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Effect of Vitamins B and G on Coccidian Infection in the Rat.

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The writers[1] recently reported their discovery that the number of oocysts eliminated by rats infected with *Eimeria miyairii* after the host had been for 9 days on a diet deficient in both vitamins B and G was only one-fourth to one-fifth that in the reference series which received a so-called normal diet. The present study was made to determine whether the observed effect was due to the absence of one or both of the vitamins.

The basal ration was as follows: Extracted casein, 18%; normal (Harris') salt mixture, 3.7%; butter, 4.0%; cod liver oil, 1.0%; bacto-agar, 2.0%; beet sugar, 71.3%. The vitamin-B deficient ration consisted of the basal ration with 12 of the 71.3 parts of sugar replaced with powdered (Fleischmann's) yeast that had been autoclaved for more than 3 hours at 120°C. and dried. The control or reference diet for the rats on the vitamin-B deficient diet consisted of the basal ration with 10 parts of the sugar replaced with the same yeast unautoclaved. The vitamin-G deficient ration consisted of the basal ration plus 1 cc. of wheat germ extract. The latter was equivalent to 2 gm. of the wheat germ. Its preparation will be described in a later paper. The reference diet for the rats receiving the vitamin-G deficient ration consisted of the basal diet with 10 parts of sugar replaced with autoclaved yeast and, likewise, one cc. of wheat germ extract each day.

Experiment I. On October 31 nine rats were started on the vitamin-B deficient ration. One November 14, 15, 16 and 18 each rat received about 2100 sporulated oocysts of *Eimeria miyairii.* The reference series was started on the control diet the same day, and infected on the same days and in the same way as the deficient series. The oocysts appeared in the feces from November 21 to November 27, and were collected in pans containing a disinfectant placed beneath the hardware cloth bottoms of the individual rat cages. The deficient series averaged 69 gm. in weight on October 31, 88 gm. on Nov. 14, 70 gm. on Nov. 21, and 102 gm. on Nov. 28. The increase in weight between November 21 and Nov. 28 is due to the

[1] Becker, E. R., and Morehouse, N. F., PROC. SOC. EXP. BIOL. AND MED., 1935, **33**, 114.

fact that the animals were placed on Steenbock's growing ration beginning Nov. 24 in order to flush from the intestine any oocysts that might be retained on account of intestinal immobility resulting from the vitamin deficiency. The reference series averaged 66 gm. on Oct. 31, 102 gm. on Nov. 14, 114 gm. on Nov. 21, and 135 gm. on Nov. 28. These rats were also placed on Steenbock's ration Nov. 24. All animals survived the experiment except one vitamin-deficient which died from an unknown cause on Nov. 24.

The deficient series eliminated, respectively, the following numbers of oocysts expressed in millions: 166, 218, 207, 279, 204, 261, 262, 328; mean, 240.63. The reference series: 255, 257, 233, 242, 222, 151, 247, 245, 269; mean, 235.67. Since the means are so close, there is no reason to believe the vitamin-B deficiency affected the infection in any way so far as the number of oocysts that were developed is concerned.

Experiment II. On November 15 nine rats were started on the vitamin-G deficient diet, and 4 more on Nov. 18. The 9 were infected on Nov. 27, 28, 29, and Dec. 1 with doses of 2100 oocysts, the 4 on Nov. 29, 30, Dec. 1 and 3 with the same dosage. The oocysts were collected from both sets of rats as described above from the seventh to the thirteenth day, inclusive, after the date of the first inoculation of each set. The deficients averaged 78 gm. when placed on the special diet, 83 gm. at the time of inoculation, 82 gm. 10 days after inoculation, when they were placed on Steenbock's growing ration in order to flush possibly retained oocysts from the intestine, and 102 gm. on the fourth day thereafter. The controls averaged 79 gm. when placed on the reference diet Nov. 15, 106 gm. when given the first infective dose on Nov. 27, 122 gm. 10 days later, when they also were placed on the Steenbock's ration, and 130 gm. the fourth day thereafter. All rats survived except one vitamin-G deficient which died on the tenth day after the first inoculating dose.

The vitamin-G deficient series eliminated by individuals the following numbers of oocysts expressed in millions: 119, 36, 89, 131, 61, 82, 69, 51, 116, 37, 58, 30; mean, 73.25. The reference series: 322, 132, 192, 337, 172, 412, 116, 196, 124; mean, 222.55. The mean for the reference series is approximately 3 times that for the deficient series.

Experiment III. This experiment was done in connection with Experiment II, and the controls are the same in both cases. Four rats were placed on the basal ration deficient in both vitamins B and G on Nov. 18. The treatment was the same as in the case of the set

of 4 rats deficient in vitamin G described in Experiment II, except that the wheat germ extract was not fed. The rats lost weight slowly on the diet, from an initial 77 gm. mean on Nov. 18 to 75 gm. on Dec. 19, when they were placed on the Steenbock ration, and 4 days later averaged 92 gm. The counts in millions of oocysts were as follows: 62, 65, 12, 47; mean, 46.5. This mean is to be compared with that of 222.55 in the controls on the complete diet, and 73.25 in the vitamin-G deficients.

Experiment III confirms our previous finding that a host on a diet deficient in both vitamins B and G is not as suitable a medium for the development of a coccidium as one receiving an adequate diet. The question arises, is this difference due to the combination of vitamin deficiencies or to one or other of the vitamins? Experiment I appears to show that it is not due to vitamin B. The three to one ratio of the mean oocyst count for the infections in the reference or normal series to the mean oocyst count for the vitamin-G deficient series indicates that all or most of the difference is attributable to vitamin G. It is true that the ratio is not so high as the previously reported 4.67 between the counts for the normal and both vitamins B and G deficient animals, but it is to be considered that the alcoholic extract of wheat germ used as a source of vitamin B probably contained some vitamin G. According to Feaster,[2] an extract prepared similarly to ours contained about one-fifth as much vitamin G as B. Hence it is apparent that the animals receiving the extract as a source of vitamin B were receiving considerably more vitamin G than those in the series receiving no material as a source of either vitamin G or B.

It is to be made clear that the work is in the early experimental stage, and that the results here reported are not to be construed as indicating practical application of vitamin-G deficiency as a control for coccidiosis. In the first place, the effects of upsetting the nutritional balance of an animal might be worse than the infection. In the second place, we have not yet determined whether the lower parasite level on the vitamin-G deficient diet sufficiently counterbalances the lowered vitality of the host to create a differential in favor of the host. The findings, however, open up a number of problems relating to the host-parasite balance in coccidiosis.

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[2] Feaster, J. F., Doctoral Thesis, Iowa State College Library.

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Preservation of Fertility in Male and Female Rats on a Supplemented Milk Diet.

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This work is the outgrowth of the studies made by the authors on the relation of iron and copper to nutritional anemia and hemoglobin synthesis. It is well known that cow's milk is inadequate for the normal maintenance of the rat. Additions of copper and iron salts to an exclusive milk diet protect animals from nutritional anemia and produce an apparent well being in the animals of the first generation from the standpoint of hemoglobin formation, growth, and reproduction. Underhill, Orten, Mugrage, and Lewis[1] have observed no abnormalities in the organs of rats fed milk, copper, and iron for periods of more than a year. Waddell[2] reported sterility in males of the first generation receiving a milk, copper, and iron diet. The sterility was marked by a progressive testicular degeneration together with an absence of motile sperm in the seminal fluid. Ferric chloride, when added to the ration, was found to produce symptoms of sterility as early as 10 weeks after administration. Keil, Keil, and Nelson[3] found the animals on a milk, copper, and iron ration grow more slowly than those on a good stock diet. Reproduction occurred in animals of the first generation, but the second generation did not reproduce. Our results on second generation rats, together with the findings of Waddell, led us to investigate both males and females of the first and second generations in order to ascertain whether or not the sterility on this diet could be ascribed to degeneration of sex organs.

All of the animals (rats) were fed whole milk obtained from Holstein cows in the dairy herd at Iowa State College. The milk was collected directly into glass jugs, in order to avoid contamina-

[1] Underhill, F. A., Orten, J. M., Mugrage, E. R., and Lewis, R. C., *J. Biol. Chem.*, 1932, **99**, 469.

[2] Waddell, J., *J. Nutr.*, 1931, **4**, 67.

[3] Keil, H. L., Keil, H. H., and Nelson, V. E., *Am. J. Physiol.*, 1934, **108**, 215.