doxorubicin (150 ng/ml) were used as the internal standards, respectively. Samples were assayed according to the procedure of Averbuch *et al.* (3), except that new columns (lot 100302) were used with the hexane–ethylene chloride–methanol–acetic acid–water mobile phase system. The assay sensitivity (signal strength of >2.5 times baseline noise) was 5 and 1 ng/ml, respectively. In both cases, the response was linear to 200 ng/ml (highest concentration tested). The correlation coefficient for six points was >0.99 for both assays. Retention times for several anthracyclines and other fluorescent compounds were determined (Table AI).

The aminocyanosilica column is effective in separating highly polar compounds and is almost free of solvent memory. It is unfortunate that a change in silica gel manufacture caused a substantial change in operating characteristics with respect to the anthracycline assay. However, if the appropriate mobile phase is used, depending on lot number, then this column continues to be useful to anthracycline research.

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Effect of Surfactant on Tetracycline Absorption across Everted Rat Intestine

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Abstract □ Absorption of tetracycline hydrochloride (500 μg/ml) from oxygenated modified Krebs buffer in randomized everted rat jejunal segments was determined alone and in the presence of calcium, polysorbate 80, and calcium plus polysorbate 80. Surfactant increased absorption of tetracycline in the presence and absence of calcium, with 0.01% (w/v) polysorbate 80 increasing transfer to the greatest extent of the concentrations examined (0.005, 0.01, 0.05, 0.1, and 1%); tetracycline hydrochloride + 12.5 m*M* $CaCl_2$, 143 ± 45 μg/ml; tetracycline hydrochloride + polysorbate 80, 389 ± 18 μg/ml; tetracycline hydrochloride + 12.5 m*M* $CaCl_2$ + polysorbate 80, 255 ± 31 μg/ml. On the premise that the effective surfactant concentration is similar to the critical micelle concentration, an absorption mechanism based on micellar solubilization is postulated.

Keyphrases □ Tetracycline—absorption, effect of polysorbate 80, everted rat intestine □ Polysorbate 80—effect on tetracycline absorption across everted rat intestine □ Surfactants—effect of polysorbate 80 on tetracycline absorption across everted rat intestine

Tetracycline is a widely used antibiotic. Its serum level should not fall below the minimum inhibitory concentration (MIC), usually between 0.1 and 1.5 μg/ml, for most bacterial strains. Occasionally, this concentration is difficult to maintain due to decreased absorption caused by other drugs or divalent and trivalent cations present in the GI tract (1–3). The intestinal absorption of most tetracyclines is rapid but incomplete, and the absorption mechanism is poorly understood (4, 5).

Physicochemical interactions in the gut are not uncommon and, at least pharmacokinetically, seem to be the most important form of interaction affecting the absorption of tetracycline derivatives. Chelation of tetracycline with polyvalent cations, which has been well documented, is the most common reaction that decreases tetracycline absorption. In addition to forming direct tetracycline–metal complexes, tetracycline binds to both nucleic acids and proteins, with the binding mediated by divalent cations such as zinc, calcium, and magnesium.

Recently, the role of endogenous and exogenous surfactants in drug absorption experiments was explored (6, 7). In some instances, the addition of surfactants enhanced drug absorption (8).

Since decreased tetracycline absorption presents a clinical problem, often requiring alteration of food and medications, a method of optimizing tetracycline absorption to avoid the present restrictions would be valuable. Surfactants may enhance tetracycline absorption to an acceptable level, even in the presence of divalent ions; if so, they may prove to be a valuable additive to oral dosage forms.

This investigation determined the effects of surfactants on tetracycline absorption from the rat everted gut sac to obtain information on the enhancement of tetracycline transport and the tetracycline transport mechanism.

EXPERIMENTAL

Intestine Preparation—An everted intestinal sac technique (9, 10) was used. The solution on the inside of the sac is referred to as the serosal solution, and the solution in which it is incubated is called the mucosal solution.

Seven male white Holtzman rats, 175–200 g, were fasted overnight (20–24 hr), but water was not withheld prior to the experiment. The animals were anesthetized with ether and then killed by stunning and cervical fracture. The jejunum was removed and rinsed immediately with several portions of cold normal saline and everted on a thin glass rod. After eversion, the jejunum was washed in cold normal saline and cut into seven segments of 5-cm length. Segments of the rat intestine were selected randomly for the different treatment groups.

Sacs of everted intestine were prepared by tying one end tightly and the other end loosely with fine thread. Then 0.5 ml of buffer was introduced inside the sac with a 1-ml syringe fitted with a blunt needle. The loose ligature was tightened, and the buffer-filled sacs then were transferred immediately to 25-ml erlenmeyer flasks containing 15 ml of buffer mixed with tetracycline hydrochloride[1], 0.5 g/liter with or without the additives. Control sacs were placed in buffer alone. The other five sacs were incubated in buffer to which 0.005, 0.01, 0.05, 0.1, or 1% (w/v) pol-

[1] Lot 6 X090-71000, Pfizer Laboratories, New York, N.Y.

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Table I—Effect of Different Polysorbate 80 Concentrations on Tetracycline Absorption across Everted Rat Gut Sac

Treatment	Tetracycline Absorbed, μg/ml
Tetracycline	241 ± 25[a]
Tetracycline + 0.005% (w/v) polysorbate 80	283 ± 19
Tetracycline + 0.01% (w/v) polysorbate 80	345 ± 23
Tetracycline + 0.05% (w/v) polysorbate 80	333 ± 14
Tetracycline + 0.1% (w/v) polysorbate 80	315 ± 25
Tetracycline + 1% (w/v) polysorbate 80	283 ± 28

[a] Mean ± *SE*.

Table II—Effect of Calcium Chloride on Inhibition of Tetracycline Transfer in Everted Rat Gut Sac

Treatment	Tetracycline Transferred, μg/ml
Tetracycline	251 ± 25[a]
Tetracycline + 12.5 m*M* $CaCl_2$	143 ± 45
Tetracycline + polysorbate 80	389 ± 18
Tetracycline + 12.5 m*M* $CaCl_2$ + polysorbate 80	255 ± 31

[a] Mean ± *SE*.

ysorbate 80[2] had been added. The solutions were gassed with oxygen, and the flasks were stoppered, transferred into a shaker-incubator[3], and incubated at 37° for 1 hr. They were taken out and opened, and the contents were placed into tubes for later tetracycline determination. The mucosal solution pH never rose above 7.7.

The buffer was pH 7.4 modified Krebs bicarbonate buffer free of calcium and magnesium as described by Mayersohn and Gibaldi (11). It contained, in millimolar concentrations: NaCl, 122; KCl, 5; KH_2PO_4, 1; and $NaHCO_3$, 26. The mucosal and serosal solutions were identical except for the presence of drug and surfactant in the mucosal solution.

Two kinds of experiments were done. In one, tetracycline absorption in the presence of calcium and surfactant was studied; the other involved the kinetics of surfactant effects on the passive transfer of tetracycline across the everted rat intestine. In the first, the mucosal solutions, except in one control experiment, contained 0.5 mg of tetracycline/ml. The solutions to which five sacs per rat were exposed were: (*a*) tetracycline alone; (*b*) tetracycline with calcium chloride, 12.5 m*M*; (*c*) tetracycline with surfactant, 0.01% (w/v) polysorbate 80; (*d*) tetracycline with both calcium chloride and polysorbate 80; and (*e*) control, containing only buffer.

Fluorometric Assays—The fluorometric assays were performed by a modification of the method of Hall (12). To the contents of each sac, 0.5 ml of 0.1 *M* HCl was added and mixed thoroughly. Then 0.5 ml of 0.75 *M* aluminum chloride[4] was added, and the tubes again were mixed thoroughly by shaking. After incubating for 15 min at room temperature, fluorescence was measured in a spectrophotofluorometer[5] at 400-nm excitation and 465-nm emission. A fluorescence standard curve was obtained by plotting fluorescence intensity against calibrator concentrations and computing the line of best fit by least-squares regression analysis ($y = 750x + 45$, $r = 0.99$). Means and standard errors of each group were determined in each experiment. Lines of best fit and correlation coefficients were determined, and the differences in transfer were compared by the Student *t* test (using *t* independent with one-tail comparisons).

The second group of experiments were carried out as follows. Eight gut sacs were prepared from each rat intestine. Twelve rats were divided into two groups of six rats each. One group had only 0.5 mg of tetracycline/ml in the mucosal solution. The other group had 0.01% (w/v) polysorbate 80 in addition to tetracycline. Sacs then were incubated in the shaker-bath for preassigned times. At 5, 10, 20, 30, 40, 50, and 60 min, one sac was removed from the shaker bath for fluorometric assay to determine the amount of tetracycline transferred during each period.

The reciprocal of tetracycline concentration and the log of tetracycline concentration were plotted against time to determine whether the absorption followed first- or second-order kinetics.

[2] Lot 11-78297, Tween 80, Sargent-Welch Scientific Co., Skokie, Ill.
[3] Shaker-bath model 50, GLA Precision Scientific Co., Chicago, Ill.
[4] Lot 45,572, J. T. Baker Chemical Co., Phillipsburg, N.J.
[5] Aminco-Bowman, American Instrument Co., Silver Spring, Md.

RESULTS

Effect of Different Polysorbate 80 Concentrations on Tetracycline Absorption across Everted Rat Gut Sac—In the presence of polysorbate 80, tetracycline movement across the intestine increased ~20–40% (Table I). The intestinal transfer rate of tetracycline increased inversely with the concentration of polysorbate 80 added. The surfactant concentration that enhanced tetracycline absorption the most across the intestine appeared to be 0.01%. A 55% increase ($p < 0.05$) in transport was observed when compared with tetracycline alone in the incubation medium after 60 min (Table II). Each difference was significantly different from tetracycline alone ($p < 0.05$) except for the 1% (w/v) concentration.

Tetracycline Transfer across Everted Rat Gut Sac in Presence of Calcium and Surfactant—Calcium chloride decreased tetracycline transfer across the intestine by 43.0% ($p < 0.05$) in the absence of surfactant and by 34.4% ($p < 0.05$) in the presence of surfactant. With calcium reducing the intestinal transfer of tetracycline and surfactant enhancing it, tetracycline transferred in the presence of both calcium and surfactant together was approximately normal (Table II).

Effect of Polysorbate 80 on Tetracycline Transfer across Everted Rat Intestine—Two kinetic studies were conducted on the data obtained on the effects of polysorbate 80 on tetracycline absorption across rat everted gut sacs. In the first, the data were plotted as a first-order reaction. In this experiment, the time for peak absorption was 60 min (Fig. 1), with further prolongation of incubation resulting in decreased absorption. Therefore, 60 min was selected as the maximal time plotted. Results of this plot correlated well with theoretical requirements of a first-order process (Fig. 1). A straight line was obtained, and computation of the correlation coefficient of the line by linear regression gave an *r* value of 0.975.

To determine goodness-of-linear-fit for second-order kinetics, the same data on tetracycline absorption were tested as a second-order kinetic process, resulting in a correlation coefficient of 0.487; thus, the goodness-of-linear-fit for a second-order process was poor.

DISCUSSION

The mechanism of intestinal tetracycline absorption has yet to be elucidated. The results of Pindell *et al.* (4) suggested that tetracycline is absorbed by passive diffusion. However, Levine *et al.* (5) observed that tetracycline absorption might not occur by passive diffusion alone.

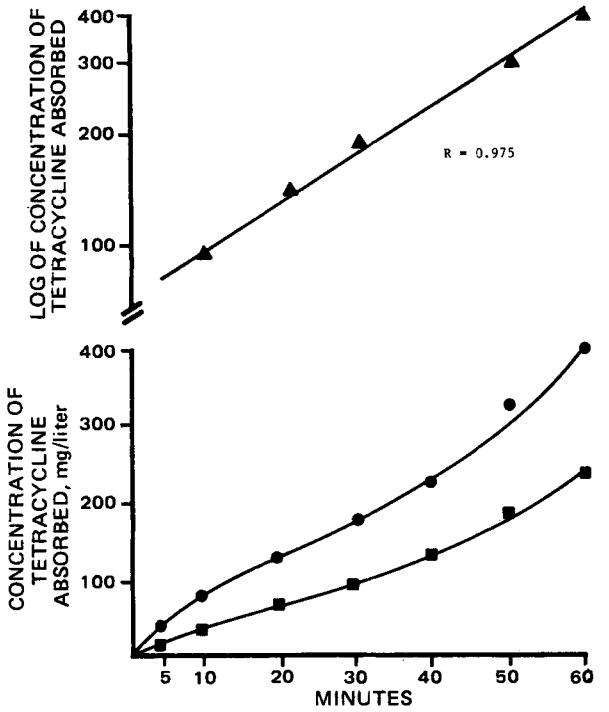

Figure 1—*Effect of 0.01% (w/v) polysorbate 80 on tetracycline absorption across the everted gut sac. Key:* ■, *tetracycline alone;* ●, *tetracycline with polysorbate 80 (0.01% w/v); and* ▲, *tetracycline with polysorbate 80 (0.01% w/v).*

Banerjee and Chakrabarti (10) suggested that the mechanism was not active transport or simple passive diffusion. They postulated that tetracycline was absorbed as its zwitterion and shared a common route with glucosamine. Glucosamine has been shown to be carrier mediated *via* facilitated passive diffusion.

Tetracycline exists mainly as the charged species over the entire pH range of the alimentary tract. Perrin and Vallner (13) stated that tetracycline is therapeutically active when given orally and, therefore, must be absorbed as a charged species. These investigators showed that tetracycline and its derivatives can be absorbed from the rat stomach as the protonated species apparently by passive diffusion. In their studies, the absorption was linked to the surface activity of tetracycline rather than to its lipid solubility. The greater the surface activity of the tetracycline derivative, the higher was the absorption rate.

In the present study, to determine further the mechanism of intestinal tetracycline absorption, dextrose was excluded from the incubation medium to eliminate or reduce greatly any active transport processes. Since absorption took place regardless of this energy source deficiency, tetracycline was transferred across the intestine by methods other than active transport.

Intestinal tetracycline absorption, when polysorbate 80 was added to the medium, was enhanced at every polysorbate 80 concentration except at 1%, with 0.01% causing optimum absorption. This low concentration approximates the critical micelle concentration (CMC) of polysorbate 80 in physiological fluids, and this finding suggests an influence of micelle formation on tetracycline absorption. The CMC for polysorbate 80 solutions was reported for distilled water (14) as 0.6×10^{-4} *M*. Further evidence of micelle-mediated absorption can be seen as a surface tension decrease during micelle formation and as an interfacial absorption increase. Above the CMC, surface tension remains relatively constant; therefore, less enhancement of tetracycline absorption at the higher polysorbate 80 concentrations tends to indicate the necessity of micelles for absorption. This postulation is in agreement with Perrin and Vallner's (13) surface activity absorption relationship and further substantiates the previous suggestion that tetracycline absorption is passive.

The enhancing effect by polysorbate 80 on tetracycline absorption may be due to the increase in drug solubility and the low viscosity in the medium. Both could be the end results of micelle formation in the medium. Said *et al.* (8) noted that the increased solubility and absorption of griseofulvin was achieved in aqueous solution by surfactant addition. Shozo *et al.* (15) found that tetracycline was better absorbed from low viscosity than from high viscosity media.

In these studies, pH probably had little effect on absorption since pH was controlled closely. The initial pH was 7.4; at the end of the experiments, it did not exceed 7.7. Tetracycline has three pKa values (16). The amount of the unionized species at pH 7.4 is 8×10^{-5} *M* for the group with a pKa of 3.3, 5.6×10^{-7} *M* for the group with a pKa of 7.7, and 3.5×10^{-5} *M* for the group with a pKa of 9.7, assuming a 1 *M* solution of total tetracycline. The ratio of the completely unionized molecules to the ionized molecules was 5.72×10^{-7}. It is unlikely that this small number of molecules could contribute much to the intestinal tetracycline transfer. Therefore, solubilization of the ionized form seems to be involved in the enhanced absorption.

It is also unlikely that the differences in the media pH contributed to differences in absorption. One reason is that the pH changes were random and could not have influenced absorption in a single direction. Another reason is that the ratio of molecules existing in the unionized forms in either case seems to be negligibly small (at pH of 7.4, 2.2×10; at pH of 7.7, 1.8×10). Thus, pH should have little effect on absorption. *In vivo*, however, the effects of pH changes may be more important.

The addition of polysorbate 80 probably does not alter any chelation between tetracycline and calcium because chelation is a direct drug–metal reaction (17). The lower tier of the tetracycline molecule contains binding sites that chelate readily with calcium to form six-membered rings. Although calcium reduced intestinal tetracycline transfer in the presence of surfactant, tetracycline was transferred in the presence of both calcium and surfactant. This result may have been due to increased tetracycline solubility, which made more drug available for absorption despite the fact that some drug had become less available due to chelation with calcium.

To reconfirm and elucidate the fact that polysorbate 80 exerts an effect on tetracycline that is absorbed, the absorption data were treated as first- and second-order reactions. A straight line was obtained on only the first-order reaction plot.

Hence, although from the previous discussion one would think that the micelle is intimately involved in tetracycline transfer across everted gut, the micelle apparently does not act as a kinetic partner with tetracycline. If it did, a second-order kinetic plot would be linear. The linearity of the first-order plot indicated a monomolecular passive transfer that is dependent only on a single species: tetracycline. This result is possible if tetracycline is picked up into the micelle and diffused out of the micelle in a purely physical fashion. The micelle then could act as a corridor for tetracycline diffusion.

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