Effect of an Inhibitor of Glucuronide Formation on Elimination Kinetics of Diphenylhydantoin in Rats

Keyphrases □ Diphenylhydantoin elimination kinetics—effect of inhibition of glucuronide formation, rats □ Elimination kinetics, diphenylhydantoin—effect of inhibition of glucuronide formation, rats □ Salicylamide inhibition of glucuronide formation—used to study diphenylhydantoin elimination kinetics, rats □ Glucuronide formation, inhibition using salicylamide—effect on diphenylhydantoin elimination kinetics, rats

Sir:

The elimination of diphenylhydantoin (I) is dose dependent in mice (1), rats (2), dogs (3), and man (4). We recently showed (5) that the elimination of I in rats cannot be described by either first-order or simple Michaelis–Menten kinetics but that it is qualitatively consistent with product inhibition of the metabolism of I. This hypothesis was supported by observations that the major metabolite of I, 5-(*p*-hydroxyphenyl)-5-phenylhydantoin (II), does indeed inhibit the biotransformation of I *in vitro* (6) and *in vivo* (7). These experiments involved the addition of II to a supernate of rat liver homogenate containing I (6) or the repetitive administration of relatively large doses of II to rats who received a single injection of I (7).

For a more definitive assessment of the role of product inhibition in the elimination of I, it was desirable to determine the effect of endogenous II (*i.e.*, II derived entirely from the biotransformation of I) on the *in vivo* elimination of I. Since II is eliminated primarily by conjugation with glucuronic acid (8), it should be possible to increase the concentration of II at sites of biotransformation of I by blocking the formation of the glucuronide of II. This, in turn, should result in the inhibition of the elimination of I if product inhibition is operative as hypothesized.

We determined the effect of salicylamide, a potent inhibitor of glucuronide formation (9), on the elimination of I. Male Sprague–Dawley rats, weighing 310–380 g., received single intravenous injections of I, 10 mg./kg. body weight. Each injection included 2-μc. ^{14}C-I (specific activity 4.65 mc./mmole). Six of these animals were also given intraperitoneal injections of salicylamide, 200 mg./kg. 30 min. before I and 100 mg./kg. every hour thereafter for a total of four doses. Another five animals received injections of 0.9% sodium chloride solution adjusted to pH 10 with sodium hydroxide. The salicylamide solution contained 10% of the drug and was prepared fresh with sufficient sodium hydroxide to yield a final pH of about 10. Blood samples were obtained every 10–30 min. for 210 min. and were assayed specifically for I by liquid scintillation spectrometry as described elsewhere (2, 7).

The results, presented as the geometric mean concentrations of I in the blood of the salicylamide-treated and control animals, are shown in Fig. 1. The elimination of I was much slower in the salicylamide-treated rats than in the control animals. Additional experiments with one-half and one-quarter of the doses of salicylamide yielded definite evidence of inhibition of I elimination with the higher doses and none with the lower doses. Single experiments (due to limited material) to determine the effect of salicylamide on the elimination of ^{14}C-II, using the experimental methods described previously (7), indicated appreciable inhibition of II elimination (terminal half-life 2.2 hr. *versus* 1.0 hr. for the control).

The results of this study provide additional support for the suggested role of product inhibition in the elimination of I. If the inhibitory effect of salicylamide on the elimination of I is due to accumulation of II, as would appear on the basis of the preliminary data presented here, then a new type of drug interaction has been demonstrated. This type of interaction consists of a primary inhibitory effect on the elimination of a drug metabolite, resulting in a decreased rate of elimination of the drug due to inhibition of the biotransformation process by the accumulated metabolite. Hydroxylated metabolites of drugs other than I are also able to inhibit the biotransformation of their precursors (10), and other drugs with dose-dependent elimination characteristics, notably dicumarol, are also biotransformed to hydroxylated metabolites. It is important, therefore, to explore

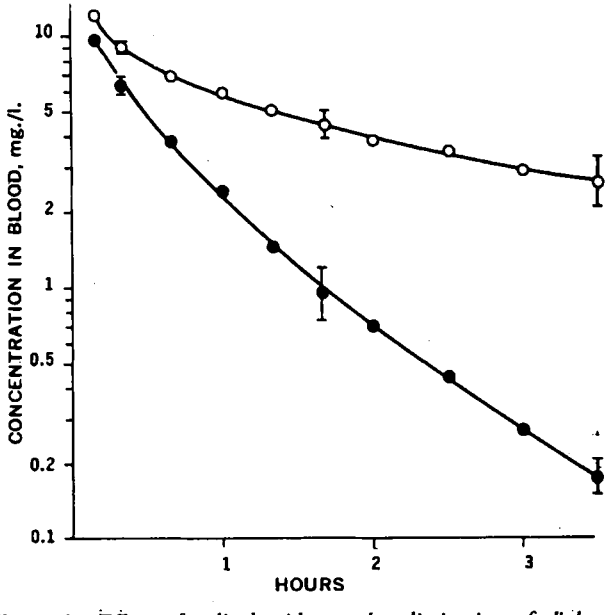

Figure 1—*Effect of salicylamide on the elimination of diphenylhydantoin, 10 mg./kg. i.v., by adult male Sprague–Dawley rats. Key: ●, control rats; and ○, rats treated with salicylamide, 200 mg./kg. 30 min. before diphenylhydantoin injection and 100 mg./kg. every hour thereafter. Vertical bars indicate standard errors of the mean of the logarithmically transformed data for five control and six salicylamide-treated animals.*

the clinical significance of product inhibition and of the type of drug interaction described here.

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Further Comments on the Existence of Spherical Micelles

Keyphrases ☐ Micelles—spherical *versus* nonspherical shape ☐ Surfactants—spherical *versus* nonspherical micelles

Sir:

A study of the shape of micelles (1), based on the combination of their measured size with geometric considerations of the extended length of the normal paraffin moiety, indicated that few surfactants having a single normal alkyl chain form spherical micelles. In Fig. 1 of a recent communication (2), Zografi and Yalkowsky superimposed the results of their calculations, based on similar geometric considerations, on Fig. 1 of *Reference 1*. The figure is a plot of z, the aggregation number or the number of surfactant molecules per micelle, *versus* the number, n, of carbon atoms in the linear hydrocarbon chain of the single-chain surfactants. Micelles with (z, n) coordinates above the curves are unlikely to be spherical. Of the 32 surfactants considered and plotted in Fig. 1, only one fulfilled the geometric prerequisites for spherical shape according to the solid line embodying the results of our original computations (1). Only 26 separate points were shown in Fig. 1 because the coordinates of another six surfactants overlapped points already plotted.

The values of z calculated according to Eq. 5 and listed in the last column of Table I of *Reference 2*[1] have the largest differences from our maximum z values consistent with spherical shape. They are represented by the uppermost of the three curves of Fig. 1. There are 18 surfactants with coordinates above that curve and 14 with coordinates below or on it, which shows that even according to the more divergent of the two analyses of Zografi and Yalkowsky (2), the majority of the surfactants under consideration are still unlikely to form spherical micelles. Moreover, the vertical axis was compressed by using a logarithmic scale for z, so that even points apparently lying close to the curve correspond to distinctly higher aggregation numbers than are consistent with spherical shape.

Of the two simplifications introduced in the original geometric considerations, the first is expressed in Eq. 4 of *Reference 1*, which correlates the molecular weight, m, of the normal alkyl chain of a single surfactant molecule with the number, n, of carbon atoms in that chain:

$$m = 14.03n + 1.01 \cong 14.03n \qquad \text{(Eq. 1)}$$

This is equivalent to neglecting the length of the carbon–hydrogen bond and the van der Waals radius of the farthest hydrogen atom of the terminal methyl group. The second approximation, designed to compensate for the previous one, is to consider n carbon–carbon bond lengths in the hydrocarbon chain instead of the actual number $n - 1$.

With the aid of Fig. 1, the length of the n-paraffin chain is now calculated rigorously and without approximations. The chain is fully extended and in the all-*trans* conformation. Segment a, representing the contribution of the farthest hydrogen atom of the terminal methyl group, consists of two parts. The first is the van der Waals radius of this hydrogen atom, 1.2 Å (3). The second part is the length of the corresponding hydrogen–carbon bond projected in the direction of the longitudinal axis of the extended chain, namely, 1.09 Å times the sine of one-half of the hydrogen–carbon–hydrogen bond angle of 111.5° (3–5), or 1.09 Å sin (111.5°/2) = 1.09 Å × 0.8266 = 0.90 Å. Adding the two parts gives a = 2.10 Å.

Segment b in Fig. 1 represents the contribution of a single carbon–carbon bond to the length of the extended chain. It equals the length of the carbon–carbon bond, namely, 1.541 Å (4, 5), multiplied by the sine of one-half of the tetrahedral angle of 109.5° (3–5), or 1.541 Å sin (109.5°/2) = 1.541 Å × 0.8166 = 1.258 Å. Alternatively, it can be calculated as 1.541 Å cos 35.25° = 1.258 Å. There are $n - 1$ such carbon–carbon bonds in a hydrocarbon chain of n carbon atoms, contributing ($n - 1$) 1.258 Å to the length of the extended chain.

[1] In their Eq. 5, Zografi and Yalkowsky omitted the exponent ½ from the parameter z. The heading of their Table I erroneously reads "Calculated Values of Maximum Radius." The table lists the maximum values for aggregation numbers z consistent with spherical shape for different values of n.