AVIAN MALARIA AND B COMPLEX VITAMINS

II. Riboflavin

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Summary

Vitamins play an important role in the host-parasite relationship of many diseases, the concentration of the vitamin affecting the onset, course, duration and severity of the infection.

Studies on the riboflavin metabolism in chick malaria infected with *P. gallinaceum* reveal a slight increase in the blood levels of riboflavin during the pre-patent period and decrease during the acute progressive stage.

Riboflavin deficiency reduces the rate of progress of parasitæmia, and lessens the severity of the infection. The supplementation of riboflavin to these deficient animals stimulates the growth and multiplication of the malarial parasite and increases the severity of the disease as indicated by the higher parasitæmic response and the shorter survival period of these birds.

These results indicate that riboflavin is an essential vitamin for the growth and multiplication of *P. gallinaceum*.

The fact that malarial parasites require vitamins has been well recognised[1] and the literature so far published on this aspect has been reviewed by Trager.[2] The requirements seem to differ from species to species and our earlier publication deals with the essential role of thiamine in *P. gallinaceum* infections in chicken.[3] The relationship of this parasitic infection with the riboflavin metabolism of the chicken is presented in this paper.

Materials and Methods

Maintenance of the strain of *P. gallinaceum*, the mode of injection and evaluation, the selection of suitable birds for experimentation and other procedural details have been described earlier.[3]

The relationship of the degree of parasitæmia to riboflavin level in blood was determined by taking the blood before and during definite intervals after infection in a group of five birds. The riboflavin levels were estimated by the microbiological procedure as outlined by Snell and Strong[4] with L. Casei as the test organism. The following procedure was adopted for the liberation of the bound vitamin prior to microbiological assay.

Blood samples were hydrolysed with 0·1 N HCl at 15 lb./sq. inch pressure for 15 minutes and later adjusted to pH 4·5 with sodium acetate and acetic acid and made up to a convenient volume. Suitable aliquots from this were added to a double strength basal medium and adjusted with distilled water to a final volume of 5 ml. Tubes were inoculated with suspension of the organism collected from a 24 hours old liquid culture after washing thoroughly with sterile saline solution over a centrifuge. The standard growth response curve was obtained by supplementing known levels of riboflavin to the basal medium. During the estimation care was taken to see that all the procedures were carried out in partial darkness or subdued light since riboflavin is destroyed by light.

The growth response to the test solutions and known amounts of riboflavin were determined both turbidimetrically at the end of 48 hours and by titration with 0·1 N alkali at the end of 72 hours. Levels of riboflavin occurring in blood were calculated by comparing the response to a standard curve and the results are presented under Table I.

TABLE I

Blood Levels of Riboflavin

mγ/c.c.

Experiment No.	Normal	Incubation period (5th day)	Parasitised
1	184	228	176
2	178	197	167
3	182	208	174
4	182	204	170
5	165	192	153·5

Effect of depletion and supplementation of riboflavin on the course of the P. gallinaceum *infection.*—The composition of the basal diet has been described earlier.[3] The vitamin deficiency was that of riboflavin in place of thiamine, which was given at a level of 170 γ/100 mg. diet. The riboflavin supplementation was done by intramuscular injections of this vitamin at three dosage levels, 30 γ, 90 γ and 300 γ per 50 gms. body weight of the bird. The injections were given daily, starting immediately after the infection, for a period of four days.

The results of riboflavin supplementation on the course of parasitæmia are presented in Fig. 1.

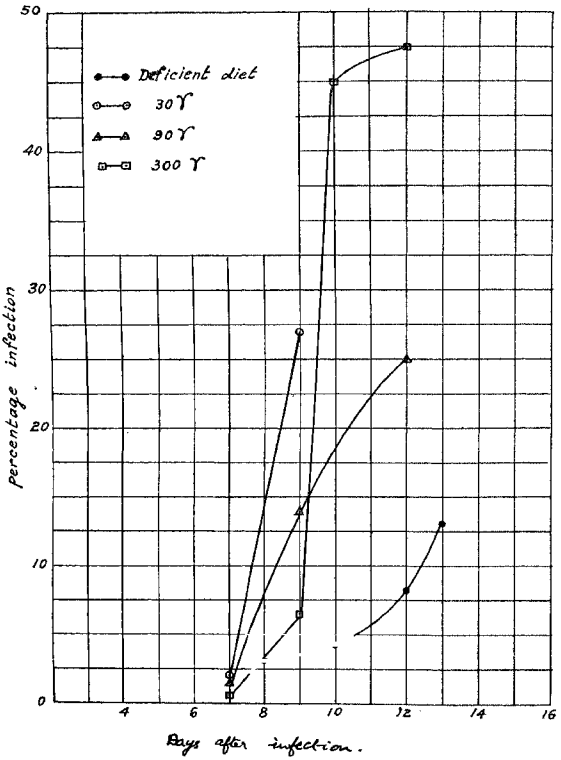

FIG. 1. Effect of Riboflavin on *P. gallinaceum* Infection in Chicken.

DISCUSSION

Figures in Table I indicate that in the incubation period, an increase in the riboflavin content of the blood is noticed. This gradually decreases with the onset of parasitæmia and apparently reaches levels below the normal during the peak of parasitæmia.

The results of riboflavin supplementation makes an interesting study. The delay in onset of parasitæmia, diminished peak of percentage infection and the

slightly prolonged survival period noticed in the riboflavin-deficient animals are all altered with the administration of this vitamin. Various dosage levels of riboflavin show varying effects. Supplementation at all levels causes increased number of red cells to be infected, higher the dosage greater being the percentage of infection. The birds given higher levels of riboflavin, 90 γ, and 300 γ, though showing higher rate of infection, survive for a longer period than the ones treated with 30 γ of the vitamin.

Riboflavin in living tissues takes part in enzyme systems regulating cellular oxidations and also pay a role in the general carbohydrate metabolism. It has an important relation to the amino acid metabolism as the *d*-amino acids are deaminated by an enzyme system containing the flavin-adenine dinucleotide. Since the above metabolic pathways are intimately connected with parasite metabolism, requirements of riboflavin will be high during rapid multiplication. This might be responsible for the slight increase in riboflavin concentration in blood during the prepatent period and low concentrations of the same during the very acute stage.

Riboflavin is an important member of the B complex group which plays an essential role in the regulation of the carbohydrate, fat and protein metabolism. It is a growth factor for animals as well as micro-organisms and as such supplementation of the same might stimulate the growth and multiplication of the parasite and also the host-cells. Since in these experiments the severity of the infection has increased, it clearly shows that the stimulatory effect of the supplement on the parasite has outweighed the beneficial effect on the host. These results also indicate that suitable antagonists to this vitamin may probably act as good anti-malarials. Observations of Madinaveitia[5] and Wright[6] that riboflavin is antagonised by a number of anti-malarials also add support to the above hypothesis.

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