fluorometric method (1, 10, 14, 22, 24). Other methods were specifically designed for the determination of I in biological specimens (1, 10) or for the determination of II and related compounds after conversion to I (1, 10, 14).

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Comparative Pharmacokinetics of Coumarin Anticoagulants XLII: Effect of Phenobarbital on Systemic Availability of Orally Administered Dicumarol in Rats with Ligated Bile Ducts

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Abstract □ The purpose of this investigation was to determine if the previously demonstrated inhibitory effect of phenobarbital treatment on the systemic availability of orally administered dicumarol in rats is related to the known effect of phenobarbital on bile output. It was found that phenobarbital had no apparent effect on the systemic availability of an aqueous dicumarol suspension in rats with ligated bile ducts. Compared to results obtained previously on normal rats, bile duct-ligated rats absorbed and eliminated dicumarol much more slowly and absorbed much less of the anticoagulant. On the other hand, the relative inductive effect of phenobarbital treatment on dicumarol elimination was similar in normal and in bile duct-ligated animals. The latter exhibited substantial serum transaminase elevations, indicative of liver damage presumably secondary to cholestasis. These results demonstrate that a drug–drug interaction can depend markedly on the pathophysiological status of the animals.

Keyphrases □ Phenobarbital—effect on dicumarol systemic availability in bile duct-ligated rats □ Dicumarol—phenobarbital effect on systemic availability in bile duct-ligated rats □ Anticoagulants—dicumarol, systemic availability, effect of phenobarbital in bile duct-ligated rats □ Bile duct—ligation, effect on rats, dicumarol systemic availability, phenobarbital □ Drug interactions—phenobarbital effect on dicumarol systemic availability in bile duct-ligated rats

The systemic availability of orally administered dicumarol in humans (1) and rats (2) is reduced by pretreatment of the subjects with a barbiturate. Oral phenobarbital sodium administration, 75 mg/kg, for 5 days before and 2 days after oral administration of dicumarol suspension to rats reduced the systemic availability of the anticoagulant from 84 ± 8 to 48.7 ± 10% (mean ± *SD*). Similar effects were observed when phenobarbital was administered intravenously (2).

Since phenobarbital treatment increased bile output (3), the inhibitory effect of phenobarbital on dicumarol absorption possibly is mediated by complexation of the anticoagulant with bile salts or by altered GI motility caused by increased bile flow. A study was initiated to determine if phenobarbital treatment affects systemic dicumarol availability in rats with ligated bile ducts.

EXPERIMENTAL

The experimental procedures were described previously (2). Briefly, adult male Sprague–Dawley rats weighing ~300 g received daily oral doses of phenobarbital sodium, 75 mg/kg, or the same volume of saline solution for 5 days. Their right jugular vein was cannulated on the 5th day to facilitate frequent blood withdrawal. In the morning of the 6th day, the rats received an intravenous tracer dose of ^{14}C-dicumarol by rapid injection and 50 mg of dicumarol/kg in aqueous suspension by gastric tube.

Blood samples were collected periodically, and daily phenobarbital treatment was continued until the end of the experiment. Food, but not water, was withdrawn for 24 hr, starting 12 hr before dicumarol administration. Plasma was assayed for ^{14}C-dicumarol and unlabeled dicumarol. The ratio of areas under the concentration–time curves for the labeled and unlabeled drug, normalized for dose, was used to calculate systemic

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Table I—Effect of Phenobarbital Treatment on Dicumarol Pharmacokinetics in Adult Male Sprague–Dawley Rats with Ligated Bile Ducts (32-hr Study)

Pharmacokinetic Constant[a]	Control Animals	Phenobarbital-Treated Animals	Statistical Significance of Difference (p)[b]
Total clearance, ml/hr/kg	4.7 ± 1.5[c]	10.0 ± 3.1	<0.005
Apparent volume of distribution, ml/kg	93 ± 7	80 ± 20	N.S.
β, hr^{-1}	0.0512 ± 0.0185	0.129 ± 0.039	<0.001

[a] Based on ^{14}C-dicumarol injected intravenously. [b] Two-sample t-test. [c] All data are mean ± SD, n = 7.

availability. Drug absorption and elimination kinetics were characterized by conventional methods (2).

In the present study, bile duct ligation was performed on the 5th day, *i.e.*, 1 day before dicumarol administration. The bile duct was exposed by a midline abdominal incision under ether anesthesia, ligatures were placed near the liver and near the intestine, and the duct was severed between the two ligatures. The incision was closed with wound clips.

Blood sampling in the first group of animals was carried out for 32 hr. When this period proved to be insufficient to determine systemic availability, a second group of animals was studied for 60 hr. At the end of the experiment, as much blood as possible was removed from the aorta of the rats under ether anesthesia and the hematocrit was measured. Serum was separated and used to determine the free fraction of dicumarol, total protein and albumin concentration (4, 5), glutamate–oxaloacetate transaminase[1] (SGOT), and glutamate–pyruvate transaminase[2,3] (SGPT). The liver was excised, excess blood was removed by mild compression between tissue paper, and the organ was weighed. The animals were weighed daily during the pretreatment period, immediately before bile duct ligation, before dicumarol administration, and at the end of the 60-hr experiment prior to sacrifice.

RESULTS

The results obtained from the first (32-hr blood sampling) study showed that dicumarol was absorbed and eliminated much more slowly than anticipated from a previous study on rats with intact bile ducts (2). Consequently, systemic availability could not be determined from the data. The drug concentration data derived from the intravenous injection of ^{14}C-dicumarol were used to characterize the drug elimination kinetics. Treatment with phenobarbital increased the total dicumarol clearance about twofold, increased the disposition rate constant (β), and had no statistically significant effect on the apparent volume of distribution (Table I). The animals appeared ill; they were inactive, they ate little, their fur became rough, and their skin acquired a yellow color.

Table II—Effect of Phenobarbital Treatment on Dicumarol Pharmacokinetics in Adult Male Sprague–Dawley Rats with Ligated Bile Ducts (60-hr Study)

Pharmacokinetic Constant[a]	Control Animals	Phenobarbital-Treated Animals	Statistical Significance of Difference (p)[b]
Total clearance, ml/hr/kg	4.4 ± 1.5[c]	8.1 ± 2.4	<0.05
Apparent volume of distribution, ml/kg	90 ± 7	76 ± 10	N.S.
β, hr^{-1}	0.0492 ± 0.020	0.105 ± 0.020	<0.01
Intrinsic clearance of free drug, ml/hr/kg × 10^{-4}	0.769 ± 0.344	1.42 ± 0.73	N.S.
Serum free fraction[d] × 10^4	7.08 ± 4.67	7.23 ± 4.36	N.S.

[a] Based on ^{14}C-dicumarol injected intravenously. [b] Two-sample t-test. [c] All data are mean ± SD, n = 4. [d] Determined at the end of the experiment.

[1] Determined with Calbiochem GOT reagent kit, Calbiochem, La Jolla, Calif.
[2] Determined with Calbiochem GPT reagent kit, Calbiochem, La Jolla, Calif.
[3] The transaminase determinations were carried out by Dr. Kenneth L. Hintze in this laboratory.

Table III—Pathophysiological Characteristics of Rats with Ligated Bile Ducts (60-hr Study)

Parameter[a]	Control Animals	Phenobarbital-Treated Animals	Statistical Significance of Difference (p)[b]
Weight loss[c], %	12.5 ± 0.6[d]	10.8 ± 3.9	N.S.
Liver weight, g/kg	42.4 ± 2.3	53.3 ± 6.7	<0.025
SGOT[e], mU/ml	627 ± 64	521 ± 76	N.S.
SGPT[e], mU/ml	263 ± 81	229 ± 9	N.S.
Total serum protein, g/100 ml	7.30 ± 0.99	6.95 ± 0.40	N.S.
Serum albumin, g/100 ml	3.18 ± 0.76	2.82 ± 0.27	N.S.
Hematocrit, %	29 ± 7	40 ± 2	<0.05

[a] All reported measurements were made at the end of the experiment. [b] Two-sample t-test. [c] Relative to body weight before bile duct ligation. [d] All data are mean ± SD, n = 4. [e] Normal value <40 mU/ml.

The dicumarol elimination kinetics observed in the second (60-hr blood sampling) study are presented in Table II. The results were almost identical to those in the first study; total clearance and β were significantly increased by phenobarbital. The average intrinsic clearance of free drug increased about twofold, but the difference was not statistically significant due to large interanimal variations. The average free fraction of dicumarol in serum of the two groups of animals was almost identical. Despite the poor physical state of the rats, the postintravenous drug concentration–time curves were "clean" in that drug concentrations declined exponentially without significant perturbations or time-dependent changes in slope (Figs. 1 and 2).

All animals exhibited appreciable weight loss and elevation of serum transaminases (Table III). There were no significant differences in these and other clinical chemical parameters between control and phenobarbital-treated rats, except that the latter had larger livers (a characteristic consequence of treatment with an enzyme inducer such as phenobarbital; Refs. 5 and 6) while the control animals, for unknown reasons, had a lower hematocrit at the end of the study (Table III).

Absorption of orally administered dicumarol was slow (Fig. 1) and in some cases persisted for the duration of the experiment (Fig. 2). Therefore, extrapolation of drug concentrations in plasma beyond the last data point was carried out by two methods representing the limiting cases of continuing absorption beyond ~60 hr (Method I) and no further absorption after ~60 hr (Method II) (Ref. 2; footnotes *b* and *c*, Table IV). The two methods yielded essentially identical systemic availability estimates for control animals but somewhat different estimates for phenobarbital-treated rats (Table IV). The latter difference was due primarily to one rat that absorbed dicumarol particularly slowly (Fig. 2). Comparing the systemic availability estimates by either Method I or II

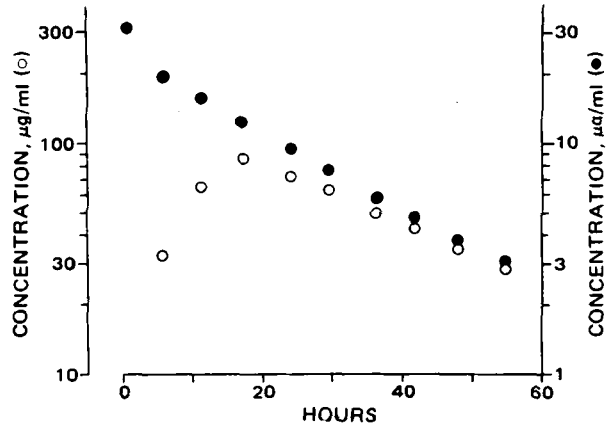

Figure 1—*Plasma dicumarol concentrations as a function of time after simultaneous administration of 50 mg/kg po (○) and 2 mg/kg iv (●) to a control rat with ligated bile duct. ^{14}C-Labeled drug was used for the intravenous injection.*

Table IV—Effect of Phenobarbital Treatment on the Systemic Availability[a] of Orally Administered Dicumarol in Rats (60-hr Study)

Control Animals				Phenobarbital-Treated Animals			
Rat	Method I[b]	Method II[c]	I/II Ratio	Rat	Method I[b]	Method II[c]	I/II Ratio
1C	46	43	1.07	1P	42	41	1.02
2C	29	27	1.07	2P	30	28	1.07
3C	49	47	1.04	3P	34	21	1.68
4C	19	17	1.12	4P	54	41	1.32
Mean	36	34	1.08		40[d]	33[d]	1.27
SD	14	14	0.03		11	10	0.30

[a] Expressed as percent of oral dose. [b] 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. [c] 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 by intravenous injection. [d] Not significantly different from the appropriate control value by two-sample *t*-test.

leads to the conclusion that phenobarbital treatment had no apparent effect on the extent of dicumarol absorption.

Figure 3 is a plot of the cumulative fraction of the dicumarol dose absorbed as a function of time after administration of the anticoagulant. Absorption kinetics are presented in Fig. 4. These data show that phenobarbital had no apparent effect on the rate or extent of dicumarol absorption by bile duct-ligated rats; the average time required to absorb 50% of the amount of drug eventually absorbed (not the administered dose) was ~11 hr, and the average amount absorbed was 33–40% of the dose.

DISCUSSION

The original intent of this investigation was to determine the effect of phenobarbital treatment on the systemic availability of orally administered dicumarol when bile was not present in the intestinal tract, *i.e.*, under conditions where an effect of phenobarbital on bile output would not influence the results. All else being equal, a lack of phenobarbital effect on dicumarol absorption in the absence of bile together with the previously demonstrated pronounced absorption inhibitory effect of phenobarbital on dicumarol in normal animals (2) would suggest strongly that the phenobarbital effect in normal animals is mediated by an altered bile output. The results of this study show, however, that bile duct ligation causes a profound change in the pathophysiological status of the animals.

The total dicumarol clearance in bile duct-ligated control rats was much lower than in previously studied normal control rats (2, 7). This finding is particularly significant since the serum free fraction of dicumarol was higher in the bile duct-ligated animals (5, 8), presumably due to accumulation of endogenous displacing agents. All else being equal, an increase in the serum free fraction should increase the total anticoagulant clearance (8). Serum transaminase elevation in the bile duct-ligated rats is indicative of hepatotoxicity, apparently caused by cholestasis. The latter condition may also account for the somewhat larger liver of the bile duct-ligated control rats as compared to the liver of normal animals (5, 6). Liver damage, in turn, is probably at least partly responsible for the decreased dicumarol clearance since the drug is eliminated by hepatic biotransformation (9). Bile acids are known to be hepatotoxic; they also block the type I binding site on cytochrome P-450 (10). Impairment of microsomal drug metabolism in cholestasis has been observed by other investigators (10).

The relative magnitude of enzyme induction caused by phenobarbital was similar in bile duct-ligated and normal animals; total dicumarol clearance was approximately doubled by phenobarbital in both types of animals (Tables I and II and Ref. 2). Therefore, phenobarbital may be potentially useful as a therapeutic tool to compensate for some biotransformation deficiencies in certain types of impaired liver function.

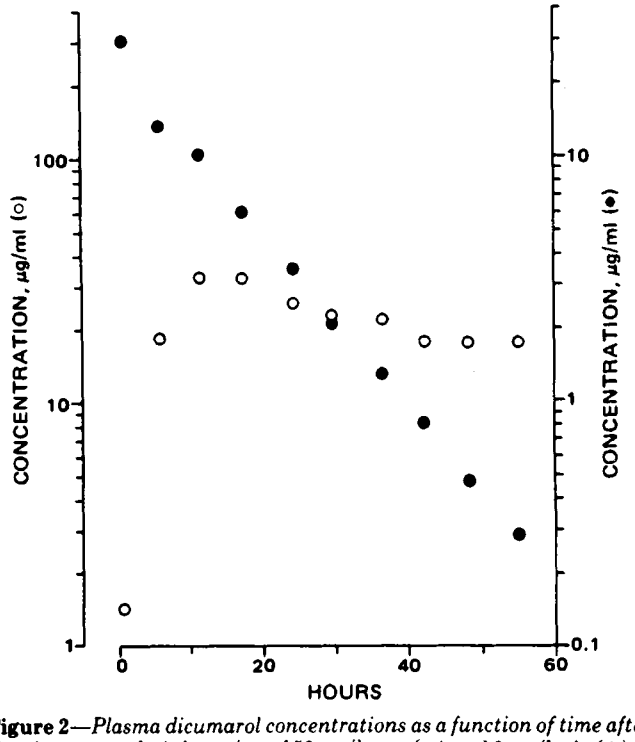

Figure 2—*Plasma dicumarol concentrations as a function of time after simultaneous administration of 50 mg/kg po (○) and 2 mg/kg iv (●) to a bile duct-ligated rat treated with oral phenobarbital sodium, 75 mg/kg/day, beginning 5 days before dicumarol administration. ^{14}C-Labeled dicumarol was used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

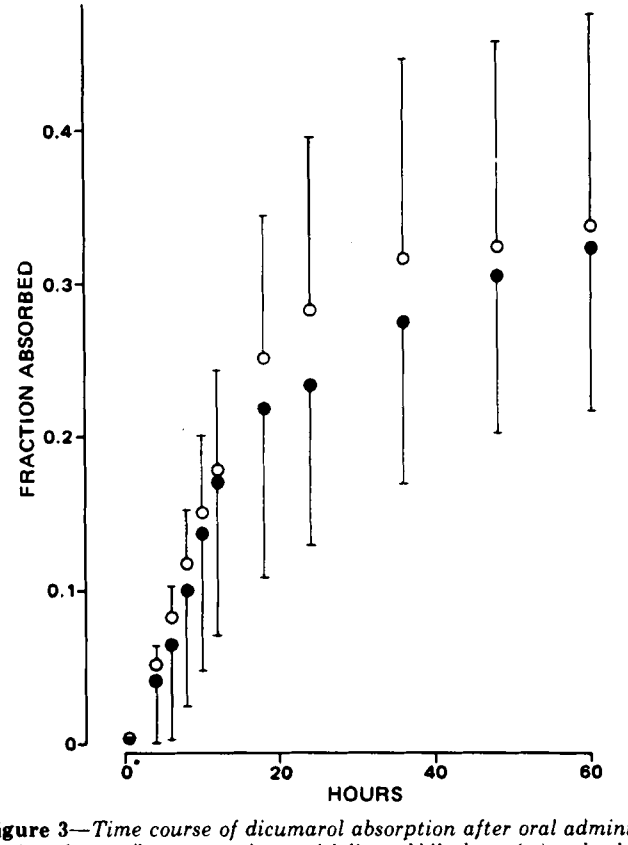

Figure 3—*Time course of dicumarol absorption after oral administration of 50 mg/kg to control rats with ligated bile ducts (○) and to bile duct-ligated rats treated orally with phenobarbital sodium, 75 mg/kg/day, beginning 5 days before dicumarol administration (●). Points are mean + or − SD of four control and four phenobarbital-treated animals.*

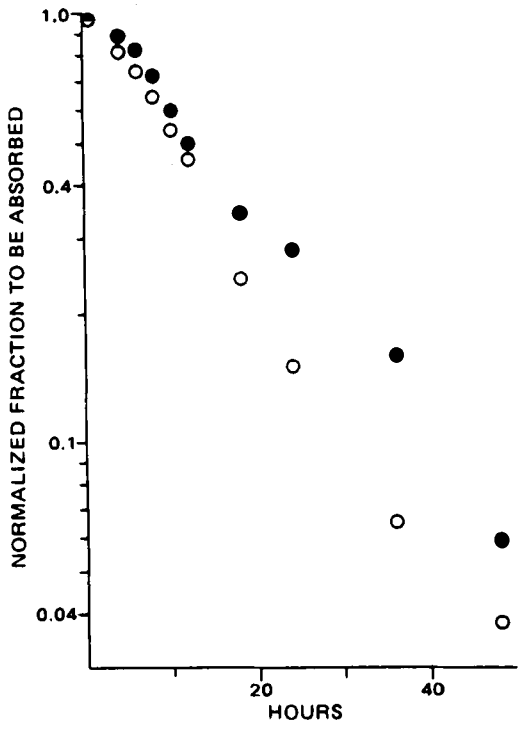

Figure 4—*Dicumarol absorption kinetics in four control (○) and four oral phenobarbital-treated (●) rats, all with ligated bile ducts. Plotted on the ordinate is the fraction of total absorbed dicumarol that was remaining to be absorbed at various times after oral administration of a 50-mg/kg dose.*

Dicumarol was absorbed much more slowly by bile duct-ligated animals ($t_{50\%} \simeq 11$ hr) than by normal rats ($t_{1/2} \simeq 3$ hr; Ref. 2). The reason for this difference is not evident from the available data. Bile duct-ligated control rats absorbed ≤40% of the oral dose while normal control rats absorbed >80% under the same experimental conditions (2). Perhaps bile enhances absorption by increasing the dissolution rate of the almost water-insoluble dicumarol. It will be of interest to determine systemic dicumarol availability in rats with exteriorized bile ducts who are receiving a concomitant intravenous bile infusion, *i.e.*, animals that are not cholestatic or bile salt deficient and presumably have normal liver function but no bile in the intestine.

Systemic dicumarol availability in normal rats was reduced from >80 to <50% by phenobarbital treatment (2). No such absorption inhibitory effect was observed in bile duct-ligated rats. Dicumarol availability is likely to be affected by many factors such as the solubilizing effect of intestinal fluids, GI motility, and gut wall metabolism. (Significant first-pass hepatic biotransformation can be excluded on theoretical grounds; Ref. 6.) Since bile duct ligation changed the pathophysiological status of the animals (rather than only preventing bile entry into the intestine), the lack of a significant phenobarbital effect on systemic dicumarol availability in bile duct-ligated rats cannot be ascribed to any one factor. However, the results of this investigation demonstrate that a drug–drug interaction such as the one between dicumarol and phenobarbital may be pronounced in normal animals and either absent or less pronounced in animals with altered pathophysiological status.

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Double Latin Square Study to Determine Variability and Relative Bioavailability of Methylprednisolone

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Abstract □ The variability and relative bioavailability of methylprednisolone tablets were evaluated utilizing a double Latin square crossover design in which each of 20 subjects was given four of five treatments. Three different lots of methylprednisolone tablets exhibited virtually identical absorption, with similar ranges and coefficients of variation of some selected bioavailability parameters indicative of lot-to-lot uniformity in bioavailability. Within-lot and between-lot uniformities in bioavailability also were similar, suggesting that the observed variability in serum methylprednisolone levels was not due to manufacturing process variables. With respect to intra- *versus* intersubject variability, no differences were found for the absorption rate or terminal half-life. In contrast, between-subject variability associated with extent of absorption was greater than that within subjects. Relative to an aqueous suspension, methylprednisolone tablets were fully bioavailable.

Keyphrases □ Methylprednisolone—bioavailability, tablet variability, commercial preparations, Latin square study □ Glucocorticoids—methylprednisolone, bioavailability, tablet variability, commercial preparations, Latin square study □ Bioavailability—commercial methylprednisolone preparations

On January 7, 1977, the Food and Drug Administration published a list of rules and regulations for conducting bioequivalency studies in humans (1). Their intent was to assure product interchangeability by demonstrating that, on the average, two or more products would exhibit similar bioavailabilities. However, the rules and regulations appear to have ignored the variability in the bioavailability of a given product; and this variability might result in thera-

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