TABLE IV. Effect of Terramycin on Growth and *C. perfringens* Count of Pig Feces.

Supplement to basal diet			Counts of black colonies/g	
Vit. B_{12}, g/kg	Terramycin, mg./kg	Avg wt at 8 wk, lbs.	1st count	2nd count
—	—	57.0	—	33700000
50	—	66.3	34300000	79400000
50	15	75.8	10	10

* 6 pigs/group.

very marked. A second count showed even more striking results. These findings that penicillin and terramycin inhibit *C. perfringens* agree with the results reported by Bliss and Warth(15) who showed penicillin and aureomycin to be inhibitory to members of this genus *in vitro* and *in vivo*.

Conclusions. In view of the inhibitory effect of penicillin and terramycin on *C. perfringens* in the intestinal tract of turkeys and of terramycin in pigs, it appears possible that these antibiotics promote growth by preventing enterotoxemia. This possibility is supported by data gathered by Merchant(16) which show that enterotoxemia in sheep is caused by *C. perfringens*.

Acknowledgement is made to:

Chas. Pfizer and Co., Brooklyn, N. Y., and Merck and Co., Rahway, N. J., for Grants-in-Aid in supplies used in partial support of this research.

Dr. C. E. Skinner, Dept. of Bacteriology and Public Health for helpful suggestions.

Dr. R. Gillespie, College of Veterinary Medicine, for use of laboratory facilities.

15. Bliss, E. A., and Warth, P. T., *Bact. Proc.*, 1950, v96.

16. Merchant, I. A., *Veterinary Bacteriology*, 3rd. Ed., Ames, Iowa State College Press, 1946.

Received November 6, 1950. P.S.E.B.M., 1951, v76.

Effect of Niacin and Tryptophan in Counteracting Toxicity of Crystalline Borrelidin for Rat.*† (18376)

J. M. COOPERMAN, S. H. RUBIN, AND B. TABENKIN.

From the Departments of Nutrition and Microbiology, Hoffman-La Roche, Inc., Nutley, N. J.

The production of pellagra-like signs in animals fed toxic materials has been described by several investigators. Stockman and Johnston(1) reported the isolation of an organic acid from corn which produced coma and death in monkeys, rabbits and guinea pigs. No attempt was made to counteract the effects with niacin. Woolley(2) concentrated a nitrogenous base from corn meal which, on addition to a synthetic ration, inhibited the growth of mice. Indolacetic acid was claimed to inhibit growth of rats(3), but the experiments were not confirmed(4). It was shown that adenine at high levels produced the symptoms of black tongue in dogs (5), but no attempt was made to counteract this effect with niacin. Krehl *et al.*(6) demonstrated the role of corn in the production of niacin deficiency in rats.

Berger, Jampolsky and Goldberg(7) have reported the isolation of a toxic crystalline antibiotic, borrelidin, from the culture broth

* A preliminary report was presented before the Fed. Am. Soc. for Exp. Biol., Apr. 1950, Atlantic City.

† Roche Publication No. 221.

1. Stockman, R., and Johnston, J. M., *J. Hygiene*, 1933, v33, 204.

2. Woolley, D. W., *J. Biol. Chem.*, 1946, v163, 773.

3. Kodicek, E., Carpenter, K. J., and Harris, L. J., *Lancet*, 1947, v2, 616.

4. Krehl, W. A., Carvalho, A., and Cowgill, G. R., *Fed. Proc.*, 1947, v6, 443.

5. Raska, S. B., *Science*, 1947, v105, 126.

6. Krehl, W. A., Teply, L. J., and Elvehjem, C. A., *Science*, 1945, v101, 283.

7. Berger, J., Jampolsky, L. M., and Goldberg, M. W., *Arch. Biochem.*, 1949, v22, 477.

TABLE I. Basal Ration.

	%
Casein	20
Sucrose	71
Salts IV*	4
Cod liver oil	3
Corn oil	2
	γ/day
Thiamine	200
Riboflavin	200
Pyridoxin	200
Calcium pantothenate	400
p-Aminobenzoic acid	1000
i-Inositol	1000
Choline	10000
Folic acid	10
Biotin	10
Vit. K	10

* Phillips, P. H., and Hart, E. B., *J. Biol. Chem.*, 1935, v109, 657.

of a species of streptomyces. Concentrates with similar *in vivo* and *in vitro* activities were found in corn steep. The experiments described in this paper were designed to test the effectiveness of niacin and tryptophan in overcoming the toxicity of crystalline borrelidin for rats.

Experimental. Sprague-Dawley rats weighing 40 to 50 g were housed in individual cages. The composition of the basal ration which was fed *ad libitum* is shown in Table I. The vitamin supplement was given by cup in sugar solutions 3 times a week and was promptly taken by the rats.

In the first experiment, 5 groups of rats were used to determine the effectiveness of niacin in counteracting borrelidin toxicity, and each received the basal ration with additional supplements. The first group, the controls, received an addition of 1 mg of niacin in the vitamin supplement. Subsequent experiments showed that niacin did not improve the growth of rats receiving the basal ration. The second group received a dose of 10 μg borrelidin per day. The borrelidin solutions were given with a syringe and blunted needle which was introduced into the rats' stomachs since it was found that the animals refused to accept borrelidin mixed with the vitamin supplement. The other 4 groups received 10 or 20 μg borrelidin with or without 1 mg niacin in the vitamin supplement as outlined in Table II. The results (Table II) indicate that niacin can partially overcome the toxicity of borrelidin when given under the conditions described above. Statistical analysis of the differences between Groups 2 and 3 showed these differences to be significant at the 1% level. In the group receiving 10 μg borrelidin per day, 2 rats succumbed during the first 2 weeks, whereas all survived in the group receiving 10 μg borrelidin plus 1 mg niacin. Nine out of 10 rats receiving 20 μg borrelidin per day died during the first 2 weeks compared to 4 out of 9 in the group receiving 1 mg niacin in addition to 20 μg borrelidin per day. It is interesting to note that borrelidin had its greatest effect during the first 2 weeks of the experiment.

In another experiment groups of rats were given 2 μg borrelidin per day. In this case, the controls gained an average of 33 g per week for the 4 week period compared to 31 g for the rats receiving 2 μg borrelidin per day and 34 g for the rats receiving 2 μg borrelidin plus 1 mg niacin per day. These differences were not significant.

Since it has been shown that tryptophan can replace the requirement for niacin in the rat(8), it was decided to determine the effectiveness of tryptophan in overcoming the toxicity of borrelidin. In these experiments, the borrelidin was added directly to the basal ration, as were the niacin and tryptophan supplements. The basal ration and vitamin supplement were identical to those employed in the first experiment. Eight groups of rats were used. Group 1 received the same ration as the controls in the first experiment. The other 7 groups received the basal ration containing 1 or 2 mg borrelidin per kilogram with or without niacin or tryptophan as outlined in Table III.

In this experiment all rats survived the 4 week test period. The results are shown in Table III. As in the first experiment the toxicity manifested itself to the greater degree during the first 2 weeks of the experiment. Although the differences between Groups 2 and 3 and between 2 and 4 were of a smaller degree for the 4 week period, statistical analy-

8. Rosen, F., Huff, J. W., and Perlzweig, W. A., *J. Biol. Chem.*, 1946, v163, 343.

TABLE II. Effect of Oral Administration of Borrelidin.

Group	No. rats	Daily supplement μg Borrelidin	Avg gain in wt, g 1st wk	2nd wk	3rd wk	4th wk	Total 4 wk	Per wk, 4 wk period
1	10	Controls	20	43	30	29	122	31
2	10	10	7.5	4(8)*	14(8)*	19(8)*	44.5(8)*	11(8)*
3	10	10 + 1 mg Niacin	12	17	28	29	86	22
4	10	20	—3	10(1)*	9(1)*	13(1)*	36(1)*	9(1)*
5	9	20 + 1 mg Niacin	7	11.5(5)*	19(5)*	22(5)*	65(5)*	16(5)*

* No. of rats in parentheses.

TABLE III. Effect of Feeding Borrelidin Mixed with the Ration.

Group	No. rats	Supplement per kilo ration, mg Borrelidin	Avg gain in wt, g 1st wk	2nd wk	3rd wk	4th wk	Total 4 wk	Per wk, 4 wk period
1	10	Controls	24	40	37	40	141	35
2	10	1	2	12	20	22	56	14
3	10	1 + 500 mg Niacin	5	24	25	26	80	20
4	10	1 + 5 g dl-Tryptophan	4	17	25	26	72	18
5	5	2	—6	3	3	7	7	2
6	5	2 + 1 g Niacin	—3	6	20	13	36	9
7	5	2 + 5 g dl-Tryptophan	—4	6	19	19	40	10
8	5	2 + 10 g Niacin	—5	8	22	19	44	11

sis revealed them to be significant at the 5% level. In the groups receiving 2 mg of borrelidin per kilogram ration, little growth occurred during the first 2 weeks. However, during the last 2 weeks, the rats receiving niacin and dl-tryptophan grew at a more rapid rate than those receiving borrelidin alone. Under these conditions the niacin and tryptophan effects were more marked.

Discussion. Although several authors have claimed to produce the effects of niacin deficiency in rats with toxic materials, they have either used non-crystalline material or have not reversed the effects with niacin. The experiments described above represent the first time that the toxicity of a crystalline, naturally occurring substance has been counteracted by niacin in the rat. It should be emphasized that complete protection was not provided by either niacin or tryptophan.

Conclusion. When crystalline borrelidin is given orally to rats at a level of 10 μg per day, poor growth results. This inhibition of growth can be partially overcome by supplementation with niacin. Two μg of borrelidin per day given orally is without effect upon the growth of rats whereas 20 μg per day results in a high mortality rate. Poor growth is also observed in rats receiving a basal ration containing levels of 1 and 2 mg borrelidin per kilo. The addition of niacin or tryptophan results in significantly better growth.

Received November 8, 1950. P.S.E.B.M., 1951, v76.