

Response of Anemic Chicks to Pteroylglutamic Acid.*

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In the course of studying the quantitative requirements of chicks for pteroylglutamic acid (folic acid),¹ information was desired regarding the ability of anemic chicks to make effective use of this substance in order to compare their response to that of anemic monkeys reported by Day and associates,² and that of human subjects described by Spies and co-workers.³

Experimental and Results. *Exp. 1.* Surviving chicks of several experiments were divided at random into 2 groups of 12 chicks each. These chicks had been reared to 4 weeks of age on Diet 653 described by Hill and associates.⁴ Microbiological assays indicated that the diet contained about 2 μ g of free pteroylglutamic acid and about 3 μ g in the conjugate form per 100 g of diet. The hemoglobin levels of these chicks averaged 2.7 g per 100 cc of blood with a range of 0.6 to 6.2 g per 100 cc.

The chicks in one group were injected intramuscularly with 100 μ g of pteroylglutamic acid;[†] those in the other group were maintained as controls. Hemoglobin values and percentage of reticulocytes were determined

at 2, 4, 6, 9, 11, 13 and 16 days following the single injection of pteroylglutamic acid.

In the group receiving the vitamin, 3 chicks died the day injections were made but no other mortality occurred during the experimental period. In the control group, 6 chicks died during the first 2 days and only 2 were alive at the end of the experiment.

The percentage of reticulocytes was strikingly greater in the group receiving the pteroylglutamic acid. Reticulocytosis reached a peak on the 6th day following injection after which it subsided rapidly to less than 10%, which is the level usually observed in untreated chicks. The peak of reticulocytosis in all cases was reached before the 9th day following the injection. These relationships are shown in Fig. 1.

Reticulocytes increased slightly in the surviving chicks in the control group. This increase may have been due to mobilization of pteroylglutamic acid in the catabolism of body tissues, or it may have been the result of normal erythropoietic stimuli occurring in this severe stage of anemia, analogous to spontaneous remission in pernicious anemia in humans.

The hemoglobin response was no less striking than the reticulocyte increase. Two days following the injection of pteroylglutamic acid, hemoglobin values had increased in most chicks. The increase was definite in all chicks by the 6th day. The peak in hemoglobin level was reached in 9 days, coincidental with the rapid decrease in reticulocytes.

Exp. 2. The purpose of this experiment was to compare oral administration with intramuscular injection of pteroylglutamic acid, and to establish the amount of the vitamin required for optimum reticulocytosis and hemoglobin formation. Seventy chicks which had been reared to 4 weeks of age on

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¹ Robertson, E. I., Daniel, Louise J., Farmer, Florence A., Norris, L. C., and Heuser, G. F., *Proc. Soc. Exp. Biol. and Med.*, 1946, **62**, 97.

² Day, P. L., Mims, V., and Totter, J. H., *J. Biol. Chem.*, 1945, **161**, 45.

³ Spies, T. D., Vilter, C. F., Koch, M. B., and Caldwell, M. H., *So. Med. J.*, 1945, **38**, 707.

⁴ Hill, F. W., Norris, L. C., and Heuser, G. F., *J. Nutrition*, 1944, **28**, 175.

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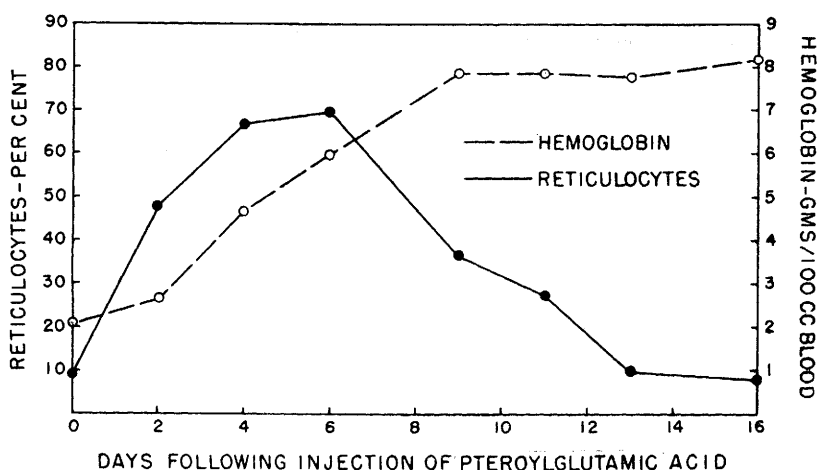


Fig. 1.

Reticulocyte and hemoglobin response of chicks to a single injection of pteroylglutamic acid.

TABLE I.
Response of Chicks to Pteroylglutamic Acid.

Groups	Days following treatment									
	Body wt (g)				Hb level (g/100 ml)				Retics. (%)	
	0	4	7	11	0	4	7	11	4	7
Basal	114	112	108	— (0) †	1.7	0.7	1.2	—	11	20
In feed*	109	129	168	220 (4)	3.1	3.2	6.4	7.3	60	47
Inj. with:										
5 μ g	115	143	140	163 (3)	2.1	2.2	3.2	3.7	40	25
10	113	144	128	155 (4)	2.4	2.8	4.1	3.4	42	26
20	109	131	148	159 (5)	2.6	2.7	4.1	3.0	59	35
30	117	156	179	198 (5)	2.2	3.2	5.0	6.1	63	41
40	117	136	131	158 (3)	2.1	3.4	6.2	7.3	72	17
50	130	165	174	192 (5)	2.3	4.4	7.5	8.0	73	39
Oral:										
5	109	131	129	132 (4)	2.2	1.6	1.4	1.3	42	11
10	125	130	145	157 (3)	2.8	1.4	1.7	1.8	20	25
20	118	131	131	145 (3)	2.2	1.9	2.7	2.8	41	39
30	122	147	153	162 (3)	1.9	2.8	3.4	3.0	56	39
40	117	144	154	156 (5)	2.7	3.7	3.8	3.5	51	26
50	114	138	146	161 (4)	1.9	3.5	4.6	4.0	75	39

* 500 μ g of folic acid per 100 g of feed from 0 to 7 days; 250 μ g thereafter.

† Numbers in parentheses indicate surviving chicks.

Diet 653 but without pteroylglutamic acid, and whose hemoglobin levels ranged between 1 and 4 g per 100 cc of blood, with weights between 100 and 150 g, were divided into 14 groups of 5 chicks each. Six groups were used for oral administration, and 6 groups for injection of pteroylglutamic acid. Of the remaining groups one was maintained as control and the other received the vitamin in its diet.

The results of Exp. 2 are presented in

Table I. Reticulocytosis was greater on the 4th than on the 7th day following treatment. This applies to the chicks which received pteroylglutamic acid in their diet, and to those to which the vitamin was administered orally or by intramuscular injection.

Hemoglobin levels were greatest in the groups which received pteroylglutamic acid by injection. When the vitamin was supplied in the feed it produced a greater response in the hemoglobin level than when admin-

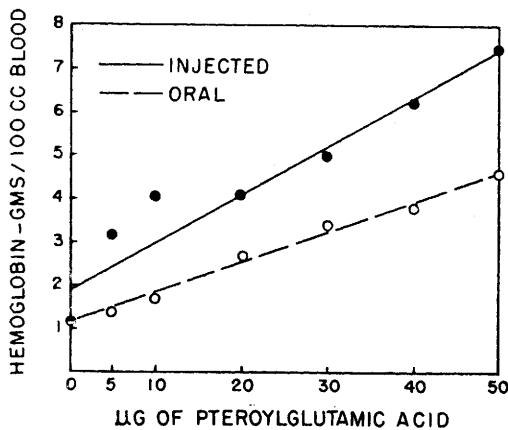


Fig. 2.

The hemoglobin response of chicks to varying levels of pteroylglutamic acid.

istered orally but separate from the feed. In all cases the response was proportional to the amount of pteroylglutamic acid administered. In the oral and injected groups the hemoglobin response was more pronounced at 7 than at 4 days following treatment. The values for the 11th day were variable due to the loss of some chicks. Hemoglobin increases after 7 days were plotted and the results are shown in Fig. 2.

Intramuscular injection of pteroylglutamic acid was approximately twice as effective in increasing hemoglobin as the same amount of the vitamin given orally. This may be due to actual destruction during the digestive processes, or to limited absorption of the vitamin.

The chicks receiving a diet containing 250 to 500 μg pteroylglutamic acid per 100 g of diet did not show increases in hemoglobin as great as that produced by the injection of 50 μg of the vitamin. Based upon feed consumption during the 7-day period the average intake per chick was estimated to be 486 μg of pteroylglutamic acid. The response of these chicks in hemoglobin increase was similar to that produced by the injection of 40 μg of the vitamin.

Discussion. The data reported for anemic

chicks indicate that their response to pteroylglutamic acid is similar to that of other species. Spies and associates³ reported peaks in reticulocyte response in 6 to 8 days following intravenous injection of folic acid in humans afflicted with macrocytic anemia in relapse. The peak of reticulocytosis rarely reached 20%. Day and co-workers² observed a 7 to 47% increase in reticulocytes between 4 and 7 days following the administration of *Lactobacillus casei* factor to vitamin M-deficient monkeys. The data reported here for anemic chicks show that reticulocyte response reached a peak 4 to 6 days following administration, and often exceeded 90% for individuals. It appears, therefore, that pteroylglutamic acid stimulates reticulocytosis in the chick the same as has been reported for man and monkey.

These data indicate that intramuscular injection of pteroylglutamic acid was more effective than oral administration in increasing the hemoglobin levels of anemic chicks. However, Goldsmith⁵ found oral administration equally or more effective in increasing hemoglobin levels of humans with macrocytic anemia. A possible explanation of this difference may be that suboptimum amounts of the vitamin were given to the anemic chicks.

Summary. Chicks deprived of pteroylglutamic acid (folic acid) for the first 4 weeks of life respond dramatically to a single administration of the vitamin given either orally or intramuscularly. The reticulocyte response preceded hemoglobin increase and reached a peak 4 to 6 days following administration. Intramuscular injection of pteroylglutamic acid was more than twice as effective as the same amount administered orally. The hemoglobin increase in 7 days was proportional to the amount of pteroylglutamic acid administered either by mouth or by intramuscular injection.

⁵ Goldsmith, Grace A., *PROC. SOC. EXP. BIOL. AND MED.*, 1947, **64**, 115.