Effects of Normal Alcohols on Intestinal Absorption of Salicylic Acid, Sulfapyridine, and Prednisolone in Rats

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Abstract □ The rates of intestinal absorption of salicylic acid, sulfapyridine, and prednisolone from solutions containing no alcohol or 0.5% ethanol, *n*-butanol, or *n*-hexanol were determined. At the concentrations used, ethanol did not significantly affect drug absorption. Butanol reduced the rate of absorption of sulfapyridine but did not significantly affect the absorption rates of prednisolone or salicylic acid. Hexanol reduced the rates of absorption of sulfapyridine and salicylic acid and increased the rate of absorption of prednisolone. The absorption-altering effects of the alcohols were concentration dependent and rapidly reversible. Histological studies indicated that the structure of the epithelium was not altered by the alcohols. While the absorption rate of water from the drug solutions was increased by the alcohols, their absorption-altering effects could not be attributed solely to increased water flux. In addition, the absorption-altering effects of the alcohols could not be attributed to formation of drug–alcohol complexes nor to alcohol-induced alterations in the extent of binding of the drugs to nondialyzable materials in the intestinal drug solution.

Keyphrases □ Alcohols, normal—effects on intestinal absorption of salicylic acid, sulfapyridine, and prednisolone, rats □ Salicylic acid—effects of normal alcohols on intestinal absorption, rats □ Sulfapyridine—effects of normal alcohols on intestinal absorption, rats □ Prednisolone—effects of normal alcohols on intestinal absorption, rats □ Absorption, intestinal—salicylic acid, sulfapyridine, and prednisolone, effects of normal alcohols, rats

Several changes in the structure and function of the epithelial lining of the small intestine may occur when it is exposed to aqueous solutions of normal alcohols. For example, ethanol inhibited the active transport of amino acids in the small intestine of humans (1), and several alcohols, including ethanol and *n*-butanol, inhibited the active transport of amino acids and glucose through the everted rat intestine (2). Acute oral administration of aqueous solutions of 5% ethanol to the rat produced hemorrhagic erosions in the intestine and reduced the activities of lactase and thymidine kinase; oxygen consumption by jejunal slices was also reduced by ethanol (3).

Such changes suggest that the capacity of the intestine to absorb drugs may be altered by exposure of the intestine to alcohols. Two percent ethanol in solutions of theophylline recirculated through segments of rat jejunum increased the rate of absorption of theophylline, apparently due to an ethanol-induced increase in the rate of water absorption (4). However, the presence of 2% ethanol in solutions of barbiturates, sulfaguanidine, and promethazine did not significantly alter the rate of drug absorption (5).

The purpose of this study was to determine the effects of low concentrations of ethanol, *n*-butanol, and *n*-hexanol on the rates of intestinal absorption of salicylic acid, sulfapyridine, and prednisolone. These drugs were used because of their diverse physicochemical properties. Salicylic acid was ionized at the pH of these experiments, while sulfapyridine and prednisolone were not. The *n*-butanol–pH 7.0 aqueous buffer partition coefficients of salicylic acid, sulfapyridine, and prednisolone were 0.5, 3, and 40, respectively.

The concentration of alcohol used was 0.5% or less. This concentration of ethanol is readily achieved in the intestinal lumen of humans following oral ingestion of relatively small quantities of ethanol. For example, oral administration of 40 g of ethanol as a 10 or 20% aqueous solution produced ethanol concentrations in the upper jejunum above 1% for 30–60 min (1). While the intestinal mucosa is exposed to a number of aliphatic alcohols following consumption of some alcoholic beverages, exposure to levels of 0.5% butanol or hexanol would only be found after accidental ingestion of preparations containing relatively high concentrations of the alcohols.

EXPERIMENTAL

Materials—Sodium salicylate[1], salicylic acid[1], sodium sulfapyridine monohydrate[2], sulfapyridine[3], prednisolone[4], and ^{3}H-prednisolone[5] were used as received. The labeled radiochemical purity (>95%) of ^{3}H-prednisolone was verified periodically by TLC on glass plates coated with silica gel G and developed with benzene–2-propanol (4:1). Reagent grade absolute ethanol[6], *n*-butanol[7], *n*-pentanol[8], and *n*-hexanol[9], 98%, were used as received. Isopropyl myristate[10] was purified by passing it through a column packed with aluminum oxide. Following purification, no free acid was detected by the USP XVII method (6).

Complex Formation—Apparent association constants for drug–hexanol complexes with assumed 1:1 stoichiometry were determined by the solubility method (7). Fifteen milligrams of prednisolone, 15 mg of sulfapyridine, or 200 mg of salicylic acid was placed in each of a series of 10-ml ampuls; then 5 ml of solutions of hexanol in isopropyl myristate (0.0–0.20 *M*) or water[11] (0.0–0.05

[1] Merck and Co., Rahway, N.J.
[2] City Chemical Corp., New York, N.Y.
[3] Matheson, Coleman and Bell, East Rutherford, N.J.
[4] Sigma Chemical Co., St. Louis, Mo.
[5] Amersham/Searle Corp., Des Plaines, Ill.
[6] U.S. Industrial Chemicals Co., New York, N.Y.
[7] J. T. Baker Chemical Co., Phillipsburg, N.J.
[8] Mallinckrodt Chemical Works, St. Louis, Mo.
[9] Aldrich Chemical Co., Milwaukee, Wis.
[10] Ruger Chemical Co., Irvington, N.J.
[11] 0.1 *M*, pH 6.5 sodium phosphate buffer for sulfapyridine.

M) were added. The ampuls were flame sealed and rotated for 72 hr in a 25 ± 0.1° water bath. After suspended drug particles were allowed to settle, the ampuls were opened, a sample of supernate was removed and filtered through a 0.25 ± 0.03-μm filter[12], and the concentration of drug was determined.

Hexanol did not interfere with the analytical methods used to determine drug concentration; when solutions of the drugs in isopropyl myristate or water were filtered, no change in drug concentration was observed. All phase solubility diagrams were type A_L, and association constants were determined from the slopes and intercepts of lines fitted to the diagrams by least squares (7).

Intestinal Clearance—Male Sprague–Dawley rats, 200–350 g, were housed in wire mesh cages in a room maintained at approximately 20° and lighted automatically 12 hr/day. The clearance of prednisolone, sulfapyridine, and salicylic acid from solutions recirculated at 6 ml/min through a 20-cm segment of *in situ* jejunum was measured by a technique described previously (8). When present, the concentration of alcohol in the recirculating drug solution was maintained constant by infusing the alcohol into the solution at the same rate that it was absorbed by the intestine.

Ethanol and butanol were infused into the recirculating drug solution as aqueous solutions. Due to its low solubility in water, hexanol was infused undiluted. Prednisolone (0.28 mM), containing 0.1 μCi/ml of ^{3}H-prednisolone, was dissolved in 154 mM NaCl solution; sulfapyridine (0.40 mM) and salicylic acid (13 mM) were dissolved in 67 mM sodium phosphate buffer at pH 6.6 and made isotonic with 82 mM NaCl. The amount of sodium chloride in the drug solutions was not reduced when alcohol was present.

The drug solution was sampled periodically and the drug concentration was determined. The drugs were absorbed by apparent first-order kinetics (Fig. 1), and absorption rate constants were determined from the slopes of lines fitted by least squares to plots of drug concentration *versus* time on semilogarithmic coordinates. The initial point was omitted from the least-squares fit to ensure stationary-state conditions; while the initial point was close to the line in the control experiments, it tended to fall above the line when the alcohols were present. This tendency for positive deviation, indicating transient rapid absorption of the drugs, increased with the molecular weight of the alcohol.

The absorption rate constants were converted to clearance per centimeter of intestine by multiplying the rate constant by the volume of perfusate, correcting for drug removed due to sampling, and dividing by the length of the intestine. The absorption rate of water per centimeter of intestine was calculated by dividing the difference between the amount of water added to the drug solution to maintain its volume and alcohol concentration constant and the amount removed as samples by the duration of the experiment and by the length of the intestine. When alcohol was present, its concentration was determined at the beginning, middle, and end of each experiment and the three values were averaged. Not more than 10% of the LD_{50} of any alcohol was absorbed during any experiment.

Drug Binding in Perfusate—Isotonic sodium phosphate buffer, alone or containing 0.3% hexanol, was recirculated through segments of intestine for 1 hr. The perfusate was placed in one chamber of a dialysis cell separated by a cellulose membrane from an equal volume of buffer containing drug and 0.3% hexanol when it was present in the perfusate. The dialysis cell was agitated in a 37° incubator, and the drug concentration on each side of the membrane was measured until successive determinations were the same. The percent of drug bound to nondialyzable substances in the perfusate was found as follows:

$$\% \text{ drug bound} = \frac{C_p - C_b}{C_p} \times 100 \qquad \text{(Eq. 1)}$$

where C_p and C_b refer to the concentration of drug in the perfusate and buffer, respectively, at equilibrium.

Histology—Segments of intestine were perfused with isotonic sodium phosphate buffer, alone or containing 0.5% ethanol, butanol, or hexanol. When present, the concentration of alcohol was maintained by infusion. After 1 hr, each segment was removed and fixed immediately in 10% buffered neutral formalin. Pieces of each segment were embedded in paraffin, and sections of 4–6-μm thickness were cut and stained with hematoxylin and eosin-phloxine (9).

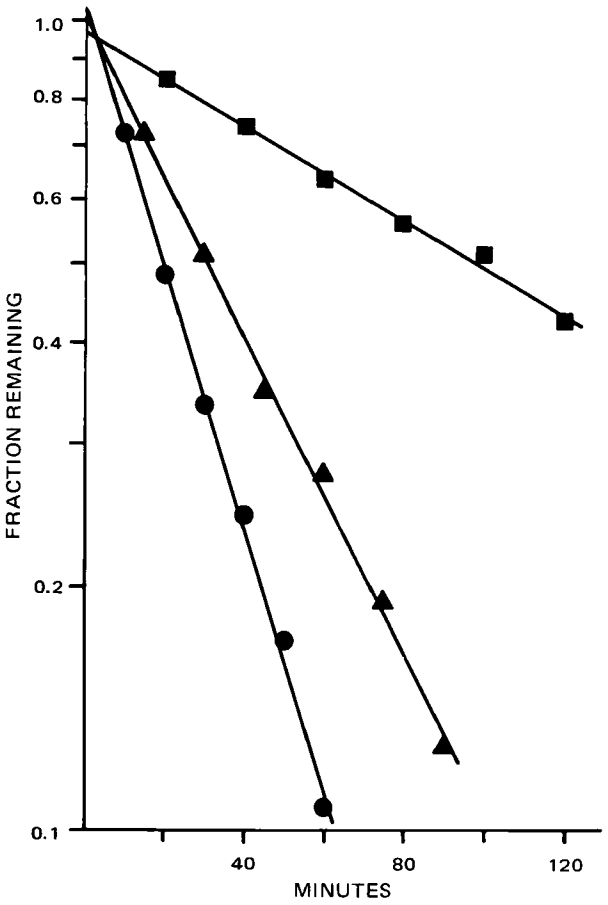

Figure 1—*Fractions of the initial concentrations of prednisolone (■), sulfapyridine (▲), and salicylic acid (●) remaining in the intestinal drug solution as a function of time. Each plot is from a single, representative experiment.*

Analytical Methods—Tritium was determined in 50-μl samples of intestinal perfusate and 1-ml samples of blood plasma by mixing with scintillation fluid[13] and counting in a liquid scintillation spectrometer[14]. For the intestinal perfusate samples, the efficiency of the counting system was constant at approximately 40% and was not affected by the small amounts of alcohol; therefore, correction for quenching was not made routinely. The counting efficiency of the plasma samples was 20–30%, and ^{3}H-toluene was used as the internal standard for quench correction. In studies on complex formation, prednisolone was determined by the USP colorimetric procedure (6). Sulfapyridine and salicylate in samples of intestinal perfusate and in studies on complex formation were determined by colorimetric methods (10, 11). The drugs were extracted from 1-ml samples of isopropyl myristate with 5 ml of 1% Na_2CO_3; 0.5 g of NaCl was added to prevent emulsification.

Ethanol in 0.1-ml samples of intestinal perfusate was determined by a GC method (12). Each sample was placed in a 10-ml serum bottle, which was then sealed with a rubber septum. The sample was incubated for 20 min in a 37° water bath and 0.50 ml of the vapor phase was injected onto a glass column, 4 mm i.d. by 1.8 m (6 ft), packed with 5% Carbowax 1500 on Haloport F (30–60 mesh). The temperatures of the injection port, oven, and flame-ionization detector were 110, 70, and 240°, respectively. The flow rates of helium, hydrogen, and air were 30, 30, and 500 ml/min, respectively. The retention time for ethanol was 3.5 min, and a plot

[12] Millipore type UG.

[13] PCS solubilizer, Amersham/Searle Corp., Des Plaines, Ill.

[14] Packard Tri-Carb model 3320, Packard Instrument Co., Downers Grove, Ill.

Table I—Effect of 0.5% *n*-Hexanol on the Distribution of Radioactivity following 30-min Perfusion of *In Situ* Rat Intestine with Solution of ^{3}H-Prednisolone

	Control[a]	*n*-Hexanol[a]	*p*
Percent of dose absorbed	21.3 (6.24)	33.2 (5.21)	<0.01
Plasma concentration[b]	4.89 (1.78)	8.70 (1.60)	<0.01
Length of intestine perfused, cm	19.4 (1.72)	19.9 (1.46)	>0.50

[a]Mean from seven experiments; *SD* in parentheses. [b](dpm/ml ÷ dpm administered) × 10^4.

of peak height *versus* concentration of ethanol was linear over the 0.1–0.5% range.

Butanol and hexanol in 0.1-ml samples of intestinal perfusate were also determined by GC. The samples were mixed with 0.1 ml aqueous 1-pentanol, which was used as an internal standard; approximately 1 μl was injected onto a stainless steel column, 0.31 cm (0.125 in.) o.d. by 1.8 m (6 ft), packed with Porapak Q, 80–100 mesh. The temperatures of the injection port, oven, and flame-ionization detector were 240, 225, and 230°, respectively. The flow rates of helium, hydrogen, and air were 40, 30, and 500 ml/min, respectively. Plots of the ratios of peak heights of butanol or hexanol to pentanol *versus* concentration were linear over the 0.1–1.0% range. No apparent alcohol was detected in samples of intestinal perfusate without alcohol.

RESULTS AND DISCUSSION

The effects of 0.5% ethanol, butanol, and hexanol on the rates of disappearance of prednisolone, sulfapyridine, and salicylic acid

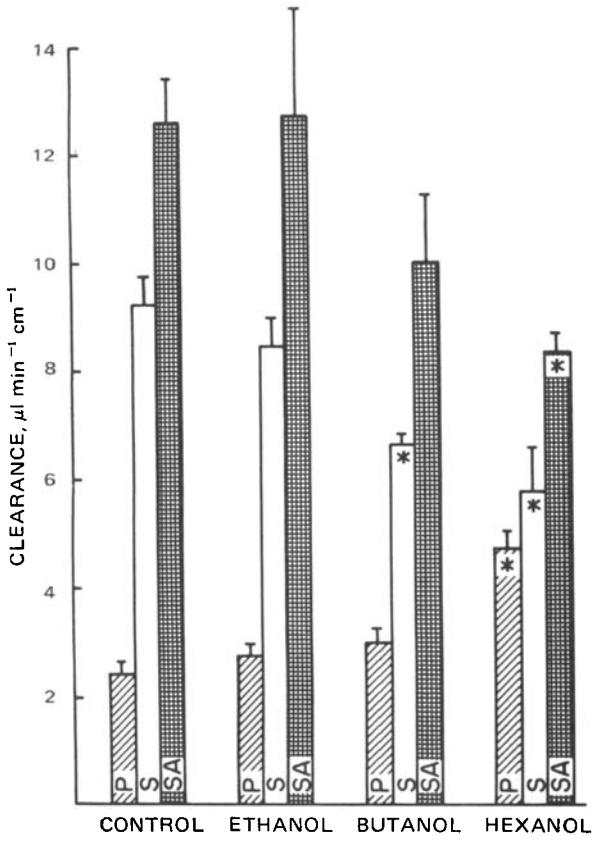

Figure 2—*Effects of constant concentrations of 0.5% ethanol, n-butanol, and n-hexanol on the clearance of prednisolone (P), sulfapyridine (S), and salicylic acid (SA) by the* in situ *rat jejunum. Each bar represents the mean from four or more experiments, lines indicate 1* SE, *and * indicates means that are significantly different (p < 0.05) from the control.*

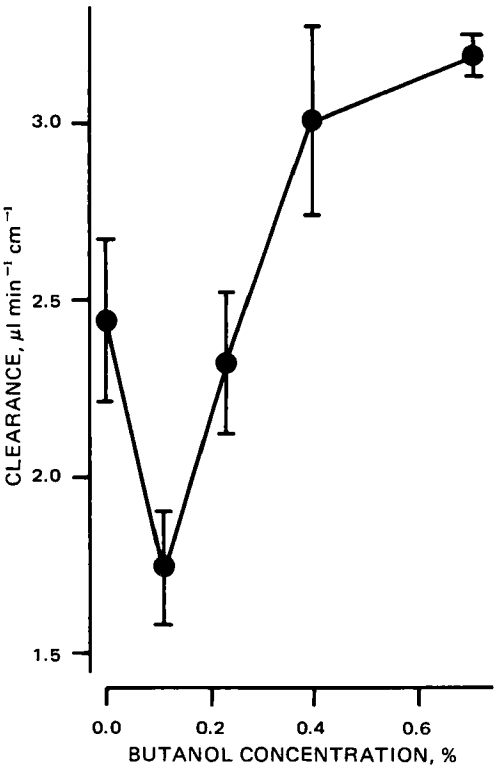

Figure 3—*Effect of various constant concentrations of butanol on the absorption of prednisolone from solutions recirculated through the* in situ *rat jejunum. Each point represents the mean from four or more experiments; bars indicate ±1* SE.

from solutions recirculated through the *in situ* rat jejunum are shown in Fig. 2. Ethanol did not significantly affect the rate of drug disappearance. The rate of disappearance of sulfapyridine was reduced by butanol, while the rates of disappearance of prednisolone and salicylic acid were not significantly altered. Hexanol increased the rate of disappearance of prednisolone and decreased the rates of disappearance of salicylic acid and sulfapyridine. The concentration dependence of the effects of butanol on the rates of disappearance of prednisolone and sulfapyridine is shown in Figs. 3 and 4.

To verify that the alcohol-induced changes in the rates of drug disappearance from the intestinal lumen reflect changes in absorption rates, the tritium concentration in the plasma was measured after perfusing intestinal segments for 30 min with a solution of ^{3}H-prednisolone and 0.5% hexanol. Compared to the control experiment in which no hexanol was present, the tritium concentration in the plasma and the amount of prednisolone absorbed were significantly increased (Table I). The increased rate of prednisolone absorption in the presence of hexanol cannot be attributed to differences in the length of intestine perfused.

A similar experiment with sulfapyridine showed no difference

Table II—Clearance of Prednisolone and Salicylic Acid by Perfused *In Situ* Rat Intestine during Two Consecutive 1-hr Periods

		Clearance[a], μl min^{-1} cm^{-1} of Intestine	
	Period[b], hr	Prednisolone	Salicylic Acid
Control	0–1	2.86 (0.304)	11.5 (2.64)
	1–2	2.60 (0.441)	9.07 (1.53)
Test	0–1	5.24 (0.485)	9.55 (0.861)
	1–2	2.69 (0.278)	6.91 (0.358)

[a]Mean from four experiments or more; *SD* in parentheses. [b]No hexanol was present during either period in the control experiment; in the test experiment, 0.5% hexanol was present only during the 0–1-hr period.

Table III—Effect of 0.5% Alcohols on pH of Solutions of Salicylic Acid and Sulfapyridine following Perfusion of Intestinal Segment

	Final pH[a]
Control	6.1 (0.03)
Ethanol	6.1 (0.07)
Butanol	6.1 (0.05)
Hexanol	6.4 (0.15)

[a]Mean from eight experiments; *SD* in parentheses; initial pH was 6.6.

between the control and hexanol in either the extent of absorption of the drug or its blood plasma concentration. The lack of effect at 30 min was apparently due to the transient rapid absorption[15] of sulfapyridine that occurred in the presence of hexanol. Such behavior illustrates the importance of characterizing the intestinal clearance of drugs by monitoring the drug concentration in the intestinal lumen over a period of time rather than making "single-point" determinations.

The absorption-altering effects of the alcohols may have resulted from damage to the intestinal epithelium exposed to the alcohols. To check this possibility, segments of jejunum were perfused for 60 min with an isotonic sodium phosphate buffer containing no alcohol or 0.5% ethanol, butanol, or hexanol. Thin sections of perfused intestine were prepared and examined by a veterinary pathologist. No differences were detected in the structure of the intestine perfused with 0.5% alcohol compared to the intestine not exposed to alcohol (Fig. 5).

In addition, the absorption-altering effects of hexanol were rapidly reversible. The clearance of prednisolone and salicylic acid was measured during two consecutive 1-hr periods in the same segment of intestine. During the first period, 0.5% hexanol was present. This solution was then removed; during the second period, the intestine was perfused with a solution of drug without hexanol.

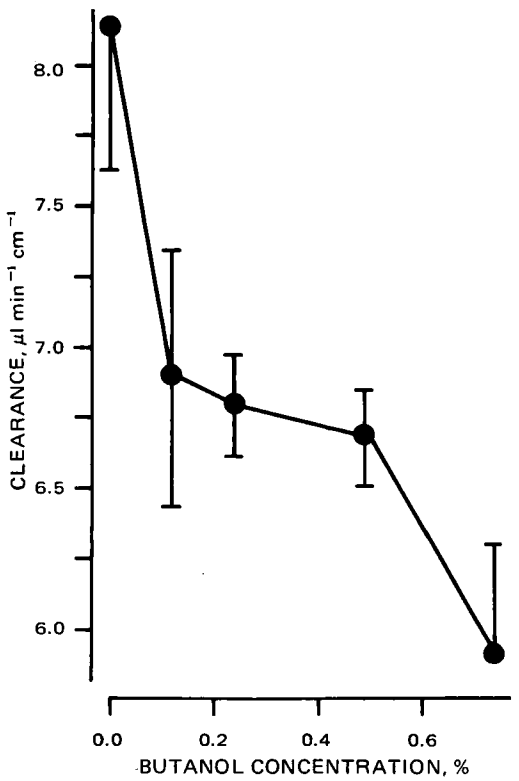

Figure 4—*Effect of various constant concentrations of butanol on the absorption of sulfapyridine from solutions recirculated through the* in situ *rat jejunum. Each point represents the mean from four or more experiments; bars indicate ±1* SE.

[15] Described under *Intestinal Clearance.*

Table IV—Apparent 1:1 Association Constants for Drug–Hexanol Complexes in Isopropyl Myristate and Water at 25°

	$K_{1:1}$[a]	
Drug	Isopropyl Myristate	Water
Salicylic acid	3.5	—[b]
Sulfapyridine	2.7	2.4
Prednisolone	4.2	2.3

[a]Liters per mole. [b]High solubility precluded measurement.

The results of this experiment (Table II) show that the increased clearance of prednisolone produced by hexanol returns immediately to the control value when hexanol is removed from the intestinal perfusate.

The clearance of salicylic acid did not return to the control value during the period following exposure of the intestine to hexanol. However, the control experiment for salicylic acid indicates that the lack of reversibility was due primarily to exposure of the intestine to salicylate rather than hexanol. Reduced absorption of salicylic acid following its repetitive instillation to the stomach in humans (13) and intestine in rats (14) was reported. Thus, it appears that the alcohols do not alter drug absorption rates by damaging the intestinal epithelium.

Salicylic acid and sulfapyridine are weakly acidic, pKa of 3.0 and 8.4, respectively (15), so an alcohol-induced change in the pH of the intestinal drug solution could explain the effects of the alcohols on the rates of drug absorption. The pH of the intestinal drug solution decreased slightly during the absorption experiments when no alcohol was present (Table III), and neither ethanol nor butanol influenced the magnitude of the decrease. Although at the end of the experiment the pH of the drug solution containing hexanol was slightly greater than the control value, it is not likely that this small difference in pH could account for the effect of hexanol on the absorption rates of salicylic acid and sulfapyridine.

The rate of drug absorption may be increased or decreased by formation of molecular complexes, depending on the relative extent of complexation of the drug in the luminal solution and the intestinal epithelium (16). To explore this possibility, the extent of complex formation between the drugs and the alcohols was determined by phase solubility analysis. The apparent 1:1 association constants for sulfapyridine–hexanol and prednisolone–hexanol complexes in water were quite low (Table IV). Not more than 9% of sulfapyridine or prednisolone would be complexed with 0.5% hexanol in the intestinal perfusate. The extent of complexation between the drugs and alcohols in isopropyl myristate was also small, indicating that formation of drug–alcohol complexes in the lipoid barrier of the intestine would be insignificant.

Thus, the lack of appreciable interaction between any of the drugs and alcohols suggests that the absorption-altering effects of the alcohols are not due to formation of drug–alcohol complexes. Another possible mechanism for the absorption-altering effects of the alcohols is an alcohol-induced change in the extent of drug binding to macromolecules released possibly from the intestinal mucosa. Dialysis studies on intestinal perfusates, however, revealed that the extent of drug binding was low and not affected significantly by hexanol (Table V).

The rate of water absorption by the intestine was shown theoretically (17) and experimentally (4, 18) to influence the rate of drug absorption by the intestine. Generally, the rate of drug absorption increases linearly with the rate of water absorption. Al-

Table V—Percent of Drug Bound in the Perfusate to Nondialyzable Material from the Intestinal Mucosa

	Percent Drug Bound[a]	
Drug	Control	Hexanol
Salicylic acid	3.64	2.32
Sulfapyridine	2.96	3.06
Prednisolone	−1.54[b]	2.12

[a]Mean of two determinations. [b]At equilibrium, concentration was lower in perfusate than in buffer.

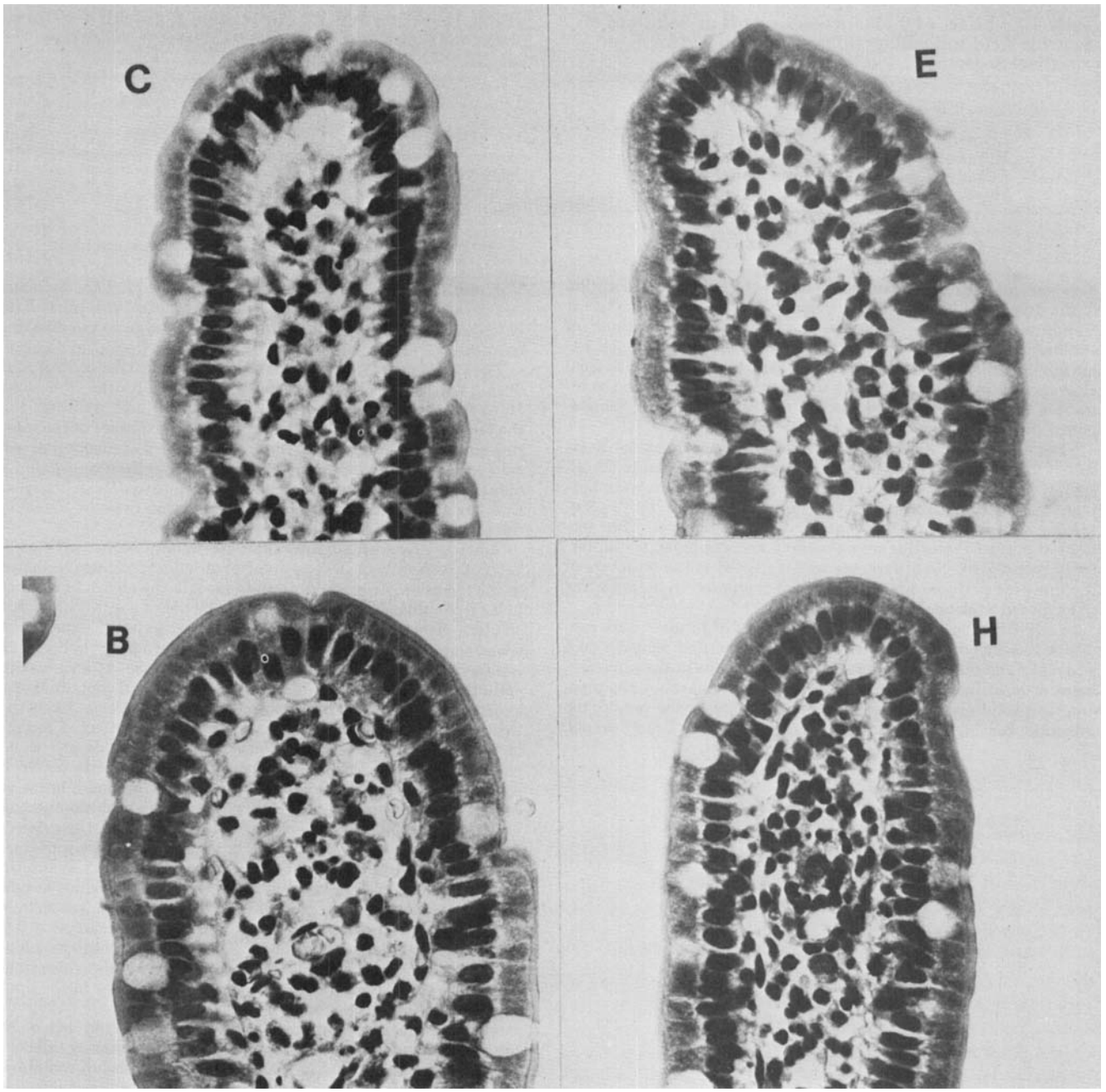

Figure 5—*Representative sections of individual villi from rat jejunum perfused for 1 hr with isotonic sodium phosphate buffer (C) or buffer containing 0.5% ethanol (E), butanol (B), or hexanol (H) (×560).*

though the presence of the alcohols increased the osmotic pressure of the drug solutions, the average rate of water absorption by the intestinal segment was increased significantly by ethanol and butanol compared to the control (Table VI). Hexanol did not appear to alter the rate of water absorption. Ethanol produced a similar increase in water absorption by the rat intestine (4), and butanol increased the permeability coefficient of water in frog eggs (19).

While the rate of water absorption probably influenced the rate of drug absorption by the intestine in this study, there was no significant correlation between drug clearance from solutions con-

Table VI—Absorption Rate of Water from the Rat Intestine Perfused with Drug Solutions Containing No Alcohol or 0.5% Alcohol

	Rate[a], μl min^{-1} cm^{-1} of Intestine			
	Control	Ethanol	Butanol	Hexanol
Salicylic acid	1.19 (0.68)	1.63 (0.45)	2.04 (0.26)	1.32 (0.25)
Sulfapyridine	0.96 (0.29)	1.97 (0.40)	1.86 (0.31)	1.24 (0.56)
Prednisolone	1.85 (0.56)	1.98 (0.24)	2.07 (0.71)	1.98 (0.36)
Weighted mean	1.43	1.88[b]	1.99[b]	1.55
Standard deviation	0.647	0.362	0.439	0.513

[a] Mean from four or more experiments; *SD* in parentheses. [b] Significantly different from control mean at the 95% level of confidence.

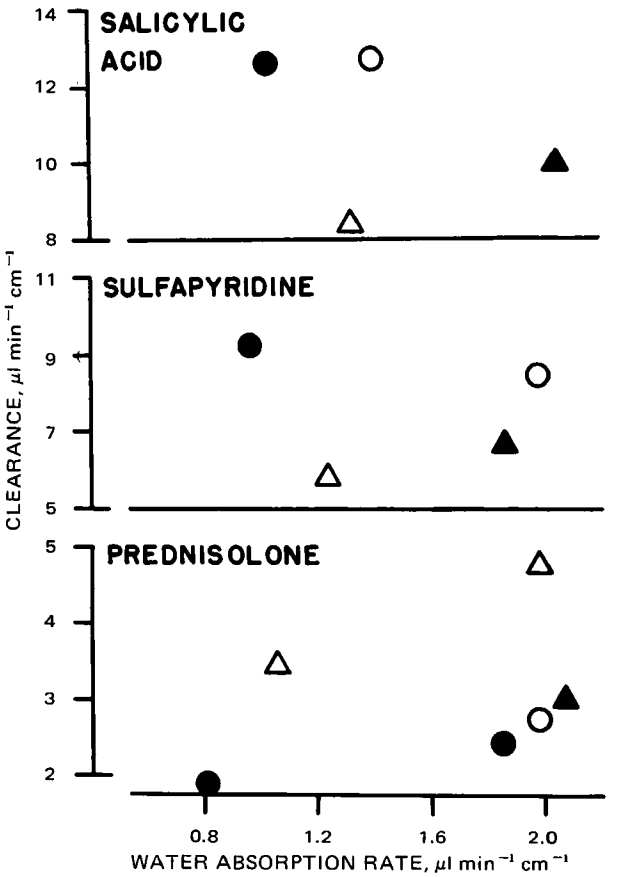

Figure 6—*Clearance of salicylic acid, sulfapyridine, and prednisolone* versus *water absorption rate from solutions containing no alcohol (●) or 0.5% ethanol (○), butanol (▲), or hexanol (△). The clearance of prednisolone from both isotonic sodium chloride and sodium phosphate solutions is shown.*

taining alcohols and the rate of water absorption (Fig. 6). It appears, therefore, that the absorption-altering effects of the alcohols did not result entirely from an alteration in the absorption rate of water. In the control experiment, the rate of water absorption from the solution containing prednisolone was greater than that from the solutions containing salicylic acid or sulfapyridine (Table VI). The latter drugs were in isotonic sodium phosphate buffer, and prednisolone was in isotonic sodium chloride solution.

When the effect of hexanol on the intestinal clearance of prednisolone from the sodium phosphate buffer was examined, hexanol was found to increase the clearance of prednisolone, as it did in the sodium chloride solution (Table VII). Thus, the ionic composition of the perfusate appears to influence the rate of water absorption.

These experiments show that normal alcohols can either increase or decrease the apparent permeability of the rat jejunal mucosa. The magnitude of the change in permeability depends on the concentration and lipophilicity of the alcohol, and the direction of change depends on the drug. The absorption-altering effects of the alcohols cannot be attributed to mucosal damage, pH changes, or complex formation. It is possible that the effects of the alcohols result from a single change in the structure or function of the intestinal mucosa.

For example, the alcohols may displace water from the lipoidal cell membrane, causing a decrease in the polarity of the membrane and thereby increasing the permeability of the membrane to prednisolone, which is relatively nonpolar, and decreasing its permeability to the relatively polar sulfapyridine and salicylate. Alternatively, if the barrier to absorption is structured water, as was suggested (20, 21), the alcohols may increase the degree of structuring of water (22), causing the permeability of the barrier toward nonpolar drugs to increase and toward polar drugs to decrease.

On the other hand, the absorption-altering effects of the alcohols may result from two or more alcohol-induced alterations in the structure or function of the intestinal epithelium. One or more

Table VII—Effect of 0.5% *n*-Hexanol on Clearance[a] of Prednisolone and Absorption Rate[a] of Water from Isotonic Sodium Chloride and Sodium Phosphate Solutions

	Sodium Chloride[b]	Sodium Phosphate[c]	*p*
Control			
Clearance	2.44 (0.613)	1.87 (0.179)	
Water absorption rate	1.85 (0.564)	0.81 (0.084)	<0.01
n-Hexanol			
Clearance	4.76 (0.646)	3.44 (0.269)	
Water absorption rate	1.98 (0.359)	1.06 (0.528)	<0.02

[a] μl min⁻¹ cm⁻¹ of intestine; mean from four or more experiments; *SD* in parentheses. [b] Sodium chloride, 154 mmoles/liter. [c] Sodium phosphate, 67 mmoles/liter; sodium chloride, 82 mmoles/liter.

of the alterations may tend to increase the permeability of the intestine, while the remaining alterations tend to decrease its permeability. The effect observed would then be the net effect of all alterations in the barrier properties of the mucosa. Whether the observed effect of the alcohols is positive or negative would depend on the relative magnitudes of all individual alterations in the intestine as they relate to the particular drug.

Possible structural or functional alterations, which could be caused by the alcohols and which may affect the apparent permeability of the intestinal mucosa, are mucosal blood flow (23), water absorption rate (4), "fluidity" of lipid bilayers (24), thickness of unstirred layers on the luminal side of the absorbing cell (25), dimensions of polar pathways through the epithelium (26), and solute drag (27). Thus, with the limited data available, it is not now possible to establish unequivocally the mechanisms by which normal alcohols alter the permeability of the intestinal epithelium to prednisolone, salicylic acid, and sulfapyridine.

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Chromatographic, Mass Spectral, and Visible Light Absorption Characteristics of Toluidine Blue O and Related Dyes

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Abstract □ Contrary to earlier literature reports, the impurities in toluidine blue O were shown by column chromatography, TLC, and mass spectrometry to be *N*-methyl homologs of 2-methylthionine rather than *N*-methyl homologs of thionine. Small amounts of 2-methyl-3-amino-7-methylaminophenothiazine and 2-methyl-3,7-diaminophenothiazine were identified in commercial samples of toluidine blue O. However, sample handling and a warm alkaline environment can cause rapid demethylation of the dye.

Keyphrases □ Toluidine blue O and related dyes—chromatography, mass spectra, and visible light absorption □ Dyes—chromatographic, mass spectral, and visible light absorption characteristics of toluidine blue O and related dyes □ Mass spectrometry—impurities, toluidine blue O and related dyes

In recent years there has been increased interest in the medical use of the dye toluidine blue O (3-amino-7-dimethylamino-2-methylphenazathionium chloride, C.I. Basic Blue 17). It recently has been used for the identification of the parathyroids during surgery (1, 2) and as an aid in the diagnosis of small oral cancers (3) and has been suggested as a potential parathyroid and pancreatic scanning agent if labeled with a suitable radionuclide (4).

BACKGROUND

During an investigation into the synthesis of radioactive analogs of toluidine blue O, some problems were encountered in obtaining pure dye samples. Elemental analysis, column chromatography, TLC, and mass spectrometry all indicated the presence of impurities which could not be separated from the dye.

Similar difficulties in analyzing samples of toluidine blue O by TLC were reported (5, 6). The investigators observed three spots for the commercial dye, two blue and one violet. They identified the violet spot as thionine (I) on the basis of its R_f value and visible light absorption spectrum. Extraction of one of the two blue spots and rechromatography again produced two blue spots, which were attributed to different ionic forms of the dye or "solvent effects." In addition, Apgar and Patel (6) reported that visible light absorption experiments indicated that toluidine blue O lost a methyl group (in this case an aryl methyl group) and was converted to azure A (III) upon standing in aqueous solution.

Normann and Normann (7) succeeded in isolating five contaminants from a sample of tritiated toluidine blue O by TLC. The resulting spots were extracted from the thin-layer chromatograms and identified by their absorption maxima (Table I). Their identification of various compounds on the basis of the absorption maxima was not consistent with earlier work (8), which is summarized in Table II.

In 1953, Ball and Jackson reported on chromatographic and spectrophotometric studies of various samples of toluidine blue O and azure A (9). Descending paper chromatograms[1] developed in butanol–acetic acid–water (40:10:50 v/v) indicated that the various samples, supplied by different manufacturers, were indeed not the same dyestuff. Column chromatography on aluminum oxide with varying solvents yielded a number of fractions.

The confusion in the assignment of consistent visible light absorption maxima to the various related dyes was disturbing. In the authors' laboratory, commercial toluidine blue O shows a significant change in absorption characteristics as the pH of the solution is changed. In saline at pH 2, toluidine blue O exhibits a λ_{max} value of 630 nm, while at pH 10 a broad absorption maximum from 550 to 630 nm is observed. Differences in reported λ_{max} values (6, 7) may have been due to sample preparation, since the pH of the solutions studied was not mentioned. In addition, the assignment of the structure of azure A (III) to the degradation product of toluidine blue O implies an aryl-demethylation rather than the more probable *N*-demethylation.

The purposes of the present study were to explain the chromatographic behavior of toluidine blue O and to clarify the interpretation of the visible light absorption data.

EXPERIMENTAL[2]

Column Chromatography—Columns [5.1-cm (2-in.) diameter] were packed with dry silica gel (70–325 mesh) to a depth of 45.7 cm (18 in.). Samples were dissolved in ethanol and applied to the tops of the dry columns. Columns were eluted with ethanol followed by

[1] Whatman No. 1.

[2] The dyes used were obtained from National Aniline and Eastman Kodak and were labeled 80–90% pure. All other chemicals were reagent grade and were used as received. Elemental analyses were performed by Schwarzkopf Microanalytical Laboratory, Woodside, N.Y., and by Strauss Microanalytical Laboratory, Oxford, England.