Antagonistic Effect of Thiocarbanilide on 4,4′-Dinitrocarbanilide, A Component of Nicarbazin. (23047)

ASHTON C. CUCKLER, DONALD POLIN AND CURT C. PORTER
(Introduced by H. J. Robinson)
Merck Institute for Therapeutic Research, Rahway, N. J.

During the course of studies on compounds related to nicarbazin, a complex of 4, 4′-dinitrocarbanilide and 2-hydroxy-4, 6-dimethylpyrimidine, it was observed that most of these compounds were ineffective as anticoccidial agents(1). Thiocarbanilide and several of its derivatives were among the ineffective compounds. Since structural analogues may competitively antagonize the pharmacological effects of certain drugs(2), we explored several compounds structurally similar to 4, 4′-dinitrocarbanilide (DNC), the active component in nicarbazin, for antagonistic effect on this anticoccidial agent. The results presented in this paper show that thiocarbanilide will competitively antagonize the anticoccidial and egg shell depigmenting(3) effects of nicarbazin.

Materials and methods. The compounds studied were supplied by Dr. L. H. Sarett and Dr. R. C. O'Neill.* The standardized methods described by Cuckler, Malanga and Ott(4) were used for evaluating anticoccidial activity of combinations of compounds. Analyses for DNC(5) were made on plasma samples from chicks on day 8 and plasma and yolk samples from hens on days 10-12. The depigmenting effect of nicarbazin on egg shell color was determined by the following method. A series of 8 shells, each graded in 0.5 units and varying in shade of color from chalk white (0) to deep brown (4) were maintained as index for color scoring. A new set of shells was prepared weekly. Shell scores for each hen were obtained during 7 to 10-day control period and during experimental period of 10-12 days. Color scores of the first 3 days of production during the experimental period were excluded from data to eliminate the period of sub-maximal drug effect on shell pigmentation.

Results. The following compounds, structurally related to 4, 4′-dinitrocarbanilide, were examined for antagonistic effect on the anticoccidial activity of nicarbazin: 4, 4′-dinitrocarbanilide; 2, 2′-dinitrocarbanilide; N, N′-diallyl-4, 4′-dinitrocarbanilide; N, N′-di(4-nitrophenyl)-p-nitrobenzamide; 4, 4′-dinitrobenzanilide; 4, 4′-dinitroazobenzene; 4, 4′-dichlorocarbanilide; 4, 4′-difluorocarbanilide; 4, 4′-disulfamylcarbanilide; thiourea; sulfathiourea; phenylthiourea; thiocarbanilide; 3, 3′-dichlorothiocarbanilide; and 4, 4′-dichlorothiocarbanilide. The last 3 compounds listed inhibited the anticoccidial effect of nicarbazin in a 1 to 1 ratio or less, whereas all other compounds were ineffective. Thiocarbanilide (sym. diphenylthiourea) was selected for further studies.

As shown in Table I, 1 part of thiocarbanilide to 4 of nicarbazin in the feed of chickens partially antagonized the protective effect of nicarbazin on mortality resulting from cecal coccidiosis. Higher concentrations of thiocarbanilide in the feed completely antagonized the anticoccidial effect of nicarbazin. The antagonistic effect of thiocarbanilide was also shown by the increased number of oocysts produced in chicks fed mixtures of thiocarbanilide and nicarbazin in ratios of 1 to 4 or less.

Thiocarbanilide may be administered parenterally and still exert its antagonistic effect on nicarbazin. For example, the data showed that thiocarbanilide, when administered in daily subcutaneous dosage of 12 mg/kg of body weight, completely inhibited the protective effect of 0.015% nicarbazin on mortality from cecal coccidiosis (Table II). A daily subcutaneous dosage of 50 mg of thiocarbanilide/kg body weight was required to antagonize the anticoccidial effect of 0.03% nicarbazin in the feed. When the amount of nicarbazin was further increased to 0.06% in the feed, thiocarbanilide in a subcutaneous

* Merck, Sharp and Dohme Research Laboratories.

TABLE I. Effect of Graded Feed Concentrations of Thiocarbanilide on Therapeutic Activity and Plasma Levels in Chicks Fed 0.015% Nicarbazin.

% thiocarbanilide in feed	% coccidiosis mortality‡		Millions of oocysts produced/chick		DNC, γ/cc plasma	
	Cont.*	NCB†	Cont.	NCB	Cont.	NCB
.0	35	0	33	<0.1	0	1.41
.0045		0		"		1.12
.0009		0		"		1.33
.0019		0		"		1.22
.0038		15		31		.68
.0075		40		29		.58
.015		15		59		.43
.030	40	60	29	62	0	

* Cont. = Non-medicated feed. † NCB = 0.015% nicarbazin in feed ‡ 20 chicks/group.

dosage of 100 mg/kg of body weight had only slight antagonistic effect. Furthermore, when thiocarbanilide was administered subcutaneously, a daily dosage of 6 mg/kg of body weight completely antagonized the suppressive effect of 0.015% nicarbazin on oocyst formation. Larger dosages of thiocarbanilide were required to antagonize the oocyst suppressing effect of 0.03 and 0.06% nicarbazin in the feed.

The antagonistic effect of thiocarbanilide for nicarbazin appears correlated with blood concentrations of DNC obtained in the chicks. When chicks were fed 0.015% nicarbazin, the plasma concentration of DNC was about 1.5 γ/ml (Tables I, II). However, by the subcutaneous administration of thiocarbanilide in a dosage of 100 mg/kg, the concentration of DNC in blood may be decreased to a nondetectable level. Likewise, simultaneous feeding of ration containing thiocarbanilide and nicarbazin in a 1 to 1 ratio reduced the concentration of DNC in the blood to about 25% of that obtained with nicarbazin alone.

Further evidence of the antagonistic effect of thiocarbanilide for nicarbazin was obtained when feeds containing both compounds in various ratios were fed to laying hens. When nicarbazin alone was fed to heavy breed hens, which normally lay brown shell eggs, the shells had considerably less pigmentation. However, when nicarbazin and thiocarbanilide were fed simultaneously in a 1 to 1 ratio, the depigmenting effect of nicarbazin was completely nullified (Table III). When feed mixtures containing thiocarbanilide and nicarbazin in ratios of 1 to 2 were fed, the depigmenting effect of nicarbazin on egg shells was only partially antagonized. Plasma levels of DNC in hens fed nicarbazin and thiocarbanilide in a 1 to 1 ratio were considerably less than in hens fed only nicarbazin (Table III). Also, hens fed rations containing both nicarbazin and thiocarbanilide produced eggs with less DNC in the yolk than in those from hens fed nicarbazin alone. Presumably, these de-

TABLE II. Effect of Subcutaneous Dosages of Thiocarbanilide on the Therapeutic Activity and Plasma Levels in Chicks Fed 0.015% Nicarbazin.

Subcut. dose thiocarbanilide, mg/kg/day*	% coccidiosis mortality‡		Millions of oocysts produced/chick		DNC, γ/ml plasma	
	Cont.†	NCB†	Cont.	NCB	Cont.	NCB
0	18	0	28	<0.1	0	1.61
1.5	30	0	20	1		1.48
3	30	0	21	1		1.01
6	30	0	25	24		.80
12	0	40	16	12		.34
25	0	20	19	12		.19
50	20	30	6	13		.13
100	60	90	8	41		.0

* Admin. daily throughout experiment. † Cont. = Non-medicated feed; NCB = 0.015% nicarbazin in feed. ‡ 20 chicks/group.

TABLE III. Effect of Thiocarbanilide on Egg Shell Color-Score and Plasma and Yolk Concentrations of DNC during Feeding of Nicarbazin.

% nicarbazin in feed	% thiocarbanilide in feed	Shell color score period Cont.	Exp.	DNC conc. Plasma, γ/ml	Yolk, γ/g
.008	None	2.9	.5	2.45	9.34
	.002	2.5	1.7	.99	11.10
	.004	3.2	2.8	1.16	5.70
	.008	2.7	2.8	.96	5.07
.0120	None	3.2	.5		20.55
	.003	2.8	.2	1.98	21.20
	.006	3.7	1.9	1.54	15.50
	.0120	2.1	2.1	.78	9.52
	.020	2.9	2.8	.33	1.25
.0125	None	3.5	.5	2.02	20.4
	.0125	3.5	3.5		
	.025	2.9	2.3	.52	1.85

creased plasma and yolk DNC levels reflected a decreased assimilation of nicarbazin by the laying hen because of the thiocarbanilide in the feed.

Discussion. The anticoccidial and egg shell depigmenting effects of nicarbazin are related to the amount of DNC absorbed from the intestinal tract. Plasma levels of DNC are correlated with both of these effects, which in turn are proportional to concentration of nicarbazin in feed(4,6). Thiocarbanilide effectively antagonized the anticoccidial activity and shell depigmenting effect of the structurally related component (DNC) of nicarbazin. The antagonism occurred either by feeding or injecting thiocarbanilide and simultaneously feeding nicarbazin. Thus, the decreased plasma and yolk DNC concentrations caused by thiocarbanilide appear to be due to an interference with nicarbazin absorption.

Summary. 1) Thiocarbanilide, in a 1 to 1 ratio or less, effectively inhibited the anticoccidial activity of nicarbazin when given parenterally as well as orally. 2) Thiocarbanilide also antagonized the effect of nicarbazin on the depigmentation of egg shell color. 3) In both instances, the antagonistic effect of thiocarbanilide was correlated with decreased plasma concentrations of dinitrocarbanilide. 4) It is suggested that thiocarbanilide may interfere with the activity of nicarbazin through competition in absorption.

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Influence of Beta-Aminopropionitrile upon Development of Connective Tissue in Croton Oil Pouches. (23048)

J. E. MIELKE, J. J. LALICH,* AND D. MURRAY ANGEVINE
Department of Pathology, University of Wisconsin Medical School, Madison.

Injection of air and dilute croton oil into subcutaneous tissues of rats produces a well-defined pouch(1). The wall of the pouch after 6 days is composed principally of fibroblasts and minimal numbers of blood vessels. When beta-aminopropionitrile (BAPN) is fed to rats daily in drinking water, the development of a croton oil pouch is retarded. Because BAPN retards development of croton oil pouches, it seemed desirable to examine gross and microscopic findings in these pouches, and to analyze some important constituents of connective tissue to resolve whether there are also differences in concentration of such components. The 2 components selected for analyses were hexosamine and hydroxyproline. These constituents were chosen because hexosamines are an im-

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