Effects of Phenobarbital on the Distribution Pharmacokinetics and Biological Half-Lives of Model Nonmicrosomal Enzyme Metabolizable Sulfonamides in Rats

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Abstract □ The pharmacokinetics of sulfisoxazole and sulfanilamide were studied in control rats and in rats treated for 5 days with a daily 100 mg/kg ip dose of phenobarbital. These drugs represent the organic anionic and nonionized drugs, respectively, whose nonmicrosomal enzymatic metabolisms were unstimulated by phenobarbital. Sulfisoxazole showed the characteristics of a two-compartment open model. However, its biological half-life and the apparent distribution volume of the central compartment were significantly lower and the intercompartmental transport rate constants and the urinary excretion rate constant were significantly greater, in phenobarbital treated rats than in control rats. The apparent steady-state distribution volume of sulfisoxazole was smaller in the phenobarbital treated rats at the 90% confidence level. Sulfanilamide showed characteristics of a one-compartment model in both the control and phenobarbital treated rats, but none of the pharmacokinetic parameters of the compound in the phenobarbital treated rats were significantly different from those in the control rats.

Keyphrases □ Phenobarbital—effect on pharmacokinetics of sulfisoxazole and sulfanilamide □ Pharmacokinetics—effect of phenobarbital on sulfisoxazole and sulfanilamide □ Sulfisoxazole—effect of phenobarbital on pharmacokinetics □ Sulfanilamide—effect of phenobarbital on pharmacokinetics □ Sulfonamides—effect of phenobarbital on the pharmacokinetics of sulfisoxazole and sulfanilamide

In a previous paper (1), it was reported that phenobarbital treatment in rats caused a decrease in the apparent distribution volumes of nonmetabolizable model organic anions which gave the body characteristics of multicompartment open models. Regardless of the model followed by the body (one-compartment or multicompartment model), the half-lives of these compounds were shorter in phenobarbital treated rats than in control rats.

BACKGROUND

It is recognized that all drugs do not possess the ideal properties described for the organic anions previously reported (1). Drugs usually are subject to metabolism and binding to plasma proteins and remain in the blood in the ionized and/or nonionized forms. Therefore, the influences of phenobarbital treatment on the distribution volumes of organic anionic drugs which are metabolized and bound to plasma proteins were investigated. However, as an extension of previous work (1), the effect of phenobarbital treatment on the apparent distribution volumes and biological half-lives of drugs, which are metabolized and bound to plasma proteins but whose metabolism and extent of protein binding will not be affected by the microsomal enzymes induced by phenobarbital, were studied. The organic anionic drug chosen was sulfisoxazole (pKa 5.1) and the nonionized drug chosen was sulfanilamide (pKa 10.1). Sulfisoxazole and sulfanilamide are metabolized mainly by acetylation by the nonmicrosomal enzymes (2), which apparently are not induced by phenobarbital treatment. Both the drugs and their metabolites are excreted by the kidney, thereby avoiding the possible influence of increased biliary flow brought about by phenobarbital treatment (3). The protein binding of sulfisoxazole is not affected by phenobarbital treatment (4). Furthermore, while sulfisoxazole is involved in renal tubular secretion, sulfanilamide is excreted in the urine due to glomerular filtration (5).

It was stated previously (1) that the apparent distribution volumes of organic anions, which give the body the characteristics of a multicompartment open model, are likely to be lower in phenobarbital treated rats than in control rats, possibly due to reduction in the aqueous pore size of tissue cell membranes brought about by phenobarbital. It was also postulated that, if the biological half-lives of compounds are shortened in treated rats due to the stimulatory effect of phenobarbital on the renal tubular secretion mechanism (1), then such effects would be expected only for compounds involved in renal tubular secretion and not those excreted in the urine due to glomerular filtration. Therefore, the apparent distribution volume(s) and biological half-life of sulfisoxazole should be reduced in the phenobarbital treated rats but not those of sulfanilamide.

EXPERIMENTAL

Materials—Sulfanilamide[1] (mp 166°), sulfisoxazole[2] (mp 198°), and phenobarbital sodium[1] were USP grade. The other chemical agents used were analytical reagent grade.

Methodology—Male Sprague-Dawley rats weighing between 175 and 210 g (most weighed ~200 g) were used in the study.

Overall urinary excretion studies of the sulfonamides were conducted to determine the effect of phenobarbital treatment on the extent of their metabolism and to select the phenobarbital pretreatment regimen. For the urinary excretion studies of sulfanilamide, 20, 50, and 100-mg/kg ip doses were tried; a given dose was administered to each rat daily for 5–10 consecutive days. The intraperitoneal doses of phenobarbital were dissolved in 5 ml of normal saline. Each control rat was treated with 5 ml normal saline for similar periods of time as the phenobarbital treated rats. Twenty-four hours after the last treatment dose of phenobarbital or normal saline, each rat received a 10-mg iv dose of sulfanilamide *via* the tail vein. The intravenous solution of sulfanilamide (2 ml) was prepared by dissolving 10 mg of the drug in water for injection, adjusting the pH of the solution to 7.4 with sodium hydroxide, and rendering the solution isotonic with sodium chloride. Food was withheld from the rats for 12–14 hr prior to the intravenous administration of the drug and during the study. The rats were anesthetized with ether for ~2 min when the drug was administered. After administering the drug, rats are transferred to urine collection cages described previously (6). Urine samples were carefully collected over the periods of 0–8 and 8–35 hr. These urine samples were analyzed for intact sulfanilamide and its metabolites. The phenobarbital treatment schedule selected was a daily 100-mg/kg ip dose for 5 consecutive days.

Overall urinary excretion studies were also conducted for sulfisoxazole in control rats and rats treated with a daily dose of 100 mg/kg of phenobarbital for 5 consecutive days. The 2 ml of pH 7.4, isotonic solution containing 7 mg of sulfisoxazole was injected intravenously *via* the tail vein to each rat 24 hr after the last dose of phenobarbital or normal saline. Urine samples were collected over a period of 0–10 and 10–52 hr and analyzed for intact sulfisoxazole and its metabolites.

In the sulfanilamide pharmacokinetic study, three sets of studies were carried out on three different days. The first two sets of studies involved six control rats and six phenobarbital treated rats, and the third set involved only 6 phenobarbital treated rats. The third study was carried out to reinforce the finding of the first two sets of studies. In each set, only one blood sample was obtained from a given rat following decapitation at the predetermined time after intravenous administration of sulfanilamide. Blood samples were collected at 0.25, 0.5, 1, 2, 3, and 4 hr from both control and phenobarbital treated rats. The procedure of obtaining one

[1] Merck and Co., Inc., Rahway, N.J.
[2] National Biochemical Corp., Cleveland, Ohio.

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Table I—Amounts of Intact Sulfanilamide and Its Metabolite(s) Recovered in the Urine in 35 hr Following Intravenous Administration of a 10-mg Dose of Sulfanilamide to Control Rats and Phenobarbital Treated Rats

	Phenobarbital Dose, mg/kg/day	Phenobarbital Treatment, days	Intact, mg	Metabolite(s), mg[a]
		Control		
1	—	—	3.62	6.38
2	—	—	3.61	6.06
3	—	—	3.15	6.01
4	—	—	3.10	6.61
5	—	—	2.92	6.33
6	—	—	3.15	6.38
7	—	—	2.96	6.84
8	—	—	3.08	6.93
9	—	—	2.80	7.00
Mean ± *SD*			3.16 ± 0.28	6.50 ± 0.36
		Phenobarbital Treated		
1	20.0	5	3.40	6.38
2	20.0	5	3.22	6.78
3	20.0	10	3.21	6.69
4	20.0	10	3.29	6.45
5	20.0	10	3.18	6.17
6	50.0	5	2.91	6.33
7	50.0	5	2.71	6.29
8	100.0	5	2.69	6.01
9	100.0	5	2.74	5.96
10	100.0	5	2.85	6.27
11	100.0	5	3.00	5.57
12	100.0	5	2.85	7.05
13	100.0	5	2.84	6.97
Mean[b] ± *SD*			2.82 ± 0.11[c]	6.30 ± 0.59[c]

[a] Expressed as the equivalent amount of sulfanilamide. [b] Mean based on data obtained in rats pretreated with 100 mg/kg/day of phenobarbital for 5 consecutive days. [c] Not significantly different from the corresponding values of the control.

blood sample per rat was described previously for other compounds (1).

In the sulfisoxazole pharmacokinetic study, two sets of studies were carried out on two different days, each set involving 12 control and 12 phenobarbital treated rats. In each set, only one blood sample was obtained from a given rat after decapitation at the predetermined time after the intravenous administration of sulfisoxazole. Blood samples were collected at 0.083, 0.166, 0.25, 0.5, 0.75, 1, 2, 3, 4, 5, 6, and 8 hr from both control and phenobarbital treated rats.

The reason multiple concentration values of a sulfonamide were obtained at a given time period was to further ascertain the assumption of the regression model: each concentration variate corresponding to the given value of time is a sample from a population of independently and normally distributed variates and that the samples along the regression line have a common variance (7).

After decapitation the blood sample from a rat was collected in a 30-ml beaker coated with 0.2 ml (40 U) of heparin to prevent coagulation. Blood samples were analyzed for the intact and total (intact plus metabolites) sulfonamide on the same day they were collected.

Assay of Sulfanilamide and Sulfisoxazole—A general method used for the analysis of a sulfonamide, first developed by Bratton and Marshall (8), was to quantitate intact sulfanilamide or sulfisoxazole and the total (intact plus metabolites) sulfanilamide or sulfisoxazole present in the urine or blood samples with minor modifications. The absorbance of the complex formed between free sulfonamide and Bratton–Marshall reagent was measured on a spectrophotometer[3] at 540 nm for sulfanilamide and 545 nm for sulfisoxazole.

An accurately measured blood sample (1.0–1.5 ml) was transferred to a 150-ml beaker, diluted with 30 ml of water, stirred for 1 min, and the proteins and blood cells precipitated by dropwise addition of 8 ml of 15% trichloroacetic acid. The precipitate was allowed to settle for 2 min and 5 ml of the supernate was transferred to a 10-ml test tube. Sodium nitrite solution (1 ml, 0.1% w/v) was added, and the contents were mixed for 2 sec. Then 1 ml of 0.5% (w/v) sulfamic acid solution was added to the mixture and stirred for 10 sec. Following this, 1 ml of a 0.1% (w/v) Marshall reagent solution was added to the mixture, and it was stirred again for 10 sec. After allowing this mixture (total volume 8.5 ml) to stand for 5 min, the colored solution was filtered and the absorbance of the complex

[3] Beckman Model 24 Spectrophotometer.

Table II—Amounts and Fractions of Intact Sulfisoxazole (f_I) and Its Metabolite (f_m) Recovered in the Urine in 52 hr Following the Intravenous Administration of a 7-mg Dose of Sulfisoxazole to Control Rats and Phenobarbital Treated Rats

	Intact, mg	Metabolite, mg[a]	f_I	f_m
		Control		
1	6.59	0.33	0.95	0.05
2	6.42	0.56	0.92	0.08
3	6.34	0.67	0.90	0.10
4	6.12	0.88	0.87	0.13
5	6.15	0.78	0.89	0.11
Mean ± *SD*	6.32 ± 0.19	0.64 ± 0.21	0.91 ± 0.03	0.09 ± 0.03
		Phenobarbital Treated		
1	5.59	0.98	0.85	0.15
2	6.07	0.87	0.87	0.13
3	6.17	0.83	0.88	0.12
4	6.17	0.43	0.93	0.07
5	6.23	0.73	0.89	0.11
Mean ± *SD*	6.05 ± 0.26	0.77 ± 0.21	0.88 ± 0.03[b]	0.12 ± 0.03[b]

[a] Expressed as the equivalent amount of sulfisoxazole. [b] Not significantly different from the corresponding values of the control.

of the sulfonamide formed was measured at the appropriate wavelength.

The amounts of intact sulfanilamide or sulfisoxazole excreted in the urine samples were determined by the procedure described for blood samples, except that protein precipitation was unnecessary for the urine samples.

The total (intact plus metabolites) amount of sulfanilamide or sulfisoxazole excreted in the urine samples was determined after hydrolyzing the conjugated metabolites of the sulfonamide by heating the samples in the boiling water bath for 2 hr with 4 *N* HCl. The total sulfonamide was then analyzed according to the procedure described above for the intact sulfonamide.

The amount of free sulfonamide present in the unhydrolyzed urine or blood sample or the hydrolyzed urine sample was calculated with a calibration curve prepared for the respective sulfonamide. To prepare a calibration curve, known quantities of a sulfonamide were added to 1-ml volumes of blood obtained from rats not treated with a sulfonamide. The standard blood samples were treated in the same way as the actual blood samples, and their absorbance values were measured on a spectrophotometer at appropriate wavelength.

The absorbance for a given amount of sulfonamide contained in 1 ml of blood or 1 ml of water was identical after treatment by the Bratton–Marshall procedure, indicating that the residual amount of the blood constituents present in the supernate of the blood samples did not contribute to the absorbance. Therefore, the calibration curve of a sulfonamide was prepared from its aqueous solution without involving blood.

The main metabolite of sulfanilamide or sulfisoxazole was reported to be the acetylated form (9, 10). The amount of metabolite (in terms of the equivalent amount of intact sulfonamide) was calculated by subtracting the amount of free sulfonamide in the unhydrolyzed urine samples from the total amount of free sulfonamide in the hydrolyzed urine samples.

pH Determination of Cumulative Urine Samples—Each of four rats was treated with 5 ml normal saline for 5 days, and each of eight rats was treated with a 100-mg/kg ip dose of phenobarbital (in 5 ml normal saline) for 5 days. Each rat received 2 ml of pH 7.4 normal saline solution *via* the tail vein 24 hr after the last treatment dose of phenobarbital or normal saline. Cumulative urine samples were then collected separately from each rat for 7 hr. The pH of each cumulative urine sample was measured with a laboratory pH-meter. The urine pH of control rats varied from 6.35 to 6.50 and that of phenobarbital treated rats varied from 6.28 to 6.45.

RESULTS

Urinary Excretion Data for Sulfanilamide and Sulfisoxazole—The overall urinary excretion data obtained following the intravenous dose of sulfanilamide to the control and phenobarbital treated rats are shown in Table I. Virtually the entire dose of sulfanilamide was recovered in the urine in the intact and the metabolic forms in 35 hours for both the control and phenobarbital treated rats. All excretable intact compound was excreted in the urine in 8 hr and all excretable metabolite(s) were

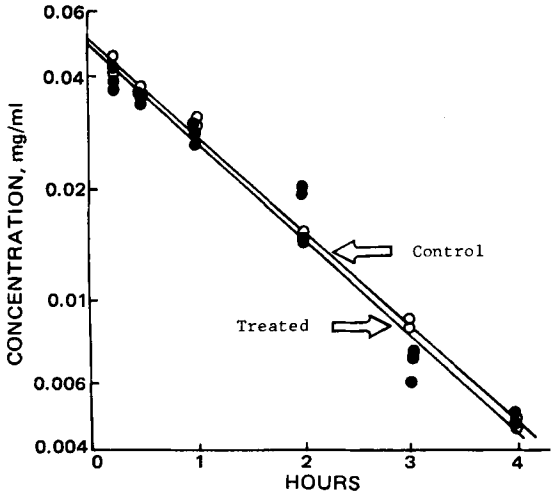

Figure 1—*Monoexponential semilogarithmic plots of blood concentrations of sulfanilamide obtained in control (○) and phenobarbital treated (●) rats. The solid lines are least-squares regression lines for the data of respective groups of rats.*

excreted in the urine in 35 hr. Of the total amount of the metabolites excreted, 80 to 85% was excreted in 8 hr.

To select the daily intraperitoneal dose of phenobarbital and the number of days to pretreat rats with this dose, the data in Table I were considered. The extent of metabolism of the compound in rats treated with a daily phenobarbital dose of 20 mg/kg for 5 days, 20 mg/kg for 10 days, 50 mg/kg for 5 days, or 100 mg/kg for 5 days was practically the same. Therefore, the regimen of phenobarbital treatment adopted in the pharmacokinetic study was a 100-mg/kg ip dose per day for 5 consecutive days. This was 5 times greater than the dose used in a previous study (1). The selection of the high phenobarbital dose was considered advisable since the compounds used in the present study are metabolized and bound to plasma proteins, unlike the nonmetabolized and nonprotein bound compounds used in a previous study (1). It was thought that, if the effects of phenobarbital treatment on sulfonamide distribution pharmacokinetics and biological half-lives were marginal at daily 20-mg/kg doses, more pronounced effects might be seen at daily 100-mg/kg doses. However, no pharmacokinetic studies were carried out at a daily 20-mg/kg dose of phenobarbital to determine if phenobarbital effects at the lower dose were less pronounced than those seen at a daily 100-mg/kg dose.

The overall urinary excretion data obtained following intravenous administration of 7 mg of sulfisoxazole to each control or phenobarbital treated rat are shown in Table II. Practically the entire administered dose of sulfisoxazole was recovered in the urine in intact and metabolic forms for the control and phenobarbital treated rats. There was no significant difference in the amounts of intact drug or metabolites recovered in the urine of the control and phenobarbital treated rats. The fractions of sulfisoxazole recovered in the intact (f_I) and metabolic (f_m) forms, based on the total amount recovered in 52 hr, are also listed in Table II. Of the total amount of sulfisoxazole recovered in the urine during the initial 10-hr period, 80–86% was in the intact form and 9–11% in the metabolic forms in the control and phenobarbital treated rats.

From the data in Tables I and II, a subject-to-subject variation among the control and phenobarbital treated rats in the extent of metabolism of the sulfonamides was minimum.

Pharmacokinetics of Sulfanilamide—The semilogarithmic plots of concentration *versus* time obtained for sulfanilamide in both the control and phenobarbital treated rats were monoexponential (Fig. 1). Therefore, the data were analyzed according to a one-compartment open model.

$$C = C_0 e^{-K_{el}t} \qquad \text{(Eq. 1)}$$

where C is the concentration of intact sulfanilamide at time t, C_0 is the concentration of intact sulfanilamide at time zero, and K_{el} is the apparent first-order rate constant of elimination of the drug. The drug concentrations plotted in Fig. 1 are normalized on the basis of a 10-mg iv dose of the drug per 200 g rat weight. The values of C_0 for the drug were determined from the intercepts obtained by extrapolating the respective least-squares line to time zero (Fig. 1). Values of K_{el} were calculated from the respective slope ($-K_{el}/2.303$) of the least-squares line. The apparent

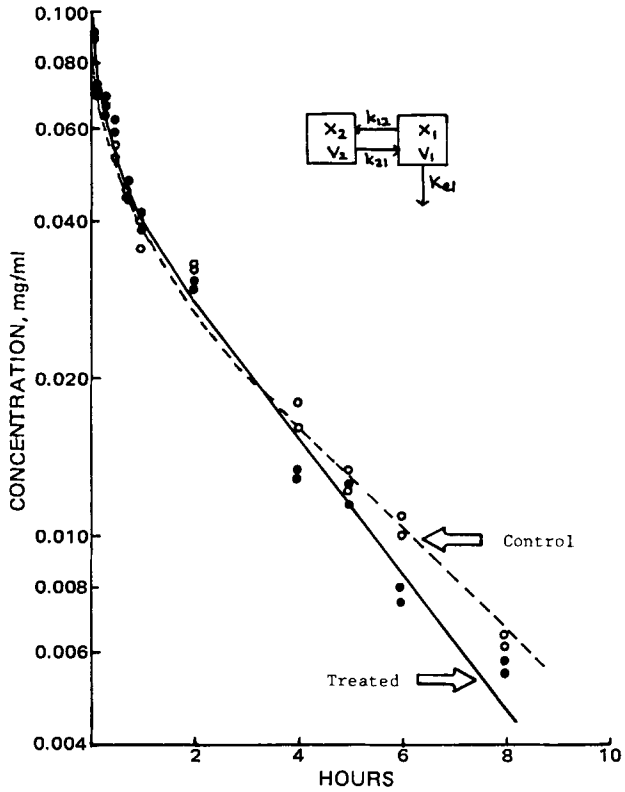

Figure 2—*Biexponential semilogarithmic plots of blood concentrations of sulfisoxazole obtained in control (○) and phenobarbital (●) treated rats. The solid and dotted lines are NONLIN least-squares regression lines for the data of respective group of rats. Insert is Scheme I.*

distribution volumes (V_d) and biological half-lives ($t_{1/2}$) of the drug were calculated in the usual manner (1). The pharmacokinetic parameters determined from these studies are listed in Table III. The standard deviations of all pharmacokinetic parameters were estimated by procedures described previously (1). The values of the parameters determined for the phenobarbital treated rats were not significantly different from those determined for the control rats. In fact, the values of all pharmacokinetic parameters observed are almost identical in the phenobarbital treated rats and the control rats, indicating that phenobarbital treatment had no effect on the distribution and elimination kinetics of sulfanilamide.

Pharmacokinetics of Sulfisoxazole—The semilogarithmic plots of concentration *versus* time for sulfisoxazole in both the control and phenobarbital treated rats indicated biexponential decline of the drug concentration in blood (Fig. 2). Therefore, the data were analyzed according to a two-compartment open model (Scheme I, Fig. 3) with elimination of the drug occurring from the central compartment:

$$C = Ae^{-\alpha t} + Be^{-\beta t} \qquad \text{(Eq. 2)}$$

where C is the concentration of intact sulfisoxazole in the blood at time t and other terms in the equation (and in Scheme I) are described in previous papers (1, 11). The drug concentrations plotted in Fig. 2 are normalized on the basis of a 7-mg iv dose of the drug per 200 g rat weight. Preliminary estimates of the intercepts (A and B) and slopes ($-\alpha/2.303$ and $-\beta/2.303$) for the two linear exponential segments (resolved by the method of residuals) were obtained by a least-squares method described previously (1). Using the preliminary estimates of A, B, α, and β, the initial estimates of V_1, k_{12}, k_{21}, and K_{el} were obtained in the manner described previously (1). 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 by analyzing the data using the NONLIN least-squares program (12). Using the computer estimated values of these parameters, estimates of V_2, V_{ss}, elimination phase ($t_{1/2}$), and body clearance ($K_{el}V_1$) were calculated as shown previously (1). The estimated and derived parameters are listed in Table IV. Standard deviation values of V_1, k_{12}, k_{21}, and K_{el} are computer estimated values and those of other parameters were estimated by the procedures described previously (1). The values of all pharmacokinetic parameters, except V_2, V_{ss}, and $K_{el}V_1$, determined for sulfisoxazole in phenobarbital treated rats were significantly different from those determined in control rats.

Table III—One-Compartment Model Pharmacokinetic Parameters of Sulfanilamide Determined in Control and Phenobarbital Treated Rats

Parameter	Control	Phenobarbital Treated	Statistical Significance of Difference (p)
C_0, mg/ml	0.054 ± 0.002	0.053 ± 0.003	*NS*[a]
K_{el}, hr^{-1}	0.598 ± 0.020	0.615 ± 0.030	*NS*
$t_{1/2}$, hr	1.16 ± 0.04	1.13 ± 0.06	*NS*
V_d, ml/kg	926.0 ± 34.3	931.0 ± 53.4	*NS*
r	0.996	0.980	

[a] No significant difference.

Rate Constants of Urinary Excretion and Metabolism of Sulfisoxazole—In Scheme I (Fig. 2), K_{el} is the sum of the apparent first-order rate constants of urinary excretion (k_{ex}) and metabolism (k_m) of sulfisoxazole. The values of k_{ex} and k_m can be calculated using the following equations (13):

$$k_{ex} = K_{el} f_I \quad \text{(Eq. 3)}$$

$$k_m = K_{el} f_m \quad \text{(Eq. 4)}$$

$$f_I = \frac{S_{e\infty}}{S_{e\infty} + M_{e\infty}} \quad \text{(Eq. 5)}$$

$$f_m = \frac{M_{e\infty}}{S_{e\infty} + M_{e\infty}} \quad \text{(Eq. 6)}$$

where $S_{e\infty}$ is the amount of sulfisoxazole excreted in the urine in infinite time and $M_{e\infty}$ is the equivalent amount of sulfisoxazole excreted in the urine as metabolite(s) in infinite time (Table II). Using the corresponding values of f_I and f_m from Table II and those of K_{el} in Table IV, the values of k_{ex} and k_m were calculated for control and phenobarbital treated rats (Table V). The standard deviation of k_{ex} and k_m were estimated according to the formulas (14):

$$k_{ex} = [\{(\sigma k_{ex}/k_{ex})^2 + (\sigma f_I/f_I)^2\}(k_{ex})^2]^{1/2} \quad \text{(Eq. 7)}$$

$$k_m = [\{(\sigma k_m/k_m)^2 + (\sigma f_m/f_m)^2\}(k_m)^2]^{1/2} \quad \text{(Eq. 8)}$$

As noted in Table V, according to the t test statistics, k_{ex}, but not k_m, was significantly greater in the phenobarbital treated rats than in the control rats.

DISCUSSION

Phenobarbital treatment did not affect the extent of nonmicrosomal enzymatic metabolism (Table II) or protein binding (4) of sulfisoxazole (which is excreted mainly by the kidney in rats). The significant decrease in the apparent distribution volume of the central compartment and the biological half-life of sulfisoxazole in the phenobarbital treated rats cannot be attributed to the increased hepatic blood flow, microsomal enzyme concentration, and biliary flow usually brought about by chronic phenobarbital treatment. The possibility of a decrease in the biological half-life of sulfisoxazole (pKa 5.1) due to a posssible increase in urine pH of rats was also evaluated. The normal pH of luminal fluids in the proximal tubules of rats was reported to be 6.82 (15). In this study, the pH of the cumulative urine samples collected separately from four control rats for 7 hr varied from 6.35 to 6.5, with an average pH of 6.42. Similarly, the pH of the cumulative urine samples collected separately from eight phenobarbital treated rats for 7 hr varied from 6.28 to 6.45, with an average pH of 6.36. Thus, the urine pH in phenobarbital treated rats was similar to that in the control rats. In the pH ranges measured, sulfisoxazole remains 94–96% in the anionic form. Therefore, the slight variation in urine pH was not expected to influence the half-life of sulfisoxazole in these rats.

Distribution Kinetics—The reduction in the distribution volume of the central compartment of sulfisoxazole in phenobarbital treated rats may be due to a possible decrease in the rate of diffusion of the anionic form of sulfisoxazole through the aqueous pores of the cell membranes of central compartment tissues. This was previously proposed (1) for the anions of mandelic acid, which also displayed the characteristics of a two-compartment model. As observed with mandelic acid, there was a tendency for V_2 and V_{ss} of sulfisoxazole to be lower in the phenobarbital treated rats, but the values of these parameters were not significantly different at the 95% confidence level from those in the control rats. However, V_{ss} of sulfisoxazole in the phenobarbital treated rats was different from that in the control rats at a 90% confidence level.

Table IV—Two-Compartment Model Pharmacokinetic Parameters of Sulfisoxazole Determined in Control and Phenobarbital Treated Rats

Parameter	Control	Phenobarbital Treated	Statistical Significance of Difference (p)
V_1, ml/kg	402.20 ± 12.00	366.35 ± 12.40	<0.05
k_{12}, hr^{-1}	0.5672 ± 0.0611	0.9716 ± 0.0814	<0.001
k_{21}, hr^{-1}	0.8308 ± 0.0845	1.6549 ± 0.1272	<0.001
K_{el}, hr^{-1}	0.4237 ± 0.0174	0.5183 ± 0.0235	<0.005
α, hr^{-1}	1.6021 ± 0.2305	2.8432 ± 0.3400	<0.01
β, hr^{-1}	0.2198 ± 0.0205	0.3017 ± 0.0239	<0.05
V_2, ml/kg	274.58 ± 41.46	215.08 ± 25.45	*NS*
V_{ss}, ml/kg	676.78 ± 43.16	581.43 ± 28.31	*NS*(p=0.1)
$K_{el}V_1$, ml/hr/kg	170.41 ± 8.69	189.88 ± 10.74	*NS*
$t_{1/2\beta}$, hr	3.15 ± 0.29	2.30 ± 0.18	<0.05
r	0.993	0.992	

As rationalized previously (1) for model organic anions, the transmembrane transport of sulfisoxazole anions is assumed to occur through the aqueous pores of cell membranes of the central and tissue compartments. It is likely that the possible increase in protein and phospholipid concentration brought about by phenobarbital pretreatment (1), probably causes a decrease in the pore size of (especially) the small size pores of cell membranes of the central and tissue compartments. This may cause a greater interaction of diffusing sulfisoxazole molecules with the proteins and phospholipids of the aqueous pore lining by intermolecular forces such as hydrogen bonding, hydrophobic bonding, and electrostatic interaction.

Interestingly, k_{12} and k_{21} for the intercompartmental transport of sulfisoxazole and mandelic acid in a previous study (1) were significantly greater in the phenobarbital treated rats than in the control rats, although the rate and extent of penetration of these anions into the deeper regions of the tissues was decreased in the phenobarbital treated rats. This may be rationalized by recognizing that k_{12} and k_{21} are the hybrid overall rate constants of anions which penetrate the tissues by diffusing through aqueous pores of small and large sizes, experiencing a greater transport barrier through the small size aqueous pores. If the very small size aqueous pores have narrowed sufficiently due to phenobarbital treatment and blocked penetration of anions through them in the deeper regions of tissues and decreased the apparent distribution volumes of accessible tissues, the remaining pores are the relatively large size aqueous pores whose resistance to penetration of the anions is not substantially affected. This situation would give a relatively rapid attainment of equilibrium of the reversible intercompartmental transport of the anions, leading to apparent increases in k_{12} and k_{21} values in phenobarbital treated rats.

Elimination Kinetics—The elimination rate constant (K_{el}) of sulfisoxazole, which is involved in renal tubular secretion (4), is increased by the phenobarbital treatment, as were the elimination rate constants of model organic anions (1) involved in renal tubular secretion in rats. Since the model organic anions were not metabolized and were eliminated from the body due to urinary excretion, K_{el} of these compounds were essentially the urinary excretion rate constants (k_{ex}). The increase in k_{ex} of model organic anions was attributed to the possible stimulatory effect of phenobarbital on the renal tubular secretory process (1). The rate constant of elimination of sulfisoxazole (K_{el}) represents the sum of k_{ex} and k_m, and, as noted in Table V, the increase in K_{el} is due to the significant increase in k_{ex} of sulfisoxazole in the phenobarbital treated rats. The increase in k_{ex} of sulfisoxazole anions may also be due to a stimulatory effect of phenobarbital on its renal tubular secretion process. This effect was shown for p-amino hippurate in phenobarbital treated rats (16).

Sulfanilamide—It was proposed (1) that a decrease in the apparent distribution volumes may be observed in phenobarbital treated rats for compounds which exist in the blood in the ionized form, exhibit multicompartment characteristics, and distribute between the compartments mainly by diffusion through the aqueous pores of the tissue cell membranes. It was also postulated (1) that such changes in the apparent distribution volumes are unlikely to be observed for compounds that exist in the blood in the nonionized form and may even display multicompartment characteristics, since they generally have greater membrane solubility and diffuse through the entire membrane surface of which aqueous pores constitute only a fractional surface area. It was further proposed that the increase in the elimination rate constants may be observed in the phenobarbital treated rats even for organic anions whose metabolic process is not stimulated by phenobarbital, but are secreted

Table V—Apparent First-Order Rate Constants of Urinary Excretion (k_{ex}) and Metabolism (k_m) of Sulfisoxazole Calculated for Control and Phenobarbital Treated Rats

	Control	Phenobarbital Treated	Statistical Significance of Difference (p)
k_{ex}, hr^{-1}	0.3838 ± 0.0204	0.4582 ± 0.0260	<0.05
k_m, hr^{-1}	0.0398 ± 0.0130	0.0601 ± 0.0156	*NS*

by the renal tubules, due to the possible stimulatory effect of phenobarbital on the renal tubular secretory mechanism of organic anions such as *p*-amino hippurate (16). As a corollary to this, it was postulated that the elimination rate constants of compounds whose metabolic process if not stimulated by phenobarbital will not be influenced in phenobarbital treated rats if the compounds are excreted in the urine by glomerular filtration.

The results (Table III) obtained for sulfanilamide are supportive of these hypotheses. The apparent distribution volume of sulfanilamide, which not only exists in the blood in the nonionized form but also shows characteristic one-compartment behavior, was not affected by the phenobarbital treatment. Also, K_{el} or $t_{1/2}$ of sulfanilamide, which is not involved in renal tubular secretion but is excreted by glomerular filtration in the urine, is not affected by the phenobarbital treatment.

CONCLUSIONS

The previous (1) and present studies demonstrate that, besides its known drug metabolizing enzyme induction effect, phenobarbital treatment may produce two additional effects on the pharmacokinetics of organic anions displaying multicompartment model characteristics. One effect is the changes in distribution space and rates reflected in such pharmacokinetic parameters as k_{12}, k_{21}, V_1, and V_{ss} of two-compartment model compounds. The other effect is on the renal tubular secretion process, reflected in such pharmacokinetic parameters as k_{ex} and K_{el} of the compounds. The fact that these pharmacokinetic parameters of drugs are independent of their elimination rate constants was pointed out previously (17).

The biological half-lives of multicompartment model compounds are derived from the disposition rate constants of the compounds and are, therefore, a function of both distribution and/or elimination (11). Therefore, the change in the biological half-lives of compounds brought about by phenobarbital treatment reflect the changes in distribution and/or elimination of the compounds.

Although the results of the previous (1) and present studies are consistent with the mechanism conceived previously (1) for the effects of phenobarbital, direct evidence of the increase in the protein and phospholipid concentration of certain target peripheral tissue cell membranes has not been obtained at this time. However, regardless of whether such direct evidence is obtained in the future, further evidence of the effects of phenobarbital treatment on the distribution and elimination pharmacokinetic parameters of other suitable organic anionic drugs should be gathered.

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