a rate of 2 ml/min after pretreatment with salicylate; the effect of pretreatment with sodium lauryl sulfate was not eliminated by washing.

After rectal administration of 0.3 ml of theophylline solution (pH 7.4, 15 mg/ml) in the presence of salicylate (15 mg/ml), the blood levels of theophylline and salicylate increased rapidly and simultaneously, reaching maximum levels (41.2 ± 15.9 μg/ml for theophylline and 41.1 ± 14.3 μg/ml for salicylate; $n = 6$) 30 min after administration. The amounts of theophylline and salicylate remaining in the solution were 25.6 ± 4.7 and 30.3 ± 7.2%, respectively. Without salicylate, maximum blood levels of theophylline were <5 μg/ml ($n = 6$).

The presence of salicylate in the perfusate (pH 4.5 or 5.0) also enhanced the disappearance of lidocaine, which is a basic drug and is not absorbed below pH 6.0, from the perfusate in the rat rectum. The effects of salicylate on the disappearance of lidocaine at various pH values are presented in Fig. 2. Thirty minutes after rectal administration of 0.3 ml of lidocaine solution (pH 4.5, 15 mg/ml) in the presence of salicylate (15 mg/ml), lidocaine and salicylate reached maximum levels in the blood (43.3 ± 4.7 μg/ml for lidocaine and 48.5 ± 6.9 μg/ml for salicylate; $n = 6$).

These results indicate that salicylate and/or its acidic form markedly enhance the absorption of various compounds from the rectum. This effect appears to be general for other drugs including cefmetazole and levodopa. Furthermore, three isomers of sodium dihydroxybenzoate (3,4-, 2,5-, and 3,5-dihydroxybenzoates) and homovanillic acid were studied as adjuvants and found to enhance the rectal absorption of both theophylline and lidocaine. These and similar compounds have significant potential as adjuvants for enhancing rectal drug absorption. Studies are continuing on the mechanism of their action.

(1) Y. Nishioka and T. Kawamura, *Yakuzaigaku*, 37, 88 (1977).

(2) D. J. A. Crommelin, J. Modderkolk, and C. J. deBlaey, *Int. J. Pharm.*, 3, 299 (1979).

Toshiaki Nishihata
J. Howard Rytting ˣ
Takeru Higuchi

Department of Pharmaceutical Chemistry
University of Kansas
Lawrence, KS 66045

Received February 8, 1980.

Accepted for publication March 18, 1980.

Supported in part by a grant from INTERx Research Corp., Lawrence, Kans., and R. P. Scherer, North America, Detroit, Mich.

Kinetic Characterization of Liposomes

Keyphrases □ Liposomes—base-catalyzed hydrolysis of *p*-nitrophenyl acetate in liposomal suspensions □ *p*-Nitrophenyl acetate—base-catalyzed hydrolysis in liposomal suspensions □ Hydrolysis—base-catalyzed degradation of *p*-nitrophenyl acetate in liposomal suspensions

To the Editor:

Liposomes have been investigated extensively for possible use as novel drug carriers for therapeutic purposes (1, 2). From a drug stability viewpoint, we studied the hydrolysis of procaine and 2-diethylaminoethyl *p*-nitrobenzoate in liposomal suspensions and compared it with spontaneous hydrolysis in aqueous bulk solutions (3, 4). The base-catalyzed hydrolysis of procaine was retarded by liposomes; with 2-diethylaminoethyl *p*-nitrobenzoate, both retardation and enhancement were observed, depending on the pH of the dispersed medium.

These ester degradations followed first-order kinetics in the liposomal suspension as well as in the corresponding aqueous bulk solution, regardless of whether retardation or enhancement was observed. This finding indicates that the substrates added in the liposomal medium partitioned into the lipid phase at so fast a rate that it was undetectable, and this partitioning was followed by quasi-first-order degradation, which can be represented by the first-order rate constants defined in the aqueous bulk and lipid phases. Consequently, the partition model was applicable for degradation of these esters.

This communication discusses the base-catalyzed hydrolysis of *p*-nitrophenyl acetate in liposomal suspensions. This compound frequently is used as a substrate in studies of micellar catalysis (5, 6) and of esterase activity of bovine serum albumin and human serum albumin (7, 8).

Figure 1 indicates that the reaction followed first-order kinetics with the ester remaining in the aqueous solution. The reaction clearly was not a continuous process in liposomal suspensions. It was enhanced in the early stage and proceeded in a manner parallel to the spontaneous degradation. The degradation rate was dependent on the lecithin concentration; the more lecithin that was present, the faster was the hydrolysis. Furthermore, enhancement was accelerated more in the unilamellar dispersion system than in the multilamellar one when the lecithin content in

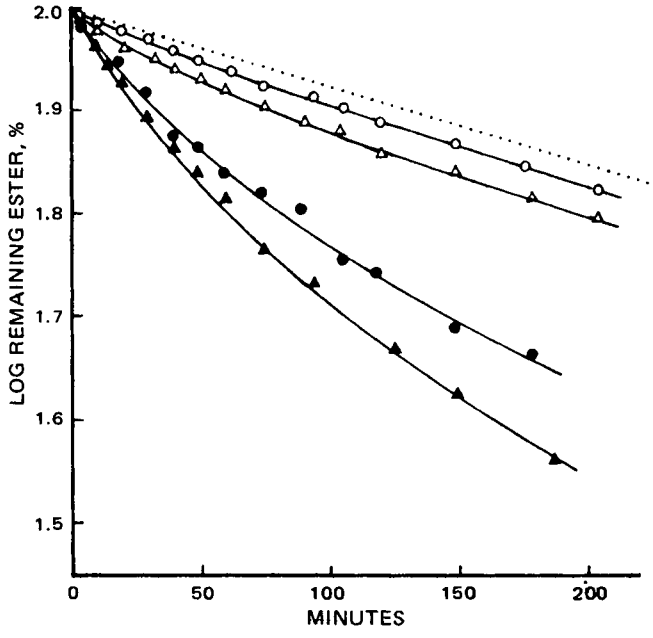


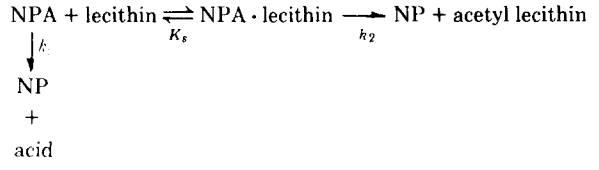
Figure 1—*First-order plots for hydrolysis of* p*-nitrophenyl acetate in aqueous solution and in liposomal suspensions. Tris(hydroxymethyl)-aminomethane buffer at pH 8.0 was used (μ = 0.3, 25°). The initial* p*-nitrophenyl acetate concentration was 5.92 × 10⁻⁴ M. The lecithin concentrations were 2.40 × 10⁻³ M (○ and △) and 8.67 × 10⁻³ M (● and ▲). Key: . . . , spontaneous degradation in water; ○ and ●, multilamellar liposome system; and △ and ▲, unilamellar liposome system. Liposomes were prepared from egg yolk lecithin as described previously (3).*

0022-3549/80/0600-0745$01.00/0
© 1980, American Pharmaceutical Association

the medium was equal in both cases.

These results suggest that partition theory, which was applied to procaine and 2-diethylaminoethyl *p*-nitrobenzoate and for the evaluation of drug entrapment in liposomes, may not apply to the present case because the liposomes appear to be exhausted by the degradation of *p*-nitrophenyl acetate. The exhaustion may be due to acetylation of the phosphate group of the lecithin molecule, which prevents further orientation of the substrate on the surface of the vesicles from being favorable for the hydroxide-ion attack. Furthermore, as seen in the difference between the rates for unilamellar and multilamellar liposomes, the effective surface area of the vesicles plays a key role, and the reaction occurring on the surface is much more predominant than that in the interior lipid phase.

Binding of *p*-nitrophenyl acetate by liposomes appears to be important at the first stage of the reaction to facilitate hydrolysis. The whole reaction can be treated in a manner analogous to that used for enzymatic catalysis:



Scheme I

where NPA and NP are *p*-nitrophenyl acetate and the product, *p*-nitrophenol, respectively; NPA · lecithin is the substrate–lecithin complex; k_1 and k_2 are the rate constants for the spontaneous and facilitated reactions, respectively; and K_s is the dissociation constant of the complex.

According to Scheme I, the apparent first-order rate constant, k_{obs}, for the appearance of NP can be expressed under the condition of an excess lecithin concentration:

$$k_{obs} = \frac{k_1 K_s + k_2 [\text{lecithin}]}{K_s + [\text{lecithin}]} \qquad \text{(Eq. 1)}$$

By converting Eq. 1 to the double-reciprocal form, a Lineweaver–Burk plot makes it possible to determine K_s and k_2 values. However, since lecithin does not exist as a molecular form in water, k_{obs} does not depend on the practical lecithin concentration but instead on the effective surface area of the vesicles formed, which could be converted to the effective concentration of the lipid based on the ratio of the k_{obs} values of unilamellar and multilamellar liposomes prepared under various conditions with an equal lecithin content.

When the lecithin concentration is moderate, k_{obs} is no longer a constant but becomes a function of the effective concentration of the lipid changing with time. Therefore, the effective concentration is an adjustable parameter that is fitted best by a nonlinear optimization procedure in addition to the k_2 value.

Thus, the surface properties of liposomes, if they are prepared under the same conditions except for size reduction procedures, may greatly affect the stability of a drug, especially when the enhancement of degradation is involved.

(1) B. E. Ryman, in "Proceedings of the Sixth International Congress of Pharmacology," vol. 5, J. Tuomisto and M. K. Paasonen, Eds., Helsinki, Finland, 1975, p. 91.

(2) G. Gregoriadis, *Ann. N.Y. Acad. Sci.*, **308**, 343 (1978).

(3) T. Yotsuyanagi, T. Hamada, H. Tomida, and K. Ikeda, *Acta Pharm. Suec.*, **16**, 271 (1979).

(4) *Ibid.*, **16**, 325 (1979).

(5) V. Gani and C. Lapinte, *Tetrahedron Lett.*, **1973**, 2775.

(6) F. M. Menger and M. J. McCreery, *J. Am. Chem. Soc.*, **96**, 121 (1974).

(7) J. T. Tildon and J. W. Ogilvie, *J. Biol. Chem.*, **247**, 1265 (1972).

(8) K. Ikeda, Y. Kurono, Y. Ozeki, and T. Yotsuyanagi, *Chem. Pharm. Bull.*, **27**, 80 (1979).

Toshihisa Yotsuyanagi ˣ
Ken Ikeda

Faculty of Pharmaceutical Sciences
Nagoya City University
Nagoya 467, Japan

Received January 15, 1980.
Accepted for publication March 24, 1980.

Quantitative Change in Metabolic Fate of Drug Related to Serum Protein Binding

Keyphrases □ Protein binding, serum—effect on drug metabolism □ Drug metabolism—effect of serum protein binding □ Biotransformation—effect of serum protein binding on drug metabolism

To the Editor:

If a drug is eliminated by renal excretion and by biotransformation, then the fraction of the dose that is metabolized, F_m, is equal to the ratio of the metabolic formation clearance to the total clearance. Thus:

$$F_m = Cl_m/Cl_T \qquad \text{(Eq. 1)}$$

If clearance is not limited or affected by the organ blood perfusion rate and the rates of the clearance processes are proportional to the concentration of free (unbound) drug in serum:

$$F_m = \frac{fCl_{m\ \text{intrinsic}}}{fCl_{T\ \text{intrinsic}}} \qquad \text{(Eq. 2)}$$

where f is the free fraction of drug in serum and the clearance terms represent intrinsic clearances of free drug (1–3). Since f cancels out, F_m is independent of serum protein binding under the stated conditions.

If the renal clearance of a drug involves glomerular filtration (the rate of which usually is proportional to the concentration of free drug in serum) as well as renal tubular secretion and if the rate of tubular secretion is proportional to the concentration of total (free plus bound) drug in serum (3), then F_m is affected by f. Specifically:

$$\text{renal clearance} = fk_g(1 - F) + k_s^*(1 - F) \qquad \text{(Eq. 3)}$$

where k_g is the glomerular filtration clearance referenced to the free drug concentration, k_s^* is the apparent renal secretion clearance referenced to the total drug concentration, and F is a dimensionless constant representing the fraction of filtered and secreted drug that is reabsorbed (3). Proportionality between the renal tubular secretion rate and the total rather than free drug concentration in plasma or serum may occur if the true renal secretion clearance is much higher than the effective renal blood flow (4).

Since total clearance is the sum of the renal and metabolic clearances for drugs eliminated entirely by those two

0022-3549/80/0600-0746$01.00/0
© 1980, American Pharmaceutical Association