THE INFLUENCE OF THIAMINE ON THE SUSCEPTIBILITY OF CHICKS TO AVIAN ENCEPHALOMYELITIS[1]

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Previous reports from our laboratories have dealt with the relation of nutrition to the resistance of rhesus monkeys and of Swiss mice to virus infections, poliomyelitis (Rasmussen, Waisman, Elvehjem, and Clark, 1944; Lichstein *et al.*, 1944; Rasmussen, Waisman, and Lichstein, 1944; Clark *et al.*, 1945; Lichstein *et al.*, 1945). The present study is directed to the possible influence of thiamine in the resistance of chicks to avian encephalomyelitis, a disease first described by Jones (1932).

EXPERIMENTAL

One-day-old white leghorn chicks maintained in electrically heated cages with raised screen bottoms were used in these experiments. The basal ration was essentially the one described by Briggs *et al.* (1945) and had the following percentage composition: dextrin 60, alcohol-extracted casein 18, gelatin 10, salts 6, and soybean oil 5. Each 100 g also contained *l*-cystine 300 mg, riboflavin 0.6 mg, pyridoxine 0.4 mg, calcium pantothenate 2 mg, niacin 5 mg, choline 150 mg, inositol 100 mg, *p*-aminobenzoic acid 5 mg, biotin 0.02 mg, folic acid 0.10 mg, ascorbic acid 100 mg, 2-methyl-1,4-naphthoquinone 0.05 mg, and α-tocopherol 0.3 mg. In addition, each chick received 3 drops of halibut liver oil (a source of vitamins A and D) weekly.

The strain of virus used was received through the courtesy of Dr. E. Jungherr of the University of Connecticut and was carried through four passages before being used in these experiments. The chicks were given 0.05 ml intracerebrally of either a 1 or 2 per cent suspension of infected chick brain and cord without ether anesthesia.

When placed on the basal ration, 1-day-old chicks usually succumbed within a week to a thiamine deficiency, and since the incubation period for the virus is 10 to 14 days, it was not feasible to use this ration by itself. The addition of 40 μg of thiamine per 100 g of this ration also proved inadequate in extending the survival period, but when the level of thiamine was raised to 60 μg per 100 grams, the chicks survived beyond the virus incubation period, although they showed poor growth and deficiency signs. The chicks were divided into three groups: group 1 received the basal ration containing 60 μg of thiamine per 100 g; group 2, the basal ration containing 90 μg of thiamine per 100 g; and group 3,

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the basal ration containing 300 μg of thiamine per 100 g. Since the minimum thiamine requirement of the chick is about 150 μg per 100 g of ration, this division allowed study of a deficient, a suboptimum, and an optimum level of the vitamin. The animals were inoculated within a week after they were placed on the diets.

The observed signs of this virus disease are a weakness of the legs manifested by an inclination to assume a sitting position. The weakness may progress after a few days to prostration and death. These signs are differentiated from thiamine deficiency by the fact that opisthotonus is absent, that the chicks do not respond to thiamine therapy, and that they do not immediately lose weight. When paralysis occurred in the groups receiving the deficient and suboptimum levels of thiamine, the animals were immediately given, *per os*, 1 mg of thiamine a day for 2 days, in order to determine whether the paralysis was due to the virus infection or to thiamine deficiency.

TABLE 1

The influence of various levels of thiamine on the susceptibility of white leghorn chicks to avian encephalomyelitis

SUPPLEMENT	60 μg B_1 PER 100 GRAMS	90 μg B_1 PER 100 GRAMS	300 μg B_1 PER 100 GRAMS
12 days after inoculation			
No. of chicks without paralysis	5	22	31
No. of chicks paralyzed	30	15	5
22 days after inoculation			
No. of chicks without paralysis	1	8	19
No. of chicks paralyzed	34	29	16

These chicks were inoculated within a week after they were placed on experiment.

In the first series, which included 12 chicks in each group, those which received the largest supplement, 300 μg thiamine per 100 g, exhibited a lower incidence of infection than those in either of the other two groups. The incubation period for the virus was longer, the paralysis was less severe, and fewer deaths occurred in the paralyzed chicks.

This series was repeated twice with similar results in each case; the composite figures are given in table 1. More than 50 per cent of the chicks receiving 300 μg of thiamine per 100 g of ration were protected from the virus infection, compared to practically no protection in the group receiving the 60 μg supplement and 22 per cent in the group receiving the 90 μg supplement.

Another series of experiments was run simultaneously in which 1-day-old chicks were given the basal ration supplemented with 300 μg of thiamine for 2 weeks. Then they were divided into 3 groups to receive the same supplements of thiamine as in the other series (60, 90, and 300 μg per 100 g of ration, respectively). After 2 weeks on this regimen, all chicks were inoculated with the virus.

In this series the chicks maintained on the optimum level of thiamine were not protected, the greatest percentage of surviving chicks being in the group receiving 60 μg of thiamine per 100 g of ration. This experiment was also repeated twice with similar results. The composite results are presented in table 2. It is interesting to note that the incubation period for the virus was reduced in the group receiving the highest level of thiamine, and in some cases paralysis was observed 5 days after inoculation. Only 4 chicks out of 34 failed to show paralysis in this group as compared with 10 out of 27 in the group receiving 60 μg of thiamine per 100 g of ration.

It is evident that the degree of protection afforded the chicks against this virus depended upon a number of factors, which included the age of the chick, the previous state of nutrition, and the state of nutrition at the time of inoculation.

Reports from this laboratory have shown that mice fed diets deficient in thiamine manifest a striking resistance both to Lansing strain poliomyelitis and to

TABLE 2

The influence of various levels of thiamine on the susceptibility of white leghorn chicks

SUPPLEMENT	60 μg B_1 PER 100 GRAMS	90 μg B_1 PER 100 GRAMS	300 μg B_1 PER 100 GRAMS
12 days after inoculation			
No. of chicks without paralysis	18	6	12
No. of chicks paralyzed	9	18	23
22 days after inoculation			
No. of chicks without paralysis	10	2	4
No. of chicks paralyzed	17	22	30

These chicks were given a ration with 300 μg of thiamine per 100 grams for 2 weeks, then depleted for 2 weeks, and then inoculated.

Theiler's encephalomyelitis (Rasmussen, Waisman, Elvehjem, and Clark, 1944), but the level of thiamine does not appear to be a crucial factor in the susceptibility of monkeys to the MV strain of poliomyelitis virus (Clark *et al.*, 1945). The results obtained with our first series of chicks, which were started immediately on the depletion diets, showed an entirely different picture from those exhibited either by the mouse or the monkey in that the higher levels of thiamine gave the greatest protection. On the other hand, the results with the chicks that were first given a complete ration and then depleted corresponded more directly with the results in mice. It is possible that the presence of the yolk sac in the younger chicks may have had some influence on the rate of thiamine depletion and hence some effect on the degree of resistance.

SUMMARY

One-day-old white leghorn chicks, divided into 3 groups, receiving a low, suboptimum, and optimum level of thiamine were inoculated with a virus suspension of avian encephalomyelitis. The chicks receiving the highest level of thiamine were protected to the greatest degree.

In another series 1-day-old white leghorn chicks were given an optimum level of thiamine in the ration for 2 weeks, then divided into 3 groups receiving levels of thiamine as indicated in the previous experiments for 2 weeks, at the end of which time they were inoculated. In this case the chicks receiving the lowest level of thiamine were protected to the greatest degree.

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