without apparent effect on the syndrome. Even vitamin K_1 at 10 mg per kg doses did not completely block the action of SN 13,936 for, although hemorrhagic lesions were absent, a significant hypoprothrombinemia was present. At 100 mg per kg doses, however, vitamin K_1 blocked development of both the hemorrhagic lesions and the hypoprothrombinemia.

Discussion. The data presented above demonstrate that, as in the case of SN 5090,† vitamin K_1 and to a lesser extent 2-methyl-1,4-naphthoquinone antagonize the hemorrhagic syndrome and hypoprothrombinemia induced by SN 5094, SN 5949 and SN 13,936. This finding would seem to strengthen the suggestion that the 2-substituted-3-hydroxy-naphthoquinones exert their hemorrhagic activity by competing with the K vitamins in whatever processes these substances serve in prothrombin formation.

This suggestion appears to offer the only tenable explanation for the hemorrhagic activity of the above naphthoquinones. The compounds do not produce severe liver injury, nor are they especially active antibacterial agents which might interfere with the synthesis of K vitamins by intestinal bacteria. Furthermore, extensive *in vitro* experiments indicate that they do not inactivate prothrombin as do the indandiones.[3] It appears, therefore, as if the relationship of the 2-substituted-3-hydroxy-1,4-naphthoquinones to vitamin K affords another example of antagonism of metabolites by structurally related compounds.

Summary. The development of the hypoprothrombinemia and hemorrhagic lesions, which occurred in the albino rat as the result of ingestion of various 2-substituted-3-hydroxy-1,4-naphthoquinones, could be prevented either partially or completely by simultaneous administration of 2-methyl-1,4-naphthoquinone or vitamin K_1. The amounts of these K vitamins required to block development of the above syndrome differed with the various hydroxy-naphthoquinone derivatives. In each instance, however, vitamin K_1 proved more effective than 2-methyl-1,4-naphthoquinone.

Observations made in this report confirm our earlier suggestion that the hemorrhagic syndrome, which results from administration of 2 - substituted - 3 - hydroxy-1,4-naphthoquinones, is due to competition between these substances and the K vitamins in processes involved in prothrombin formation.

[3] Kabat, H., Stohlman, E. F., and Smith, M. I., *J. Pharm. and Exp. Therap.*, 1944, **80**, 160.

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Action of 3,3′ Methylenebis (4-Hydroxycoumarin) (Dicumarol) on Thromboplastic Activity of Rabbit Brain.*

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Several observations suggest that other factors besides the interference with the formation of prothrombin[1] must be considered in the pathogenesis of the bleeding tendency produced by dicumarol. Widespread dilatation of capillaries, arterioles and venules has been found, indicating that the hemorrhagic condition is due, at least in part,

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[1] Quick, A. J., *The Hemorrhagic Diseases*, pp. 284-285, C. C. Thomas, Springfield, Ill., 1942.

TABLE I.

Influence of Dicumarol Poisoning on Thromboplastic Activity of Brain Tissue (Rabbit) When Tested Against Normal Plasmas.

Thromboplastin preparation from Rabbit No.	Prothrombin time (in sec)													
	Human plasma					Rabbit plasma				Dog plasma				
	N.*	1†	2†	6†	8†	N.	1	2	6	8	N.	1	2	6	8
$CaCl_2$ concentrations															
.02 Molar	12	14	12½	13½	14	6	7	10½	9	9	6	11	8	8½	8
.01 "	11½	12½	12½	12½	13½	6	7½	11	9	9	6	11	8½	9	8
.005 "	11½	12½	12½	12	14	6	8	11	9½	9½	6	11	9½	9½	8½
.0025 "	11	13½	13½	11½	15	5½	9	11	10	10½	5½	11	9	10	9
.00125 "	14½	19½	20	16	22½	6	13	12	12	12½	6	11	9½	12	10
.0006 "	26	72	39	48	43	8½	20	24	19	18	8½	14	13	17	16
.0003 "	42	115	69	127	75	10½	28	31	22	27	12	19	22	21	20

* N.: Normal control.

† Rabbit 1: Killed on 4th day (dicumarol by stomach tube) Prothrombin time at time of death using 0.01 M $CaCl_2$

" 2: " " 7th " " " " 28.5 sec.

" 6: " " 7th " " " " 8 "

" 8: " " 7th " " intravenously) 82 min.

to vascular damage.[2] Moreover, other factors of the normal mechanism of coagulation have been found to be affected by dicumarol. Adhesiveness of the platelets is decreased[3,4] and the minimum requirement of calcium for optimum prothrombin time is increased[5,6] during the administration of the drug.

Evidence has been obtained which indicates that dicumarol causes changes in the thromboplastic activity of tissues. Dyckerhoff[7] claims that dicumarol inhibits the first phase of coagulation by depressing the activity of thrombokinase. Recently Bose[8] has shown that thromboplastin prepared from desiccated brain of rabbits which have been fed 10 mg of dicumarol for 3 days is less active than that prepared from normal brains when tested on normal rabbit plasma using the prothrombin time technic.

In the present report, thromboplastic activity of rabbit brain obtained from animals severely poisoned by dicumarol has been studied by the prothrombin time method of Quick, but using a standard series of dilutions of $CaCl_2$. Plasma from men and from normal and dicumarol-treated dogs and rabbits was employed.

Experimental. Ten full grown male rabbits in good state of nutrition were given dicumarol [3,3′-methylenbis(4-hydroxycoumarin)] until the prothrombin level was so depressed that they either died from hemorrhage or their prothrombin time and coagulation time were so delayed that neither one could any longer be determined satisfactorily. The drug was administered in doses of 5 mg per kg of body weight per day. In some animals the drug was administered orally as a gum acacia suspension by stom-

[2] Bingham, J. B., Meyer, O. O., and Pohle, F. J., *Am. J. Med. Sc.*, 1941, **202**, 563.

[3] Spooner, M., and Meyer, O. O., *Am. J. Physiol.*, 1944, **142**, 299.

[4] Wright, H. P., *J. Path. Bact.*, 1945, **57**, 382.

[5] Jaques, L. B., and Dunlop, A. P., *Am. J. Physiol.*, 1945, **143**, 355.

[6] Quick, A. J., *Am. J. Physiol.*, 1947, **148**, 211.

[7] Dyckerhoff, H., *Bioch. Z.*, 1944, **316**, 397, (*Chem. Abstracts*, 1945, p. 551).

[8] Bose, A. N., *Ann. Biochem. Exp. Med.*, 1945, **5**, 105.

TABLE II.

Influence of Dicumarol Poisoning on Thromboplastic Activity of Brain Tissue (Rabbit) When Tested Against Plasmas of Animals Treated with Dicumarol (Single Intravenous Dose of 5 mg per Kilo Weight).

	Prothrombin time (in sec.)																			
	Dog* plasma																		Rabbit plasma	
Days after injection	2		6			7		9		8			15			1				
Animal No.	5		5			2		2		6			6			8				
Thromboplastin preparation from Rabbit No.	N.†	3‡	N.	3	4	N.	1	N.	3	N.	5	6	N.	7	9	N.	5	6		
$CaCl_2$ concentrations																				
.02 Molar	18	24	10	13	13½	7	11½	7	9½	20½	37½	36	8½	12	13	9	13	14		
.01 "	18½	25	10½	12½	13½	7	11	7	10	20	43	48	8	10½	12	9	13	13		
.005 "	19	26	10½	14	14	6½	11	7	10	24	53	54	8½	11	12	9	13	14		
.0025 "	23	31	11	15	15	6½	11	6½	10	26	59	68	9	11	12	10½	14	15		
.00125 "	45	52	12	24	16	6	12½	6	11½	59	121	166	9	13	14	13	22	22		
.0006 "	132	207	17	50	43	11	30	10	28	69	184	192	13	17	19	42	94	103		
.0005 "	—	—	20	67	55	12	35	—	—	—	—	—	17	20	27	45	141	192		

* The above are representative figures from 7 dogs.

† Normal control.

‡ Rabbit 3: Killed 7th day (dicumarol by stomach tube)	Prothrombin time at time of death using .01 M $CaCl_2$
" 4: " 6th " " intravenously)	∞
" 5: " 7th " " "	102 min.
" 7: Died 7th " " "	20 "
" 9: Killed 7th " " stomach tube)	∞
	108 "

ach tube; in others intravenously as the sodium salt and in a third group both the oral and the intravenous routes were used. Thromboplastin was prepared by the acetone dehydration procedure of Quick.[9] One-tenth of 0.1 M. sodium oxalate per rabbit brain was added before triturating with acetone. By this means any calcium in the brain tissue was removed, since no detectable amount of this element was found by ordinarily chemical methods in 0.2 g of the dried rabbit brain powder. The prothrombin time of oxalated human, dog and rabbit plasma was determined by the procedure of Quick. A standard series of dilutions of $CaCl_2$ solutions was used since the lower concentrations have been shown to be more sensitive to minimum changes of the prothrombin time.

Results and Discussion. It is evident from the results in Table I that the thromboplastin prepared from the brains of rabbits poisoned with dicumarol was less active than that obtained from normal animals. With dogs' and rabbits' plasmas this difference is obtained with the various dilutions of calcium used but becomes more accentuated in the lower concentrations. With human plasma this difference is obtained only in the lower concentrations.

In comparing the thromboplastin from dicumarolized rabbits with that of normal animals on rabbit and dog plasmas in which the prothrombin was depressed by dicumarol, the difference in activity was found to have a wider spread (Table II) but followed essentially the pattern of normal oxalated plasma in regard to the influence of calcium. Occasionally when the prothrombin is presumably normal as measured with fully active thromboplastin, a distinct delay is brought out by dicumarol thromboplastin as shown in the case of dog No. 2 on the 9th day after a single dose of dicumarol intravenously.

It is somewhat surprising that dicumarol should decrease the activity of the thromboplastin in brain tissue and this naturally leads to the question whether the thromboplastin occurring in blood is similarly affected. If this be the case, then the delayed coagulability due to dicumarol would have a 3-fold cause: decrease of prothrombin level, inadequate calcium for optimum prothrombin activity and less active thromboplastin.

What importance the latter factor has is difficult to evaluate especially since the diminution in thromboplastic activity is not particularly marked. In view of the fact that the nature of thromboplastin is poorly understood and the action of dicumarol is not completely known, it would be futile to attempt to offer any possible explanation for the results presented in this paper.

[9] Quick, A. J., *Science*, 1940, **92**, 113.

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Grisein, a New Antibiotic Produced by a Strain of *Streptomyces griseus*.*†

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Streptomyces griseus represents a large and heterogeneous group of actinomycetes, widely distributed in nature. Cultures belonging to this species or species-group have been isolated from a great variety of substrates, largely soils, peats, composts and animal contents. Since the first report[1] on the production of streptomycin by 2 cultures of *S. griseus* obtained from 2 different substrates,

* Journal Series Paper, New Jersey Agricultural Experiment Station, Rutgers University, Department of Microbiology.

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[1] Schatz, A., Bugie, E., and Waksman, S. A., PROC. SOC. EXP. BIOL. AND MED., 1944, **55**, 66.