Effect of Plasma Protein Binding on Elimination of Bilirubin

Keyphrases □ Bilirubin—effect of plasma protein binding on elimination □ Protein binding, plasma—effect on elimination of bilirubin

To the Editor:

It is generally appreciated that interindividual differences in plasma protein binding can have a pronounced effect on the elimination kinetics of extensively protein bound drugs, but relatively little is known about the quantitative aspects of this relationship. Levy and Yacobi (1) recently presented an analysis of the pharmacokinetic aspects of plasma protein binding, which led to the prediction of a linear relationship between total plasma (or serum) clearance (P.C.) and the free fraction (f) of a highly protein bound drug in the plasma:

$$\text{P.C.} = k''f \qquad \text{(Eq. 1)}$$

where k'' is an intrinsic clearance constant for the free drug. Experimental support for this hypothesis was presented by demonstrating an excellent linear relationship between the total serum clearance of warfarin and the free fraction of this drug in the serum of individual rats (1).

We now report the results of a study of the relationship between plasma protein binding and total plasma clearance of bilirubin, another highly protein bound substance and one that is eliminated by conjugative pathways (2) rather than by oxidative processes [as is the case with warfarin (3)]. Seven adult male Sprague–Dawley rats, 335–374 g, received an infusion of ^{14}C-bilirubin (4) into the right jugular vein. An infusion rate of 0.8 mg/kg/min was maintained for 150–240 min. Three animals received an initial "loading" infusion of 2 mg/kg/min for 15 min.

Figure 1—*Relationship between total plasma clearance of bilirubin and the fraction of free bilirubin in the plasma of individual rats. The regression line, obtained by the vector least-squares method (7), is described by the equation P.C. = 0.19 + 7880f.*

Blood samples (0.6 ml) were obtained at 2, 3, and 4 hr or at 1, 2, and 2.5 or 3 hr and were assayed for free (5) and total (6) unconjugated bilirubin. The total plasma clearance of bilirubin was calculated by dividing the infusion rate by the steady-state concentration of total bilirubin in plasma.

Essentially constant concentrations of bilirubin in plasma were obtained within 60–120 min after the start of infusion. Steady-state concentrations were determined by averaging the concentrations of the three plasma samples; they differed by less than 6% on the average in individual animals. The steady-state concentrations of bilirubin in the plasma ranged from 11.7 to 20.7 mg/100 ml, and the total plasma clearance ranged from 3.9 to 6.8 ml/kg/min in the seven rats.

The free fraction of bilirubin in plasma, representing the mean of three separate determinations for each animal, ranged from 4.3×10^{-4} to 9.0×10^{-4}, equivalent to from 99.910 to 99.957% protein binding. There was a strong ($r = 0.82$) and statistically highly significant ($p < 0.02$) linear correlation between plasma clearance and free fraction of bilirubin in the plasma of individual rats (Fig. 1).

The results of this investigation are consistent with the theoretical model of Levy and Yacobi (1) and show that the elimination rate of bilirubin is proportional to the concentration of free rather than total (*i.e.*, free and protein bound) bilirubin in plasma.

(1) G. Levy and A. Yacobi, *J. Pharm. Sci.*, **63**, 805(1974).

(2) B. H. Billing and F. H. Jansen, *Gastroenterology*, **61**, 258(1971).

(3) M. Ikeda, A. H. Conney, and J. J. Burns, *J. Pharmacol. Exp. Ther.*, **162**, 338(1968).

(4) P. V. D. Barrett, F. X. Mullins, and N. I. Berlin, *J. Lab. Clin. Med.*, **68**, 905(1966).

(5) J. Jacobsen and O. Fedders, *Scand. J. Clin. Lab. Invest.*, **26**, 237(1970).

(6) R. Broderson and I. Vind, *ibid.*, **15**, 107(1963).

(7) J. B. Scarborough, "Numerical Mathematical Analysis," Johns Hopkins Press, Baltimore, Md., 1930, pp. 380–383.

Svein Øie
Gerhard Levy [x]

Department of Pharmaceutics
School of Pharmacy
State University of New York
at Buffalo
Buffalo, NY 14214

Received March 28, 1975.
Accepted for publication May 19, 1975.
Supported in part by Grant GM 19568 from the National Institute of General Medical Sciences, National Institutes of Health, Bethesda, MD 20014

[x] To whom inquiries should be directed.