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NATURE OF *EIMERIA NIESCHULZI*-GROWTH PROMOTING POTENCY OF FEEDING STUFFS. 4. RIBOFLAVIN AND NICOTINIC ACID

ELERY R. BECKER

Observations on the effect of certain vitamins on the multiplication of *Eimeria nieschulzi* in its rat host have indicated that under certain conditions the infection may be significantly modified by feeding these materials. Vitamin B_1 had a moderately restraining effect on the development of the parasite, vitamin B_6 strongly enhanced its growth, while the two vitamins fed together limited its increment considerably more stringently than vitamin B_1 alone (Becker and Dilworth, 1941). Pantothenic acid, like vitamin B_6, enhanced the coccidium-growth-promoting potency of the otherwise unsupplemented ration, and neutralized the growth-inhibiting properties exhibited by the combination of vitamins B_1 and B_6 (Becker and Smith, 1942). The present study on the effects of riboflavin and nicotinic acid supplements to a particular ration were made in further prosecution of the general problems.

Procedure. Details relating to material and methods have been fully described elsewhere (Becker and Dilworth, 1941), but a few of the more pertinent are herewith repeated. The hosts were "Wistar A" white rats, raised free from coccidial infection and started on the experimental rations when of about 70 grams. On the tenth and twelfth days each rat was innoculated through a stomach tube with an estimated 3000 sporulated oocysts of *Eimeria nieschulzi*. Elimination of oocysts commenced about 7 days later and continued for about 6 days. During this period the droppings were collected, and the number of oocysts estimated by a dilution method. The numbers appearing in the table thus represent an estimation of the number of oocysts eliminated during such an experimental infection. The rats were practically immune to *Eimeria nieschulzi* when elimination of oocysts ceased. The control ration was made up in parts by weight as follows: beet sugar, 69; fine cellulose, 2; medium fine unextracted (crude) casein, 10; soy bean oil meal, expeller process, 10; normal salt mixture (Harris), 4; cod liver oil, 2; lard, 3.

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TESTS WITH NICOTINIC ACID

Nicotinic acid was thoroughly mixed with the control ration in the proportion of 1:10,000. The vitamin was a synthetic product marketed in powder form.

The results of the experiment appearing in Table 1, involving in all 11 rats on the control ration and the same number on the supplemented ration, show quite definitely that the vitamin was without effect on the oocyst count. The counts of tests and controls in the two groups, viz., 178.6 and 177.2 millions, and 225.8 and 205 millions, respectively, are unusually close and make it appear that further tests would be unwarranted. The rats receiving nicotinic acid out-gained the controls in weight, but since to what extent rats utilize this vitamin is still undetermined, it is unsafe to draw conclusion on this point from so few experiments.

Table 1. Mean oocyst counts and weight gains for 2 groups of rats receiving control ration and for test rats receiving same mixed with nicotinic acid.

	Group 1		Group 2	
	Oocysts (millions)	Wt. gain (gm.)	Oocysts (millions)	Wt. gain (gm.)
Controls	178.6 (5 rats)	15	225.8 (6 rats)	22
Nicotinic acid recipients	177.2 (5 rats)	22	205 (6 rats)	28

TESTS WITH RIBOFLAVIN

The riboflavin used in these experiments was a synthetic crystalline product manufactured by a reputable and nationally known drug concern. The daily dose of 20 μ per rat was administered through a stomach tube, in 1 c.c. of water. It seemed to have little or no effect on the growth of the rat during the time required by the experiment, a result which would probably be expected since crude casein, which was used in the counted ration, is known to contain large amounts of riboflavin.

Since the object of the experiment was to determine whether the control series or the test series would eliminate the larger

number of oocysts, a number of groups including both controls and tests were utilized in the experiment. The results, appearing in Table 2, show that in every one of five groups the riboflavin recipients eliminated fewer oocysts than the controls. The probability of obtaining 5 positives in 5 tests with chances equal is .031, a figure which indicates that we may have found a significant difference in the two series. When the differences of the five means are treated statistically according to the paired sample method (Fisher, 1930) a P-value less than .01 is obtained, so that the difference between the results is clearly significant.

Conclusions

1. Riboflavin supplements to the ration definitely curbed the multiplication of *Eimeria nieschulzi* in its host when numbers of oocysts eliminated were used as the measure of the parasite's increment.
2. Nicotinic acid appeared to be without effect on the multiplication of the coccidium, at least in the ration employed.

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Table 2. Mean oocyst counts and weight gains for 5 groups of rats receiving control ration and for test groups receiving 20μ riboflavin daily per rat.

	Group 1		Group 2		Group 3		Group 4		Group 5	
	Oocysts (millions)	Wt. gain (gm.)	Oocysts (millions)	Wt. gain (gm.)	Oocysts (millions)	Wt. gain (gm.)	Oocysts (millions)	Wt. gain (gm.)	Oocysts (millions)	Wt. gain (gm.)
Controls	25.3 (3 rats)	19	20 (4 rats)	24	28.8 (4 rats)	20	63 (4 rats)	21	81 (2 rats)	18
Riboflavin recipients	14.7 (3 rats)	25	15.5 (4 rats)	20	13.8 (4 rats)	23	47.3 (4 rats)	23	67 (3 rats)	21