acetone to yield light-tan crystals (homogeneous on TLC, ethyl acetate, R_f 0.40), mp 244–245° dec.; IR (KBr): 3420, 3340, broad absorption from 3200 to 2600, 1675, 1635, 1600, 1570, 1545, 1470, 1435, 1380, 1370, 1355, 1270, 1240, 1155, 955, 805, and 630 cm^{-1}; NMR (dimethyl sulfoxide-d_6): δ 1.19 (t, 6H, $-CH_3$ of diethylamino), 2.08 (s, 6H, $-CH_3$ at C_4 and C_5), 3.14 (q, 4H, methylenes of diethylamino), 4.05 (broad s, 2H, $COCH_2$), 6.55 (broad s, 2H, $CONH_2$), 9.90–10.50 (broad s, 1H, N^+H), 10.70 (broad s, NH of amide at C_2 or N_1H), and 10.95 (broad s, 1H, N_1H or NH of amide at C_2) ppm. (See Table III for analyses.)

Pharmacology—*Antiarrhythmic Activity*—With the method of Lawson (5), fibrillations were induced in 20–30-g male mice by exposure to chloroform vapor until respiration ceased. The heart was exposed, and the cardiac rate was determined with a binocular microscope. Mice with cardiac rates >200 beats/min were considered unprotected (Table IV).

Local Anesthetic Activity—The guinea pig wheal method of Bülbring and Wajda (6) was used to determine the activity. The back of the guinea pig was shaved 1 day prior to the test, and 0.25 ml of the aqueous drug solution was administered intradermally at two sites along the midline. The resulting wheals were tested by pricking the area six times with a pin at 5-min intervals for 1 hr. Local anesthesia was present if the pinprick did not elicit a skin twitch. The number of pinpricks that failed to elicit a response was then recorded at each time interval (Table V).

REFERENCES

(1) R. W. Johnson, T. H. Keenan, J. W. Kosh, and J. W. Sowell, Sr., *J. Pharm. Sci.*, **68**, 317 (1979).

(2) R. Gewald, *Z. Chem.*, **1**, 349 (1961).

(3) R. W. Johnson, R. J. Mattson, and J. W. Sowell, Sr., *J. Heterocycl. Chem.*, **14**, 383 (1977).

(4) V. I. Shvedov, M. V. Mezentseva, and A. N. Grinev, *Khim. Geterotsikl. Soedin.*, **9**, 1219 (1975); through *Chem. Abstr.*, **84**, 59299 (1976).

(5) J. W. Lawson, *J. Pharmacol. Exp. Ther.*, **160**, 22 (1968).

(6) E. Bülbring and I. Wajda, *ibid.*, **85**, 78 (1945).

ACKNOWLEDGMENTS

Adapted in part from theses submitted by R. W. Johnson and T. H. Keenan to the Graduate School, University of South Carolina, in partial fulfillment of the Master of Science degree requirements.

Comparative Pharmacokinetics of Coumarin Anticoagulants XLI: Effect of Phenobarbital on Systemic Availability of Orally Administered Dicumarol in Rats

JAMES W. CROW, MILO GIBALDI, and GERHARD LEVY ×

Received December 8, 1978, from the *Department of Pharmaceutics, School of Pharmacy, State University of New York at Buffalo, Amherst, NY 14260.* Accepted for publication January 26, 1979.

Abstract □ The purpose of this investigation was to determine the effect of phenobarbital on the systemic availability of orally administered dicumarol in rats. Adult male Sprague–Dawley rats, matched for dicumarol free fraction in serum, received either phenobarbital sodium, 75 mg/kg, or saline solution, orally or intravenously, daily for 7 days. On Day 6, they also received ^{14}C-dicumarol, 2 mg/kg iv, and unlabeled dicumarol, 50 mg/kg po, in aqueous suspension. Venous blood samples were obtained serially over 32 hr through an indwelling cannula. Systemic dicumarol availability was determined from the dose-normalized ratio of areas under the plasma concentration–time curves. Phenobarbital treatment almost doubled the total clearance of dicumarol and the intrinsic clearance of free dicumarol, with no significant difference between the inductive effects of oral and intravenous doses of phenobarbital. Systemic dicumarol availability in control rats (mean ± *SD*) was 84 ± 8% (n = 10) and 84 ± 10% (n = 6) in the oral and intravenous phenobarbital studies, respectively. The systemic dicumarol availability in phenobarbital-treated rats was appreciably lower: 48 ± 7% (n = 10) and 61 ± 12% (n = 6) for orally and intravenously treated animals, respectively. The effect of oral phenobarbital on systemic dicumarol availability was more pronounced than that of intravenous phenobarbital ($p < 0.025$). The apparent first-order absorption rate constants for the fraction of the dose available systemically were similar for control and treated animals. There was a positive correlation between systemic dicumarol availability and total dicumarol clearance in control animals ($p < 0.001$). Proper matching of control and treated animals is, therefore, important for this type of study. The rat appears to be a good model for investigating the mechanism of the inhibitory effect of phenobarbital on dicumarol absorption observed previously in humans.

Keyphrases □ Phenobarbital—effect on systemic availability of oral dicumarol, comparison of oral and intravenous doses □ Bioavailability—dicumarol in rats, effect of oral and intravenous phenobarbital □ Dicumarol—bioavailability, effect of oral and intravenous phenobarbital □ Anticoagulants—dicumarol, effect of oral and intravenous phenobarbital on systemic availability

The bioavailability of orally administered dicumarol in humans is reduced by pretreatment of the subjects with an orally administered barbiturate (1). Similar effects have been observed with respect to two other poorly water-soluble drugs, griseofulvin and diethylstilbestrol. The bioavailability of orally administered griseofulvin in humans is reduced by pretreatment with orally administered phenobarbital (2). Such a reduction in griseofulvin bioavailability has also been observed in rats (3). Pretreatment with phenobarbital has been reported to decrease diethylstilbestrol absorption from the rat intestine (4).

Little is known about the mechanism of the barbiturate effect on drug absorption. A study was designed to determine the effect of orally and intravenously administered phenobarbital on GI absorption of dicumarol in rats. The results of this investigation have bearing not only on the specific interaction under study but also on the design of studies to determine the bioavailability of drugs that are subject to enzyme induction and that exhibit pronounced interindividual differences in pharmacokinetic characteristics.

EXPERIMENTAL

The studies were carried out on adult male Sprague–Dawley rats weighing 250–375 g. Groups of animals were screened for plasma free fraction values of dicumarol, using serum obtained from ~5 ml of blood withdrawn from the tail artery under light ether anesthesia. The free fraction determinations were performed in duplicate by dialyzing, to

0022-3549/79/0800-0958$01.00/0

Table I—Pharmacokinetic Constants for Dicumarol in Adult Male Sprague–Dawley Rats Used to Determine the Effect of Oral Phenobarbital on Dicumarol Absorption

Pharmacokinetic Constant[a]	Control Animals	Phenobarbital-Treated Animals	Control–Phenobarbital Ratio	Statistical Significance of Difference (p)[c]
Total clearance, ml/hr/kg	18.6 ± 4.9[b]	31.4 ± 13.2	0.655 ± 0.242	<0.025
Apparent volume of distribution, ml/kg	150 ± 20	153 ± 71	1.16 ± 0.47	N.S.
β, hr^{-1}	0.123 ± 0.026	0.214 ± 0.054	0.593 ± 0.119	<0.001
Intrinsic clearance of free drug, ml/hr/kg × 10^{-4}	12.4 ± 7.3	21.9 ± 12.8	0.596 ± 0.226	<0.005
Serum free fraction × 10^4	2.09 ± 1.27[d]	1.89 ± 1.17[d]	1.12 ± 0.14	—[e]

[a] Based on ^{14}C-dicumarol injected intravenously. [b] All data are mean ± SD, n = 10. [c] Paired two-tailed t-test. [d] Determined before phenobarbital and dicumarol administration. [e] Not analyzed statistically because animals were matched with respect to serum free fraction.

Table II—Pharmacokinetic Constants for Dicumarol in Adult Male Sprague–Dawley Rats Used to Determine the Effect of Intravenous Phenobarbital on Dicumarol Absorption

Pharmacokinetic Constant[a]	Control Animals	Phenobarbital-Treated Animals	Control–Phenobarbital Ratio	Statistical Significance of Difference (p)[c]
Total clearance, ml/hr/kg	19.5 ± 6.5[b]	38.6 ± 11.1	0.513 ± 0.138	<0.005
Apparent volume of distribution, ml/kg	189 ± 42	190 ± 57	1.03 ± 0.18	N.S.
β, hr^{-1}	0.102 ± 0.016	0.206 ± 0.031	0.512 ± 0.168	<0.005
Intrinsic clearance of free drug, ml/hr/kg × 10^{-4}	13.1 ± 9.3	24.4 ± 16.4	0.518 ± 0.128	<0.025
Serum free fraction × 10^4	2.00 ± 0.96[d]	2.02 ± 0.92[d]	0.993 ± 0.107	—[e]

[a] Based on ^{14}C-dicumarol injected intravenously. [b] All data are mean ± SD, n = 6. [c] Paired two-tailed t-test. [d] Determined before phenobarbital and dicumarol administration. [e] Not analyzed statistically because animals were matched with respect to serum free fraction.

equilibrium (20–24 hr), 1-ml samples of serum with 30 μg of added ^{14}C-dicumarol[1] against an equal volume of isotonic Sorensen phosphate buffer, pH 7.4, at 37° (5). The serum and buffer phases were assayed after selective extraction (6).

Each group of 20 rats yielded, on the average, four pairs of animals, with the members of any one pair having similar serum free fraction values. One member of each pair was assigned to the control group, and the other was assigned to the treatment group. Treatment with either normal saline solution or phenobarbital was started 1–2 weeks after screening. Phenobarbital sodium, 75 mg/kg, or saline solution was administered intravenously or orally daily for 7 days. The volumes of the intravenous and oral solutions were 0.75 and 1.8 ml/kg, respectively. Rats that received intravenous treatments had the solutions injected through a right femoral vein catheter exteriorized at the back of the neck, which was implanted 1 day before the treatment period. On the 5th day of the treatment period, the right external jugular vein was cannulated (7) to facilitate blood withdrawal, and the rats were placed in individual metabolism cages in a room maintained at 21°. Food, but not water, was withdrawn for 24 hr, starting that evening at about 9 pm.

On the 6th day at about 9 am, the animals received an intravenous injection of ^{14}C-dicumarol (2 mg/kg in Sorensen buffer solution containing 1.3 mg/ml) and an oral dose of dicumarol[2], 50 mg/kg in aqueous suspension, by gastric tube. On the day of dicumarol administration, the daily phenobarbital dose was given immediately after the anticoagulant.

Dicumarol suspensions were prepared with 0.25% aqueous methylcellulose[3] solution. The solution was made by sprinkling the suspending agent on the water, refrigerating, and gently stirring the cold solution with a glass rod. Dicumarol was incorporated on the day of dosing by making a paste with a small volume of the solution and then adding the remaining solution. This was done with a spatula in a beaker, and care was taken not to grind the dicumarol particles. Pairs of control and phenobarbital-treated animals always received the same lot of dicumarol suspension.

Blood samples (0.5 ml) were obtained from the intravenous cannula at about 0.5, 2, 4, 6, 8, 10, 14, 16, 18, 24, 28, and 36 hr and were transferred to heparinized micro-blood collecting tubes. These tubes were centrifuged, and duplicate 0.1-ml plasma samples were collected and quick frozen pending assay.

Plasma was assayed simultaneously for radiolabeled and nonlabeled dicumarol (6, 8). Duplicate 0.1-ml plasma portions were adjusted to pH 3.1 and extracted into heptane. One portion of the extract was added to scintillation fluid and counted. The other portion was extracted into sodium hydroxide solution, which was assayed spectrophotometrically. The concentration of unlabeled dicumarol was determined by subtracting the concentration of radiolabeled drug, determined by scintillation spectrometry, from the concentration determined by spectrophotometry.

Pharmacokinetic constants were obtained in the usual manner (5, 9, 10) from the ^{14}C-dicumarol (intravenous) concentrations in plasma. Intrinsic clearance of free drug was calculated by dividing total clearance by the serum free fraction of dicumarol. The area under the nonlabeled (orally administered) dicumarol plasma concentration–time curve (AUC) was obtained by the trapezoidal method (11). The area beyond the last concentration point was obtained by two methods: (a) extrapolating the apparently exponential concentration decay phase to zero concentration (Method I), and (b) dividing the last concentration by the β value obtained concomitantly in the same animal from the plasma ^{14}C-dicumarol concentrations (Method II). Systemic availability was calculated by dividing AUC_{oral} by AUC_{iv} after multiplying the latter by the oral/intravenous dose ratio. The cumulative fraction of the oral dose absorbed as a function of time was determined by a literature method (12) modified such that the denominator was $\beta \cdot AUC_{iv}$ (oral dose/intravenous dose).

RESULTS

Initial screening of a large group of rats yielded 17 pairs with similar dicumarol serum free fraction values in any given pair. Eleven pairs were assigned to the experiment with orally administered phenobarbital, and the other six pairs were assigned to the experiment with intravenous phenobarbital. One member of the 11-pair group was lost during the study, so the reported results for the experiment with orally administered phenobarbital are based on 10 pairs of animals.

The serum free fraction values for the group of 10 pairs ranged from 7.24×10^{-5} to 4.40×10^{-4}, and the correlation coefficient between free fraction values for members of each pair was 0.99 ($p < 0.001$). The range of serum free fraction values in the group of six pairs was 8.08×10^{-5}–3.39×10^{-4}, with a correlation coefficient of 0.96 ($p < 0.001$). In view of the excellent match of serum free fraction values in any one pair and the consequent similarity of dicumarol total clearance within each pair [total clearance of dicumarol is proportional to the drug's free fraction in serum (13)], statistical analyses of observed differences in pharmacokinetic parameters due to phenobarbital treatment were performed by paired t-test.

Figures 1 and 2 show typical results obtained when ^{14}C-dicumarol was injected intravenously and unlabeled dicumarol in an aqueous suspension was administered orally to control and phenobarbital-treated rats. Prolonged absorption was often evident in both control and treated animals during the entire experimental period (*i.e.*, for more than 30 hr), as reflected by the difference in the log concentration *versus* time slopes of ^{14}C-dicumarol and unlabeled drug in plasma.

Pharmacokinetic constants for dicumarol disposition were determined from plasma concentrations of ^{14}C-dicumarol following intravenous injection of that drug (Tables I and II). Phenobarbital caused approxi-

[1] ^{14}C-Dicumarol, 71.4 μCi/mg, labeled in the methylene position, radiochemical purity > 98%, New England Nuclear Corp., Boston, Mass.

[2] Lot 4210, particle diameter 0.002–0.01 mm, Nutritional Biochemical Corp., Cleveland, Ohio.

[3] Methylcellulose 60 HG, Premium, 4000 cps, Dow Chemical Corp., Midland, Mich.

Table III—Effect of Oral Phenobarbital on Systemic Availability of Oral Dicumarol in Adult Male Sprague-Dawley Rats

Rat Pair	Systemic Availability, % of dose					
	Control Animals			Phenobarbital-Treated Animals		
	Method I[a]	Method II[b]	I–II Ratio	Method I	Method II	I–II Ratio
1	0.78	0.78	1.00	0.41	0.39	1.05
2	0.78	0.78	1.00	0.50	0.50	1.00
3	0.79	0.78	1.01	0.60	0.59	1.02
4	0.81	0.81	1.00	0.48	0.42	1.14
5	0.91	0.89	1.02	0.57	0.49	1.06
6	0.79	0.78	1.01	0.41	0.41	1.00
7	0.90	0.87	1.03	0.45	0.45	1.00
8	0.75	0.75	1.00	0.54	0.48	1.12
9	0.91	0.91	1.00	0.39	0.38	1.03
10	0.97	0.97	1.00	0.51	0.49	1.04
Mean	0.84	0.83	1.01	0.48[c]	0.46[c]	1.05
SD	0.08	0.07		0.07	0.06	

[a] Method I = area under plasma concentration–time curve of orally administered drug (AUC_{oral}) determined by extrapolation of apparently exponential concentration decay phase from last data point to zero concentration. [b] Method II = AUC_{oral} determined by assuming that concentrations after the last data point decline at the same relative rate as the postdistribution concentrations of ^{14}C-dicumarol administered intravenously. [c] Significantly different from the appropriate control value ($p < 0.05$).

Table IV—Effect of Intravenously Administered Phenobarbital on Systemic Availability of Oral Dicumarol in Adult Male Sprague-Dawley Rats

Rat Pair	Systemic Availability, % of dose					
	Control Animals			Phenobarbital-Treated Animals		
	Method I[a]	Method II[b]	I–II Ratio	Method I	Method II	I–II Ratio
1	0.74	0.74	1.00	0.49	0.48	1.02
2	0.71	0.71	1.00	0.52	0.52	1.00
3	0.83	0.83	1.00	0.56	0.53	1.06
4	0.92	0.89	1.03	0.80	0.79	1.01
5	0.87	0.82	1.06	0.68	0.59	1.15
6	0.97	0.94	1.03	0.59	0.59	1.00
Mean	0.84	0.82	1.02	0.61[c]	0.58[c]	1.04
SD	0.10	0.09		0.12	0.11	

[a,b] See Table III. [c] Significantly different from the appropriate control value by paired two-tailed t-test ($p < 0.005$).

mately twofold increases in total clearance, β, and in intrinsic clearance but had no significant effect on the apparent distribution volume. There was no statistically significant difference between the total and intrinsic clearance ratios, control/phenobarbital, of dicumarol in the oral and intravenous phenobarbital studies. Thus, the enzyme inductive effect of intravenous and oral phenobarbital was of similar magnitude.

Figure 3 shows the average time course of dicumarol absorption by control rats and by rats pretreated orally with phenobarbital. The individual dicumarol systemic availability data obtained in that experiment are listed in Table III. Calculation of the *AUC* of unlabeled plasma dicumarol was complicated by prolonged absorption. Two methods were used for the extrapolation of the plasma concentration *versus* time curve from the last assayed concentration (36 hr) to infinity. Method I involved the extrapolation of the apparently exponential terminal concentration decay phase. This implied that absorption was continuing in a predictable manner until the dicumarol concentration approached zero. Method II involved extrapolation of postexperimental plasma concentrations such that these concentrations declined at the same relative rate as the post-

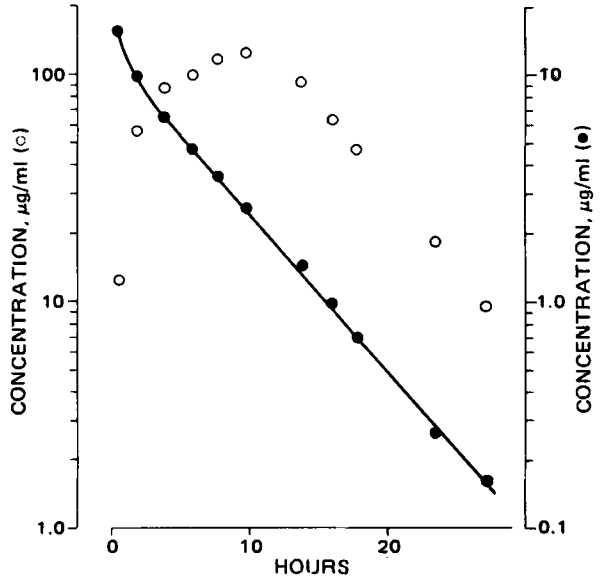

Figure 1—*Plasma dicumarol concentrations as a function of time after simultaneous administration of 50 mg/kg po (○) and 2 mg/kg iv (●) to a control rat. ^{14}C-Labeled drug was used for the intravenous injection.*

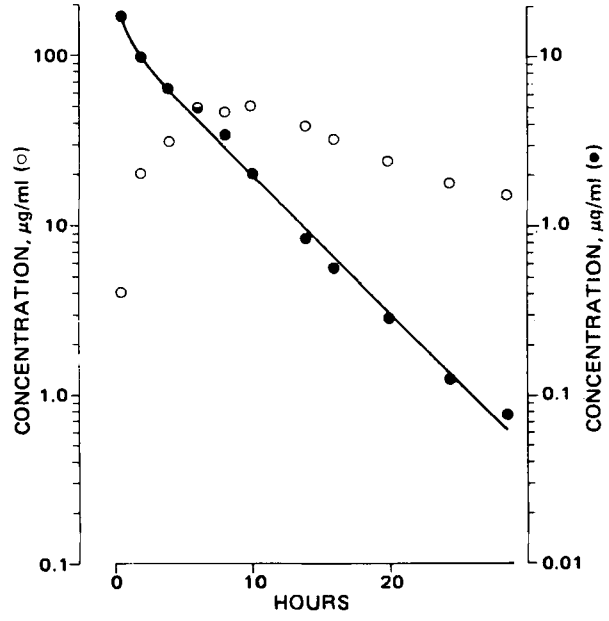

Figure 2—*Plasma dicumarol concentrations as a function of time after simultaneous administration of 50 mg/kg po (○) and 2 mg/kg iv (●) to a rat treated with phenobarbital sodium, 75 mg/kg/day iv, beginning 5 days before dicumarol administration. ^{14}C-Labeled drug was used for the intravenous injection.*

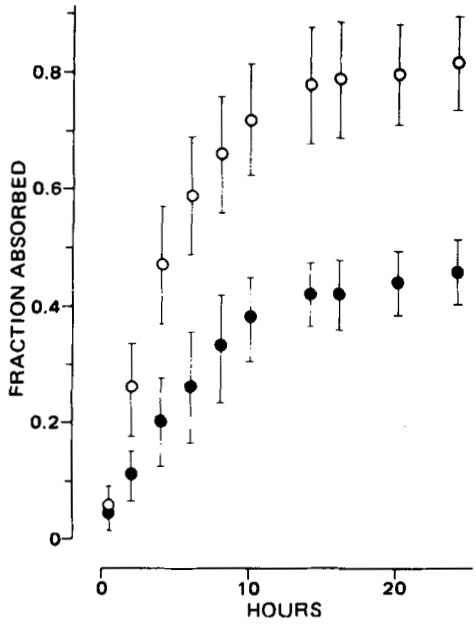

Figure 3—*Time course of dicumarol absorption after administration of 50 mg/kg po to control rats (○) and to rats treated with phenobarbital sodium, 75 mg/kg/day po, beginning 5 days before dicumarol administration (●). Points are means ± SD of 10 control and 10 phenobarbital-treated animals.*

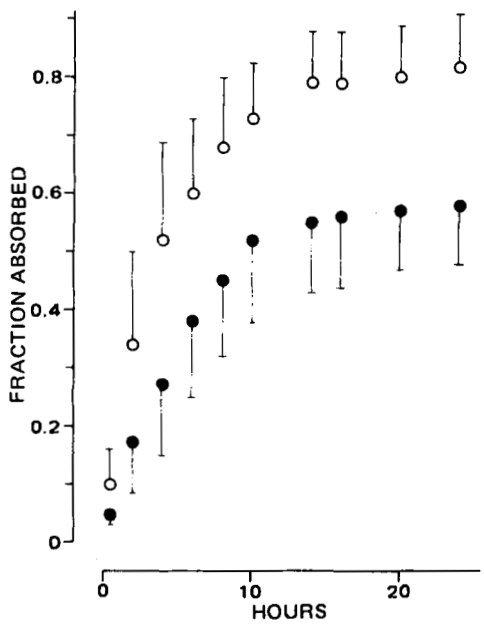

Figure 5—*Time course of dicumarol absorption after administration of 50 mg/kg po to control rats (○) and to rats treated with phenobarbital sodium, 75 mg/kg/day, iv beginning 5 days before dicumarol administration (●). Points are means ± SD (in one direction) of six animals each.*

distribution concentrations of intravenously injected ^{14}C-dicumarol. As evident from Table III, the results from the two methods were virtually identical.

Oral phenobarbital treatment reduced the absorption of orally administered dicumarol from 84% (control animals) to 48% on the average. While phenobarbital treatment reduced the amount of dicumarol absorbed, it had little or no effect on absorption kinetics. The drug was absorbed by apparent first-order kinetics, and the absorption rate constant *for the amount absorbed* apparently was not affected by phenobarbital (Fig. 4).

Figure 5 shows the time course of dicumarol absorption in control rats and in rats treated with intravenous phenobarbital. Individual systemic availability data for this experiment are listed in Table IV. Like the control animals for the oral phenobarbital experiment, the intravenous controls absorbed 84% of the oral dicumarol dose on the average. Intravenous phenobarbital reduced the extent of absorption to 61% of the dose, *i.e.*, to a lesser degree than the 48% average following oral phenobarbital. This difference is statistically significant ($p < 0.005$). Again, phenobarbital treatment had no apparent effect on the dicumarol absorption kinetics (Fig. 6).

Additional examination of the data revealed a statistically significant positive correlation ($r = 0.76$; $p < 0.001$) between the systemic availability and the total dicumarol clearance in control animals but not in phenobarbital-treated animals (Fig. 7).

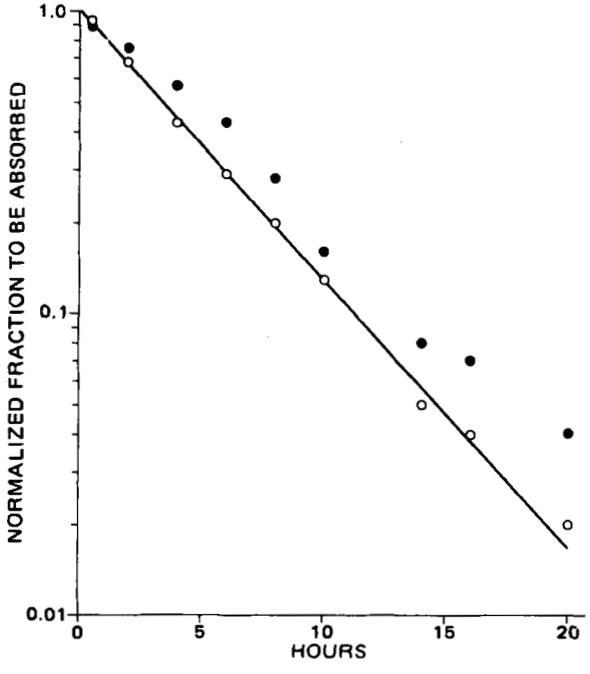

Figure 4—*Apparent first-order dicumarol absorption kinetics in 10 control (○) and 10 oral phenobarbital-treated rats (●). Plotted on the ordinate is the fraction of the total amount of dicumarol eventually absorbed that is remaining to be absorbed at various times after a 50-mg/kg po dose.*

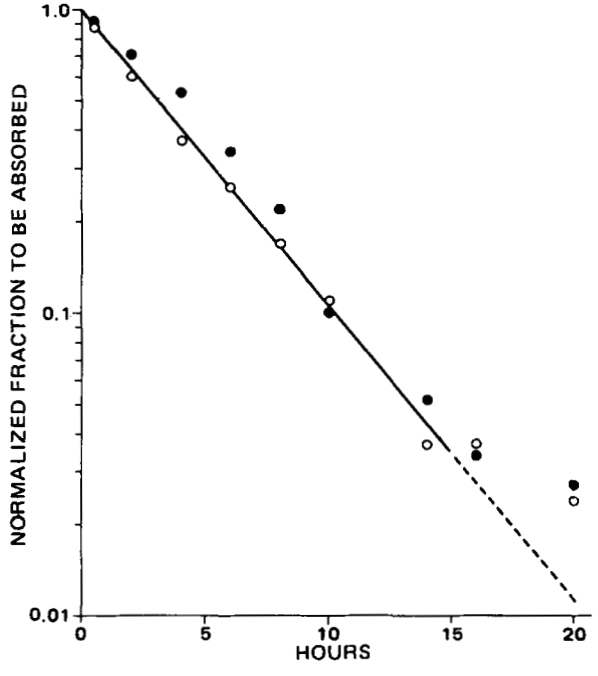

Figure 6—*Apparent first-order dicumarol absorption kinetics in six control (○) and six intravenous phenobarbital-treated rats (●). See Fig. 4 for further explanation.*

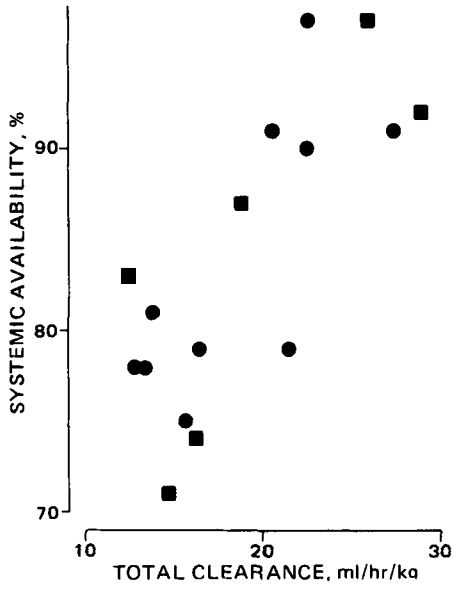

Figure 7—*Relationship between total clearance of intravenous dicumarol and systemic availability of oral dicumarol in 16 rats. Ten rats were control animals in the oral phenobarbital study (●), and six rats were control animals in the intravenous phenobarbital study (■). Correlation coefficient = 0.76, p < 0.001.*

DISCUSSION

Assessment of the effect of phenobarbital on the systemic availability of orally administered dicumarol requires consideration of numerous experimental design variables. There are pronounced interindividual differences in dicumarol pharmacokinetics in rats (5, 14) and humans (15). Phenobarbital is an enzyme inducer known to enhance dicumarol elimination (16). This enzyme induction may be prolonged, making crossover studies in rats difficult if not impossible. In addition, the results of the present investigation reveal that the systemic dicumarol availability in control rats increases with increasing total clearance. The reason for this correlation is not known.

To deal with these complicating factors, the systemic availability of orally administered dicumarol was determined by simultaneous administration of unlabeled dicumarol orally and ^{14}C-dicumarol intravenously as an "internal standard." Since crossover studies could not be performed, the control and phenobarbital-treated groups were matched with respect to their serum free fraction values of dicumarol. It was found previously that serum protein binding is the major determinant of interindividual differences in dicumarol elimination by rats (13).

A problem encountered in the pharmacokinetic assessment of dicumarol systemic availability was the very prolonged drug absorption, which had been observed also in humans (1). This prolonged absorption complicated calculations of the *AUC* values of orally administered drug. Fortunately, plasma concentrations were monitored long enough to encompass most of the *AUC*. Consequently, extrapolations based on the assumption of two different limiting cases yielded essentially identical *AUC* and systemic availability estimates.

The slow and prolonged absorption of dicumarol can cause "flip-flop" kinetics (11) in animals that eliminate the drug rather rapidly. This may have been the case in a recent study (17), which was commented upon subsequently (18, 19).

Plasma dicumarol concentrations decline triexponentially with time after rapid intravenous injection (10). However, the so-called distribution phase is so small that the apparent volume of distribution estimated by the area method and by the intercept ("single-compartment") method are almost identical [159 *versus* 166 ml/kg in a recent study (10) on 10 animals]. The data obtained in the present study were insufficient for a multiexponential characterization of the time course of dicumarol elimination (Figs. 1 and 2) because the number of blood samples during the first hours after drug administration had to be minimized to permit blood withdrawals for 36 hr without serious depletion of the blood volume. Therefore, absorption kinetics were estimated by the Wagner–Nelson method, which is based on the assumption of monoexponential elimination kinetics. The error introduced by that data treatment probably is minor.

Phenobarbital had a pronounced inductive effect on dicumarol elimination kinetics. It was established previously that phenobarbital treatment does not affect the serum protein binding of dicumarol in rats (20). There was no significant difference in the inductive effect of identical intravenous and oral phenobarbital doses.

Consistent with previous observations in humans (1), phenobarbital had a pronounced inhibitory effect on dicumarol absorption from an oral aqueous suspension in rats. The effect of oral phenobarbital was somewhat greater than that of intravenous phenobarbital, perhaps due to greater exposure of gut tissue to the orally administered drug. Little or no phenobarbital is excreted as such in the bile of rats (21); thus, intravenous phenobarbital-treated animals have little or no phenobarbital in their GI tracts. Considering this fact and the absorption inhibition by intravenous phenobarbital, it is our opinion that the inhibition of dicumarol absorption is unlikely to be the result of a direct physicochemical interaction between dicumarol and phenobarbital.

An "hepatic first-pass" effect on dicumarol in the rat can be excluded (16). However, the possibility must be considered that phenobarbital caused induction of dicumarol metabolizing enzyme systems in the gut wall. The results obtained in the present study are consistent with dissolution rate-limited absorption and subsequent partial biotransformation of some absorbed drug as it passes through the intestinal wall. However, studies in humans have shown that the amount of unmetabolized dicumarol found in the stool after oral administration of the anticoagulant increased following treatment with a barbiturate (1). It cannot be excluded, in our view, that some of the drug found in the stool of humans was derived from the hydrolysis of a conjugate formed by, or excreted into, the intestine.

Phenobarbital treatment affects bile flow (22) and, particularly in the large doses used in this study, may affect GI motility. The role of these variables cannot be determined from the present investigation. The possible role of bile in dicumarol absorption will be examined in a subsequent report.

REFERENCES

(1) P. M. Aggeler and R. A. O'Reilly, *J. Lab. Clin. Med.*, **74**, 229 (1969).

(2) S. Riegelman, M. Rowland, and W. L. Epstein, *J. Am. Med. Assoc.*, **213**, 426 (1970).

(3) F. Jamali and J. E. Axelson, *J. Pharm. Sci.*, **67**, 466 (1978).

(4) J. L. Lasker and D. E. Richert, *Pharmacologist*, **18**, 8 (1976).

(5) A. Yacobi, C.-M. Lai, and G. Levy, *J. Pharm. Sci.*, **64**, 1995 (1975).

(6) L. B. Wingard and G. Levy, *J. Pharmacol. Exp. Ther.*, **184**, 253 (1973).

(7) J. R. Weeks and J. D. Davis, *J. Appl. Physiol.*, **19**, 540 (1964).

(8) R. Nagashima, G. Levy, and E. Nelson, *J. Pharm. Sci.*, **57**, 58 (1968).

(9) E. Jähnchen, L. B. Wingard, and G. Levy, *J. Pharmacol. Exp. Ther.*, **187**, 176 (1973).

(10) A. Yacobi, C.-M. Lai, and G. Levy, *J. Pharm. Sci.*, **66**, 1741 (1977).

(11) M. Gibaldi and D. Perrier, "Pharmacokinetics," Dekker, New York, N.Y., 1975.

(12) J. G. Wagner and E. Nelson, *J. Pharm. Sci.*, **53**, 1392 (1964).

(13) C.-M. Lai and G. Levy, *ibid.*, **66**, 1739 (1977).

(14) *Ibid.*, **67**, 337 (1978).

(15) E. S. Vesell and J. G. Page, *J. Clin. Invest.*, **47**, 2657 (1968).

(16) C.-M. Lai, A. Yacobi, and G. Levy, *J. Pharmacol. Exp. Ther.*, **199**, 74 (1976).

(17) L. Putcha, P. Nandiwada, S. Feldman, J. V. Bruckner, and W. G. Kramer, *Res. Commun. Chem. Pathol. Pharmacol.*, **19**, 67 (1978).

(18) G. Levy, *ibid.*, **20**, 609 (1978).

(19) S. Feldman, *ibid.*, **20**, 611 (1978).

(20) A. Yacobi, J. T. Slattery, and G. Levy, *J. Pharm. Sci.*, **66**, 941 (1977).

(21) C. D. Klaassen, *Br. J. Pharmacol.*, **43**, 161 (1971).

(22) C. D. Klaassen, *J. Pharmacol. Exp. Ther.*, **176**, 743 (1971).

ACKNOWLEDGMENTS

Supported in part by Grant GM 20852 and by postdoctoral fellowship 5 F32 GM05653 (J. W. Crow) from the National Institute of General Medical Sciences, National Institutes of Health.

For the previous paper in this series, see A. Jain, F. G. McMahon, J. T. Slattery, and G. Levy, *Clin. Pharmacol. Ther.*, **25**, 61 (1979).