

# AVIAN MALARIA AND B. COMPLEX VITAMINS

## 1. Thiamine

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### SUMMARY

Besides their powerful metabolic effects, vitamins play an important role in the host parasite relationship in diseases. The effects of vitamin deficiency and supplementation vary with different kinds of infections.

Investigations carried out on the role of thiamine in avian malaria (*P. gallinaceum* in chicken) has shown a marked depletion of the thiamine content of the blood during the acute phase of the infection, this tendency becoming evident even in the prepatent and exo-erythrocytic stages.

Thiamine depletion adversely affects the multiplication of the malarial parasites as also the host as shown by earlier mortality of the latter; supplementation of thiamine at low dosages prolongs the survival time of the infected birds while checking the parasitemia; in high dosages, rapid multiplication of the malarial parasites, quicker and higher peak of parasitemia and diminution of survival periods are observed.

The implications of these findings are discussed.

Vitamins existing in relatively small concentrations perform specific and vital functions in living organisms. They also exert very powerful influence on metabolism and their biological activities are partly traceable to co-enzymic effects. Specific relationships have been reported to exist between the vitamin levels of the host and the degree of susceptibility to an infectious agent. In many instances, vitamin deficiencies have been reported to lower the resistance of the host to pathogenic micro-organisms whereas in certain other infections high levels of vitamins have been reported to increase the severity of the disease. A few specific relationships between the vitamin level of the host and the degree of susceptibility to an infectious agent have been reported by several investigators and Table I presented below indicates how this type of biochemical alteration in the system renders the animal either more susceptible or more resistant to manifestations of certain viruses, bacteria and protozoa.

TABLE I  
Influence of nutrition on disease

	Disease	Experimental animal	Susceptibility
Well fed animal	Rou's Sarcoma	Rats	+
	Foot and Mouth Disease	Guinea pigs	+
Starvation	Virus Disease	Rabbits	-
	Malaria	Human	-
Vitamin A deficiency	<i>Trichinella spiralis</i> <sup>1</sup>	Rats	+
Riboflavin	Malaria	Chicks	-
"	Typhus <sup>8</sup>	Rats	-
Thiamine	Leprosy <sup>2</sup>	"	+
Riboflavin	Pneumonia	Mice	+
Thiamine	<i>Poliomyelitis</i> <sup>4</sup> (Lancing)	"	-
"	" (Theiler)	"	±
"	Pneumonia	"	+
Pantothenic Acid	"	Rats	-
	<i>Poliomyelitis</i> (Theiler)	Mice	+
	* (Lancing)	"	-
Vit. C administration	Malaria	Chicks	+
Fat-free diet	Enteritidis	Mice	-

+ More susceptible (Resistance lowered).

- Less susceptible.

#### Relationship between vitamins and malarial infections

Maegraith<sup>5</sup> *et al.* have observed that if a milk diet was given to rats and infected with *P. berghei* the parasites failed to develop. This effect was found to have nothing to do with low iron contents of milk but with some other factors present in milk. Hawking<sup>6</sup> later on reported that the suppressive action of milk might be due to its deficiency in *para*-amino-benzoic acid (PABA) which is known to be a growth factor for many malarial parasites especially *P. berghei* and *P. knowlesi*. The course of blood induced infections in starved rats was very mild and the reversal

of the effect was achieved by feeding methionine and PABA to these animals.<sup>7</sup> Again ascorbic acid-deficient condition has been found to be very unfavourable for progress of *P. knowlesi* infection in monkeys and supplementation of this vitamin increased the severity of the disease.<sup>8</sup>

In an attempt to understand the relationship between the *P. gallinaceum* infections in chicken and certain vitamins especially of the B. Complex group, the effect of infection on the blood level of thiamine in chicken and the influence of depletion and supplementation on the course of infection have been investigated and presented in this communication.

#### MATERIALS AND METHODS

##### *Blood levels of thiamine and malarial infection*

Three to four weeks old chicken were used for these experiments. They were divided into seven groups with two birds in each and were kept in individual cages on the usual laboratory diet. Blood samples (1 c.c. from each bird of each group and pooled) were drawn from the normal experimental birds after 24 hours starvation and the vitamin contents were determined. The above groups of birds were infected intramuscularly each with 16 million parasites and blood samples were drawn on different days after infection for the vitamin assay.

Blood thiamine was estimated by the microbiological procedure outlined by Arnol Peter and Meiklejohn<sup>9</sup> using *Phycomyces blakesleeanus* (sex - ve) as the test organism. Briefly, the procedure involved direct incorporation of the various blood samples into a double strength basal medium, consisting of asparagine and inorganic salts and making up to a final volume with distilled water. Sterilization was carried out at controlled temperature (107° for 10 minutes) and inoculation with a uniform suspension of spores from a 2-week-old malt-agar slant. Incubation was carried out in the dark for a period of 10 days at 30° C. at the end of which period the mycelia were harvested, washed by centrifugation before drying and weighing to constant weight. The growth response of the organism to the test solution was compared with a standard curve obtained using standard amounts of thiamine hydrochloride (0.05  $\gamma$  to 0.3  $\gamma$  per 10 ml.) and the thiamine levels calculated.

#### RESULTS

Results got with blood of the chicken under normal, incubation period and under parasitised conditions are presented in Table II.

##### *Effect of vitamin depletion and supplementation on malarial infection*

In these series of experiments, the effect of supplementation of different levels of thiamine was studied. A group of chicken 5-days old and 12 in number were chosen and were fed on a synthetic diet (deficient in thiamine) prepared as follows:—

TABLE II

Changes in the blood levels of thiamine during the normal and infected conditions in the chick

( $\gamma$ /100 ml.)

Group No.	Normal	Prepatent period (5th day)	Parasitised condition
1	28.66	12.8	10.5
2	24.08	13.40	11.84
3	27.39	12.46	10.34
4	28.22	20.62	14.83
5	30.42	18.32	12.47
6	26.90	14.68	11.32
7	20.35	24.96	18.94

*Composition and preparation of the synthetic diet*

*Vitamin-free casein.*—This was prepared by extracting 1 lb. of casein (about 3 times) with 750 ml. of water, 750 ml. of ethyl alcohol and 7.5 ml. of concentrated hydrochloric acid. This was kept at 70° C. for 3 hours and then filtered with a piece of cloth, washed with water and some more alcohol and dried.

*Vitamin-free corn starch.*—Corn starch was devitaminised after refluxing with alcohol for 5 hours and then filtered and dried.

*Salt mixture.*—McCollum and Davis salt mixture was used for the experiment and it consisted of the following constituents:—

*Composition of salt mixture:*

Calcium lactate..	..	..	35.15 per cent.
Ca (H <sub>2</sub> PO <sub>4</sub> ) <sub>2</sub> H <sub>2</sub> O	..	..	14.6 „
K <sub>2</sub> HPO <sub>4</sub>	..	..	25.78 „
NaH <sub>2</sub> PO <sub>4</sub> H <sub>2</sub> O	..	..	9.38 „
NaCl	..	..	4.67 „
MgSO <sub>4</sub> (Anhydrous)	..	..	7.19 „
Iron citrate	..	..	3.19 „

*Composition of the diet used:*

Vitamin-free casein	..	..	15 per cent.
Corn starch	..	..	71 ..
Fat as cocoanut oil	..	..	10 ..
Salt mixture	..	..	4 ..
and vitamins as follows: (Requirements of chick as reported in "Vitamins and Hormones")			
Vitamin A	..	..	260 I.u. per 100 gm. of diet
Vitamin D	..	..	(A.O.A.C.U.) 25 I.u. per 100 gm. of diet
Riboflavin	..	..	300 $\gamma$
Pantothenic acid (Calcium)	..	..	1000 $\gamma$
Niacin	..	..	1500 $\gamma$
Pyridoxin HCl	..	..	300 $\gamma$
Biotin	..	..	10 $\gamma$
Cholin chloride	..	..	130 mg.
Vitamin K	..	..	40 $\gamma$

The experimental birds were fed for a period of 10 days with the above deficient diet to deplete the animals of this vitamin. They were then divided into 4 groups of 3 in each and infected intramuscularly with the standard dose of malarial parasites (16 million). One group was kept as the control and the other groups were injected intramuscularly with 30  $\gamma$ , 80  $\gamma$ , 160  $\gamma$  (per 100 gm. body weight) of thiamine respectively daily once in the mornings for 4 days. Blood smears were taken from the second day onwards daily, stained with J.S.B. stain and the degree of parasitæmia determined until the death of the birds. Results of the above experiment are presented in Fig. 1.

## DISCUSSION

From Table II it is clear that thiamine levels go on decreasing along with the progress of parasitæmia. Even from the fifth day of infection thiamine levels decrease and at the stage of acute infection it is reduced to just about 40 per cent. of the initial level present in the blood of the chick. Marked depletion of this vitamin in blood even before the appearance of the parasites in the peripheral circulation indicates the need of the vitamin during the exo-erythrocytic and prepatent periods.

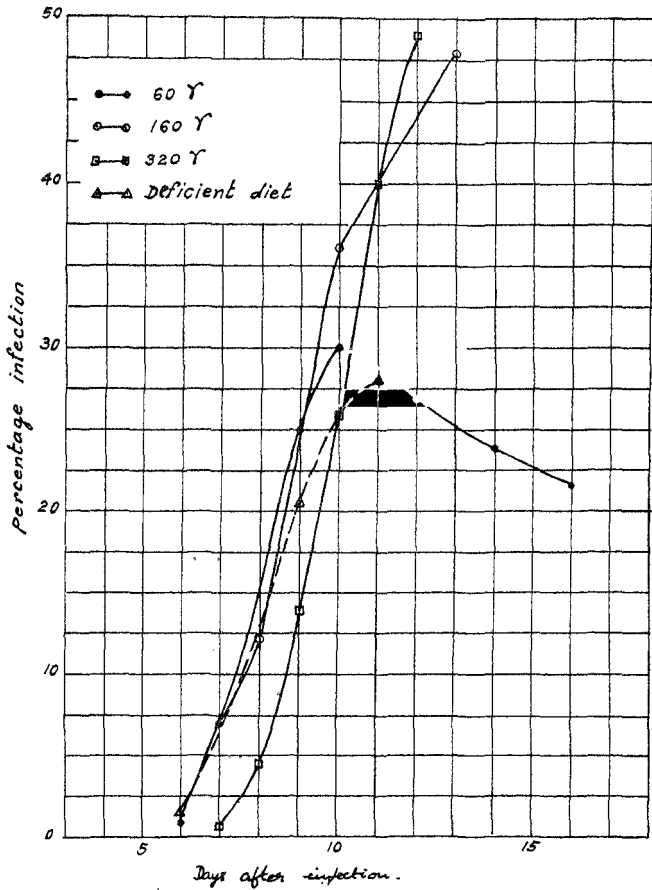


FIG. 1. Effect of Thiamine on *P. gallinaceum* infection in Chicken.

Effect of depletion of thiamine and its supplementation at various levels shown in Fig. 1 reveal that thiamine depletion and low thiamine administration markedly influence the multiplication of the parasites as compared with the high dosage of thiamine. While complete depletion of thiamine retards the multiplication of the parasites, this severe deficiency affects the host also adversely and the birds of this group die earlier without marked degree of parasitemia. The low dosage of thiamine supplementation (60  $\gamma$  per day) on the other hand retards the parasite multiplication with prolongation of the survival time. The high dosages, 160  $\gamma$  and 320  $\gamma$  per day, actually assist the parasite in its growth, these animals exhibiting a higher peak of parasitemia much earlier and causing mortality quicker. This also indicates the possibility of the absence of internal synthesizing mechanism of this vitamin in the parasite and also dependence of the parasite entirely on the external supply for its requirements. It can thus be concluded, that administration of larger doses of thiamine actively assist the progress of parasitemia and adversely affects the host in acute blood induced infections of *P. gallinaceum* in chicken. Thiamine stands out as being very much involved in the metabolism of carbohydrates, as the coenzyme, co-carboxylase, which takes part in the carbohydrate metabolism is none other than a pyrophosphoric ester of thiamine. In *P. gallinaceum* infection carbohydrate metabolism is greatly affected and the parasitized erythrocyte has been shown to utilise glucose nearly 60 times greater than normal erythrocyte.<sup>10</sup> This might also be the reason for the infection being mild in thiamine-deficient condition and becoming severe when high doses of thiamine are supplemented to the deficient diet.

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