# Influence of Polysorbate 20 and Sodium Cholate on Uptake of *p*-Hydroxybenzoates by the Frog, *Rana pipiens*

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**Abstract** □ The frog, *Rana pipiens*, was found to take up certain *p*-hydroxybenzoates when partially immersed in a solution of these substances. The order of decreasing uptake rates for the benzoates was butyl, propyl, ethyl, and methyl. The presence of a nonionic surfactant, polysorbate 20, reversed the order; however, a biological surfactant, sodium cholate, appeared to increase the rate of uptake of the parabens. The process was first order.

**Keyphrases** □ Polysorbate 20—influence on uptake of *p*-hydroxybenzoates by frog □ Sodium cholate—influence on uptake of *p*-hydroxybenzoates by frog □ *p*-Hydroxybenzoates—influence of polysorbate 20 and sodium cholate on uptake by frog □ Surfactants—influence of polysorbate 20 and sodium cholate on uptake of *p*-hydroxybenzoates by frog

The purpose of this study was to screen a number of drugs for uptake by the frog and, for those found, to determine the effect of two types of surfactants on the process. The screened drugs included the weak bases procaine, acetanilid, and 4-aminoantipyrine and the weak acids phenobarbital, sulfacetamide, and four benzoic acid esters. Of these drugs, only the benzoates were taken up to any detectable degree. Previously, salicylic acid was shown (1) to be taken up readily, mainly in the undissociated form, from solution by the frog, thus conforming to the proposed pH–partition theory of passive absorption of some drugs as offered by Shore *et al.* (2). This study supports the evidence of this theory since the frog took up the higher molecular weight benzoates which had greater oil solubility more readily than the ones with lower molecular weight.

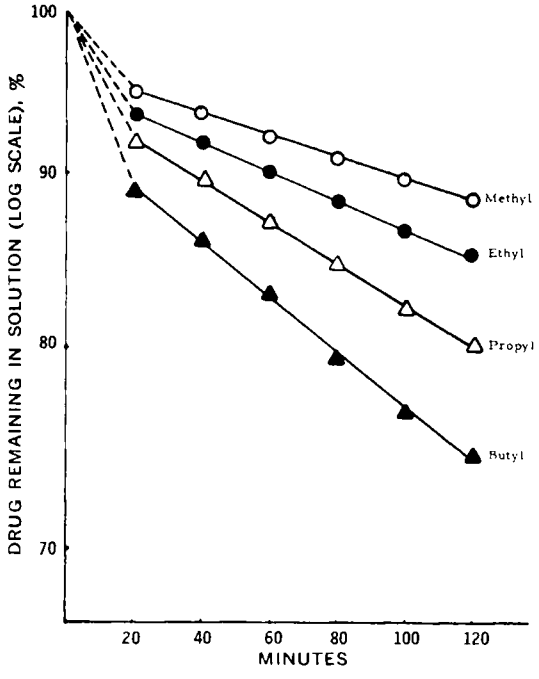


**Figure 1**—*Plot of average percent of parabens remaining in solution over 2 hr. when five frogs were each placed in 500 ml. of aqueous drug solution. Each solution was initially 5.0 × 10⁻⁵ M in the paraben.*

**Table I** Rate Constants (min.⁻¹ × 10⁴)

| Additive | Paraben | | | |
|---|---|---|---|---|
| | Methyl | Ethyl | Propyl | Butyl |
| None (control) | 1.53 | 1.92 | 2.84 | 3.78 |
| Polysorbate 20 (0.5%) | 1.74 | 1.42 | 0.747 | 0.625 |
| Polysorbate 20 (1.0%) | 1.42 | 1.21 | 0.671 | 0.499 |
| Sodium cholate ($5.0 \times 10^{-5}$ *M*) | 1.64 | 2.06 | 3.18 | 4.48 |

## EXPERIMENTAL

The technique employed in this study was used previously (1, 3, 4). Five frogs, 30–35 g. each, were used in the determinations. Each frog was placed in 500 ml. of drug solution contained in a 2-l. beaker which permitted immersion to the neck. At 20-min. intervals, about 5 ml. of drug solution was withdrawn and assayed for drug content and returned to the container to maintain uniform volume. The solutions were buffered to pH 4.0 with hydrochloric acid in 0.05 *M* glycine. This pH is well tolerated by frogs and was used in previous studies (1, 3, 4) to minimize the effect of ionization changes

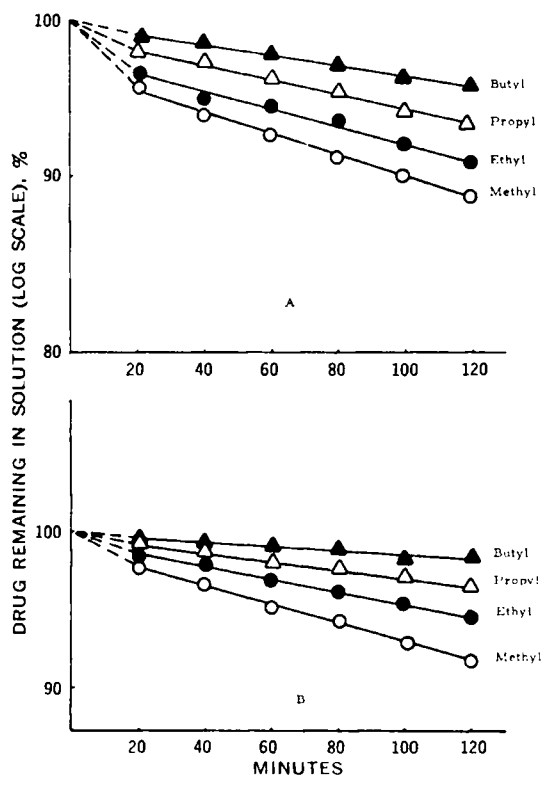


**Figure 2**—*Plot of average percent of parabens remaining in solution over 2 hr. when five frogs were each placed in 500 ml. of aqueous drug solution, each containing a paraben in a concentration of 5.0 × 10⁻⁵ M and polysorbate 20 (0.5%, upper curves A; 1%, lower curves B).*

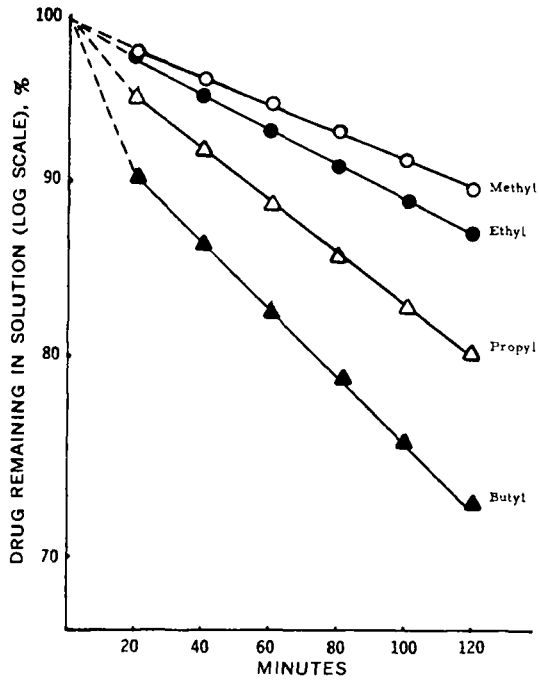


**Figure 3**—*Plot of average percent of parabens remaining in solution over 2 hr. when five frogs were each placed in 500 ml. of aqueous drug solution, each containing a paraben in a concentration of 5.0 × $10^{-5}$ M and sodium cholate, 5.0 × $10^{-5}$ M.*

on drug uptake. Distilled water was used as the solvent, and the solutions of parabens were 5.0 × $10^{-5}$ *M*. The concentration of benzoate remaining in solution was determined by absorbance readings[1] at a wavelength of 255 nm.

The partition coefficient was determined by allowing each paraben to equilibrate with stirring for 3 hr. at 25° between 100 ml. of *n*-hexane–distilled water (50:50), adjusted to a pH of 2.0 with hydrochloric acid. The aqueous layer was assessed for paraben content, and the partition coefficient was calculated from:

$$P = \frac{\text{concentration in } n\text{-hexane}}{\text{concentration in water}} \qquad \text{(Eq. 1)}$$

## RESULTS AND DISCUSSION

As seen in Figs. 1–3, the data can be linearized satisfactorily by plotting the log of the percent of paraben remaining in solution against time, thus indicating a first-order process. In each case, however, the relationship does not become linear until after the first 20 min. During this initial period, the frog takes up the drug considerably faster than during the remainder of the 2 hr., as noted in a previous study (4).

The linear equation is:

$$\log C = -\frac{kt}{2.303} + \log C_0 \qquad \text{(Eq. 2)}$$

where $C$ = percent of benzoate remaining in solution, $C_0$ = initial percent of benzoate in solution (100), $k$ = rate constant, and $t$ = time in minutes. The rate constants were calculated from the slope, $-K/2.303$, of the line and show the rate of disappearance of drug.

Figure 1 shows that the frog, *Rana pipiens*, takes up the parabens with higher molecular weights more readily than those with lower molecular weights, in the order butyl > propyl > ethyl > methyl.

[1] Beckman DU spectrophotometer.

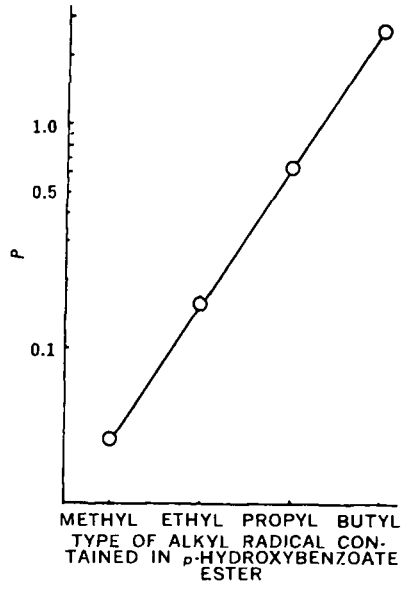


**Figure 4**—*Partition coefficient* (P) *of parabens at pH 2.0.* P = *concentration in* n-*hexane/concentration in water.*

This is consistent with previous findings (1) that the greater the lipid solubility of the drug the greater is the rate of uptake by the frog for these drugs. Figure 4 shows paraben partition coefficient data which correlate molecular weight and lipid solubility.

Surfactants have been shown to influence drug absorption (4, 5). Figure 2 shows the effect of two concentrations of polysorbate 20 on the uptake of parabens. This nonionic surfactant decreased the uptake of most of the parabens and also reversed the order of the sorption process with respect to molecular weight (Table I).

Sodium cholate is a bile salt having surfactant properties, and its effect on the uptake of parabens by the frog is seen in Fig. 3. A comparison of rate constants (Table I) indicates that sodium cholate slightly increased the rate of uptake of each paraben.

The mechanisms by which such additives influence drug uptake is not well understood. The effect of polysorbate 20 in decreasing uptake is perhaps due to complexation. The surface tension-lowering ability of the sodium cholate probably increases drug uptake by permitting better wetting action by the drug solution on the skin of the frog. Bile salts were found to increase the permeability of everted rat intestine to salicylate (6).

The frog, *Rana pipiens*, has been found to take up relatively few types of drugs using an immersion technique. The partition coefficient of the drugs taken up plays an important role in determining the rate.

## REFERENCES

(1) C. W. Whitworth, *J. Pharm. Sci.*, **54**, 463(1965).

(2) P. A. Shore, B. B. Brodie, and C. A. M. Hogben, *J. Pharmacol. Exp. Ther.*, **119**, 361(1967).

(3) C. W. Whitworth and L. D. Yantis, *J. Pharm. Sci.*, **56**, 1661 (1967).

(4) C. W. Whitworth and E. R. Carter, *ibid.*, **58**, 1285(1969).

(5) G. Levy, K. E. Miller, and R. H. Reuning, *ibid.*, **55**, 394 (1966).

(6) S. Feldman and M. Gibaldi, *ibid.*, **58**, 425(1969).

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