THE INFLUENCE OF BARBITURATES ON COUMARIN PLASMA LEVELS AND PROTHROMBIN RESPONSE *

By PETER G. DAYTON, YAVUZ TARCAN, THEODORE CHENKIN AND MURRAY WEINER

(From the New York University Research Service, Goldwater Memorial Hospital, Welfare Island, New York, N. Y.)

(Submitted for publication April 25, 1961; accepted May 19, 1961)

Wide individual variations in response to drugs is a common clinical problem, and is especially evident with coumarin anticoagulant therapy (1–3). A possible contributing factor to such variations could be the influence of another presumably unrelated drug upon the response to the coumarin anticoagulant. Reports from several laboratories (4–7) indicate that barbiturates can reduce the prothrombin response to coumarin anticoagulants. This report presents initial results of studies designed to determine the manner by which barbiturates alter the response to coumarins in man, guinea pig and dog.

MATERIAL AND METHODS

Guinea pigs. Diet, method of handling guinea pigs, and blood sample collection have been reported (8). Drugs were administered to guinea pigs intraperitoneally, barbital and acenocoumarin as aqueous solutions of their sodium salts in 0.5 to 1 ml, and chlorobutanol in 1.0 ml of peanut oil.

Dogs. Dogs were maintained on Kibbled dog food (Big Red Co.). They were given biscoumacetate (75 mg per kg) intravenously and barbital orally (50 mg per kg).

Human subjects. Ambulatory, hospitalized subjects free from known liver, kidney or gastrointestinal disease, on a regular hospital diet were studied. Their age ranged from 40 to 60 years. Heptabarbital and acenocoumarin were administered orally. Dicumarol (bishydroxycoumarin) and biscoumacetate were administered either orally or intravenously.

Chemical methods. The procedures for the estimation of prothrombin time (9), Dicumarol (10), and biscoumacetate (11) have been described. Acenocoumarin was determined by the method used for biscoumacetate except that the final extraction was made into a smaller volume (2 ml) of NaOH, and optical density was measured at 302 mμ. Barbital and heptabarbital did not interfere with the analyses of Dicumarol, biscoumacetate and acenocoumarin.

* Presented in part before the American Heart Association, Philadelphia, Pa., October 25, 1959. This investigation was supported in part by Research Grant H-4347 (C 1), National Heart Institute, United States Public Health Service, Bethesda, Md., and the National Vitamin Foundation, New York, N. Y.

RESULTS

Studies with guinea pigs. Administration of several daily doses of 140 mg per kg of sodium barbital to guinea pigs had no effect on prothrombin time (Table I). Administration of acenocoumarin produced the expected hypo-prothrombinemia observed previously (8). However, in animals which had received acenocoumarin after pretreatment with barbital, no appreciable hypoprothrombinemia was noted. At this dosage level of barbital the effect was consistent; with lower doses (100 mg per kg) block of acenocoumarin effect was less complete. Pretreatment with three daily doses of chlorobutanol (125 mg per kg) also antagonized the hypo-prothrombinemia induced by acenocoumarin. The average prothrombin time in the acenocoumarin-treated control group was 70 seconds (whole plasma) and 53 seconds in the chlorobutanol- and acenocoumarin-treated group. The corresponding time was 145 seconds and 84

TABLE I

Prothrombin response of guinea pigs to acenocoumarin, barbital, and acenocoumarin and barbital

	Plasma prothrombin time*			
	Whole		Saline diluted (12.5%)	
	Average	Range	Average	Range
	sec		*sec*	
Saline (1 ml)	38	32–45	54	44–67
Barbital†	38	32–43	62	55–70
Acenocoumarin‡	138	60–250	>200	97–420+
Acenocoumarin‡ and barbital†	41	33–61	80	55–128

* Blood collected on Day 5; nine animals in each group.
† Sodium barbital, 140 mg/kg, i.p. daily on Days 1–4.
‡ Sodium acenocoumarin, 4.5 mg, i.p. on Days 3 and 4 in 1 ml water.

TABLE II

Effect of pretreatment with barbital on plasma levels of acenocoumarin in guinea pigs *

Time	No pretreatment			Pretreated†		
	No. of animals	Plasma level		No. of animals	Plasma level	
		Mean	Range		Mean	Range
hrs		*mg/L*			*mg/L*	
0.5	5	40	25–56	8	26	18–42
1				7	16	7–24
1.5	11	34	16–51	9	6.6	2–12

* All animals were injected i.p. with 33 mg/kg of sodium acenocoumarin, and sacrificed at the indicated time.
† Injected with single daily i.p. doses of 140 mg/kg of sodium barbital beginning 4 days prior to the experiment.

seconds for diluted plasma. Chlorobutanol by itself had no effect on prothrombin time.

Barbital has a half-life in plasma of about 3 days in guinea pigs, and is not appreciably metabolized (12). The half-life of acenocoumarin, as measured by the fall in plasma concentration in guinea pigs, is much shorter than that of barbital. Considerably lower levels of acenocoumarin were found in barbital-pretreated animals than in control animals (Table II). In both groups of animals detectable amounts of acenocoumarin were absent in the plasma at the time of prothrombin estimation (24 hours after dosage).

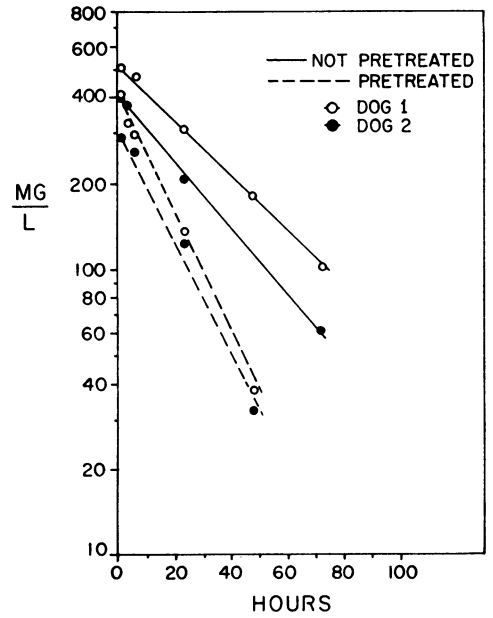

FIG. 1. PLASMA LEVELS IN TWO DOGS FOLLOWING AN INTRAVENOUS DOSE (75 MG/KG) OF BISCOUMACETATE. The same dose was repeated in the same animal several weeks later, 2 hours after an oral dose of barbital (50 mg/kg).

Studies in dogs. Plasma levels of biscoumacetate after a single intravenous dose of the drug were compared with levels resulting from the same dose after pretreatment of the same animals with barbital (Figure 1). Pretreatment accelerated the decline of the plasma biscoumacetate level and reduced the hypo-prothrombinemic response. Diluted (12.5 per cent) plasma prothrombin time 48 hours after dosage was 75 seconds (Dog 1) and 66 seconds (Dog 2) without pretreatment, and 65 and 48 seconds with pretreatment.

Studies in man. The prothrombin response was measured in the same human subjects after: *1*) an oral dose of a coumarin anticoagulant only;

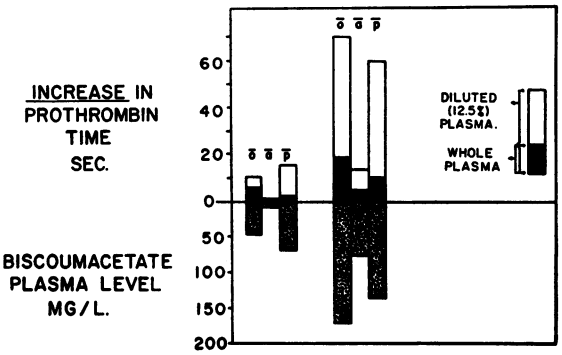

FIG. 2. MAXIMAL PROTHROMBIN RESPONSE (UPPER BARS) AND BISCOUMACETATE PLASMA LEVEL 5 HOURS AFTER A SINGLE ORAL DOSE OF THE DRUG (LOWER BARS) IN TWO ILLUSTRATIVE HUMAN SUBJECTS. The first set of bars presents values obtained in a relatively resistant subject (Wi), and the second set in a sensitive subject (M). The first bar in each set ($\bar{o}$), results without barbiturate; the center bar ($\bar{a}$), pretreated with 400 mg heptabarbital 1 hour before the anticoagulant; the last bar ($\bar{p}$), heptabarbital 5 hours after the anticoagulant.

2) pretreatment with an orally administered barbiturate (heptabarbital) followed by an orally administered coumarin; *3*) an orally administered coumarin, followed by an orally administered barbiturate; *4*) an intravenous dose of a coumarin only; *5*) pretreatment with an orally administered barbiturate followed by an intravenously administered coumarin. Experiments on individual subjects were carried out at 2- to 3-week intervals.

Results of experiments with biscoumacetate are shown in Figure 2 and Table III; with Dicumarol in Table IV and with acenocoumarin in Table V. Pretreatment with heptabarbital, followed by an *oral* dose of any of the three coumarin anticoagulants resulted in lower plasma levels of the cou-

TABLE III

Effect of heptabarbital on biscoumacetate plasma levels and prothrombin response in man

Subject	Drugs administered: Biscoumacetate Dose	Biscoumacetate Route	Heptabarbital (400 mg, oral) Pretreatment	Heptabarbital (400 mg, oral) Posttreatment	Maximum increase in prothrombin time: Whole plasma	Maximum increase in prothrombin time: 12.5% Saline diluted plasma	Plasma level of biscoumacetate after dose at: 1 hr	4 hrs	5 hrs	24 hrs
	mg		*hrs before*	*hrs after*	*sec*		*mg/L*			*mg/L*
Wi	1,800	Oral	0	0	5.8	10.2			44	<1
	1,800	Oral	1	0	1.2	0.5			4.8	<1
	1,800	Oral	0	5	3.0	15.0			66	4.6
M	1,500	Oral	0	0	16.2	68.0			170	9.3
	1,500	Oral	1	0	4.5	13.0			75	4.1
	1,500	Oral	0	5	10.0	58.0			136	9.4
S	1,500	Oral	0	0	5.5	15.0			137	4.0
	1,500	Oral	1*	0	2.0	9.0			63	3.0
	1,500	i.v.	0	0	2.0	22.5	152	83		24
	1,500	i.v.	1	0	3.0	22.0	180	110		31
Wr	1,500	i.v.	0	0	4.0	13.5	138		51	24
	1,500	i.v.	1*	0	3.5	11.0	136		47	23

* Besides the heptabarbital dose given 1 hour before the coumarin, 400 mg/day was given for 3 to 5 days before the day of the experiment.

marin and a decreased prothrombin response, compared with experiments in which the coumarin was given alone, either orally or intravenously. Administration of the barbiturate 5 hours after rather than before the coumarin, had a slight effect in some instances, and none in others. Blood levels of acenocoumarol were low (less than 3.5 mg per L) in all subjects without pretreatment,

TABLE IV

Effect of heptabarbital on Dicumarol plasma levels and prothrombin response in man

Drugs administered	Time of collection	Prothrombin times: Subject B Whole	Subject B Dil.	Subject C Whole	Subject C Dil.	Plasma conc. of Dicumarol: Subject B	Subject C
	hrs	*sec*		*sec*		*mg/L*	
Dicumarol, 500 mg p.o.	Control	13.0	29.5	14.5	33.0		
	24	13.0	35.0	15.0	51.5	23.1	29.2
	48	20.0	51.5	19.5	61.5	19.2	8.9
	72	19.5		14.5		17.1	5.7
	96					11.8	5.0
Dicumarol, 500 mg p.o.	Control	14.5	24.5	14.5	23.0		
Heptabarbital, 400 mg/day	24	15.5	27.5	15.0	33.5	14.5	1.8
for 3 days and 1 hr	48	17.0	29.5	14.0	30.0	14.1	2.2
before Dicumarol	72	16.0	30.5	14.5	27.0	6.9	3.0
	96	14.0	34.5	13.0	28.5		<0.9
Dicumarol, 500 mg p.o.	Control	13.5	26.0	15.5	29.5		
Heptabartital, 400 mg	5					21.4	8.9
5 hours after Dicumarol	24	19.5	37.5	18.5	40.0	13.6	12.5
	48	24.0	48.0	18.0	44.0	12.0	8.6
	72	20.5	43.5	15.0	31.5	10.0	4.4
Dicumarol, 500 mg i.v.	Control	13.5	32.0	13.0	31.5		
	5					35.0	63.5
	24	17.0	37.5	19.5	39.5	30.0	31.8
	48	23.0	31.0	24.5	52.0	24.0	22.6
	72	16.5		16.2		23.0	18.8
	96	17.5	33.0	17.0	35.0	18.7	11.9
Dicumarol, 500 mg i.v.	Control	13.0	22.5	14.5	23.5		
Heptabarbital, 400 mg/day	5					49.6	43.0
for 3 days and 1 hr before	24	17.0	35.0	19.5	40.0		
Dicumarol	48	23.0	43.5	22.5	50.5	17.0	19.2
	72	28.0		26.0		16.0	11.8
	96	15.5		20.5		14.0	8.7

TABLE V

Influence of heptabarbital on maximal prothrombin response to acenocoumarin in 3 human subjects

Subject*		Peak prothrombin time	
		Whole plasma	Diluted (12.5%) plasma
		sec	
E	No pretreatment	27	56
	Pretreated	22	44
F	No pretreatment	22	60
	Pretreated	19	37
H	No pretreatment	30	72
	Pretreated	25	45

* Each subject was given a single oral dose of 50 mg of acenocoumarin (no pretreatment). Three weeks later each subject received an oral dose of 400 mg of heptabarbital daily for 4 days. The last dose was followed in 1 hr by a single 50 mg oral dose of acenocoumarin.

and undetectable with pretreatment. There were individual differences in the response to coumarins, but these variations are well known from previous studies (2, 11). Pretreatment with a barbiturate had no appreciable effect on plasma levels of the anticoagulant or prothrombin time response to an *intravenous* dose of biscoumacetate.

It was noted that after an intravenous dose of biscoumacetate the plasma level at 24 hours was 20 to 30 mg per L, whereas after oral dosage the level was less than 10 mg per L (Table III). Since biscoumacetate is rapidly metabolized (about 25 per cent per hour) in man, and absorption is delayed 1 to 2 hours (11), the plasma levels of the drug 4 to 5 hours after oral dosage are consistently higher than those after intravenous administration of the same dose. Yet, the data in Table III show that at 24 hours there are higher levels following intravenous dosage than after oral dosage. The readings obtained by the method of analysis employed may represent, at least in part, a metabolite of biscoumacetate rather than the drug itself. In any event, it is apparent from these data that the metabolic fate of the drug after oral administration differs from that after intravenous administration. It is therefore conceivable that the observed difference in effect of barbiturates on orally versus intravenously administered biscoumacetate in man may be a metabolic effect rather than an effect on absorption. It has previously been shown (13) that the metabolic pathway of biscoumacetate is quite different in various species.

DISCUSSION

During studies with human subjects, Avellaneda (6) noted that a variety of orally administered barbiturates (including heptabarbital) decreased the hypo-prothrombinemic response to biscoumacetate in about two-thirds of the cases studied. The mechanism of this effect was not explained. In the present study, measurements of plasma levels of the anticoagulants revealed that barbiturate-induced reduction of hypo-prothrombinemic effect was correlated with lower plasma levels of the anticoagulant in man and in other species. The effect was observed not only with biscoumacetate but also with Dicumarol and acenocoumarin. It was shown that the relative timing of dosage and the route of administration of the coumarin derivatives were important. Barbiturate pretreatment had no appreciable influence on plasma levels or hypo-prothrombinemic effect of *intravenously* administered coumarin anticoagulants in man. Barbiturate administered *after* the coumarins also had little or no effect.

These findings exclude a vitamin K-like mechanism to explain the barbiturate effect. Unlike barbiturates, vitamin K_1 is effective even when given hours or days after the coumarin, and the action is independent of the route of administration of the coumarin; furthermore, Vitamin K does not affect the plasma levels of coumarin anticoagulants (14). Barbiturates themselves do not affect coagulation (8, 15). The barbiturates could, however, influence the physiological disposition of the coumarin. The finding that a drug can influence the disposition of another apparently unrelated drug is not unique in man (16) or experimental animals (17).

The question arises whether barbiturates affect the absorption of coumarin anticoagulants. It is known that absorption of Dicumarol is slow and sometimes incomplete (2), possibly because of the low aqueous solubility of the drug at the pH range of the gastrointestinal tract. However, acenocoumarin forms a sodium salt which is quite soluble at pH 7 (8) and biscoumacetate is rapidly absorbed from the small intestine (11).

Do barbiturates influence the rate of metabolism of the coumarin? Such an explanation may at first be considered unlikely, since in man the effect was not observed when the coumarin was

given intravenously. However, these findings in man are different from those in the guinea pig and dog. Pretreatment of guinea pigs with barbital (and also with chlorobutanol in adequate dosage) completely inhibited the hypo-prothrombinemic action of intraperitoneally administered acenocoumarin. This species difference may be related to several factors. The doses of barbiturate employed in the experiments with human subjects were small compared with those given to the guinea pigs. In the dog, pretreatment with barbiturate results in lower biscoumacetate levels after intravenous administration of the anticoagulant. Species differences in the metabolic pathway of some coumarins have been clearly demonstrated (13). The data in man suggest differences in the metabolic pathways of oral versus intravenous biscoumacetate. It is therefore possible that, in man, barbiturate pretreatment may influence the metabolic fate (other than absorption) of orally administered biscoumacetate, even though there is no detectable effect on the fate of intravenously administered biscoumacetate.

The results obtained in guinea pigs (antagonism of acenocoumarin by barbital and chlorobutanol) and dogs (antagonism of biscoumacetate by barbital) are similar to the earlier observations of Baumann, Field, Overman and Link (4, 5) (antagonism of Dicumarol by chlorobutanol, aminopyrine, and so forth) in rats. Several of the drugs that produce this antagonism, including barbiturates, were known to increase the biosynthesis, excretion and tissue levels of L-ascorbic acid in dogs and rats (18). In fact, Link and co-workers studied the effect of the "vitamin C stimulators" on Dicumarol-induced hypo-prothrombinemia, because they had previously observed that L-ascorbic acid can influence and even antagonize coumarin-induced hypo-prothrombinemia (19, 20).

Might the coumarins themselves, like the barbiturates, stimulate the enzymes responsible for coumarin metabolism? After prolonged continuous therapy with biscoumacetate, the plasma level pattern upon discontinuation is the same as that following the initial dose (3). Repeated intravenous doses of Dicumarol or biscoumacetate given to the same subject result in similar plasma level curves (2, 13). In fact, in the same subject the half-life of a large dose of Dicumarol or biscoumacetate is longer than that of a small dose (2, 11). Thus, at least in man, coumarins do not accelerate their own metabolism in therapeutic dosage.

Coumarin anticoagulant therapy is frequently accompanied by the administration of other drugs, including barbiturates. This may add to various other difficulties (1–3, 11) in controlling the hypoprothrombinemic effect of coumarins in the treatment of thromboembolic diseases. It may be necessary in the future to pay more attention to the mutual influence of concomitant therapy on the physiological disposition of the drugs employed.

SUMMARY

Results obtained in guinea pig, dog and man show that pretreatment with barbiturates may antagonize the hypo-prothrombinemic effect of coumarin anticoagulants. This effect has been correlated with lower plasma levels of the anticoagulant drugs. In man, route and timing of dosage significantly influence this barbiturate effect.

ACKNOWLEDGMENT

We wish to thank Drs. J. M. Steele, J. J. Burns and B. B. Brodie for valuable suggestions in the preparation of this manuscript, and Mrs. D. Moses, Mr. Gus John and Mrs. Marcellette Snell for technical assistance.

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