Quinidine and lidocaine are drugs that have intermediate-to-high hepatic extraction ratios and metabolites formed by the hepatic mixed-function oxidase system (10). Therefore, their clearances should be dependent both on liver blood flow and drug-metabolizing enzyme activity (18). The most likely explanation for the decreased clearances observed in this study is inhibited drug metabolism; however, decreased liver blood flow may play a role since a single dose of cimetidine has been shown to reduce hepatic blood flow 25% in humans (4). Since quinidine and lidocaine are drugs with narrow therapeutic ranges, decreases in their clearances could potentially lead to drug accumulation with resultant toxicity. Evaluated blood levels of quinidine have been associated with cinchonism, arrhythmias, and syncope, while increased lidocaine levels have been linked to confusion, seizures, and respiratory arrest (10). Therefore, frequent measurement of quinidine and lidocaine serum levels are recommended when cimetidine is prescribed concurrently, pending human pharmacokinetic studies on these interactions.

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Acetaminophen–Aluminum Hydroxide Interaction in Rabbits

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Received March 25,1982, from the **Department of Pharmaceutics, College of Pharmacy, University of Florida, Gainesville, FL 32610*, and the ‡*Pharmaceutics Department, College of Pharmacy, University of Houston, Houston, TX 77030.* Accepted for publication July 8, 1982.

Abstract □ Acetaminophen–aluminum hydroxide interaction was investigated in a crossover study using six rabbits. Blood samples were collected at various time intervals for up to 6 hr following the oral administration of acetaminophen alone or in combination with aluminum hydroxide. Aluminum hydroxide at a 40-mg/kg dose did not appear to affect the rate and extent of acetaminophen absorption. The influence of aluminum hydroxide on gastric emptying could be compromised by gastric absorption of acetaminophen, resulting in a negligible effect on the overall bioavailability of acetaminophen.

Keyphrases □ Acetaminophen and aluminum hydroxide interaction—crossover study in rabbits, pharmacokinetics □ Aluminum hydroxide—effect on rate and extent of acetaminophen absorption in rabbits □ Pharmacokinetics—rate and extent of acetaminophen absorption in rabbits with and without aluminum hydroxide administration

Retarded drug absorption in the presence of aluminum hydroxide has been demonstrated in animals and humans (1). This pharmacokinetic interaction probably results from a slowed gastric emptying (1). *In vitro*, aluminum ion inhibits the contractile response of human and rat gastric strips to acetylcholine (2). This effect is possibly due to the antagonization by aluminum of calcium influx into smooth muscle cells during depolarization, leading to a delay in muscle contractions (3).

Acetaminophen (weak acid, pK_a 9.5) is a common, nonprescription, analgesic drug. It is not clear whether acetaminophen is completely free of damaging effects on the gastric mucosa, although minimal or no gastric structural damage has been found to be induced by acetaminophen in marked contrast to aspirin (4). Since acetaminophen is believed to cause less damaging effects, it has replaced aspirin as the analgesic of choice in many situations. However, acetaminophen is frequently used in combination with aspirin in nonprescription drugs (5), necessitating the use of antacids to avoid the gastric damage induced by aspirin, or perhaps acetaminophen.

Table I—Mean Plasma Acetaminophen Concentrations Following Oral Administrations of Acetaminophen Alone (100 mg/kg) and in Combination with Aluminum Hydroxide (40 mg/kg) to Rabbits

Time, hr	Acetaminophen		Acetaminophen Plus Aluminum Hydroxide	
	Mean[a]	*SEM*	Mean[a]	*SEM*
0.25	35.77	2.38	31.37	3.15
0.50	27.39	1.65	27.17	1.18
0.75	23.53	2.05	22.89	1.79
1.0	18.77	1.51	16.95	1.86
1.5	11.54	1.16	11.06	1.40
2.0	7.84	0.62	7.65	0.96
3.0	4.74	0.60	4.65	0.62
4.0	2.82	0.47	2.57	0.36
5.0	1.77	0.28	1.81	0.26
6.0	1.29	0.24	1.41	0.21

[a] Mean data of six rabbits.

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Table II—Distribution Parameter (α), Elimination Parameter (β), and AUC of Acetaminophen Following Treatments with Acetaminophen Alone and in Combination with Aluminum Hydroxide

Rabbit	Acetaminophen Alone			Acetaminophen Plus Aluminum Hydroxide		
	α, hr^{-1}	β, hr^{-1}	AUC, $\mu g \cdot hr/ml$	α, hr^{-1}	β, hr^{-1}	AUC, $\mu g \cdot hr/ml$
1	1.93	0.38	38.30	3.03	0.36	31.83
2	3.06	0.38	51.99	2.50	0.29	42.44
5	1.90	0.38	43.72	2.02	0.44	35.18
6	1.27	0.31	55.45	1.83	0.40	55.80
9	2.12	0.53	43.46	1.15	0.34	43.80
12	1.55	0.26	39.20	1.77	0.54	50.53
Mean[a]	1.97	0.37	45.39	2.05	0.40	43.26
SEM	0.25	0.04	2.83	0.27	0.04	3.68

[a] Mean data of six rabbits. Statistical difference of the mean was tested by Student's paired t test, $p > 0.8$ for α, $p > 0.75$ for β, and $p > 0.55$ for AUC.

The delayed absorption of salicylic acid from tablets buffered with aluminum hydroxide has been documented previously (6). A recent review of drug interactions with acetaminophen and aspirin was presented by Hayes (7). This study is undertaken to verify the drug interaction between acetaminophen and aluminum hydroxide using the rabbit model.

EXPERIMENTAL

Materials—Acetaminophen[1] and aluminum hydroxide[2] were reagent grade and were used without further purification. Appropriate strengths of acetaminophen alone or in combination with aluminum hydroxide were prepared in warm deionized water (37°) prior to oral administration to rabbits.

Animal Studies—The validity of the rabbit model for drug absorption has been discussed in a previous publication (8). Six male New Zealand rabbits weighing 2.8–4.6 kg were studied in a crossover design, with a 2-week washout period allowed between studies. Rabbits were fasted for 38–42 hr prior to the experiment, but water was allowed *ad libitum*. Food and water were withheld during the experiment. Acetaminophen (100 mg/kg) alone or in combination with aluminum hydroxide (40 mg/kg) was dissolved in 70 ml of warm deionized water and was administered orally to the rabbit by intubation. The intubation line was flushed with 30 ml of warm water to ensure complete delivery of the drugs to the stomach. Blood samples (0.5 ml each) were collected from the ear vein in heparinized tubes[3] at 0 (just before experiment), 0.25, 0.5, 0.75, 1.0, 1.5, 2, 3, 4, 5, and 6 hr. After centrifugation of the blood samples, plasma aliquots were obtained for analysis of acetaminophen content.

Drug Assay—Plasma acetaminophen was assayed by a high-performance liquid chromatographic method (9). Vanillin[4] was used as an internal standard. The assay method provided a minimum detectable concentration of 0.5 μg/ml in plasma. However, since 0.2-ml aliquots of plasma were the maximal volume available for analysis, the minimum detectable quantity was 0.1 μg.

RESULTS AND DISCUSSION

Acetaminophen, alone or in combination with aluminum hydroxide, was rapidly absorbed in rabbits, with the peak plasma concentration occurring within 15 min of drug administration. Mean plasma data obtained from the two crossover studies are presented in Table I. Following the administration of acetaminophen alone and in combination with aluminum hydroxide, similar plasma levels of acetaminophen were observed at corresponding time intervals for all the rabbits. Since plasma concentrations of acetaminophen appeared to decay biexponentially, data were fitted to a biexponential function using the NONLIN program (10). The distribution parameter (α) and the elimination parameter (β) thus obtained from the curve fitting are presented in Table II. The mean parameter values are not significantly different from each other ($p > 0.8$ for α; $p > 0.75$ for β). The lack of difference in mean values of α and β was expected, since aluminum hydroxide presumably affected drug absorption but not the distribution and/or elimination.

Values for area under the plasma concentration–time curve (AUC) were estimated according to the trapezoidal rule and the extrapolation method. Values for mean AUC were similar, 45.39 μg hr/ml for acetaminophen alone and 43.26 μg hr/ml for acetaminophen in combination, indicating no significant difference in the extent of acetaminophen absorption between the two treatments ($p > 0.55$). Since blood sampling was not carried out during the first 15 min after drug administration, it was not clear whether aluminum hydroxide affected the rate of acetaminophen absorption. However, a drug interaction of this magnitude would be of minimal clinical significance if it indeed occurred. By the same token, since areas covering 0–15 min constituted <10% of the total estimated areas, the effect of aluminum hydroxide, if indeed seen in the first 15 min, would not affect the overall bioavailability of acetaminophen. Therefore, it is to be concluded, based on these data, that aluminum hydroxide affected neither the rate nor the extent of acetaminophen absorption.

Aluminum salts have been known to delay the absorption of various drugs in animals and in humans. *In vitro* experiments have demonstrated that aluminum ion antagonizes the contractile response of human and rat gastric strips to acetylcholine. Although *in vitro* experiments demonstrating the antagonizing effect of aluminum ion have not been conducted in rabbits, a similar conclusion to that found for the human and rat would be drawn. At the 40-mg/kg dose used in this study, aluminum hydroxide did not appear to affect the rate and extent of acetaminophen absorption. This dose, on a weight basis, was approximately three times the therapeutic dose of the antacid used in humans. Acetaminophen is an acidic compound, a fact that favors its absorption from the stomach according to the pH partition theory. Although the gastric emptying might be delayed in the presence of aluminum hydroxide, the effect could be compromised by the gastric absorption of acetaminophen. The acetaminophen dose used in this study, 100 mg/kg, was approximately three times the recommended maximal daily dose for humans; whereas, the 40-mg/kg dose of aluminum hydroxide was similar to that used in a drug–antacid interaction study in humans (11).

Hurwitz (1) proposed that only drug products in which absorption is not rate limited by dissolution are subject to a significant gastric emptying effect. Acetaminophen is in this category. To eliminate the dissolution factor, a solution of acetaminophen instead of solid dosage forms was used in this study. Therefore, any interaction found in this study was the exclusive effect of gastric emptying and gastric absorption of the interactants. Since acetaminophen is available as solid dosage forms for human use, direct extrapolation of rabbit data to the human situation is not deemed appropriate. However, it is felt that any acetaminophen–aluminum hydroxide interaction, if indeed one occurs in humans, will be of minimal clinical significance based on results of this study in rabbits. The proof awaits further investigation in humans.

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High-Yield Synthesis of Warfarin and Its Phenolic Metabolites: New Compounds

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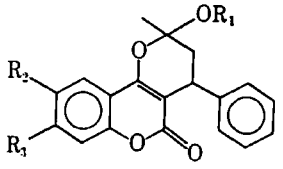
Received April 15, 1982, from the *Department of Medicinal Chemistry, University of Washington, Seattle, WA 98195.* Accepted for publication June 29, 1982.

Abstract □ A novel synthesis of warfarin and phenolic warfarin metabolites is presented which results in higher yields than previous methods.

Keyphrases □ Warfarin—phenolic metabolites, new high-yield synthetic method □ Synthesis—warfarin and its phenolic metabolites, new high-yield method

The oral anticoagulant warfarin [4-hydroxy-3-(1-phenyl-3-oxobutyl)-2*H*-1-benzopyran-2-one] (I) has found extensive clinical use in the treatment of such pathological conditions as thrombophlebitis, pulmonary emboli, and myocardial infarction (1). It is also used widely as a rodenticide to help control rat populations (2) and more recently has been used as a probe to investigate the multiplicity and catalytic activity of microsomal and purified cytochrome P-450 preparations (3–7). Because of its clinical and pharmacological importance, considerable effort has been expended to develop analytical methods to quantitate both warfarin and its metabolites from biological matrices (8–11). However, one of the impediments to progress in this area has been the lack of a high-yield synthetic procedure for these materials. Since mechanistic work in this laboratory on cytochrome P-450 required the synthesis and optical resolution of specifically deuterated warfarin analogues as substrates and multideuterated metabolites as GC–MS assay internal standards, the need for a reproducible, high-yield synthesis for these compounds was evident.

Although successful synthetic routes to these materials are documented in the literature, the reported yields are poor to moderate at best (12). Warfarin has been synthesized by the Michael addition of 4-hydroxycoumarin to benzalacetone under a number of acid- or base-catalyzed conditions (13). Traditionally, the reaction has most often been run in water containing a catalytic amount of triethylamine (~5 mole %). Hermodson *et al.* (12), using essentially the same conditions, extended this synthesis to the phenolic metabolites of warfarin by condensing benzalacetone with the appropriately substituted 4-hydroxycoumarin. Fasco *et al.* (14) followed a similar route, but to obtain a homogeneous system substituted dioxane as the solvent and piperidine as the catalyst. A further refinement was reported by Cook *et al.* (15) who, in their synthesis of 3′-bromowarfarin, heated a solution of *m*-bromobenzalacetone and 4-hydroxycoumarin in pyridine at reflux. However, rarely was the overall yield of warfarin or hydroxywarfarin >65%. In the case of 7-hydroxywarfarin, it was invariably much lower.



		R_1	R_2	R_3
I:	Warfarin	H	H	H
II:	Warfarin Ethyl Ether	C_2H_5	H	H
III:	Cyclocoumarol	CH_3	H	H
IV:	6-Hydroxywarfarin	H	OH	H
V:	7-Hydroxywarfarin	H	H	OH
VI:	6-Hydroxycyclocoumarol	CH_3	OH	H
VII:	7-Hydroxycyclocoumarol	CH_3	H	OH

RESULTS AND DISCUSSION

Numerous attempts in this laboratory to improve previous yields by alternate reaction pathways failed. A new approach was conceived when it was found that warfarin was quantitatively converted to its ethyl ether (II) by refluxing in absolute ethanol. This observation suggested that in absolute ethanol, the acidity of 4-hydroxycoumarin might be sufficient to catalyze its condensation with benzalacetone to generate warfarin. Once formed, the reaction would be driven to completion by the subsequent and essentially irreversible formation of the ethyl ether under these conditions. Initial experiments investigating this possibility proved successful. Complete removal of the ethanol, however, proved to be difficult; therefore, methanol was substituted as the solvent, with equal success. This extended the necessary reaction time, presumably because of the lower reaction temperature; however, the lower temperature also allowed the relatively unstable starting material (4,7-dihydroxycoumarin) to be converted to the warfarin methyl ether (VII) in high yield.

The reaction involved stirring an appropriate coumarin analogue with benzalacetone in refluxing methanol. After 4–24 hr, as determined by TLC, the corresponding methyl ether was obtained in high yield. The ether can be quantitatively converted back to the warfarin analogue by acid hydrolysis. Typically, overall yields are >70%, and often yields as high as 95% are obtained. This dramatic increase in reaction yields should significantly aid in the development of warfarin as a tool to probe metabolic pathways.

EXPERIMENTAL[1]

Warfarin [4-Hydroxy-3-(1-phenyl-3-oxobutyl)-2*H*-1-benzopyran-2-one] (I)—4-Hydroxycoumarin (1.0 g, 0.0069 mole) was stirred

[1] Melting points were determined on a Thomas-Hoover melting point apparatus and are uncorrected. NMR spectra were recorded on a Varian EM-360A spectrometer using tetramethylsilane as internal standard. 4-Hydroxycoumarin was purchased from the Aldrich Chemical Co., and benzalacetone was purchased from MCB Reagents. 4,6- and 4,7-Dihydroxycoumarin were gifts from Dr. Lawrence Low, University of Washington. TLC was performed on EM Reagents analytical silica gel chromatography plates with flourescent indicator (no. 5539). All other solvents and reagents were of reagent purity.

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