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The Folic Acid Requirement of Chicks for Growth, Feathering and Hemoglobin Formation.*

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Pfiffner and associates[1] isolated vitamin B_c (folic acid) in crystalline form from liver, and observed that when this substance was fed to chicks at the level of 250 μg per 100 g of deficient diet, they grew normally and exhibited no anemia at 4 weeks of age. Campbell, Brown, and Emmett[2] found that a vitamin B_c-deficient diet caused poor feath-

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1 Pfiffner, J. J., Binkley, S. B., Bloom, E. S.,

ering, retardation in growth, and a macrocytic, hypochromic anemia. Some improvement in growth was provided by 20 μg of vitamin B_c, but 100 μg were necessary for normal growth. They stated that 40 μg of vitamin B_c completely corrected the hemoglobin level, although their data indicated that even the 100 μg level was not equivalent to the positive controls.

The same authors in a subsequent paper[3] found that by injecting vitamin B_c the same responses were obtained as when the factor was given orally. They concluded that the intestinal flora was not affected by folic acid, directly at least, but that the effect on growth, feathering, and blood cell components was caused by vitamin B_c *per se*. From the data of Pfiffner and associates[4] it appears that 25 μg of vitamin B_c per 100 g of diet were adequate for growth, but more than 25 μg were needed for maximum hemoglobin levels. Campbell, McCabe, Brown, and Emmett,[5] while studying the relationship of vitamin B_c to the cellular elements of chick blood, concluded that 20 to 200 μg of vitamin B_c were needed to maintain normal hematopoiesis in the growing chick for the first 4 weeks of life.

Briggs, Luckey, Elvehjem, and Hart[6] used the fermentation *L. casei* factor of Hutchings, Stokstad, Bohonos, and Slobodkin[7] at levels of 5, 20, and 50 μg per 100 g of diet. Succinylsulfathiazole, added to the 20 μg level of *L. casei* factor, was found to decrease the growth response about 60 g and the hemoglobin level 0.9 g per 100 cc of blood at 4 weeks of age. Their data indicated that 50 μg of *L. casei* factor were not adequate for normal growth and hemoglobin formation. This finding has been substantiated in this laboratory by Charkey and associates[8] who suggest that between 50 and 100 μg of *L. casei* factor per 100 g of diet (probably closer to the lower value) are needed for optimum response from this factor.

Angier and associates[9] using synthetic *L. casei* factor (folic acid) found that 50 μg per 100 g of diet gave as good a response as did 100 μg, indicating that the requirement for this factor is not greater than 50 μg.

Petering, Marvel, Glausier, and Waddell,[10] using a yeast extract and a liver fraction to supply vitamin B_c, observed complete prevention of deficiency symptoms when the amounts of the vitamin present were 22 and 44 μg per 100 g of diet, respectively. They concluded that the level of vitamin B_c required by the chick is probably less than 50 μg per 100 g.

Adequate data have been reported to establish the qualitative role of folic acid in promoting normal growth and hemoglobin formation in the chick and to suggest its relationship to normal feathering. In this paper 3 experiments are described in which the quantitative folic acid requirements of chicks for growth, feathering, and hemoglobin formation were determined.

Experimental. Single Comb White Leghorn cockerels, 15 to a lot, were housed in electrically-heated batteries placed in a room where the temperature was thermostatically controlled. All chicks were on wire mesh floors, and feed and water were supplied *ad libitum*.

The chicks were fed purified diet 653 as

Brown, R. A., Bird, O. D., Emmett, A. D., Hogan, A. G., and O'Dell, B. L., *Science*, 1943, **97**, 404.

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10 Petering, H. G., Marvel, J. P., Glausier, C. E., Jr., and Waddell, J., *J. Biol. Chem.*, 1946, **162**, 477.

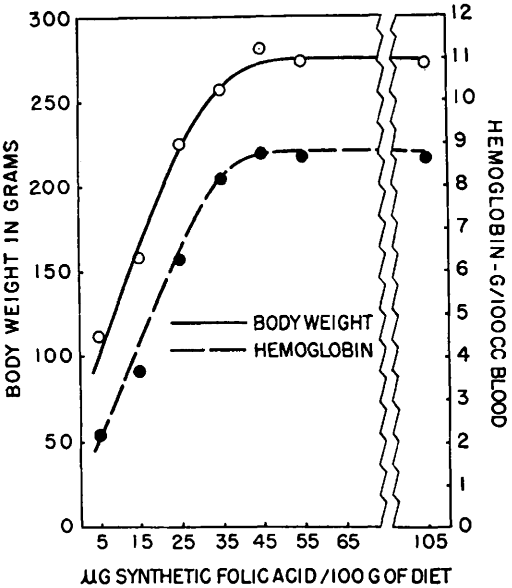

Fig. 1.

Growth and hemoglobin responses of chicks 4 weeks of age to synthetic folic acid. Each point represents the average of 75 chicks, except at the 5 μg and 105 μg points which are the averages of 30 chicks.

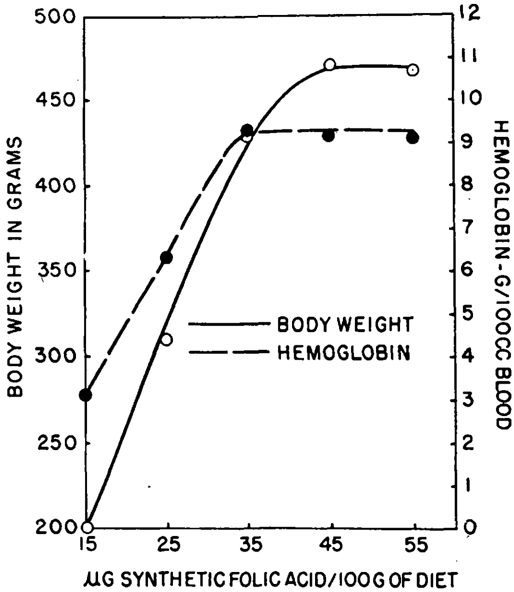

Fig. 2.

Growth and hemoglobin responses of chicks 6 weeks of age to synthetic folic acid. Each point represents the average of 45 chicks.

reported by Hill, Norris, and Heuser[11] plus a factor S concentrate equivalent to 5% yeast.† In Experiment 1, in which 50 μg of 2-methyl-3-hydroxy-4-carboxy-5-hydroxymethlypyridine (B-pyracin) were added per 100 g of diet, the basal diet was found to contain 5 μg of folic acid per 100 g by microbiological assay following chick liver incubation. In Experiment 2 the same amount of B-pyracin was added and the inositol level was reduced to 50 mg per 100 g of diet. No B-pyracin was included in the basal diet of Experiment 3. The folic acid content of the basal diets used in Experiments 2 and 3 was 15 μg per 100 g. The difference between the folic acid contents of the diets used is due to the factor S preparation added in Experiment 1 having only one-third the amount of folic acid as the preparations added in Experiments 2 and 3. The preformed folic acid in all basal diets was approximately 2.0 μg per 100 g.

Experiment 1 was divided into 2 parts: (a) folic acid fed at levels of 10, 20, 30, 40, 50, and 100 μg per 100 g of diet; and (b) folic acid at these same levels plus 1% succinylsulfathiazole. In Experiment 2 the supplements were: (a) 10, 20, 30, and 40 μg of folic acid per 100 g of diet; and (b) folic acid at these same levels plus 2% succinylsulfathiazole. The design of Experiment 3 was the same as 2 (a). Levels greater than 40 μg per 100 g were not added to the basal diets fed in Experiments 2 and 3, because this amount of folic acid plus the 15 μg already present in the diet, exceeded the minimum amount found necessary in Experiment 1.

Body weights were recorded weekly, and the hemoglobin levels were determined at the 2d, 3d, and 4th weeks in Experiment 1, and in Experiments 2 and 3 at the 3d, 4th, 5th, and 6th weeks. Hemoglobin was determined by the oxyhemoglobin method using a Coleman spectrophotometer to read the color produced, according to a method de-

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

† The authors are indebted to Anheuser-Busch, Inc., St. Louis, Mo., for Strain S dried brewers' yeast; to Lederle Laboratories, Inc., Pearl River, N.Y., for synthetic *L. casei* factor (folic acid); and to Merck and Co., Rahway, N.J., for crystalline B-pyracin.

TABLE I.
Results of Feeding Synthetic Folic Acid to Chicks for 6 Weeks.

Folic acid added per 100 g diet µg	Avg wt 4 wk g	Avg wt 6 wk g	Hemoglobin 4 wk g per 100 cc	Hemoglobin 6 wk g per 100 cc	Feather score 6 wk %
		Experiment 2 (a)			
None*	153 (11)†	202 (5)	3.9‡	2.4	25‡
10	225 (15)	345 (12)	5.1	6.9	37
20	249 (15)	412 (15)	8.0	9.5	77
30	288 (15)	476 (15)	8.3	9.2	83
40	274 (14)	461 (14)	8.7	9.3	98
		Experiment 2 (b) + 2% succinylsulfathiazole			
None	153 (8)	173 (2)	3.2	1.6	0
10	206 (12)	310 (11)	4.8	6.6	36
20	248 (14)	431 (14)	7.3	9.1	81
30	282 (14)	469 (14)	8.2	9.5	89
40	281 (14)	470 (14)	8.5	9.1	96

* Basal diet 653 + factor S concentrate equivalent to 5% yeast contained 15 µg of folic acid per 100 g.

† Numbers in parentheses indicate the number of surviving chicks.

‡ Hemoglobin values and feather scores were determined on all chicks.

veloped in this laboratory.

In Experiments 2 and 3 feathering was scored on the chicks at 6 weeks of age as follows: 4—excellent; 3—good; 2—fair; 1—poor; 0—very poor. Growth, smoothness and condition of the feathers were taken into consideration in determining feather score which was calculated for each lot by the formula:

$$\frac{\text{Sum of scores of individual chicks}}{4 \times \text{No. of chicks}} \times 100 = \text{degree of feathering } (\%)$$

Results. The data obtained at 4 weeks of age from the 3 experiments are presented in combined form in Fig. 1. In Experiments 1 and 2 the data obtained from the sulfonamide and non-sulfonamide diets were not significantly different. It was, therefore, considered justifiable to combine the results from all groups in determining the requirements.

From Fig. 1 it is evident that the amount of folic acid required for optimum growth and hemoglobin formation to 4 weeks of age under the experimental conditions is approximately 45 µg per 100 g of diet. A straight-line response was obtained in growth and hemoglobin levels from 5 µg to almost 45 µg of folic acid per 100 g of diet.

Data from Experiments 2 and 3 which show growth and hemoglobin response at 6 weeks of age are presented in Fig. 2. The requirement of folic acid for maximum growth was found to be 45 µg per 100 g of diet, the same as at 4 weeks of age. However, the requirement to maintain normal hemoglobin values was 35 µg per 100 g of diet, showing that as the chicks became older less folic acid was needed.

The results of Experiment 2 are presented in Table I. These data are included because this experiment is typical of those conducted to determine the folic acid requirement of the chick. In addition, the data demonstrate that succinylsulfathiazole had no effect on the response to folic acid under the conditions of the experiment, and that folic acid was directly concerned with feathering. Hemoglobin values and body weights at 4 and 6 weeks of age, and feather scores at 6 weeks of age on sulfonamide and non-sulfonamide diets are given.

Perosis occurred in each of the experiments. A report was made by Daniel, Farmer, and Norris[12] on the effect of folic acid on perosis. The perosis was more severe on the sulfonamide diet than on the non-sulfonamide diet. This was especially noticeable at the lower levels of folic acid. Because of

[12] Daniel, L. J., Farmer, F. A., and Norris, L. C., *J. Biol. Chem.*, 1946, **163**, 349.

the severity of perosis many of the chicks were unable to secure food and water and died before the end of the 6-week experimental period. As a result the 6-week weights and hemoglobin values of the first 2 lots on the sulfonamide diet are somewhat lower than the corresponding lots on the non-sulfonamide diet.

The feather scores indicated that succinylsulfathiazole included at 2% of the basal diet had no detrimental effect on feathering. The feather score in the basal lot of Experiment 2(b) was not considered typical since it represented the scores of only 2 chicks. There was no difference in the feather scores obtained on the 2 diets indicating that folic acid *per se* is concerned with feathering and that intestinal synthesis of a feather factor is not probable. The requirement of folic acid for good feather development appears to be not less than 55 μg per 100 g of diet.

Survival of chicks to 6 weeks of age in Experiment 2 required approximately 25 μg of folic acid per 100 g of diet. Data from Experiments 1 and 3 confirm this estimate.

Summary. The folic acid requirement of chicks for growth, feathering, and hemoglobin formation has been determined using a purified diet containing 2.0 μg of preformed folic acid per 100 g of diet. The total folic acid content of the basal diets as determined microbiologically following incubation with chick liver was 5 μg per 100 g of diet for Experiment 1 and 15 μg for Experiments 2 and 3.

The following amounts of folic acid, which include the amount in the basal diet, were required for its several functions: for survival to 6 weeks of age, approximately 25 μg per 100 g; for growth and hemoglobin formation at 4 weeks of age, 45 μg; for growth at 6 weeks, 45 μg, but for hemoglobin formation at 6 weeks, 35 μg; and for feathering at 6 weeks of age, not less than 55 μg per 100 g of diet.

In these experiments the addition of succinylsulfathiazole at 1 or 2% of the diet did not have any effect on growth, feathering, or hemoglobin formation in chicks to 6 weeks of age.

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Milk as a Source of the Monkey Anti-Anemia Factor.*

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Previous studies[1,2] have shown that whole liver powder, raw and lyophilized liver are good sources of the monkey anti-anemia factor. In this paper we wish to report experiments carried out to determine if milk contains this factor.

Experimental. The method of handling the monkeys has been described previously.[3] The assay monkeys consisted of animals which failed to show complete recovery from a riboflavin deficiency after riboflavin therapy,[2] and animals made deficient in the monkey anti-anemia factor by the prolonged feed-

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