

AVIAN MALARIA AND B-COMPLEX VITAMINS

III. *Para-Amino-Benzoic Acid*

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SUMMARY

A study of the biochemical alterations with particular reference to B-complex vitamins in the blood of *P. gallinaceum* infected chicken has shown that *para*-amino-benzoic acid level is increased during the incubation period and registered a fall during the parasitæmic stage thus indicating the need and its utilisation by the parasite. This is made more evident by following the course of infection in PABA deficient and supplemented chicken infected with *P. gallinaceum*. The appearance of the parasite in the peripheral blood is delayed in the chicken kept on deficient diet while an earlier manifestation of parasitæmia and increased severity of the disease is noticed in animals supplemented with high doses of PABA.

Several investigators^{1,2,3,4} have postulated that PABA is an essential metabolite of all micro-organisms susceptible to the action of sulphanamides. While some of these organisms are capable of supplying their needs by internal synthesis, others require this vitamin in a preformed state in the medium of growth.

Since PABA is an essential requisite for the invading organisms susceptible to sulphanilamides, the final outcome of the host parasite relationship as regards the utilisation of PABA is a delicately balanced one. PABA accelerates the onset of disease in experimental typhoid of mice⁵ while mitigating the severity of experimental tuberculosis in guinea pigs.⁶

Amongst the protozoal infections, PABA has been shown to be an essential vitamin for the growth and multiplication of *Plasmodium knowlesi* in *in vitro* cultures.^{7,8} Several workers have suggested that suppression of both blood and sporozoite induced *P. cynomolgi* and *P. berghei* infections in monkeys and rodents respectively, by milk diet, might be due to the low and inadequate level of PABA in the diet, as the reversal of the suppression could be induced by the supplementation of PABA.⁹

Though the need for PABA, for the growth of those various species of plasmodia has been shown, the evidence is only an indirect one. Since quantitative determination of PABA in the blood of normal and malaria infected birds might throw some light on the intimate relationship between the need for PABA and the progress of parasitæmia this investigation has been undertaken in the *P. gallinaceum*

infection of chicks. Besides noting these biochemical alterations, the influence of depletion and supplementation of PABA on the course and severity of this infection has also been studied.

EXPERIMENTAL

The details regarding the maintenance of the strain of *P. gallinaceum*, the selection of chicks, the mode of infection and evaluation, and other procedural details in these studies have been same as described earlier.^{10,11}

Estimation of PABA.—Influence of the progress of infection on blood concentration of PABA was investigated on the blood samples drawn from a group of five chicken under normal, prepatent and parasitised conditions. A microbiological procedure using *Neurospora crassa* was followed for the estimation of PABA and this, as described below, was in all essentials the same as outlined by Thompson.¹²

The method for the assay involved hydrolysis of blood samples over a water-bath with 4 N HCl for an hour, adjustment of the pH to 4 and making up to a convenient volume with distilled water. The disc-plate method was used for the assessment of PABA levels. The growth response of the organism to the test solutions as measured by the diameter of zone of growth was compared to the standard obtained with known amounts of vitamin and the PABA levels in samples were calculated.

Results of these experiments are presented under Table I.

TABLE I

Changes in the blood levels of PABA in chicken infected with P. gallinaceum
(γ /100 ml.)

Bird No.	Normal	Incubation period	Parasitised condition
64	15.0	23.0	12.6
65	15.6	23.0	12.6
66	13.5	20.8	11.4
67	15.2	18.4	22.31
68	16.2	21.7	15.8

EFFECT OF PABA SUPPLEMENTATION ON THE COURSE OF INFECTION

Experimental details regarding the selection of birds, the basal diet used, mode of infection were in all aspects the same as described under thiamine¹⁰ except

that riboflavin and thiamine were added in the concentration of 300 γ and 170 γ respectively per 100 gm. of the synthetic diet free from PABA.

Three groups of chicks, three in each were chosen and fed with the above synthetic diet for a period of 10 days, after which period they were infected intramuscularly with 16 million parasites. One group was then kept as the control and was fed with the deficient diet. The second group received PABA (50 γ /100 gm. B. wt.) intramuscularly daily once in the mornings for four days. The third group was maintained on the natural laboratory diet for purposes of comparison. Blood smears of these birds were taken from the fourth day onwards and an estimate of the degree of parasitæmia was carried out as described earlier. The results of the findings are presented in Fig. 1.

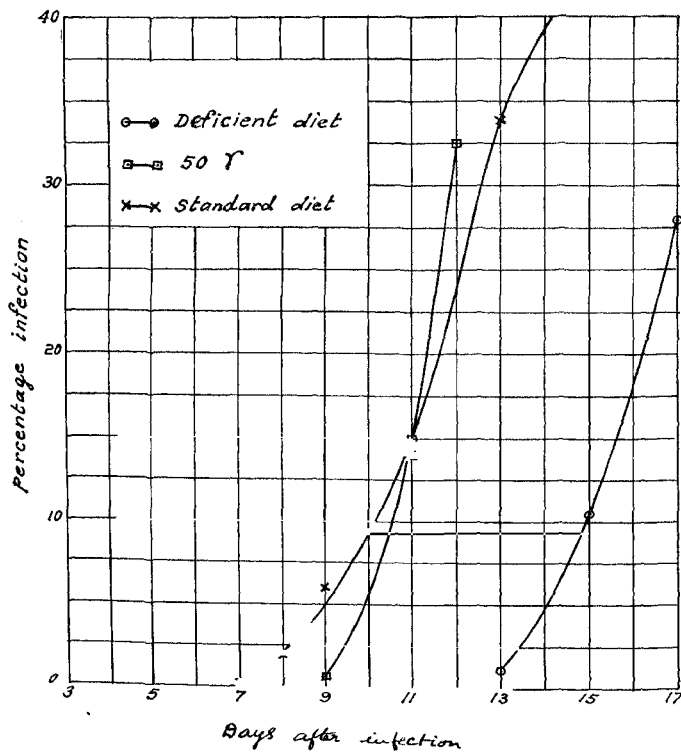


FIG. 1. Effect of PABA on *P. gallinaceum* infection in Chicken.

DISCUSSION

A definite increase in the blood levels of PABA is noticed in the incubation period, while during the height of parasitæmia it reaches levels much below the normal in the majority of the infected birds (Table I). PABA deficient diet prolongs the period of incubation and the day of appearance of parasites in the peripheral blood. Supplementation of large doses of PABA on the other hand reduces the prepatent period and increases the severity of the infection, as compared to the deficient group.

These results evidently show that PABA is an essential nutrient both during the exo-erythrocytic and the erythrocytic phases of this parasite development. The evidence available in the literature suggests that PABA plays a prominent part in the synthesis of purine, thymine, methionine and possibly other amino acids and resemble vitamin B₁₂ and folic acid.¹³ Since these substances are essential for the formation of nucleoprotein and hence the growth and multiplication of cells, a similar role might be assigned to PABA in its relation to the *P. gallinaceum*, depletion causing a retarded growth and supplementation affecting a rapid multiplication.

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