TABLE I—MOLECULAR WEIGHT OF 1% SARAMYCETIN SOLUTIONS IN VARIOUS SOLVENTS AS DETERMINED BY APPROACH TO EQUILIBRIUM SEDIMENTATION

Solvent	Mol. Wt.
1 *M* acetate buffer, pH 4.05	55,000 ± 2,000
0.2 *M* NaCl–0.02 *M* phosphate buffer, pH 6.85	56,000 ± 2,000
2 *M* NH_4OH–2 *M* NH_4Cl buffer, pH 9.4	55,000 ± 2,000
Isotonic phosphate buffer containing K^+, Na^+, Ca^{++}, Mg^{++}, Cl^-, and $SO_4^=$	54,000 ± 2,000
0.68 *M* borate buffer, pH 8.6	6,000–20,000
8 *M* urea in NaCl–phosphate buffer	7,000 ± 1,000
90% ethanol (95%)–10% NaCl–phosphate buffer	2,100 ± 100

Sedimentation of 1% saramycetin in 8 *M* urea–NaCl–phosphate buffer diminished the apparent molecular weight to 7000 ± 1000 daltons, indicating a disruption of the aggregate.

Borate buffer, pH 8.6, 0.68 *M*, also affected the aggregate molecular weight with several components found with uncorrected molecular weights of from 6000 to 20,000 daltons.

By diminishing the concentration of the phosphate–NaCl buffer to concentrations of $^1/_{100}$ of the original, a molecular weight of 5500 ± 1000 daltons was found ($S = 1.37 \times 10^{-13}$ cm. sec.$^{-1}$). The physical effect of diluting the buffer is to diminish the apparent molecular weight of the saramycetin aggregate. The *in vitro* microbiological activity of 0.1% saramycetin solutions varied inversely with the ionic strength of the buffer (7). This indicates that the biological effect of using a more dilute buffer is to increase the *in vitro* antifungal activity of saramycetin solutions.

In summary, saramycetin, molecular weight 2100, may form aggregates in solution. The molecular weight of these aggregates may range upward to 55,000 depending on the solvent and on electrolyte ionic strength.

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Communications

Mathematics of the Three-Phase *In Vitro* Absorption Models

Sir:

Several reports on three-phase models for drug absorption have recently appeared in the literature (1–3). However, it appears that volume terms should be included in the rate equations as well as in the equilibrium equations to obtain more meaningful values for the transfer rates. In these models, the drug (usually weakly basic or weakly acidic) is transferred from an aqueous buffer (*A*) of a pH found in the alimentary tract, through a water immiscible organic phase (*B*) acting as the "membrane," to a buffer of blood pH (*C*). Assuming that only the unionized drug is soluble in the organic phase, then the equilibrium concentrations (C_A, C_B, C_C) can be expressed in the equations derived below in terms of the initial concentration of the drug (a_0), the true distribution coefficient of the drug between the organic layer and water (D), the pH's of the buffers (pH_A and pH_C), the pKa of the drug, and the volumes of the phases (V_A, V_B, and V_C).

For a monobasic acid

$$C_B = \frac{a_0 D}{1 + 10^{pH_A - pK_A} + \frac{V_C}{V_A}(1 + 10^{pH_C - pK_A}) + \frac{V_B D}{V_A}}$$

$$C_A = \frac{C_B}{D}(1 + 10^{pH_A - pK_A})$$

$$C_C = \frac{C_B}{D}(1 + 10^{pH_C - pK_A})$$

For a mono-acidic base

$$C_B = \frac{a_0 D}{1 + 10^{pK_A - pH_A} + \frac{V_C}{V_A}(1 + 10^{pK_A - pH_C}) + \frac{V_B D}{V_A}}$$

$$C_A = \frac{C_B}{D}(1 + 10^{pK_A - pH_A})$$

$$C_C = \frac{C_B}{D}(1 + 10^{pK_A - pH_C})$$

In the kinetics of the three-phase distribution

experiment, volume terms should be included to obtain a mass balance. Consider the transfers

$$A \underset{k_{-1}}{\overset{k_1}{\rightleftarrows}} B \underset{k_{-2}}{\overset{k_2}{\rightleftarrows}} C$$

then

$$V_A \frac{dA}{dt} = -V_A k_1 A + V_B k_{-1} B$$

$$V_B \frac{dB}{dt} = V_A k_1 A - V_B k_{-1} B - V_B k_2 B + V_C k_{-2} C$$

$$V_C \frac{dC}{dt} = V_B k_2 B - V_C k_{-2} C$$

where A, B, C are the concentrations in the three compartments at any instant. These equations are perfectly general and all transfers in the three-phase model should obey them.

Estimates of the values of the transfer constants k_1, k_{-1}, k_2, and k_{-2} can be obtained with or without the aid of a computer. These transfer constants are dependent on the pH of the solutions, the pKa of the drug, the true distribution coefficient, and the interfacial area, but not on the volumes of the phases. Often several of the terms are negligible (substitution in the equilibrium equations given above will aid in predicting this), so simplifying solution of the equations. The simplest case observed (3) and the one possibly nearest to an *in vivo* absorption situation is the transfer of a weakly acidic drug from a buffer of low pH, in which ionization is negligible, through an organic solvent, in which the drug is much more soluble than the unionized entity is in water, to a buffer of pH 7.4 ("blood"), in which the drug is fully ionized. In this case the back reactions are negligible and if $V_A = V_C$ then on integration we have

$$A = a_0 e^{-k_1 t}$$

$$B = \frac{V_A}{V_B} \frac{a_0 k_1}{k_2 - V_A k_1 / V_B} \left(e^{-V_A k_1 t / V_B} - e^{-k_2 t}\right)$$

$$C = a_0 \left[1 - \frac{1}{k_2 - V_A k_1 / V_B} \left(k_2 e^{-k_1 t} - k_1 e^{-k_2 t} - \frac{V_A}{V_B} k_1 e^{-k_1 t} + k_1 e^{-V_A k_1 t / V_B}\right)\right]$$

Many of the other special cases for this type of transfer have been reported by Doluisio and Swintosky (2); here the authors have a different approach, avoiding the use of volumes in the differential equations. However, if these equations are integrated and/or used in the comparison of theoretical with experimental data, then volume terms must be introduced at some stage.

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