Effect of Polysorbate 80 on Apparent Partition Coefficient of Salicylic Acid and Its Absorption from the Rat Intestine

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Abstract □ The effect of polysorbate 80 on the apparent partition coefficient of salicylic acid between chloroform and a 0.05 *M* phosphate buffer at pH 6.5 was investigated. Three different concentrations of the surfactant were studied: 0.001, 0.01, and 2.0% (w/v). Concentrations of polysorbate 80 equal to or above the CMC significantly increased the apparent partition coefficient of salicylic acid. A lower surfactant concentration did not produce a significant change in the apparent partition coefficient of the acid. Polysorbate 80 at the same three concentrations produced no significant change in the percent of salicylic acid absorbed from the rat intestine *in situ* when dissolved in a 0.05 *M* phosphate buffer at pH 6.5. When 2.0% (w/v) polysorbate 80 in the phosphate buffer was left inside the rat intestine for 1 hr, the permeability of the intestine to salicylic acid was not significantly changed.

Keyphrases □ Polysorbate 80—effect on apparent partition coefficient and absorption of salicylic acid, rat intestine □ Salicylic acid—apparent partition coefficient and absorption, rat intestine, effect of polysorbate 80 □ Surfactants—polysorbate 80, effect on apparent partition coefficient and absorption of salicylic acid, rat intestine □ Absorption, intestinal—salicylic acid, effect of polysorbate 80, rats □ Partition coefficient—salicylic acid, effect of polysorbate 80, rats

In recent years, several nonionic surfactants have been incorporated in pharmaceutical formulations to influence drug availability (1). Numerous studies also have dealt with various aspects of the effects of surfactants on drug absorption (2–18). Many observations reported in these studies seem contradictory. The source of this conflict might have been in the particular drug, surface-active agent, biological system, or experimental technique utilized.

To the best of these authors' knowledge, the effect of surfactants on the apparent partition coefficient of drugs has not been documented.

In the present study, an attempt was made to elucidate the effect of a nonionic surfactant (polysorbate 80) on the apparent partition coefficient of salicylic acid and to correlate this effect with the effect on the rate of absorption of salicylic acid from an *in situ* rat intestine. The influence of the surfactant on the permeability of the rat intestine to salicylic acid also was investigated.

EXPERIMENTAL

All chemicals and reagents were used as obtained without further purification.

Determination of Apparent Partition Coefficient—Phosphate buffer solution (0.05 *M*) and chloroform were first saturated, each with the other phase, by shaking[1] at room temperature for 24 hr. Salicylic acid[2], 30, 40, 50, 60, and 70 mg, was accurately weighed and placed in 125-ml glass-stoppered bottles. Forty milliliters of chloroform was added to each bottle, followed by 40 ml of the aqueous phase [0.05 *M* phosphate buffer containing 0.001, 0.01, or 2.0% (w/v) polysorbate 80[3]]. One bottle contained no salicylic acid and served as a blank; another bottle contained only a standard solution of salicylic acid (1 mg/ml) in the aqueous phase.

All bottles were placed on a horizontal shaker[4] and shaken at a speed of approximately 250 rpm for 24 hr. A portion of the aqueous layer (approximately 10 ml) was then transferred to a test tube and centrifuged[5] for 1 hr to break the emulsion formed in the polysorbate 80 solution. One milliliter of the clear aqueous layer was withdrawn for assay.

The concentration of salicylic acid in the chloroform layer was determined by difference, and the apparent partition coefficient was determined by dividing the concentration in chloroform by the concentration in the aqueous layer.

Absorption of Salicylic Acid from Rat Intestine—The technique used to study the absorption of salicylic acid from the rat intestine was similar to that developed by Doluisio *et al.* (19). Male albino rats, 200–450 g, were fasted for at least 24 hr, with water being allowed *ad libitum*. The rats were anesthetized with pentobarbital sodium[6], 50 mg/kg ip. The animals were kept warm during the experiment with an IR lamp.

Details of the cannulation and of the sample withdrawal procedures were reported previously (19). The cannulated intestine was flushed with 400–500 ml of normal saline before the salicylic acid solution was introduced. A 1.0-ml sample of the absorption solution was withdrawn at 10-min intervals for assay. Net water absorption was determined in separate experiments using phenolsulfonphthalein as a marker, and absorption values were corrected accordingly. Six animals were used for each phase of the experiment.

The following solutions were tested:

1. Salicylic acid, 1 mg/ml in a 0.05 *M* phosphate buffer at pH 6.5 (measured before addition of salicylic acid, changed to 6.2 after the acid was added).
2. Salicylic acid, 1 mg/ml in the phosphate buffer containing the following concentrations of polysorbate 80: 0.001, 0.01, and 2.0% (w/v).

Effect of Polysorbate 80 on Intestinal Membrane of Rats—To study the effect of polysorbate 80 on the intestinal membrane of the rat, a solution containing 2.0% (w/v) polysorbate 80 in the 0.05 *M* phosphate buffer was introduced into the cannulated intestine and left for 1 hr. The intestine was then flushed with normal saline, and the absorption of salicylic acid (1 mg/ml) in the phosphate buffer at pH 6.5 was studied as already described.

Treatment of Data—The Student *t* test was used to determine the significance of the differences observed in the apparent partition coefficients. A two-way analysis of variance was used to determine the significance of differences observed in the intestinal absorption data.

Assay of Salicylic Acid—The following procedure was used to determine salicylic acid, both in the partition coefficient experiments and the absorption experiments. One milliliter of the solution to be assayed was diluted with 20 ml of water, and 5.0 ml of Trinder's reagent (20) was added. The absorbance of the resulting solution was then measured at 526 nm[7]. In the intestinal absorption experiments, it was necessary to centrifuge the solutions after adding Trinder's reagent and to use the supernatant liquid for measuring the absorbance.

RESULTS

Table I lists the apparent partition coefficients of salicylic acid

[1] Eberbach shaker, Eberbach Corp., Ann Arbor, Mich.
[2] Fisher Scientific Co., Houston, Tex.
[3] Atlas Chemical Industries, Wilmington, Del.
[4] Shaker bath model 25, Precision Scientific Co., Chicago, Ill.
[5] Clinical centrifuge, International Equipment Co., Boston, Mass.
[6] Nembutal Sodium, Abbott Laboratories, North Chicago, Ill.
[7] Spectronic-20, Bausch and Lomb, Rochester, N.Y.

Table I—Apparent Partition Coefficient of Salicylic Acid in the Presence of Polysorbate 80

Polysorbate 80, % (w/v)	Apparent Partition Coefficient[a] (±SE)
None	0.021 (±0.002)
0.001	0.023 (±0.002)
0.01	0.031 (±0.003)
2.0	0.085 (±0.002)

[a] Chloroform–0.05 *M* phosphate buffer at pH 6.5.

in the 0.05 *M* phosphate buffer. The Student *t* test showed that the increases in the apparent partition coefficient produced by 0.01 and 2.0% (w/v) polysorbate 80 were significant ($p < 0.05$)(t values for $N_1 = N_2 = 5$ were 2.567 and 21.428, respectively); there was no significant change ($p > 0.05$) in the apparent partition coefficient in the presence of 0.001% (w/v) surfactant ($t = 0.670$).

The results of the intestinal absorption studies are given in Table II. A two-way analysis of variance showed that the differences observed in the percent absorbed with and without polysorbate 80 were not significant ($p > 0.05$) [F ratios for $\nu = 1/10$ were 0.920, 0.015, and 0.014 for 0.001, 0.01, and 2.0% (w/v) surfactant, respectively]. Therefore, polysorbate 80 at concentrations below, equal to, or above the critical micelle concentration (CMC) had no significant effect on the percent of salicylic acid absorbed from the rat intestine. Furthermore, a 2.0% (w/v) polysorbate 80 solution in phosphate buffer at pH 6.5, when left in the rat intestine for 1 hr, had no significant effect on the permeability of the intestine to salicylic acid (F ratio = 0.003).

DISCUSSION

The relationship between the apparent partition coefficients of drugs and their rates of diffusion across biological membranes has long been established (21). Drugs diffuse through membranes at rates directly proportional to their lipid–water partition coefficients. Chloroform often has been used to simulate the lipid phase (21).

Surfactants influence the absorption of drugs from solutions through various mechanisms (15). There are possible interactions between the surfactant and drug as well as between the surfactant and biological membrane. Drug–surfactant interactions seem to be related to the lipid solubility of the drug (2–4). Lipid-soluble drugs are more likely to interact with surfactant micelles than are lipid-insoluble drugs. In particular, surfactant concentration, whether it is below or above the CMC, is another determinant of drug–surfactant interactions (11).

Retardation of the absorption of lipid-soluble drugs in the presence of micellar concentrations of surfactants is postulated to be according to the following model (15):

1. A micellar solution consists of two phases, a micellar phase and the surrounding aqueous phase.
2. The partition ratio of drug between the micellar phase and the aqueous phase is constant, independent of drug concentration.
3. Absorption of the drug incorporated in the micelle is negligible.

Since the drug in the micellar phase is unavailable for absorption, the effective concentration of the drug is less than the apparent concentration, and a decreased absorption rate is observed.

Interactions between surfactants and biological membranes are related to the ionic nature of the surfactant (15) and, possibly, to the type of membrane studied. Anionic and cationic surfactants seem to have a greater influence on membrane permeability than do nonionic surfactants.

The current data (Table I) show that polysorbate 80 at concentrations equal to or higher than the CMC significantly increased the apparent partition coefficient of salicylic acid. This finding indicates that a complex is formed between the drug and the surfactant that is more soluble in chloroform than the drug alone. The fact that a surfactant concentration below the CMC did not significantly change the apparent partition coefficient of the drug indicates that the complex formed is most probably micellar in nature. Micellar complexation has been shown to occur between salicylic acid and another nonionic surfactant, polysorbate 60, in 0.1 *N* HCl (8).

Intestinal absorption data (Table II) indicate that concentrations of polysorbate 80 of up to 2.0% (w/v), considerably higher than the CMC of 0.01% (w/v), produced no significant change in the percent absorbed of salicylic acid. It is possible that the complex between polysorbate 80 and salicylic acid under the present experimental conditions is not very stable. A complex may dissociate at such a rate as not to be absorption rate limiting. The intestinal membrane itself also may have contributed to the rapid dissociation of the complex. In one study (22), complex formation between certain drugs (*e.g.*, codeine and aminopyrine) and dyes (*e.g.*, bromthymol blue and eosine B) markedly increased the apparent partition coefficients of the dyes but did not influence their absorption from an everted rat intestine. The intestinal membrane may have had a dissociating effect upon the complexes formed (22).

Another possibility is that micellar complexation did not occur under the present experimental conditions. Salicylic acid has a pKa of 3.0 (23) and would exist mostly in the unionized form in 0.1 *N* HCl. However, it exists mostly as salicylate anions in a buffer system at pH 6.5. It was demonstrated previously (13) that significant micellar complexation occurred between polysorbate 80 and the unionized form of secobarbital but not with the ionized form. However, despite the difference in complexation, the absorption of the ionized and unionized forms of secobarbital was influenced to the same extent by polysorbate 80 (13).

Surfactants also can alter the permeability of biological membranes. This effect seems to vary with the type of surfactant and the type of membrane studied. Polysorbate 80 enhanced the absorption of 4-aminoantipyrine in the goldfish by a direct effect on the biological membrane (14). However, polysorbate 80 had no effect on the permeability of the rat intestine to salicylamide (10). In the present study, when 2.0% (w/v) polysorbate 80 in the phosphate buffer at pH 6.5 was left in the rat intestine for 1 hr, the permeability of the membrane to salicylic acid was not significantly changed. The percent of salicylic acid absorbed in the pretreated intestine was almost the same as that without pretreatment (Table II).

Table II shows that there was a slight reduction in the percent absorbed in the presence of 0.001% (w/v) surfactant and that the animal-to-animal variation was large. The reduction is not statistically significant, and there is no explanation for the apparent large variation.

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Table II—Percent of Salicylic Acid Absorbed over 60 min from the Rat Intestine[a]

Minutes	Polysorbate 80, % (w/v) None	0.001	0.01	2.0	None[b]
10	37.23 (1.20)	31.92 (4.33)	37.25 (1.38)	41.26 (1.44)	35.10 (2.10)
20	57.79 (1.55)	47.67 (7.81)	57.16 (2.31)	58.90 (2.21)	58.48 (3.87)
30	71.77 (1.03)	59.05 (8.62)	72.48 (2.09)	72.70 (1.21)	73.27 (3.20)
40	82.03 (1.03)	68.62 (7.17)	80.45 (2.05)	78.44 (0.79)	81.37 (3.31)
50	87.73 (1.07)	75.44 (5.80)	84.61 (1.87)	83.47 (0.87)	86.10 (1.79)
60	91.91 (0.70)	80.71 (4.28)	87.90 (1.36)	85.90 (0.97)	90.09 (1.34)

[a] Mean of six rats (±SE). [b] After a 2.0% polysorbate 80 solution was left in the intestine for 1 hr.

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ACKNOWLEDGMENTS AND ADDRESSES

Received September 6, 1974, from the *College of Pharmacy, University of Houston, Houston, TX 77004*

Accepted for publication July 8, 1975.

Supported in part by a Limited Grant-in-Aid from the Faculty Research Support Program, University of Houston.

The authors thank Dr. James R. Brown, Quantitative Management Sciences, University of Houston, for assistance with the statistical analysis.

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New Compounds: Synthesis of Aliphatic Seleno Amino Acids as Potential Pancreatic Imaging Agents

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Abstract □ External imaging of the pancreas is made possible by taking advantage of that organ's large requirement for exogenous amino acids. The only successful approach thus far has been the substitution of selenium for the sulfur atom in amino acids naturally containing sulfur, specifically selenomethionine labeled with selenium-75. The synthesis of a class of selenium-containing amino acids has been undertaken where the selenium atom replaces a methylene group of common amino acids that do not contain a sulfur atom. Reported here are the synthesis and toxicological evaluation of one such analog, 3-[(2-aminoethyl)selenyl]alanine (L-4-selenalysine).

Keyphrases □ Seleno amino acids, aliphatic—synthesis of 3-[(2-aminoethyl)selenyl]alanine, potential pancreatic imaging agent, toxicological evaluation □ Amino acids, aliphatic—selenium substituted, synthesis of 3-[(2-aminoethyl)selenyl]alanine, potential pancreatic imaging agent, toxicological evaluation □ Pancreatic imaging agents, potential—3-[(2-aminoethyl)selenyl]alanine synthesized, toxicological evaluation

It is well known that the pancreas has a large requirement for exogenous amino acids. This specific characteristic was the basis for choosing radiolabeled amino acids to detect pancreatic tumors. Hansson (1) demonstrated this feature by using ^{14}C- and ^{35}S-labeled amino acids and proved that these compounds localized in the exocrine portion of the organ.

To obtain gamma-emitting amino acids for external detection and visualization of the pancreas, it was proposed to replace the sulfur atom in sulfur-containing amino acids such as methionine and cysteine. ^{75}Se-Selenomethionine was subsequently synthesized (2) but has achieved only limited clinical utility as a pancreatic imaging agent due to the unfavorable pancreas to liver concentration ratio. Several studies comparing ^{14}C-labeled amino acids showed that methionine has one of the poorest pancreas to liver ratios of all naturally occurring amino acids (3).

DISCUSSION

The sulfur analogs of numerous natural and synthetic amino acids have been synthesized but no attempts have been made to substitute selenium for the hetero sulfur atom. Compounds I–III are prime examples of reported sulfur analogs of common amino acids.

Compound I is a derivative of alanine, 3-[(2-aminoethyl)thio]alanine, but can also be considered as a hetero analog of lysine (4-thialysine). This compound has been shown to be a potent inhibitor of lysine utilization (4). Compound II, 3-methyl-3-(methylthio)alanine, is an isostere of isoleucine (4-thiaisoleucine and 4-thiaalloisoleucine) (5). α-Aminoadipic acid is a lysine precursor, and Compound III, 3-[(carboxymethyl)thio]alanine, can be viewed as a sulfur-containing α-aminoadipic acid (4-thia-α-aminoadipic acid) (6).