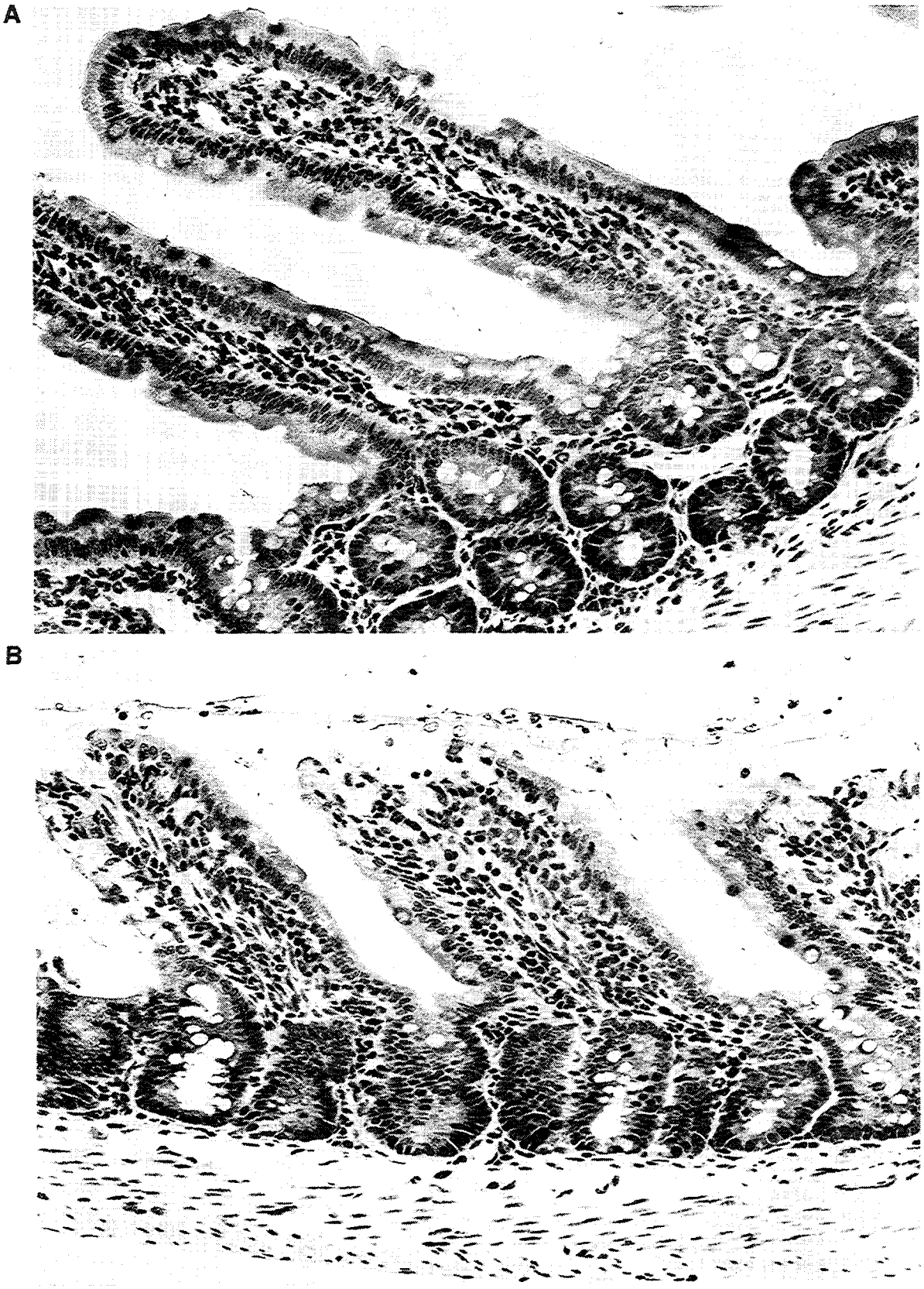

Figure 1. Light micrographs of rat intestinal villi after (A) 4 hr perfusion with phenol red, (B) 1 hr perfusion with phenol red plus 1% NP-POE-10.5, and (C) 1 hr perfusion with phenol red plus 1% NP-POE-10.5 followed by a 3 hr perfusion with phenol red alone (313× magnification).

Figure 1. Continued.

ence of basophilic material and lysed cells in the lumen. Villous shortening refers to apparent retraction of villi. Erosion refers to loss of epithelium, exposing the lamina propria, without ulceration. Swollen epithelial cells are an indication of cytoplasmic fluid gain. Flat epithelial cells refers to cells which are short, and have spread laterally in an apparent attempt to cover voids in the epithelium. [Goblet cells] (concentration of goblet cells) is an indication of relative loss of columnar epithelial cells. In general, goblet cells appeared to be more resistant than the absorptive enterocytes to surfactant-induced damage. The average total scores demonstrate that PS-80 and TC cause minimal damage, while TDC is more damaging. NP-POE-10.5 and SDS were the most damaging of the materials tested. The observed rank order of histological damage was similar to that obtained by study of biochemical damage markers.

Effect of Surfactant Concentration. The effects of TDC concentration on K_A, plasma phenol red, LDH release, and lipid phosphate release were studied. Table IV demonstrates that K_A and plasma phenol red increase with increasing TDC concentration, as do perfusate LDH and lipid phosphate. The limited number of TDC concentrations studied does not permit an analysis of whether sharp increases in these values occur at TDC concentrations in the vicinity of the critical micellar concentration, which has been reported to be in the range 0.1–0.6% (21,22). The effects of NP-POE-10.5 concentration on K_A, plasma phenol red, LDH, and lipid phosphate are also presented in Table IV. All four parameters increase with increasing NP-POE-10.5 concentration. The limited number of NP-POE-10.5 concentrations studied precludes analysis of whether sharp increases in K_A, plasma phenol red, LDH, or lipid phosphate occur at the NP-POE-

Table III. Histological evaluation of rat small intestinal mucosa after 1 hr perfusion with phenol red in the absence and presence of various surfactants (1%, w/v). See text for details.

	Score* for Treatment with					
	Control	PS-80	NP-POE-10.5	TC	TDC	SDS
(# rats)	(2)	(2)	(2)	(3)	(3)	(2)
Mucus/Debris	1, 1	2, 1	2, 2	2, 1, 2	2, 2, 2	2, 2
Villous Shortening	0, 0	0, 0	1, 1	0, 0, 0	2, 0, 1	2, 1
Erosion	0, 0	0, 0	3, 3	0, 0, 0	0, 1, 0	3, 3
Swollen Epithelial Cells	0, 0	0, 0	1, 0	0, 0, 0	0, 0, 0	0, 0
Flat Epithelial Cells	0, 0	0, 0	0, 1	0, 0, 0	1, 2, 0	0, 0
[Goblet Cells]	0, 0	0, 0	1, 1	0, 0, 0	0, 0, 0	0, 0
Total	1, 1	2, 1	8, 8	2, 1, 2	5, 5, 3	7, 6
Average Total Score	1	1.5	8	1.7	4.3	6.5

* Scores range from 0 (no effect) to 3 (severe effect).

Table IV. Dependence on surfactant concentration of phenol red absorption enhancement and biochemical markers of intestinal wall damage. Experimental conditions as in Tables I and II.

Treatment	n_A*	K_A ($\times 10^3$ min^{-1})	n_B†	Plasma Phenol Red (microgm/ml)	LDH (Units)	Lipid Phosphate (Micromoles)
Control[a]	8	2.3 ± 0.4	8	0.35 ± 0.12	2.26 ± 1.04	0.17 ± 0.17
0.01% TDC	4	2.2 ± 1.0	4	0.34 ± 0.11	1.14 ± 0.80	0.12 ± 0.04
0.1% TDC	4	3.1 ± 2.2	4	1.14 ± 0.43	7.15 ± 1.99	0.17 ± 0.07
0.5% TDC	4	9.6 ± 2.5	4	9.70 ± 6.36	32.31 ± 10.98	1.84 ± 0.52
1.0% TDC	5	12.1 ± 1.8	4	11.75 ± 1.48	39.81 ± 10.19	1.75 ± 0.89
0.01% NP-POE-10.5	4	2.1 ± 0.3	4	0.52 ± 0.35	1.95 ± 0.79	0.10 ± 0.04
0.1% NP-POE-10.5	4	6.8 ± 3.3	4	13.13 ± 7.94	10.51 ± 4.11	0.38 ± 0.20
0.5% NP-POE-10.5	4	9.4 ± 2.0	4	28.21 ± 12.38	20.66 ± 5.15	0.78 ± 0.22
1.0% NP-POE-10.5	6	9.6 ± 2.0	5	31.06 ± 9.50	30.72 ± 9.12	1.16 ± 0.33

* Number of rats averaged for KA, LDH, and lipid phosphate.
† Number of rats averaged for plasma phenol red.
[a] Phenol red alone.

10.5 CMC (which is suggested to be in the vicinity of 0.02% by surface tension data provided by the manufacturer).

II. Reversibility of Permeability Enhancement and Acute Local Toxicity

Phenol red absorption. Figure 2 presents the phenol red absorption rate constant K_A (measured over 15 min intervals) during the course of a 4 hr rat intestinal perfusion with phenol red (negative control). A steady K_A of $\sim 2 \times 10^{-3}$ min^{-1} is observed over the course of the control experiment.

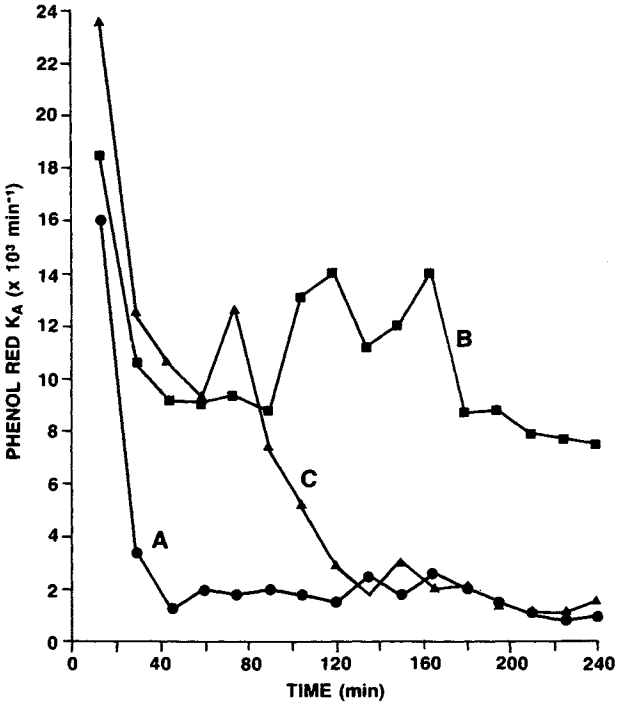

Figure 2. Phenol red absorption rate constant as a function of time during rat intestinal perfusions. Perfusates contained (A) phenol red alone for 4 hr; (B) phenol red plus 1% (w/v) TDC for 4 hr; (C) phenol red plus 1% (w/v) TDC for 1 hr followed by phenol red alone for 3 hr. Each point is an average of determinations in 3 rats for (A) and (B), and in 4 rats for (C).

Figure 2 also presents the phenol red K_A measured during the course of a 4 hr co-perfusion of phenol red with 1% (w/v) sodium taurodeoxycholate (TDC) (positive control). K_A ranges from $\sim 8-14 \times 10^{-3}$ min^{-1} during the TDC perfusion. Figure 2 also presents K_A for a 1 hr co-perfusion of phenol red and TDC, followed by a 3 hr perfusion of phenol red alone. It is clear that the phenol red K_A falls to control values within 1 hr of the cessation of TDC perfusion. A similar experiment was carried out to assess the reversibility of phenol red absorption enhancement by 1% NP-POE-10.5. Figure 3 demonstrates that, following a 1 hr perfusion with NP-POE-10.5, the phenol red K_A returns to control values within approximately 2 hr.

Table V presents the plasma phenol red concentration at the end of a 1 hr or 4 hr perfusion with phenol red, in the presence and absence of 1% TDC. Table V also presents the plasma phenol red concentration at the end of a 4 hr phenol red perfusion during which 1% TDC was present only during the first hour. It is clear that the plasma phenol red concentration returns to near control values within 3 hr of cessation of TDC treatment. Table V also presents data which demonstrated that 1% NP-POE-10.5 enhances the absorption of phenol red, and that the enhanced phenol red plasma level falls to near control within 3 hr of the cessation of NP-POE-10.5 treatment.

Biochemical markers of intestinal wall damage. Perfusate LDH and lipid phosphate content were measured during the course of each 4 hr perfusion, as markers of intestinal wall damage. Figure 4 presents the time course of perfusate LDH content for three sets of perfusions: (A) phenol red alone for 4 hr, (B) phenol red with 1% TDC for 4 hr, and (C) phenol red with 1% TDC for 1 hr followed by phenol red alone for 3 hr. Figure 4 demonstrates that TDC causes release of LDH into the intestinal lumen, and that the perfusate LDH content returns to the control value within approximately 2 hr of the cessation of TDC treatment. In the 4 hr phenol red/TDC perfusion (positive control), it appears that the perfusate LDH content is also slowly returning toward control values. This may be due to depletion of the LDH content of the intestinal epithelium or to homeostatic mechanisms which limit the extent of epithelial damage.

Figure 5 presents the time course of perfusate lipid

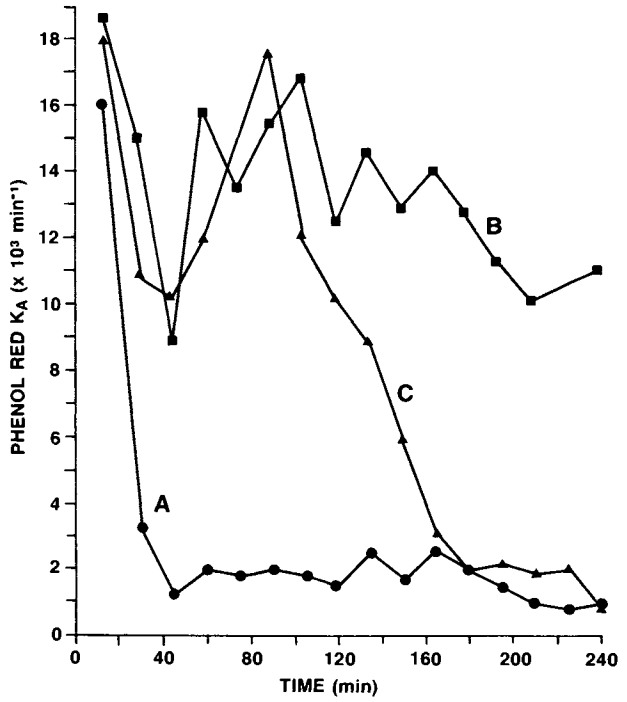

Figure 3. Phenol red absorption rate constant as a function of time during rat intestinal perfusions. Perfusates contained (A) phenol red alone for 4 hr; (B) phenol red plus 1% (w/v) NP-POE-10.5 for 4 hr; (C) phenol red plus 1% (w/v) NP-POE-10.5 for 1 hr followed by phenol red alone for 3 hr. Each point is an average of determinations in 3 rats for (A) and (B), and in 4 rats for (C).

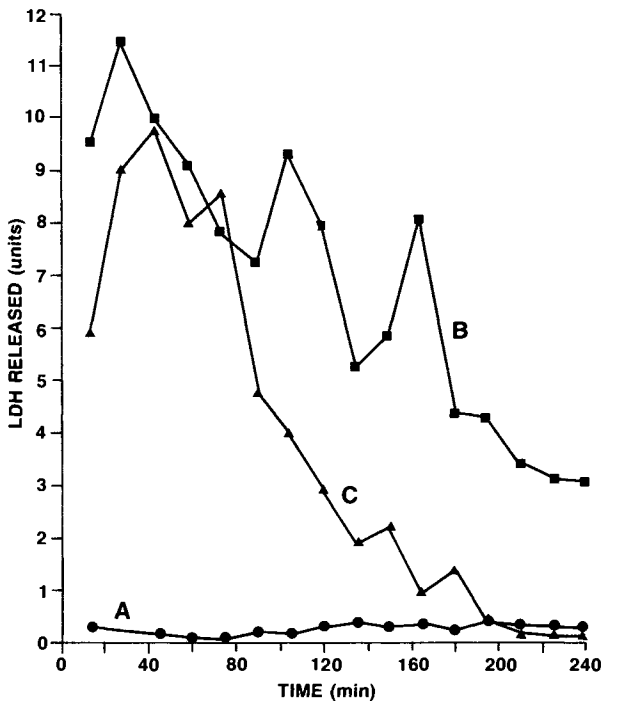

Figure 4. LDH release into perfusate as a function of time (15 min intervals) during rat intestinal perfusions. Perfusates contained (A) phenol red alone for 4 hr; (B) phenol red plus 1% (w/v) TDC for 4 hr; (C) phenol red plus 1% (w/v) TDC for 1 hr followed by phenol red alone for 3 hr. Each point is an average of determinations in 3 rats for (A) and (B), and in 4 rats for (C).

phosphate content for the TDC negative control, positive control, and reversibility experiments. The perfusate lipid membrane content returns to the control value within approximately 1 hr of the cessation of TDC treatment.

The release of biochemical damage markers into the perfusate was also studied for perfusions containing the nonionic surfactant NP-POE-10.5. Figure 6 presents the time course of perfusate LDH content for three sets of perfusions: (A) phenol red alone for 4 hr, (B) phenol red with 1% NP-POE-10.5 for 4 hr, and (C) phenol red with 1% NP-POE-10.5 for 1 hr followed by phenol red alone for 3 hr. The perfusate LDH content increases in the presence of NP-POE-10.5, and falls to control values within approximately 2 hr after the cessation of NP-POE-10.5 treatment. Figure 7 presents the time course of the perfusate lipid phosphate content for the NP-POE-10.5 negative control, positive control, and reversibility experiments. It is clear that the perfusate lipid phosphate content returns to the control value within approximately 1 hr of the cessation of NP-POE-10.5 treatment.

Table V. Plasma phenol red concentration following intestinal perfusion (rat) of phenol red in the presence and absence of the permeability enhancers taurodeoxycholate (TDC) or nonylphenoxy-(polyoxyethylene-10.5) (NP-POE-10.5).

Perfusion Solution/Duration	*n*	Plasma Phenol Red (microgm/ml)
Phenol red (1 hr)	8	0.34 ± 0.12
Phenol red (4 hr)	3	0.60 ± 0.08
Phenol red plus 1% TDC (1 hr)	5	11.75 ± 1.48
Phenol red plus 1% TDC (4 hr)	3	13.28 ± 2.18
Phenol red plus 1% TDC (1 hr), followed by phenol red alone (3 hr)	4	0.93 ± 0.28
Phenol red plus 1% NP-POE-10.5 (1 hr)	6	31.06 ± 9.50
Phenol red plus 1% NP-POE-10.5 (4 hr)	3	34.95 ± 11.80
Phenol red plus 1% NP-POE-10.5 (1 hr), followed by phenol red alone (3 hr)	4	3.25 ± 0.94

Histology. Histological evaluation was carried out on segments of small intestine which had been perfused with (A) phenol red for 1 hr or 4 hr, and (B) phenol red and either 1% TDC or 1% NP-POE-10.5 for 1 hr or 4 hr, and (C) phenol red and either 1% TDC or 1% NP-POE-10.5 for 1 hr followed by phenol red alone for 3 hr. Figure 1B presents a cross section of intestine perfused with phenol red and 1% NP-POE-10.5 for 1 hr. The epithelial covering on the tips of the villi has been lost, the villi are contracted, and mucus and cell debris have accumulated around the villi. Figure 1C presents a cross section of intestine perfused for 1 hr with 1% NP-POE-10.5 and phenol red, followed by 3 hr with phenol red alone. The damage seen in Figure 1B is reversed, and the epithelial covering of the villi is again intact.

Histological sections were scored for a variety of measures of epithelial damage. The data in Table VI indicate that TDC causes damage to the intestinal wall, as assessed by histological markers. The histological data also indicate that TDC-induced damage returns to control values within 3 hr of

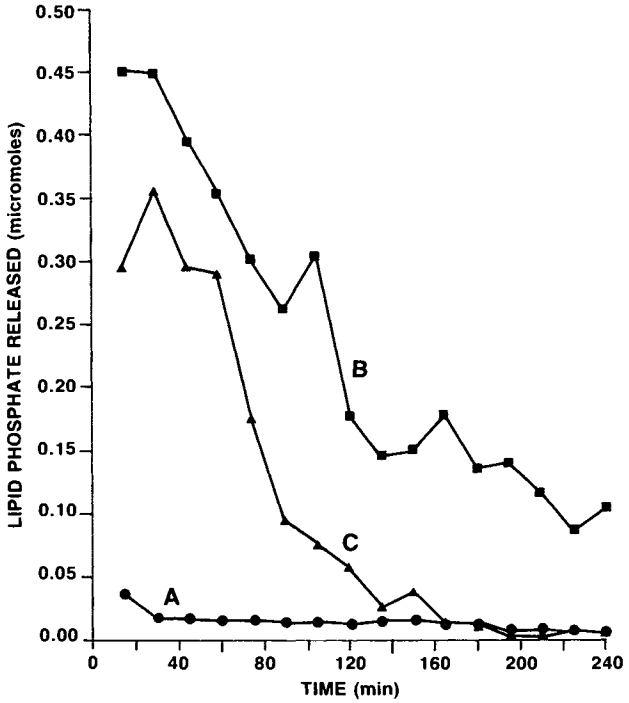

Figure 5. Lipid phosphate release into perfusate as a function of time (15 min intervals) during rat intestinal perfusions. Perfusates contained (A) phenol red alone for 4 hr; (B) phenol red plus 1% (w/v) TDC for 4 hr; (C) phenol red plus 1% (w/v) TDC for 1 hr followed by phenol red alone for 3 hr. Each point is an average of determinations in 3 rats for (A) and (B), and in 4 rats for (C).

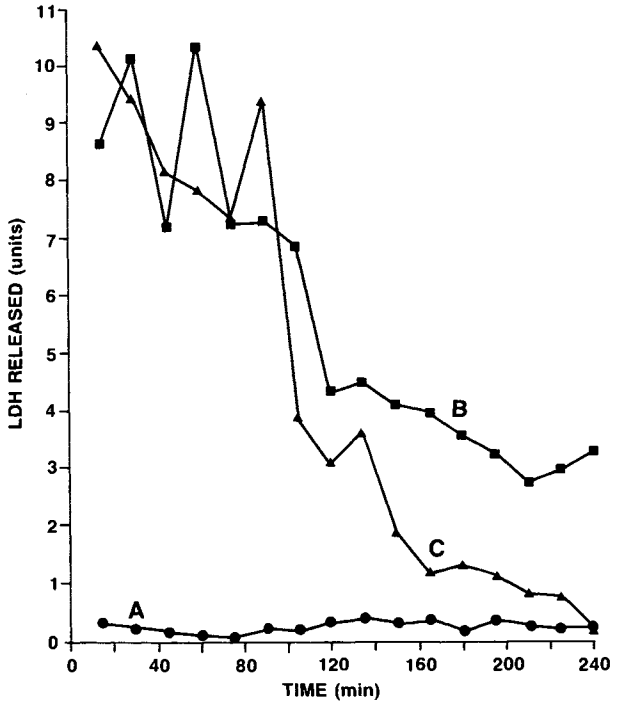

Figure 6. LDH release into perfusate as a function of time (15 min intervals) during rat intestinal perfusions. Perfusates contained (A) phenol red alone for 4 hr; (B) phenol red plus 1% (w/v) NP-POE-10.5 for 4 hr; (C) phenol red plus 1% (w/v) NP-POE-10.5 for 1 hr followed by phenol red alone for 3 hr. Each point is an average of determinations in 3 rats for (A) and (B), and in 4 rats for (C).

the cessation of TDC treatment. Table VI also presents histological evaluation of the effects of NP-POE-10.5 on the intestinal wall. NP-POE-10.5 acutely damages the intestinal wall as assessed by histological markers. Within 3 hr of the cessation of treatment with NP-POE-10.5, intestinal wall damage has returned to control values.

DISCUSSION

The rat intestinal perfusion system described here provides a useful method for concurrent observation of absorption enhancement and damage to the intestinal wall. The trihydroxy bile salt TC was a relatively ineffective absorption enhancer, while the less polar dihydroxy bile salt TDC was more effective, consistent with previous reports on nasal and intestinal absorption (23–26). The nonionic surfactant PS-80 was ineffective, while the nonionic NP-POE-10.5 was an effective absorption enhancer in this model. The anionic surfactant SDS was also effective. In the presence of 1% NP-POE-10.5 or 1% SDS, the plasma phenol red concentration was enhanced by 89-fold or 106-fold, respectively.

The release of LDH and lipid phosphate into the lumen suggests that some intestinal wall damage occurs in the presence of surfactant absorption enhancers. Figure 8 presents the correlation of phenol red K_A with LDH released into the perfusate, for each individual perfusion experiment. It is clear that the phenol red absorption rate constant is correlated with release of the biochemical damage marker. Figure 9 presents the correlation of phenol red K_A with lipid phosphate released into the perfusate, for each individual perfusion experiment. It is clear that K_A is correlated with this second marker of intestinal wall damage. TLC analysis revealed that monoglycosylceramide, a brush border membrane lipid, was released from the intestinal wall during perfusion with SDS.

Histological evaluation, though less quantitative, demonstrates that the release of these cellular markers is associated with tissue damage, and is not merely the consequence of increased intestinal wall permeability. Intestinal wall damage by bile salts (6–9), nonionic surfactants (10), and SDS (11,12) has been reported previously. The present work clearly demonstrates that absorption enhancement and intestinal wall damage are correlated phenomena. It is possible that enhancement and damage are independent sequelae of the interaction of surfactants with the brush border membrane. Alternatively, the increased intestinal wall permeability may occur at the regions of membrane damage.

Biochemical and histological markers of intestinal wall damage are shown in the present report to normalize rapidly after removal of a surfactant absorption enhancer from the intestinal lumen. This has been demonstrated for two structurally different surfactants: the bile salt TDC and the nonionic NP-POE-10.5. Rapid restitution of mucosal integrity has been reported previously by Nakanishi *et al.* (27), who observed recovery of the rat rectal epithelium within 2 hr of cessation of treatment with deoxycholate, sodium dodecyl sulfate, ethylenediaminetetraacetate, or polyethyleneglycol-400. Erickson (28) has reported that mucosal injury to the rat small intestinal mucosa by the bile salt chenodeoxycholate was reversed within 1 hr of cessation of bile

Table VI. Histological Evaluation of Rat Small Intestinal Mucosa after Perfusion (1 hr or 4 hr) with Phenol Red in the Absence or Presence of the Permeability Enhancers TDC (1%, w/v) or NP-POE-10.5 (1%, w/v). Reversibility Experiment Consisted of a 1 hr Perfusion with Phenol Red Plus Surfactant Permeability Enhancer (1%, w/v) Followed by a 3 hr Perfusion with Phenol Red Alone.

	Score*							
Perfusate:	Phenol Red		Phenol Red plus TDC			Phenol Red plus NP-POE-10.5		
Perfusion Duration: (# Rats): Histological Marker	1 hr (2)	4 hr (1)	1 hr (3)	4 hr (1)	Reversal (2)	1 hr (2)	4 hr (2)	Reversal (3)
Mucus/debris	1, 1	3	2, 2, 2	1	1, 1	2, 2	3, 3	0, 1, 0
Villous Shortening	0, 0	0	2, 0, 1	1	0, 0	1, 1	2, 2	0, 0, 0
Erosion	0, 0	0	0, 1, 0	1	0, 0	3, 3	2, 2	0, 0, 0
Swollen Epithelial Cells	0, 0	0	0, 0, 0	0	0, 0	1, 0	1, 0	0, 0, 0
Flat Epithelial Cells	0, 0	0	1, 2, 0	1	0, 0	0, 1	2, 1	0, 0, 0
[Goblet Cells]	0, 0	0	0, 0, 0	0	0, 0	1, 1	0, 1	1, 1, 0
Total Score	1, 1	3	5, 5, 3	4	1, 1	8, 8	10, 9	1, 2, 0

* Scores range from 0 (no effect) to 3 (severe effect).

salt treatment. Recently, Moore *et al.* demonstrated that repair of Triton-X-100-induced focal epithelial denudation is repaired rapidly (29,30). These authors suggested that this rapid repair involves (a) villus shortening which reduces the surface area of injury and (b) epithelial cell migration to cover the injured area. Nusrat and Madara studied repair of mechanical wounds in epithelial cell monolayers, and observed that wound closure was due largely to migration and spreading of existing cells (31).

Restitution of the stomach wall after chemical injury has been widely studied because of interest in ulcer formation and cure. Restitution of the rat gastric mucosa after ethanol-induced damage has been shown by Ito and colleagues to be almost complete in 1 hr (32,33). Frog gastric mucosal restitution was shown to be slower, requiring 4–6 hr (32). Recovery of the gastric epithelium after aspirin-induced damage occurs via migration of preexisting mucosal cells (34). Rapid epithelial restitution has also been shown to occur after acid-induced damage of the human colonic epithelium,

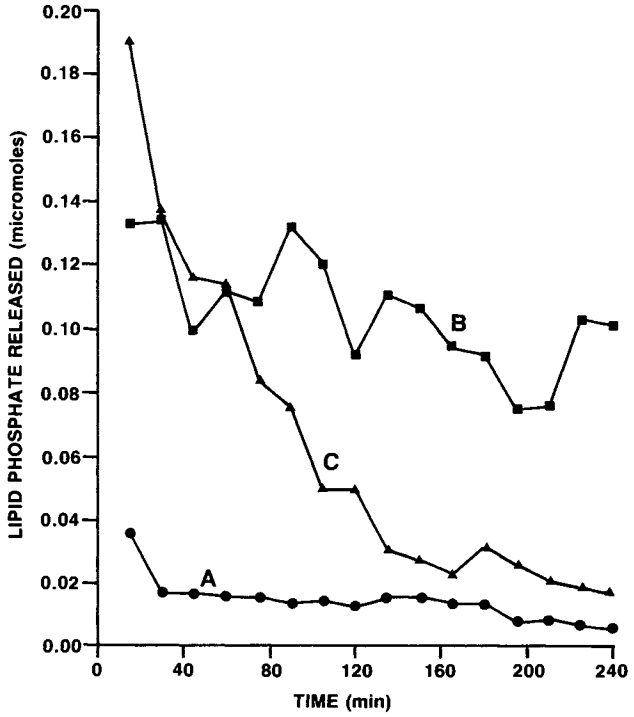

Figure 7. Lipid phosphate release into perfusate as a function of time (15 min intervals) during rat intestinal perfusions. Perfusates contained (A) phenol red alone for 4 hr; (B) phenol red plus 1% (w/v) NP-POE-10.5 for 4 hr; (C) phenol red plus 1% (w/v) NP-POE-10.5 for 1 hr followed by phenol red alone for 3 hr. Each point is an average of determinations in 3 rats for (A) and (B), and in 4 rats for (C).

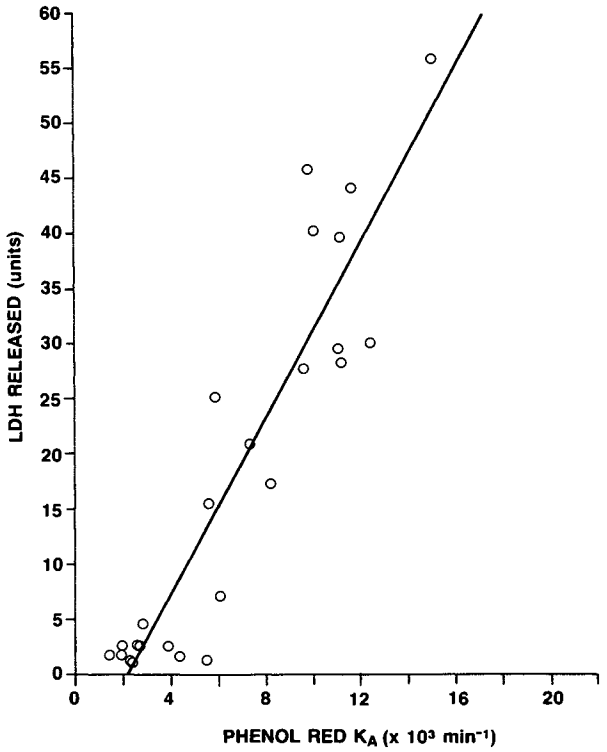

Figure 8. Relationship between phenol red K_A and LDH released into the lumen, for 1 hr rat intestinal perfusions with phenol red and various surfactant permeability enhancers.

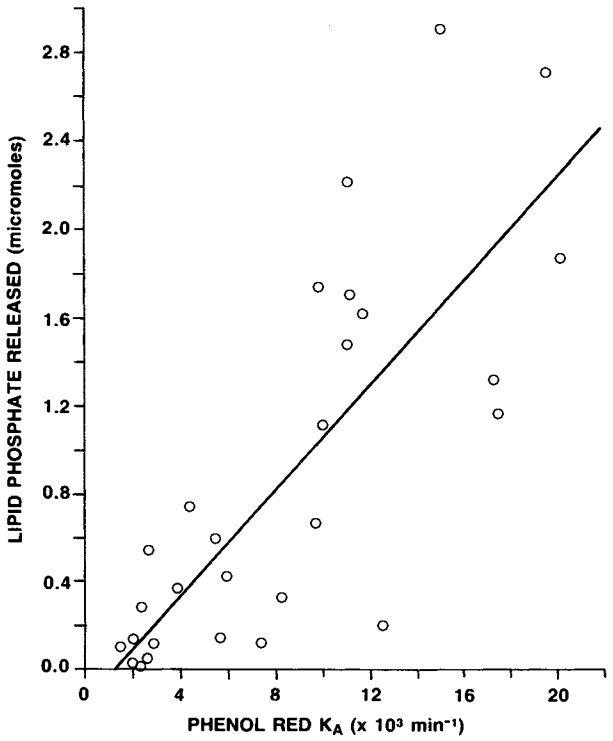

Figure 9. Relationship between phenol red K_A and lipid phosphate released into the lumen, for 1 hr rat intestinal perfusions with phenol red and various surfactant permeability enhancers.

and this restitution involves migration of the undamaged epithelial cells (35). Thus, facile restitution of superficial mucosal injury appears to be characteristic of the entire GI tract.

In summary, surfactant-induced intestinal permeation enhancement and acute epithelial damage are correlated phenomena, and the present work demonstrates that both reverse rapidly after removal of the surfactant from the intestinal lumen. This rapid reversal may reflect repair mechanisms which evolved as a response to the osmotically variable harsh chemical environment of the intestinal lumen. The toxicity of chronically dosed intestinal permeation enhancers can only be properly evaluated in chronic oral toxicology studies in appropriate species. The present work suggests that the short-term use of surfactants as intestinal permeation enhancers should not be prematurely discarded because of potential toxicity concerns.

NOTATION

CMC, critical micellar concentration; LDH, lactate dehydrogenase; NP-POE-10.5, nonylphenoxy (polyoxyethylene) 10.5; PS-80, polysorbate 80; SDS, sodium dodecyl sulfate; TC, sodium taurocholate; TDC, sodium taurodeoxycholate; MGC, monoglycosylceramide.

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