JOURNAL OF PHARMACEUTICAL SCIENCES

MARCH 1982
VOLUME 71 NUMBER 3

RESEARCH ARTICLES

Effects of Phenobarbital on the Distribution Pharmacokinetics and Biological Half-Lives of Model Nonmetabolizable Organic Anions in Rats

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Abstract □ The blood level pharmacokinetics of model nonmetabolizable and nonprotein bound anionic compounds (pKa 3.3–3.4), namely, benzoylformic acid (I), D-(−)-mandelic acid (II), and *p*-methyl benzoylformic acid (III), which are excreted in the urine in the unchanged form, were studied in control and phenobarbital-treated rats to determine the effects of phenobarbital on distribution and elimination pharmacokinetic parameters. These compounds were used, also, because they represent three different compartment models in the untreated rats: a three-compartment open model (I), a two-compartment open model (II), and a one-compartment open model (III). The three-compartment open model (I) in the control rats was reduced to a two-compartment open model with only one kinetically distinguishable peripheral tissue compartment in the phenobarbital-treated rats. The pharmacokinetics of II were described by a two-compartment open model in both control and phenobarbital-treated rats but the apparent distribution volumes of the central and peripheral compartments were found to be significantly smaller in phenobarbital-treated rats. One-compartment open model pharmacokinetics were observed for III in both the control and phenobarbital-treated rats with no significant difference in apparent distribution volumes. The transmembrane transport of these compounds, which exist in the body fluids as anions, was previously reported to occur *via* the aqueous pores of the tissue cell membranes. The decrease in the apparent distribution volumes of these organic anions in the phenobarbital-treated rats was explained in terms of the possible effect of phenobarbital in increasing the protein and phospholipid concentrations of the cell membranes of tissues other than liver. This narrows the size of the aqueous pores of the central and tissue compartment cell membranes and hinders the diffusion of these compounds through the aqueous pores. Regardless of the compartment model displayed by these compounds, the biological half-life of each compound was significantly shorter in the phenobarbital-treated rats than in the control rats. This effect was attributed to the possible induction effect of phenobarbital on the renal tubular secretion carrier mechanism of these compounds in rats.

Keyphrases □ Phenobarbital—effect on pharmacokinetics of nonmetabolizable organic anions, rats □ Pharmacokinetics—effect of phenobarbital, nonmetabolizable organic anions, rats □ Nonmetabolizable organic anions—effect of phenobarbital on pharmacokinetics, rats

It has been shown in humans and animals that concomitant administration of many metabolizable drugs with phenobarbital results in a decrease in their biological half-lives as well as in pharmacological activity (1, 2). In most instances these effects are attributed to the enzyme-inducing property of phenobarbital which results in the increased rate of metabolism of concomitantly administered drugs. The enzyme-inducing property of phenobarbital is due to its ability to increase the synthesis of drug-metabolizing enzymes in the liver (1–3).

BACKGROUND

Phenobarbital was shown to increase general protein synthesis by its direct action on the hepatic endoplasmic reticulum (4, 5) and to increase the concentration of microsomal phospholipids by reducing their rate of catabolism (6) and/or by increasing their rate of biosynthesis (5). The liver appears to have been extensively investigated for these effects of phenobarbital and, in keeping with the objective of such studies, mainly metabolizable drugs have been used in these investigations. The kidney has been infrequently investigated to show the effect of phenobarbital (7).

In addition to the effect in the liver and kidney, phenobarbital may also increase protein and phospholipid concentrations in other tissues. Since proteins and phospholipids are the major components of tissue plasma membranes, it is conceivable that chronic administration of phenobarbital might alter the transmembrane transport or distribution of certain concomitantly administered drugs into various tissues. Consequently, this may decrease the biological half-lives of these drugs and affect pharmacological responses. This consideration is important because, for a drug to exert pharmacological activity, it must first reach its site(s) of action.

The extent of pharmacological response to a drug is dependent not only on the drug–receptor interaction but also on the drug concentration at the receptor site(s). It is known that the distribution rate of a drug to the site(s) of action is governed by many processes occurring simultaneously; these processes include metabolism, binding to plasma and/or tissue proteins, and renal and nonrenal excretion.

Studies of the effect of phenobarbital on the distribution pharmacokinetics of drugs would be difficult if conducted with concomitantly administered drugs that are subject to metabolism and binding to plasma and/or tissue proteins. These factors may obscure and complicate identification of the effect of phenobarbital on the distribution pharmacokinetic parameters of the drugs.

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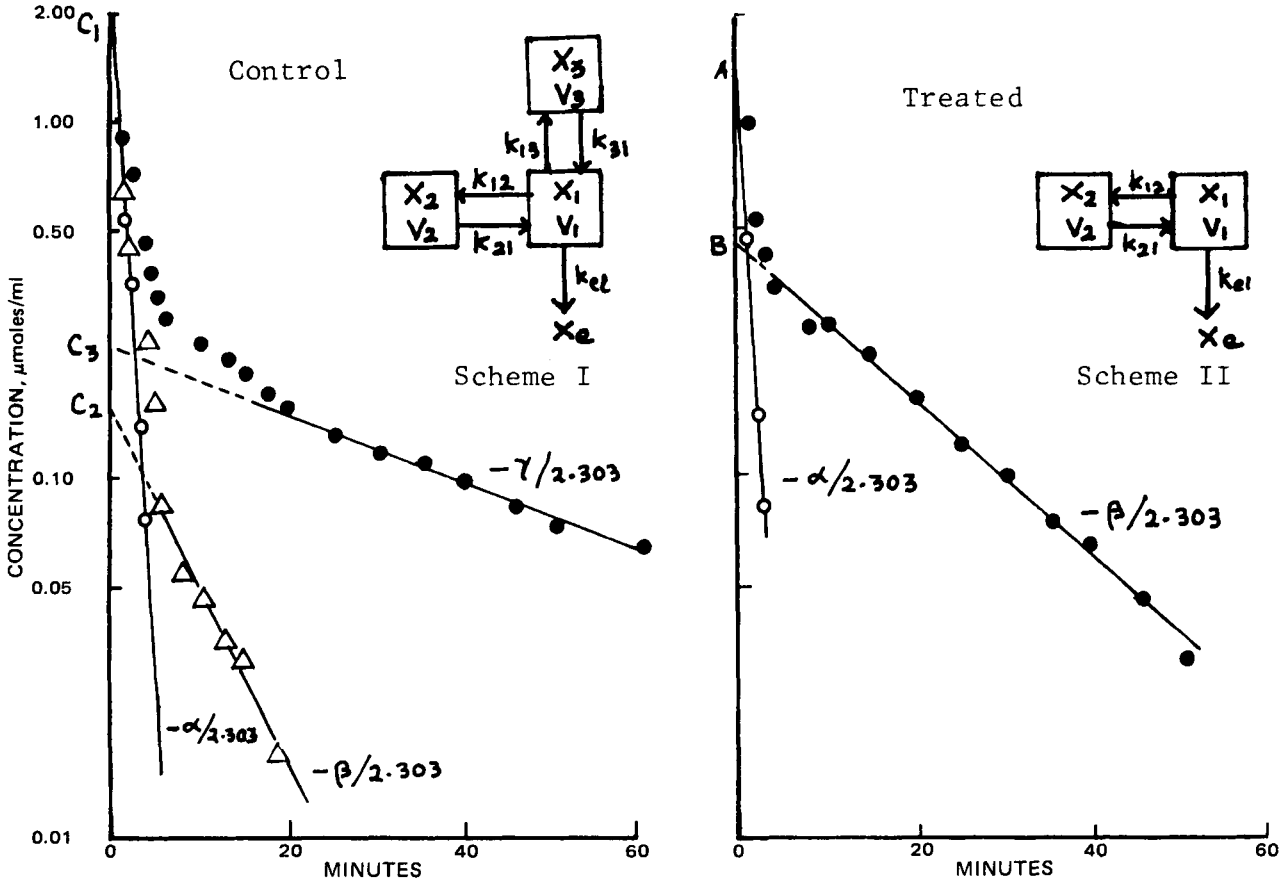

Figure 1—*Semilogarithmic plots showing blood concentrations of benzoylformic acid declining triexponentially in control rats and biexponentially in phenobarbital-treated rats following intravenous administration.*

Scheme I—Three-compartment open model; Scheme II—Two-compartment open model. Key: ●, observed data points; △, ○, data points obtained upon feathering by the residual method.

To demonstrate the effect of phenobarbital on the distribution and biological half-lives of certain compounds, the pharmacokinetics of benzoylformic acid (I), D-(−)-mandelic acid (II), and *p*-methyl benzoylformic acid (III) were studied. These compounds served as model compounds since they are neither metabolized, bound to plasma proteins, nor reabsorbed in the renal tubules. They are excreted in the urine of rats entirely in the unchanged form (8–10). Second, these compounds (pKa 3.3–3.4, referred to in this study as organic anions because they remain completely ionized in the blood) distribute between the body compartments by diffusion through the "aqueous pores" of cell membranes, which presumably are lined with proteins and phospholipids (10). Consequently, it was reasonable to assume that any alteration in the composition of tissue cell membranes might reduce the transmembrane transport of these anions due to a possible decrease in mean pore size. Third, these compounds are represented by three distinctly different compartment models in untreated rats. The pharmacokinetics of I, II, and III have been described (10) by a three-compartment open system, a two-compartment open system, and a one-compartment open system, respectively.

EXPERIMENTAL

Materials—The following were used: D-(−)-mandelic acid[1], mp 131–133°, $[\alpha]_D^{25°}$ − 153°; benzoylformic acid[1], mp 67–69°; *p*-methyl benzoylformic acid, mp 97–99°, synthesized by the method of Kindler *et al.* (11); and sodium phenobarbital[2] USP, granular.

Methodology—Male Sprague–Dawley rats weighing between 170 and 230 g (most weighed ~200 g) were used in the study. The pharmacokinetics of I, II, and III were studied in phenobarbital-treated rats and in control rats. To pretreat the rats, 2 ml of isotonic solution containing a 20-mg/kg dose of sodium phenobarbital was administered intraperitoneally daily to each rat for 5 days in the study of II, 4 days in the study of I, and 3 days in the study of III. The control rat used in each study was pretreated daily with 2 ml normal saline intraperitoneally for 5, 4, and 3 days, respectively.

Food, but not water, was withheld from the rats 12–14 hr prior to the study as well as during the course of the experiment. A given compound was injected intravenously to the rats 24 hr after the last dose of sodium phenobarbital in each study. Five milliliters of normal saline was administered intraperitoneally to each rat 20 min prior to the intravenous administration of the substrate compound being studied to be consistent with previous procedures (8–10). The rat was anesthetized with ether for less than a minute prior to the intravenous injection of a fixed dose of the compound in a 2-ml isotonic solution. The fixed doses of I, II, and III used were 7.5 mg (~250 μmoles/kg), 5 mg (~165 μmoles/kg), and 5 mg (~151 μmoles/kg), respectively. Each compound was injected as its sodium salt by adding equivalent amounts of sodium hydroxide. The solutions were made isotonic with sodium chloride. One blood sample was obtained from each rat upon its decapitation at predetermined time after the administration of the compound. After decapitation, blood was collected in 30-ml beakers which were previously coated with 0.2 ml (40 U) of heparin to prevent coagulation. The blood samples were analyzed on the same day that they were collected.

Because of the voluminous work involved in extracting a compound from numerous blood samples and then analyzing it by GC, blood samples for the pharmacokinetic studies of I and II were collected in two or three portions on two or three different days, each portion involving 5–8 rats. Each portion represented a proper distribution of blood sampling times. For instance, in the control study of II, one study portion included 2-, 4-, 8-, 10-, 15-, 25-, and 50-min blood sampling times and the other study portion included 3-, 5-, 20-, 30-, and 40-min blood sampling times. In the control study of I, one study portion included 2-, 4-, 6-, 8-, 15-, 25-, 45-, and 60-min blood sampling times, the second portion included 1-, 3-, 5-, 10-, and 40-min blood sampling times, and the third study portion included 13-, 18-, 20-, 30-, 35-, and 50-min blood sampling times. For each study portion, a standard curve for the compound was prepared for quantitative determination of the blood samples. In the case of III, all

[1] Aldrich Chemical Co., Milwaukee, Wis.
[2] Merck and Co., Rahway, N.J.

Table I—Pharmacokinetic Parameters for Benzoylformic Acid Estimated and Derived by NONLIN Least-Squares Fitting in Control Rats (Three-Compartment Model) and 4-Day Phenobarbital-Treated Rats (Two-Compartment Model)

Parameter	Control (Three-Compartment)	Treated (Two-Compartment)	Statistical Significance of Difference (p)
V_1, ml/kg	178.95 ± 13.15	151.30 ± 10.35	N.S.
k_{12}, min^{-1}	0.2474 ± 0.0640	0.5312 ± 0.0413	
k_{21}, min^{-1}	0.1821 ± 0.0410	0.2706 ± 0.0139	
k_{13}, min^{-1}	0.1051 ± 0.0241		
k_{31}, min^{-1}	0.0761 ± 0.0281		
K_{el}, min^{-1}	0.1041 ± 0.0083	0.1574 ± 0.0112	<0.001
α, min^{-1}	0.5812	0.9125 ± 0.1465	
β, min^{-1}	0.1068	0.0466 ± 0.0063	
γ, min^{-1}	0.0234 ± 0.0029		
V_2, ml/kg	278.90	297.00 ± 34.25	
V_3, ml/kg	344.65		
V_{ss}, ml/kg	802.50	448.30 ± 35.78	
$K_{el}V_1$, ml/min/kg	18.63 ± 2.02	23.81 ± 2.35	N.S.
$t_{1/2}$ elimination phase, min	29.62 ± 3.67	14.87 ± 2.01	<0.005
Correlation, r	0.997	0.998	

blood samples were obtained in a single study portion since each group of control and phenobarbital-treated rats involved only seven blood samples.

GLC Analysis—I, II, and III were quantitatively determined by GC using a flame ionization detector[3]. These procedures were essentially the same as those described previously (10).

Phenobarbital Pretreatment—*Mandelic Acid (II)*—A 5-day pretreatment schedule for rats with phenobarbital was chosen because numerous literature reports have indicated that a maximum drug metabolizing enzyme induction effect of phenobarbital is produced within ~3 days of pretreatment with a daily 20–100-mg/kg ip dose of phenobarbital. It was felt that if there was an effect of phenobarbital, a 5-day treatment period would be adequate to produce a maximum effect on the pharmacokinetic behavior of II.

Since phenobarbital treatment influenced the pharmacokinetics of II, it was of interest to determine if the maximum effect of phenobarbital on the pharmacokinetics of the compound was produced in a 5-day treatment. Therefore, the pharmacokinetics of II were also studied in rats treated with a daily 20-mg/kg ip dose of phenobarbital for 1 day and for 10 days.

Benzoylformic Acid (I)—It was noted that a substantial effect of phenobarbital on the pharmacokinetics of II could occur in rats even after a 1-day treatment and that a maximum effect occurred after a 5-day treatment. Possibly, the maximum effect of phenobarbital on the pharmacokinetics of the compound might have occurred after treating rats with phenobarbital for less than 5 days. To determine this possibility with I, the pharmacokinetics were studied in 4-day phenobarbital-treated rats. Additional pharmacokinetic studies of the compound were carried out in 6- and 10-day phenobarbital treated rats where each rat received a daily 20-mg/kg ip dose of phenobarbital.

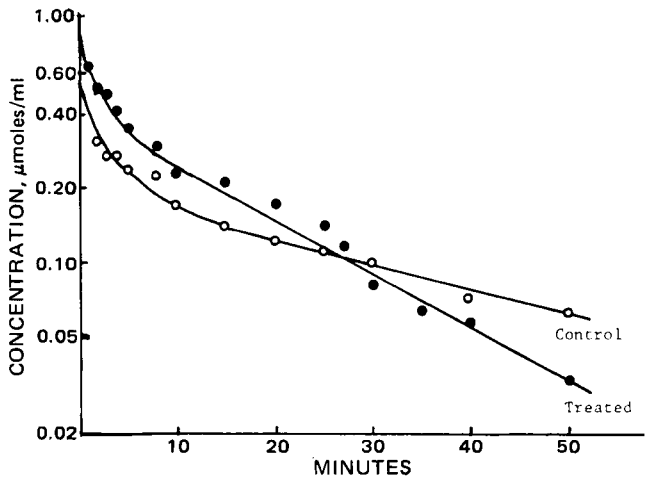

Figure 2—*Biexponential semilogarithmic plots of blood concentrations of mandelic acid obtained in control (○) and phenobarbital-treated (●) rats. The solid lines are the NONLIN least-squares regression lines for the data of respective groups of rats.*

[3] Hewlett-Packard Model 5720.

p-Methyl Benzoylformic Acid (III)—Since the maximum effects of phenobarbital on the pharmacokinetic parameters of II and I were observed in rats after 4 to 5 days of phenobarbital treatment, it was thought possible that the maximum effect of phenobarbital treatment on the pharmacokinetic parameters of III would be observed in less than 4 days of phenobarbital treatment. Therefore, the pharmacokinetics of III were studied in 3-day phenobarbital-treated rats.

To determine if the maximum effect on the pharmacokinetic parameters of the compound was produced after a 3-day phenobarbital treatment, additional pharmacokinetic studies of the compound were carried out in 5-day phenobarbital-treated rats. In that study, each rat also received a daily 20 mg/kg ip dose of phenobarbital.

Overall Urinary Recovery of the Tested Compounds in Phenobarbital-Treated Rats—Studies were done to determine if intravenous doses of II (5 mg), I (7.5 mg), and III (5 mg) are recovered in the urine of phenobarbital-treated rats entirely in the unchanged form. After administering intravenous doses of II, I, and III to rats treated with phenobarbital (20 mg/kg ip) for 5, 4, and 3 days, respectively, urine samples were collected for 12–24 hr for II and I and for 3–5 hr for III.

Binding of Tested Compounds by Blood from Phenobarbital-Treated Rats—The fact that I, II, and III are negligibly bound to whole blood of untreated rats has been demonstrated previously (8, 10). Therefore, the binding of these compounds was studied only with blood from rats treated with phenobarbital for 3–5 days. These determinations were carried out at 37° by the equilibrium dialysis method previously described for these compounds (10). The amount of a given compound used in these studies was calculated on the basis of its apparent distribution volume of the central compartment determined in the phenobarbital-treated rats from the blood level studies described later.

RESULTS

In previous demonstrations (8, 10), all of the intravenously administered doses of I, II, and III were recovered unchanged in untreated rat

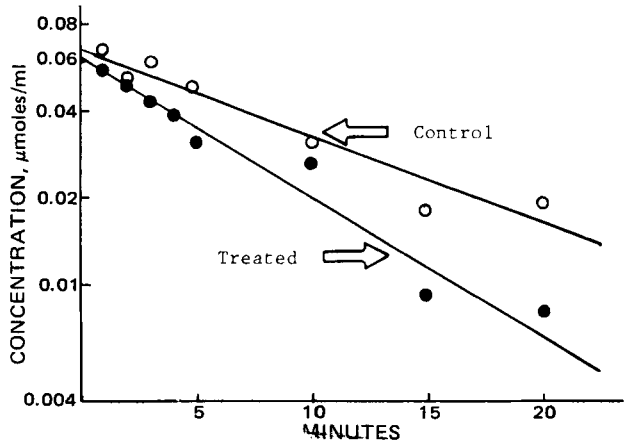

Figure 3—*Monoexponential semilogarithmic plots of blood concentrations of p-methyl benzoylformic acid obtained in control (○) and phenobarbital-treated (●) rats. The solid lines are the least-squares regression lines for the data of respective groups of rats.*

Table II—Comparison of Two-Compartment Model Pharmacokinetic Parameters Determined for Benzoylformic Acid in 4-, 6-, and 10-Day Phenobarbital-Treated Rats

Parameter	Duration of Phenobarbital Treatment		
	4 Days	6 Days	10 Days
V_1, ml/kg	151.30 ± 10.35	145.60 ± 10.80	150.25 ± 10.60
k_{12}, min^{-1}	0.5312 ± 0.0413	0.4593 ± 0.0431	0.3777 ± 0.0563
k_{21}, min^{-1}	0.2706 ± 0.0149	0.2214 ± 0.0109	0.2339 ± 0.0253
K_{el}, min^{-1}	0.1574 ± 0.0112	0.1646 ± 0.0107	0.1571 ± 0.0093
α, min^{-1}	0.9125 ± 0.1465	0.7998 ± 0.0674	0.7176 ± 0.1249
β, min^{-1}	0.0466 ± 0.0063	0.0456 ± 0.0010	0.0512 ± 0.0063
V_2, ml/kg	297.00 ± 34.25	302.05 ± 39.03	242.62 ± 47.85
V_{ss}, ml/kg	448.30 ± 35.78	447.65 ± 40.49	392.87 ± 49.00
$K_{el}V_1$, ml/min/kg	23.81 ± 2.35	23.96 ± 2.36	23.60 ± 2.16
$t_{1/2\beta}$, min	14.87 ± 2.01	15.19 ± 0.33	13.53 ± 1.66
Correlation, r	0.998	0.997	0.994

urine. In the present study, virtually 100% of the dose of each compound administered to phenobarbital-treated rats was recovered unchanged in the urine, indicating negligible metabolism of the compounds in the rats. The urinary recoveries of II, I, and III were 99.13 ± 0.12(n = 3), 99.47 ± 0.3(n = 4), and 98.1 ± 1.0%(n = 5), respectively.

Negligible binding of the tested compounds to plasma proteins in untreated rats has been demonstrated previously (8, 10). From the equilibrium dialysis studies, the binding of these compounds to the whole blood of phenobarbital-treated rats varied from 0.4 to 3.3%, which is negligible.

Pharmacokinetics of I in Control and Phenobarbital-Treated Rats—The blood level data obtained for I in the control and phenobarbital-treated rats were plotted on semilogarithmic graph paper. Since these plots indicated multiexponential concentration decline of the compound, they were resolved into all possible linear exponential segments upon feathering the data by the method of residuals. While the data for the control rats could be resolved into three linear exponential segments, the data for the phenobarbital-treated rats could be resolved into only two linear exponential segments. This indicates that I shows characteristics of a three-compartment model in the control rats (Scheme I, Fig. 1), as was observed previously in saline-untreated rats (10). But I shows characteristics of a two-compartment model in the phenobarbital-treated rats (Scheme II, Fig. 1).Therefore, the blood levels of the compound obtained in the control rats and the phenobarbital-treated rats were analyzed according to the three-compartment model kinetics and two-compartment model kinetics, respectively. As shown in Schemes I and II, the fact that elimination of I occurs from the central compartment only by urinary excretion (10) is asserted by demonstrating that the intravenous dose of the compound is completely recovered in the urine.

In Schemes I and II, X_1, X_2, and X_3 represent the amounts of I at time t in the central compartment, tissue compartment I, the tissue compartment II, respectively; V_1, V_2, and V_3 represent the apparent distribution volume for the compound in the respective compartments. The variable X_e is the amount of the compound excreted in the urine up to time t. The variable K_{el} is the apparent first-order rate constant of elimination of the compound from the central compartment, and k_{12}, k_{21}, k_{13}, and k_{31} are the apparent first-order rate constants for the transfer of the compound between the given compartments.

The general solution for the three-compartment open model is given by the following equation (12):

$$C = C_1 e^{-\alpha t} + C_2 e^{-\beta t} + C_3 e^{-\gamma t} \qquad \text{(Eq. 1)}$$

where C is the concentration of the compound in the blood at any time t. The concentrations in Fig. 1 are normalized on the basis of the intravenous dose of 7.5 mg of I/200 g rat body weight. The preliminary estimates of the intercepts (C_1, C_2, and C_3) and slopes ($-\alpha/2.303$, $-\beta/2.303$, and $-\gamma/2.303$) due to the three linear exponential segments (Fig. 1) were obtained by the least-squares method. Using the preliminary estimates of C_1, C_2, C_3, α, β, and γ, the initial estimates of K_{el}, k_{12}, k_{21}, k_{13}, and k_{31} were obtained using the appropriate well-known equations (12). The preliminary estimate of V_1 was obtained from V_1 = dose/$C_1 + C_2 + C_3$. Using the initial estimates of these rate constants and V_1, the blood level data of the control rats were analyzed by the NONLIN least-squares program (13) and refined estimates of V_1, k_{12}, k_{21}, k_{13}, k_{31}, and K_{el} with their standard deviations and those of α, β, and γ without their standard deviations were obtained. Using the estimated values of these parameters, the values of V_2 and V_3 were calculated using the appropriate equations (12). Values of V_{ss} ($V_1 + V_2 + V_3$), blood clearance or body clearance ($K_{el}V_1$), and elimination phase ($t_{1/2} = 0.693/\gamma$) were calculated. The estimated and derived pharmacokinetic parameters are listed in Table I. The apparent volume of the central compartment (V_1) refers to the blood volume and the fluid volume of highly perfused tissues which are readily accessible to the compound, and V_2 and V_3 refer to the apparent volumes of fluids of the "shallow" and "deep" tissues, respectively.

The general solution for the two-compartment open model is given by the following equation:

$$C = A e^{-\alpha t} + B e^{-\beta t} \qquad \text{(Eq. 2)}$$

where C is the concentration of the compound in the blood at time t. Preliminary estimates of the intercepts (A and B) and slopes ($-\alpha/2.303$ and $-\beta/2.303$) for the two linear exponential segments (Fig. 1) were obtained by the least-squares method. Using the preliminary estimates of A, B, α, and β, the initial estimates of k_{12}, k_{21}, and K_{el} were obtained by using known equations (12). The preliminary estimate of V_1 was obtained from V_1 = intravenous dose/$A + B$. Using the initial estimates of k_{12}, k_{21}, K_{el}, and V_1, the blood level data of I obtained in the phenobarbital-treated rats were analyzed by the NONLIN least-squares program (13) and the refined estimates of V_1, k_{12}, k_{21}, and K_{el} with their standard deviations and those of α and β without their standard deviations were obtained. Using the estimated values of these parameters, V_2 [V_1 (k_{12}/k_{21})] was calculated. The values of V_{ss} ($V_1 + V_2$), elimination phase ($t_{1/2} = 0.693/\beta$), and body clearance ($K_{el}V_1$) were also calculated. The estimated and derived pharmacokinetic parameters of the compound obtained in the 4-day phenobarbital-treated rats are listed in Table I and those obtained in the 6- and 10-day phenobarbital-treated rats are listed in Table II.

Pharmacokinetics of II—The semilogarithmic graphical analysis

Table III—Pharmacokinetic Parameters Estimated and Derived for the Two-Compartment Model by NONLIN Least-Squares Fitting for Mandelic Acid in Control and 5-Day Phenobarbital-Treated Rats

Parameter	Control	Treated	Statistical Significance of Difference (p)
V_1, ml/kg	447.85 ± 15.60	190.15 ± 26.85	<0.001
k_{12}, min^{-1}	0.0535 ± 0.0075	0.1912 ± 0.0479	<0.001
k_{21}, min^{-1}	0.0854 ± 0.0080	0.2542 ± 0.0385	<0.001
K_{el}, min^{-1}	0.0377 ± 0.0021	0.0949 ± 0.0114	<0.001
α, min^{-1}	0.1561 ± 0.0458	0.4913 ± 0.1343	<0.05
β, min^{-1}	0.0206 ± 0.0056	0.0491 ± 0.0095	<0.05
V_2, ml/kg	280.55 ± 48.31	143.00 ± 46.47	<0.05
V_{ss}, ml/kg	728.40 ± 50.77	333.15 ± 53.67	<0.001
$K_{el}V_1$, ml/min/kg	16.88 ± 1.11	18.04 ± 3.34	N.S.
$t_{1/2\beta}$, min	33.64 ± 8.40	14.11 ± 2.73	<0.05
Correlation, r	0.995	0.989	

Table IV—Comparison of Two-Compartment Model Pharmacokinetic Parameters Determined for Mandelic Acid in 1-, 5-, and 10-Day Phenobarbital-Treated Rats

Parameters	Duration of Phenobarbital Treatment		
	1 Day	5 Days	10 Days
V_1, ml/kg	162.35 ± 15.55	190.15 ± 26.85	165.90 ± 11.45
k_{12}, min^{-1}	0.1937 ± 0.0270	0.1912 ± 0.0479	0.2019 ± 0.0191
k_{21}, min^{-1}	0.1120 ± 0.0121	0.2542 ± 0.0385	0.1927 ± 0.0114
K_{el}, min^{-1}	0.0973 ± 0.0095	0.0949 ± 0.0114	0.1093 ± 0.0071
α, min^{-1}	0.3739 ± 0.0713	0.4913 ± 0.1343	0.4580 ± 0.0532
β, min^{-1}	0.0292 ± 0.0036	0.0491 ± 0.0095	0.0460 ± 0.0035
V_2, ml/kg	280.75 ± 56.34	143.00 ± 46.47	173.80 ± 22.73
V_{ss}, ml/kg	443.10 ± 58.43	333.15 ± 53.67	339.70 ± 25.47
$K_{el}V_1$, ml/min/kg	15.79 ± 2.16	18.04 ± 3.34	18.13 ± 1.71
$t_{1/2\beta}$, min	23.73 ± 2.92	14.11 ± 2.73	15.06 ± 1.15
Correlation (r)	0.997	0.989	0.996

Table V—Comparison of the One-Compartment Model Pharmacokinetic Parameters Determined for *p*-Methyl Benzoylformic Acid in Control Rats and in 3- and 5-Day Phenobarbital-Treated Rats

Parameters	Control	Duration of Phenobarbital Treatment		Statistical Significance of Difference (p)	
		3 Days	5 Days	3-Day[a]	5-Day[a]
V_d, ml/kg	222.35 ± 25.85	251.28 ± 29.65	242.47 ± 11.18	N.S.	N.S.
K_{el}, min^{-1}	0.0689 ± 0.0092	0.1053 ± 0.0106	0.1049 ± 0.0047	<0.05	<0.01
$K_{el}V_d$, ml/min/kg	15.33 ± 2.71	26.46 ± 4.10	26.45 ± 1.69	<0.05	<0.01
$t_{1/2}$, min	10.06 ± 1.34	6.58 ± 0.66	6.61 ± 0.29	<0.05	<0.05
Correlation (r)	0.971	0.982	0.993		

[a] Compared to the control.

of blood level data obtained for II both in the control and the phenobarbital rats indicated that blood levels of the compound declined biexponentially (Fig. 2). Therefore, these data were analyzed according to two-compartment model kinetics, with the elimination of the compound occurring from the central compartment. The blood concentrations in Fig. 2 are normalized on the basis of the 5 mg iv dose/200 g rat body weight; the blood level data were treated with Eq. 2. The preliminary estimates of the intercepts (A and B) and slopes ($-\alpha/2.303$ and $-\beta/2.303$) due to the two resolved linear exponential segments were obtained by the least-squares method. Using the estimates of A, B, α, and β, the initial estimates of V_1, k_{12}, k_{21}, and K_{el} were obtained by the method described previously. The refined estimates of V_1, k_{12}, k_{21}, and K_{el} with their standard deviations and those of α and β without their standard deviations were obtained using the NONLIN least-squares program (13). Using computer estimated values of these parameters, the estimates of V_2, V_{ss}, elimination phase ($t_{1/2}$), and $K_{el}V_1$ were calculated. The estimated and derived pharmacokientic parameters of II obtained in the control rats and the 5-day phenobarbital-treated rats are listed in Table III and those obtained in the 1-day and 10-day phenobarbital-treated rats are listed in Table IV.

Pharmacokinetics of III—The blood levels of III in both the control rats and the phenobarbital-treated rats declined monoexponentially (Fig. 3), indicating a one-compartment open model. The concentrations in Fig. 3 are normalized on the basis of the 5 mg iv dose of III/200 g rat body weight. The blood level data were treated according to the following equation:

$$\log C = \log C_0 - K_{el}t/2.303 \qquad \text{(Eq. 3)}$$

where C is the concentration of the compound at time t, C_0 is the concentration of the compound at zero time, and K_{el} is the apparent first-order rate constant of elimination of the compound. Values of C_0 for the compound were determined from the intercepts obtained by extrapolating the respective least-squares lines to zero time (Fig. 3). The volumes of distribution and the biological half-lives ($t_{1/2}$) were calculated according to the equations V_d = intravenous dose/C_0 and $t_{1/2} = 0.693/K_{el}$. The value of K_{el} was calculated from the slope ($-K_{el}/2.303$) of the least-squares lines. The pharmacokinetic parameters thus determined for the compound in the control rats as well as in the 3- and 5-day phenobarbital-treated rats are listed in Table V. The body clearance ($K_{el}V_d$) of the compound was also calculated.

Statistical Considerations and Treatment—Since only one blood sample was obtained from a rat at a given time following the intravenous administration of the compound under study, each data point in Figs. 1–3 and other pharmacokinetic studies represents the concentration of the compound in a single rat. This procedure was the same as that employed in previous pharmacokinetic studies (10) of these compounds in saline-untreated rats where the correlation coefficient observed between the concentration and time values was >0.991. There were reasons for using this procedure, instead of performing the study in a single rat by collecting the blood samples over the duration of the study following intravenous administration of a compound.

If a complete pharmacokinetic study of a compound were performed in a single rat, the total volume of blood that would have been withdrawn would have amounted to as high as 6 ml. The sensitivity of the assay procedures for these compounds at low intravenous doses would have necessitated the withdrawal of 0.2–0.3 ml of blood in each sampling during the initial period and 0.5–0.7 ml of blood in each sampling during the later period of the kinetic study. This would have been true especially in the case of I and II which confer upon the body the characteristics of multicompartment models. Withdrawal of such a high blood volume (about 40% of the total blood volume of a 200 g rat) would have been undesirable. In conjunction with the blood level pharmacokinetic studies carried out previously (10), the pharmacokinetics of these compounds were also studied from urinary excretion data (14) by performing (unlike in the blood level studies) complete pharmacokinetic studies in individual rats, since the sensitivity of the same assay procedure used for blood samples was not prohibitive in the quantitative determination of the amounts of compounds excreted in the urine. In these urinary excretion studies (14) a minimum subject-to-subject variation was observed in the excretion rates and elimination phase half-lives of these compounds, thereby supporting the assumption that rats showed minimum variations in the blood concentrations and half-lives of the compounds.

Therefore, in each pharmacokinetic study, a single blood concentration value of the compound was obtained per rat per time with the basic assumptions of the regression model that the single concentration variate corresponding to a given time is independently and normally distributed and the samples along the regression line have a common variance (15). The high correlation coefficient values between the two variates, concentration and time, support this assumption.

The standard deviation values indicated in Tables I–IV for the pharmacokinetic parameters V_1, k_{12}, k_{21}, k_{13}, k_{31}, and K_{el} of I and V_1, k_{12}, k_{21}, and K_{el} of II are the computer estimated values. According to the NONLIN least-squares program, the computer also estimated the values of α, β, and γ, but without their standard deviations. The standard deviations of these parameters and other derived pharmacokinetic parameters of I and II were estimated by the following procedures:

To estimate the standard deviation of β, the data points of a given study obtained exclusively in the elimination β-phase were plotted according to the equation $\ln C = \ln B - \beta t$. The standard deviation ($\hat{\sigma}c, t$) about the least-squares regression line thus obtained was estimated by the following formula (16):

$$\hat{\sigma}c,t = \left[\frac{\Sigma(\ln C)^2 - \frac{(\Sigma \ln C)^2}{n} - \beta^2\left\{\Sigma t^2 - \frac{(\Sigma t)^2}{n}\right\}}{n-2}\right]^{1/2}$$

The standard deviation of the slope ($\hat{SD}\beta$) was then calculated by the formula (16):

$$\hat{SD}\beta = \frac{\hat{\sigma}c,t}{\sqrt{\Sigma t^2 - \frac{(\Sigma t)^2}{n}}}$$

The standard deviations of α ($= K_{el}k_{21}/\beta$), V_2 ($= k_{12}V_1/k_{21}$), body clearance ($BC = K_{el}V_1$), V_{ss} ($= V_1 + V_2$), and $t_{1/2\beta}$, (0.693/β) were estimated by the general approximate formulas (17, 18):

$$\sigma\alpha = [\{(\sigma K_{el}/K_{el})^2 + (\sigma k_{21}/k_{21})^2 + (\sigma\beta/\beta)^2\}(\alpha)^2]^{1/2}$$

$$\sigma V_2 = [\{(\sigma k_{12}/k_{12})^2 + (\sigma V_1/V_1)^2 + (\sigma k_{21}/k_{21})^2\}(V_2)^2]^{1/2}$$

$$\sigma BC = [\{(\sigma K_{el}/K_{el})^2 + (\sigma V_1/V_1)^2\}(BC)^2]^{1/2};\ \sigma V_{ss} = [\sigma V_1^2 + \sigma V_2^2]^{1/2}$$

and

$$\sigma t_{1/2\beta} = 0.693 \cdot \sigma\beta/\beta^2$$

In the case of I a direct comparison of V_2, V_3, α, and β, the pharmacokinetic parameters of a three-compartment model in control rats, cannot be made with V_2, α, and β, the parameters of a two-compartment model in phenobarbital-treated rats. For this reason, and because of the complex equations from which V_2 and V_3 are obtained (12) for the three-compartment model, no attempt was made to determine the standard deviations of V_2, V_3, α, and β for I in control rats. However, because it is appropriate to compare the three-compartment parameters γ, $t_{1/2\gamma}$, and body clearance ($K_{el}V_1$) with the equivalent two-compartment parameters β, $t_{1/2\beta}$ and body clearance ($K_{el}V_1$), respectively, the standard deviations of these parameters were determined as described and used for determining statistical differences between the corresponding parameters (Table I).

In the case of III, which followed one-compartment model kinetics, the standard deviation of K_{el} was obtained as follows. First, the standard deviation ($\hat{\sigma}c,t$) about the least-squares regression line from the plot of ln$C$ *versus* t was obtained by (16):

$$\hat{\sigma}c,t = \left[\frac{\Sigma(\ln C)^2 - \frac{(\Sigma \ln C)^2}{n} - K_{el}^2\left\{\Sigma t^2 - \frac{(\Sigma t)^2}{n}\right\}}{n-2}\right]^{1/2}$$

The standard deviation of the slope ($\hat{SD}_{K_{el}}$) was then calculated from (16):

$$\hat{SD}_{K_{el}} = \frac{\hat{\sigma}c,t}{\sqrt{\Sigma t^2 - \frac{(\Sigma t)^2}{n}}}$$

The standard deviation of concentration of the compound at zero time (C_0) was estimated from the standard deviation of the slope of a least-squares regression line obtained by plotting C *versus* $e^{-K_{el}t}$ according to the equation $C = C_0e^{-K_{el}t}$.

For the three compounds used in the study, statistically significant differences between corresponding pharmacokinetic parameters obtained in the control and phenobarbital-treated rats were determined using t test statistics at $p \leq 0.05$.

DISCUSSION

Benzoylformic Acid (I)—The three-compartment model noted for I with two kinetically distinguishable peripheral tissue compartments in the control rats was reduced to a two-compartment model with one kinetically distinguishable peripheral tissue compartment in the 4-day phenobarbital-treated rats as well as in 6- and 10-day phenobarbital-treated rats. The pharmacokinetic parameters determined for the compound in 6- and 10-day phenobarbital-treated rats (Table II) were very similar to those determined in 4-day phenobarbital-treated rats. This indicates that the maximum effects of phenobarbital treatment on the pharmacokinetic parameters of the compound were indeed produced after a 4-day treatment with phenobarbital.

Since different compartment models are observed in the control and phenobarbital-treated rats, V_1, K_{el}, γ, $k_{el}V_1$, and $t_{1/2}$ elimination phase are the only pharmacokinetic parameters obtained for the compound in control rats which may be compared with the corresponding pharmacokinetic parameters in the phenobarbital-treated rats. It was observed that K_{el} and β of I in phenobarbital-treated rats were significantly greater than K_{el} and γ in the control rats. The $t_{1/2}$ of the compound in phenobarbital-treated rats was significantly shorter than that in control rats. Although there was a tendency for V_1 to decrease and $K_{el}V_d$ to increase in the phenobarbital-treated rats, these changes were not significant ($p = 0.05$) (Table I).

Mandelic Acid (II)—Table III shows that, except for $K_{el}V_1$, all pharmacokinetic parameters of II in the phenobarbital-treated rats were significantly different from those in the control rats. The parameters V_1, V_2, V_{ss}, and $t_{1/2\beta}$ decreased, and K_{el}, α, and β increased in the phenobarbital-treated rats compared with the corresponding parameters in the control rats.

The comparison of the pharmacokinetic parameters (Table IV) of the compound in the 1- and 10-day phenobarbital-treated rats with those of the 5-day treated rats indicated that the effects of phenobarbital treatment are seen on certain parameters even after a 1-day treatment with phenobarbital, but the maximum effects on all parameters were certainly obtained after a 5-day phenobarbital treatment. The pharmacokinetic parameters obtained in the 10-day phenobarbital-treated rats were not significantly different from those obtained in the 5-day phenobarbital-treated rats. Some of the pharmacokinetic parameters noted in the 10-day phenobarbital-treated rats are very similar to those in the 5-day phenobarbital-treated rats.

***p*-Methyl Benzoylformic Acid (III)**—Table V shows that the pharmacokinetic parameters of III from the 5-day phenobarbital-treated rats were very similar to those obtained in the 3-day phenobarbital-treated rats, indicating that the maximum pharmacokinetic effects were produced in 3-day phenobarbital-treated rats.

Except for V_d, all pharmacokinetic parameters of III determined in 3-day phenobarbital-treated rats were significantly different from those determined in the control rats. The values of K_{el} and $K_{el}V_d$ increased and $t_{1/2}$ decreased in phenobarbital-treated rats as compared with control rats.

Effects of Phenobarbital Treatment on the Pharmacokinetic Parameters of the Compounds—The effect of phenobarbital treatment on some pharmacokinetic parameters of the compounds studied seemed to depend on whether the compound conferred the characteristics of a one-, two-, or three-compartment model on the body. The effect on some other pharmacokinetic parameters appeared to be model independent. These effects are as follows:

1. The biological half-lives ($t_{1/2}$) of all compounds were significantly shorter in the phenobarbital-treated rats than those in the control rats.
2. The elimination rate constants (K_{el}) of the compounds from the central compartment were significantly greater in phenobarbital-treated rats than in the control rats.
3. The disposition rate constants (β) in phenobarbital-treated rats, which are a function of elimination and distribution of the compounds, were significantly greater than the corresponding disposition rate constants in the control rats (γ in the case of I and β in the case of II).
4. The distributive phases observed for I and II were shorter in the phenobarbital-treated rats than in the control rats.
5. A reduction in the apparent distribution volumes of the peripheral tissue compartments for I in phenobarbital-treated rats became evident by the fact that a three-compartment model in the control rats was reduced to a two-compartment model in the phenobarbital-treated rats. In the case of II (which displayed two-compartment model characteristics in both control and phenobarbital-treated rats), the apparent distribution volume of the peripheral tissue compartment (V_2) was significantly smaller in the phenobarbital-treated rats than that in the control rats.
6. The apparent distribution volume of the central compartment (V_1) of II was significantly smaller in the phenobarbital-treated rats than in the control rats. However, V_1 of I (three-compartment in control rats) and V_d of III observed in the phenobarbital-treated rats were not found to be significantly different from those observed in the control rats.
7. The body clearance ($K_{el}V_d$) of III was significantly greater in phenobarbital-treated rats than in the control rats. In the case of I and II, although there was a tendency for body clearance ($K_{el}V_1$) to be greater in the phenobarbital-treated rats, such increases were not significantly different, most probably because the increase in K_{el} was offset by a simultaneous decrease of V_1.

Thus, the major effects produced by the phenobarbital treatment were to shorten the biological half-lives of the compounds used, irrespective of the pharmacokinetic model displayed, and to reduce the apparent distribution volumes of the peripheral tissue compartments of the compounds which displayed multicompartment model pharmacokinetics.

The known *in vivo* effects of phenobarbital treatment are to induce drug metabolizing microsomal enzymes (1, 3), to increase the bile flow (19, 20), and increase the liver (21) and kidney (7) blood flows. These effects potentially can shorten the biological half-lives of compounds, but since the compounds used in this study were not metabolized and were excreted in the urine entirely in the intact form by the phenobarbital-treated rats, the enzyme induction effect was not expected to influence their biological half-lives. The increase in the biliary flow and liver blood flow was also expected to have little or no effect on the kinetic dispositions of these compounds. Since the increased blood flow to the kidney is compensated by the autoregulation of the kidney to yield a constant blood flow to the glomerulus (7), no effect on the glomerular filtration rates of the compounds is expected. However, recent reports (7) have shown that the renal tubular secretion rates of *p*-aminohippuric acid increased in phenobarbital-treated rats. This effect was attributed to the increase in the renal tubular transport system available for the compound in rats. Since I, II, and III are secreted by the renal tubules of rats (9, 22), the contribution of this factor in increasing K_{el} of the compounds or in shortening their biological half-lives is possible.

The effects of phenobarbital treatment on the distribution pharmacokinetic parameters of the compounds that displayed multicompartment pharmacokinetics appear to be consistent with the hypothesis that phenobarbital treatment of rats may also increase the concentration of proteins and phospholipids in the membranes of tissue cells other than those of liver and kidney. Consequently, the rate of diffusion of the ionic species of the compounds through the aqueous pores of the tissue cell membranes decreases. One mechanism which has been widely recognized (23–29) for transmembrane transport of solutes is diffusion through aqueous pores. Previous pharmacokinetic studies (10, 22) of I, II, and several of their *para*-alkylated homologs, showed that the alkyl groups in these model organic anions decreased the apparent distribution volumes of the peripheral tissue compartments in rats, instead of increasing such distribution volumes as might have been expected due to the presence of lipophilic alkyl groups. Therefore, it was proposed (10, 22) that the distribution of these organic anions from the central compartment to the peripheral tissue compartments occurs mainly through aqueous pores of the tissue cell membranes.

No direct evidence has been obtained to show that the concentration of proteins and phospholipids of various tissue cell membranes increased resulting in reduction of the aqueous pore size of the membranes. However, the rationalization of the results of this study on the basis of the following considerations supports this possible effect of phenobarbital treatment in rats. It was previously indicated(10, 22) that the anionic forms of the compounds studied interact with the components (proteins and phospholipids) of the lining of aqueous pores while diffusing through them by means of intermolecular forces such as electrostatic interaction, hydrogen bonding, and hydrophobic bonding. By considering the slight differences in the chemical structures of these anions, the variations in the composition of cell membranes of various tissue cells (30), the possible differences in the degree of intermolecular interactions with aqueous pore lining, and the heteroporosity (31–33) of the membranes, the existence of a three-compartment open model for I and a two-compartment open model for II was explained (10).

It is also recognized that the membranes of most organs are heteroporous, with pore size ranging from 8 to 320 Å, with a mean pore size range of 70–120 Å (31–33), and that only about one-fourth of the total pore size constitutes the effective space for the diffusion of solute molecules through a pore (34). Therefore, the possible phenobarbital treatment induced reduction in the aqueous pore size would be expected to cause greater intermolecular interactions of the anions with the constituents of the pore lining and cause a decrease in the penetration of the anions into the tissue. This may decrease the apparent distribution volume occupied by the anions in that tissue.

The two peripheral tissue compartments noted for I in the control rats, were thought to be due to those organs whose aqueous pores exhibit weak hydrogen bonding with the I molecules and due to those organs whose aqueous pores exhibit strong hydrogen bonding with the I molecules (10, 22). In the phenobarbital-treated rats, only one peripheral tissue compartment was kinetically distinguishable. This may be due to the phenobarbital affecting the size of aqueous pores in the two tissue compartments such that the strengths of hydrogen bonding of the I anions with the aqueous linings of one tissue compartment were similar to those of the other tissue compartment.

It may be pointed out that the decrease in the distribution volumes observed in the phenobarbital-treated rats is for the compounds that display multicompartment characteristics and exist in the blood in the ionized form. Such changes in the distribution volume may not be observed for multicompartment model compounds that exist in the blood in the nonionized form. These compounds generally have greater membrane solubility and are expected to diffuse through the entire membrane surface of which aqueous pores constitute only a fractional surface area. Furthermore, if the shortening of the biological half-lives of these compounds is due to the stimulation by phenobarbital of their renal tubular secretion mechanism, changes in the biological half-lives may not be observed for compounds not involved in renal tubular secretion but are excreted in the urine due to glomerular filtration. Studies with model drugs supporting these hypotheses are presented elsewhere (35).

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ACKNOWLEDGMENTS

Abstracted in part from a dissertation submitted by D. J. Szymanski to the Graduate School, Wayne State University, in partial fulfillment of the Doctor of Philosophy degree requirements.