Effect of Trichloroacetate Anion on Partition Behavior of Tetracyclines

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Abstract □ The influence of the trichloroacetate anion on the *n*-octyl alcohol–aqueous phosphate buffer apparent partition coefficients of various tetracyclines was determined. At acidic pH values, the presence of trichloroacetate caused a significant increase in the apparent partition coefficients of most of the tetracycline analogs studied, presumably through intermolecular ion-pairing between the positively charged tetracycline moiety and the trichloroacetate anion. From intermediate to mildly alkaline pH values, trichloroacetate had essentially no effect on partitioning. The transfer of compounds such as minocycline and 9-dimethylamino-6-demethyl-6-deoxytetracycline to the *n*-octyl alcohol phase was not as significantly altered by the addition of trichloroacetate, apparently because the effect of two charged moieties in each of these analogs was not as easily overcome by ion-pair formation. The alteration of the apparent partition coefficients of the tetracycline antibiotics by intermolecular ion-pair formation is discussed in terms of its relationship to the absorption of these compounds.

Keyphrases □ Trichloroacetate—effect on tetracycline partition coefficients, ion-pair formation □ Tetracycline partition coefficients —trichloroacetate effect, ion-pair formation □ Ion-pair formation —effect of trichloroacetate on tetracycline partition behavior

Irwin *et al.* (1) defined ion-pairs as neutral species formed by electrostatic attraction between oppositely charged ions in solution, which are often sufficiently lipophilic to dissolve in nonaqueous solvents. The concept of ion-pairs was introduced in 1926 to account for the low conductance of strong electrolytes in nonaqueous solutions (2). Kraus (3) elaborated on the ion-pair concept and discussed in mathematical terms the electrostatic forces holding ions together in a pair. Ions that unite in some fashion to form a pair essentially cancel all or part of the charge of each ion, and thus there are fewer species in the solution having an exposed charge.

Since the pH–partition hypothesis does not readily explain the absorption of highly ionized compounds, such as quaternary ammonium compounds, sulfonic acids, dextromethorphan, and the tetracyclines, Irwin *et al.* (1) postulated that ion-pair formation and subsequent transport across the lipid GI barrier may constitute an important mechanism for drug absorption. By increasing the lipophilic character of isopropamide through ion-pair formation, they showed increased mydriatic activity in mice. Through the selection of appropriate ion-pair formers, the efficiency, rate, and uniformity of absorption from the GI tract of other highly ionized drugs such as the tetracyclines may also be significantly enhanced. The objectives of this investigation were: (*a*) to study the effect of the trichloroacetate anion on the apparent partition coefficients of the various tetracyclines, and (*b*) to consider the possibility of ion-pair formation between trichloroacetate anion and the various ionic forms of the tetracyclines that exist at different pH values.

EXPERIMENTAL

The experimental design was similar to that used in determining the relative lipophilicity of the different ionic forms of the tetracyclines (4). The tetracycline analogs were obtained from the same sources and were of the same purity as those used previously. Phosphate buffers containing trichloroacetate anion were freshly prepared. Trichloroacetate was prepared by neutralizing a solution of trichloroacetic acid[1] with a solution of sodium hydroxide[2]. To force the equilibrium to the direction favoring ion-pair formation, the concentration of trichloroacetate anion in the buffer solutions was 50 times the molar concentration of the tetracycline analog in the study, unless specified otherwise. The ionic strength was maintained at 0.1 for all buffers by the addition of sodium chloride[3], if necessary. The total molar buffer concentration was 0.050 for buffers of pH 2.1, 3.0, 3.9, and5.6, but it was reduced for buffers of pH 6.6, 7.5, and 8.5 to 0.042, 0.027, and 0.025, respectively, so as not to exceed the ionic strength of 0.1. The ion content of the water used in these experiments was below 0.1 p.p.m. (as sodium chloride) as determined by a conductivity meter[4].

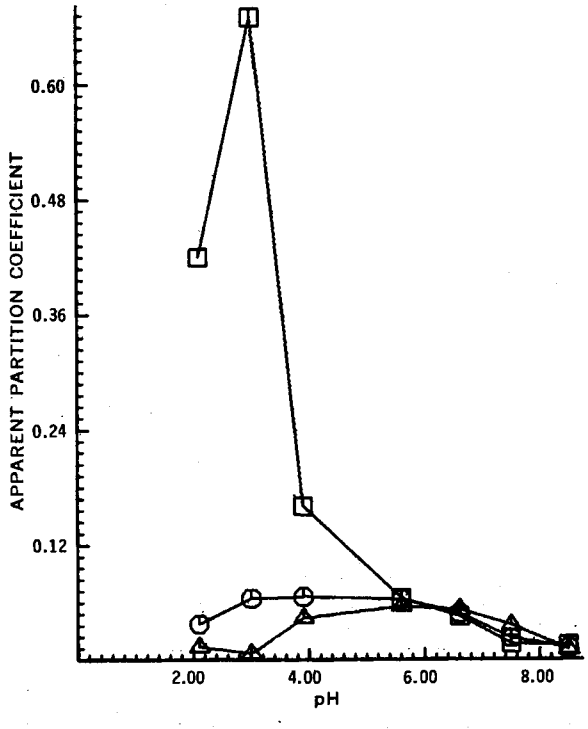

Figure 1—*Comparison of the effect of pH and various concentrations of trichloroacetate anion on the* n*-octyl alcohol–aqueous phosphate buffer apparent partition coefficients for 0.00052* M *tetracycline hydrochloride at 25°. Key:* □, *tetracycline hydrochloride and 0.02600* M *trichloroacetate;* ○, *tetracycline hydrochloride and 0.00520* M *trichloroacetate; and* △, *tetracycline hydrochloride (no trichloroacetate).*

[1] Analytical reagent grade, Mallinckrodt Chemical Works, St. Louis, Mo.
[2] Analytical reagent grade, Baker Chemical Co., Phillipsburg, N. J.
[3] Certified reagent grade, Fisher Scientific Co., Fair Lawn, N. J.
[4] Barnstead Still and Sterilizer Co., Boston, Mass.

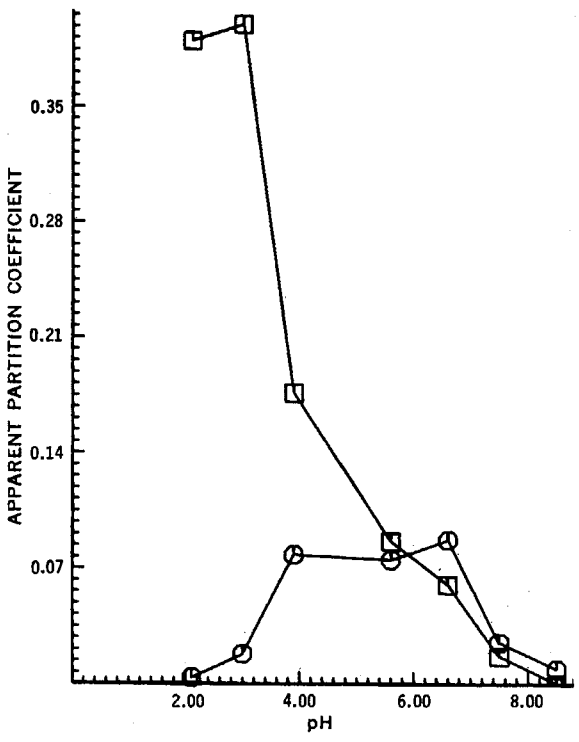

Figure 2—*Plot showing the effect at 25° of 0.02600* M *trichloroacetate on the* n*-octyl alcohol–phosphate buffer apparent partition coefficients of 0.00052* M *oxytetracycline hydrochloride at various pH values. Key:* □, *oxytetracycline hydrochloride and trichloroacetate; and* ○, *oxytetracycline hydrochloride.*

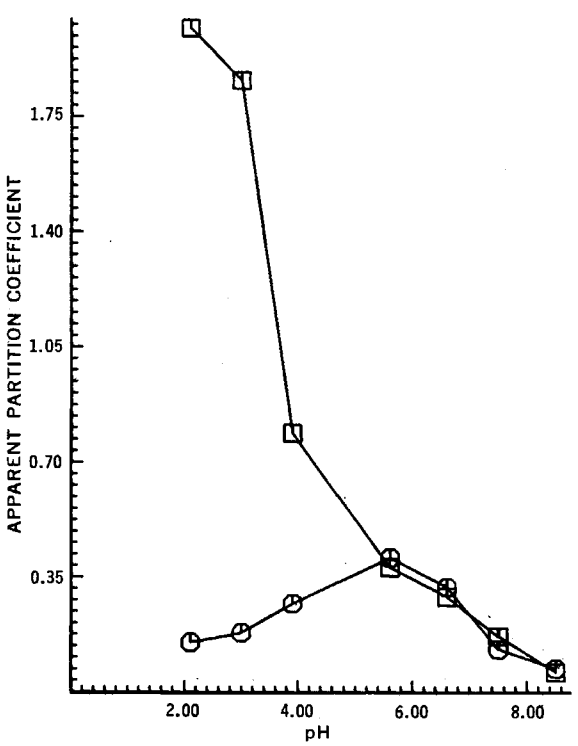

Figure 3—*Plot showing the effect at 25° of 0.02600* M *trichloroacetate on the* n*-octyl alcohol–phosphate buffer apparent partition coefficients of 0.00052* M *chlortetracycline hydrochloride at various pH values. Key:* □, *chlortetracycline hydrochloride and trichloroacetate; and* ○, *chlortetracycline hydrochloride.*

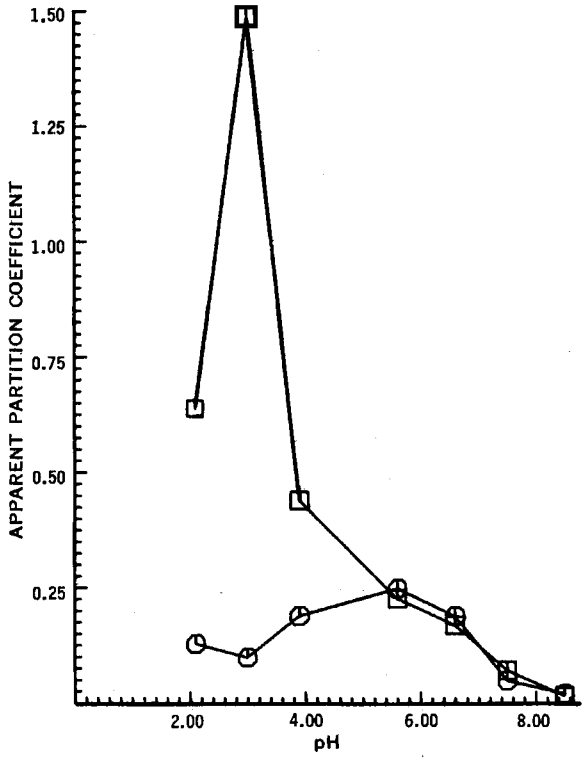

Figure 4—*Plot showing the effect at 25° of 0.02600* M *trichloroacetate on the* n*-octyl alcohol–phosphate buffer apparent partition coefficients of 0.00052* M *demeclocycline hydrochloride at various pH values. Key:* □, *demeclocycline hydrochloride and trichloroacetate; and* ○, *demeclocycline hydrochloride.*

The apparent partition coefficients of the tetracycline analogs in the presence of trichloroacetate anion were determined as previously described (4) for the tetracycline analogs. The procedure was carried out for each of four test flasks containing 10 ml. *n*-octyl alcohol saturated buffer solution, which was 5.20×10^{-4} *M* in tetracycline analog and 2.60×10^{-2} *M* in trichloroacetate anion. The blank consisted of *n*-octyl alcohol saturated buffer solution containing trichloroacetate anion.

RESULTS AND DISCUSSION

Partitioning Experiments—Figures 1–8 illustrate the effects of trichloroacetate on the apparent partition coefficients of selected tetracycline analogs. At the acidic experimental pH values of 2.1 and 3.0, tetracycline hydrochloride, oxytetracycline hydrochloride, chlortetracycline hydrochloride, demeclocycline (demethylchlortetracycline) hydrochloride, methacycline hydrochloride, and doxycycline hyclate exhibited a marked increase in partitioning in the presence of a 50 *M* excess of trichloroacetate anion (Figs. 1–6). On the other hand, minocycline (7-dimethylamino-6-demethyl-6-deoxytetracycline hydrochloride) and 9-dimethylamino-6-demethyl-6-deoxytetracycline were not greatly affected at any of the experimental pH values (except possibly for minocycline at pH 3.9), as Figs. 7 and 8 illustrate.

The very significant increase in the apparent partition coefficients at the acidic pH values of 2.1 and 3.0 (Figs. 1–6) can be rationalized on the basis of the percent of the cationic form of tetracycline analog present. As shown in Fig. 8 of *Reference 4*, the tetracycline molecule exists predominantly as a cation at the experimental pH values of 2.1 and 3.0. Due to the larger concentration of cations, there is greater potential for interaction with trichloroacetate. More cations would be participating in an ion-pair formation, thus imparting greater lipophilicity to the tetracycline system.

The possible role of intermolecular ion-pair formation in enhancing lipophilicity or membrane transfer of tetracycline analogs, as reflected by changes in the magnitude of apparent partition coefficients, depends upon the molar concentration of the anion available for ion-pair formation, as illustrated in Fig. 1 for tetracycline, and

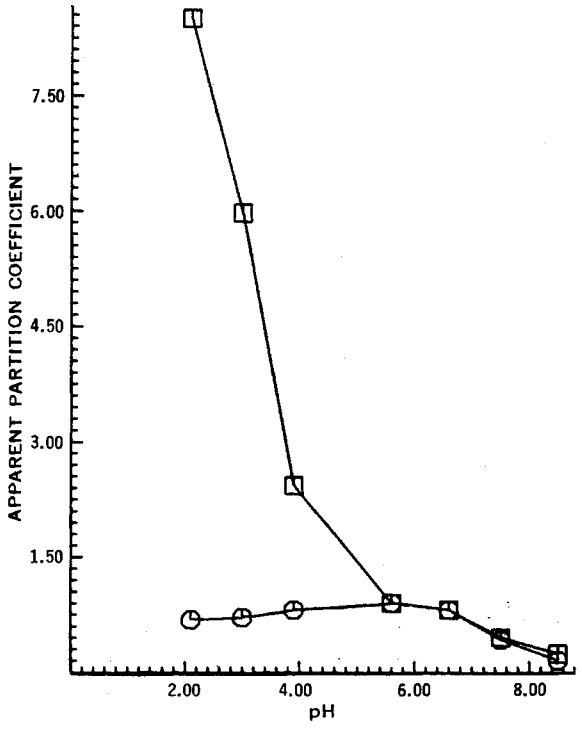

Figure 5—*Plot showing the effect at 25° of 0.02600* M *trichloroacetate on the* n*-octyl alcohol–phosphate buffer apparent partition coefficients of 0.00052* M *methacycline hydrochloride at various pH values. Key:* ⊡, *methacycline hydrochloride and trichloroacetate; and* ○, *methacycline hydrochloride.*

upon the ability or tendency of the analog to pair with other ions. In the case of minocycline and 9-dimethylamino-6-demethyl-6-deoxytetracycline, it appears that the presence of the two dimethylamino moieties in each of these analogs apparently limits the effectiveness of intermolecular ion-pair formation in producing a more lipophilic compound (Figs. 7 and 8).

In the intermediate pH region (beginning at pH 5.6), the trichloroacetate anion appears to have essentially no effect upon the apparent octyl alcohol–aqueous buffer partition coefficients of tetracycline analogs. As noted previously (4), in the absence of the trichloroacetate anion, the maximum apparent partition coefficients of the various tetracycline analogs occurred at the experimental pH values of 5.6 and 6.6, where the existence of isoelectric species of the tetracyclines is favored.

In the absence of trichloroacetate anion, tetracycline methiodide exhibited essentially no transfer into the *n*-octyl alcohol phase (4). In the presence of trichloroacetate anion, however, transfer was exhibited at all pH values studied except at pH 7.5 and 8.5[5].

Biological Implications—Hogben *et al.* (5) and Schanker (6) indicated that the rate of absorption of various weak electrolytes parallels the lipid–aqueous partition coefficients of the unionized form of a drug. However, a drug must be in the solution state to be absorbed. If a significant concentration of dissolved drug in the aqueous contents of the GI tract cannot be achieved, absorption may be negligible in spite of a favorable partition coefficient. For optimum absorption of dissolved drug, the partition coefficient should not be so high that the drug accumulates in lipophilic phases and is denied access to aqueous barriers, nor should it be so low that the drug cannot enter a lipophilic membrane such as the intestinal wall at a sufficient rate to provide an adequate flux. In the case of the tetracyclines, it was suggested (4) that there may be a direct relationship between apparent octyl alcohol–aqueous buffer partition coefficients and quantity of each analog absorbed or quantity of each analog necessary to obtain comparable blood levels. The authors also noted that the pH values of highest apparent partition coefficient for the various analogs correspond to the pH values at which optimum antimicrobial activity has been reported (7). This seems to indicate that it might be possible to enhance absorption and/or activity by increasing the apparent partition coefficients of the various analogs.

A low gastric pH is essential for rapid dissolution of tetracycline. The aqueous solubility of tetracycline is about 100 times greater at pH 1–3 than at pH 5–6 (8). The oral administration of a 2-g. dose of sodium bicarbonate has been shown to reduce significantly the rate and the cumulative amount of tetracycline excreted in the urine of humans. This observation has been attributed to the fact that the gastric pH increased above pH 4, which is less favorable for the dissolution of tetracycline (8).

By maintaining normal gastric pH, a more rapid and complete dissolution of tetracycline may be achieved. However, at low pH values the apparent partition coefficients of many of the tetracycline analogs are markedly lower and this could cause absorption to be significantly lower. Thus, it would seem that if the apparent partition coefficients of the tetracyclines could be increased at lower pH values without greatly affecting dissolution, absorption of the analogs from the stomach would be enhanced beyond that suggested by the work of Pindell *et al.* (9) in dogs and of Perrin and Vallner (10) in rats.

Certain anions, such as perfluorobutyrate, perfluoropropionate, trichloroacetate, and citrate (11, 12) have been shown to promote the absorption of the tetracyclines, although the mechanism has not been completely elucidated. Irwin *et al.* (1) showed that trichloroacetate anion increases both the lipid solubility and the absorption of a quaternary ammonium compound, isopropamide, presumably through ion-pair formation. This same phenomenon likely accounts for the increased partition coefficients of the tetracyclines observed in the present study at lower pH values in the presence of trichloroacetate anions. The negative charge of the trichloroacetate anion combines with the positive charge of the cationic form of the tetra-

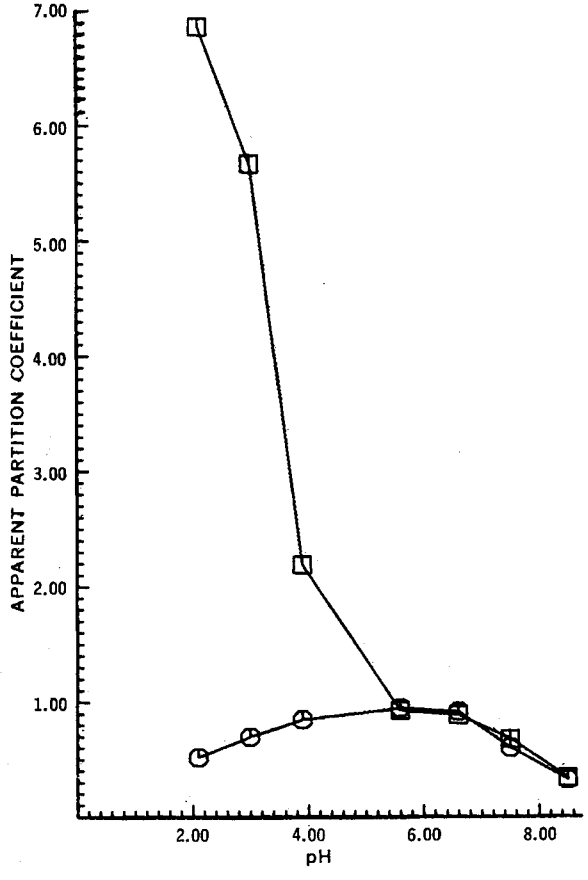

Figure 6—*Plot showing the effect at 25° of 0.02600* M *trichloroacetate on the* n*-octyl alcohol–phosphate buffer apparent partition coefficients of 0.00052* M *doxycycline hyclate at various pH values. Key:* ⊡, *doxycycline hyclate and trichloroacetate; and* ○, *doxycycline hyclate.*

[5] Apparent partition coefficients for tetracycline methiodide in the presence of trichloroacetate anion were 0.47 at pH 2.1, 6.23 at pH 3.0, 0.17 at pH 3.9, 0.02 at pH 5.6, 0.01 at pH 6.6, and zero at the remaining two pH values.

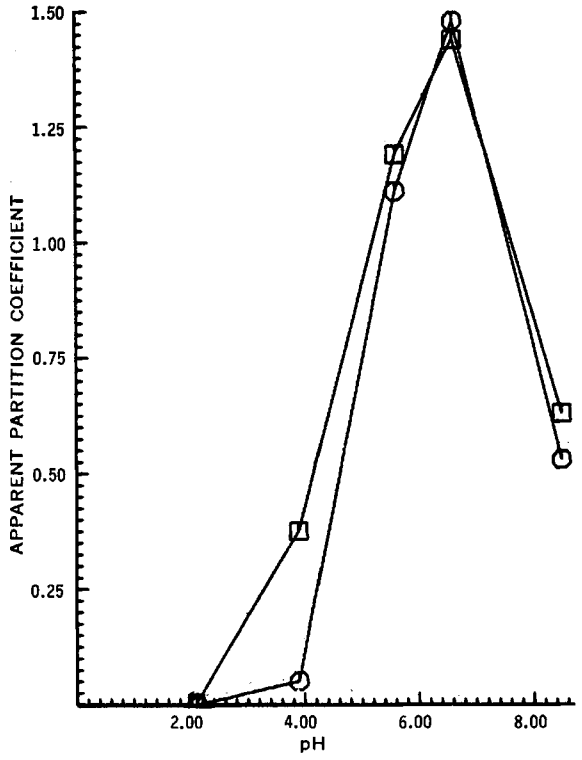

Figure 7—*Plot showing the effect at 25° of 0.02600* M *trichloroacetate on the* n*-octyl alcohol–phosphate buffer apparent partition coefficients of 0.00052* M *minocycline hydrochloride at various pH values. Key:* □, *minocycline hydrochloride and trichloroacetate; and* ○, *minocycline hydrochloride.*

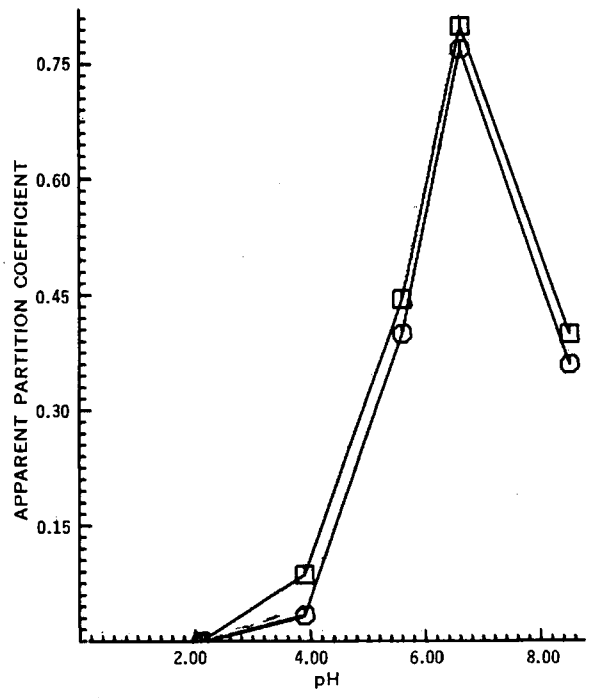

Figure 8—*Plot showing the effect at 25° of 0.02600* M *trichloroacetate on the* n*-octyl alcohol–phosphate buffer apparent partition coefficients of 0.00052* M *9-dimethylamino-6-demethyl-6-deoxytetracycline at various pH values. Key:* □, *9-dimethylamino-6-demethyl-6-deoxytetracycline and trichloroacetate; and* ○, *9-dimethylamino-6-demethyl-6-deoxytetracycline.*

cycline molecule, thus forming an intermolecular type of ion-pair which is more lipid soluble by reason of being more thermodynamically stable in nonaqueous environments.

Perrin and Vallner (10) utilized ^{14}C-labeled trichloroacetate to study the absorption of tetracycline from various buffer systems. These authors showed that less trichloroacetate was absorbed into the phosphate buffer from the stomach pouch of the rat when tetracycline was added to the mucosal fluid, even though both the extent and rate of tetracycline absorption were increased by trichloroacetate. They concluded that tetracycline was not being absorbed in conjunction with trichloroacetate.

In the presence of trichloroacetate, one can expect increased partitioning of tetracycline at the lower pH values in the biological system due to ion-pair formation. One cannot, on the other hand, account for the general effects of the formed complex on the integrity of the barrier. If partitioning is increased by a factor of 10 but permeability is decreased by 25%, there is a net 7.5-fold increase in tetracycline flux. Since the permeability of the free acid species would experience a similar decrease due to changes in general membrane integrity but, unlike tetracycline, the membrane concentration would not be substantially increased by ion-pair formation due to the huge molar excess at which trichloroacetate is present in the lumen, there would be a decrease in trichloroacetate flux. That such general effects on membranes occur is well documented for cholesterol, anti-inflammatory steroids, *etc.* Therefore, Perrin and Vallner's experiment does not rule out partitioning as the dominant effect and, based on other considerations, their surface tension theory is questionable. One should point out here that surface excesses resulting in lowered surface tensions are determined by the same free energy factors that determine partitioning between aqueous and lipid phases.

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