17133. Interrelationship of Vitamin B_{12} and Choline. II. Effect on Growth of the Chick.*

A. E. SCHAEFER, W. D. SALMON, AND D. R. STRENGTH.

From the Laboratory of Animal Nutrition, Agricultural Experiment Station of the Alabama Polytechnic Institute, Auburn.

In the accompanying paper it was reported that the severity and incidence of renal hemorrhage in weanling rats fed diets low in choline and methionine were significantly decreased by supplementing the diet with vitamin B_{12}.[1] In concurrent experiments the interrelationship of the vitamin B_{12} and choline requirements for growth of the chick was studied.

Experimental. Day-old Leghorn chicks of the A.P.I. strain were divided into 3 weight classes ranging from 34 to 42 g. The selected chicks were then distributed, 8 to 12 per group, so that the group average was about 38 g. The chicks were wing-banded and housed in standard commercial, electrically heated batteries, which were equipped with raised wire floors. Feed and water were supplied *ad libitum* and the chicks were weighed weekly. The basal diet was composed of extracted peanut meal 30,[2] sucrose 36.9, extracted casein 6,[3] L-cystine .1, cod liver oil 1, lard 19, $Ca_3(PO_4)_2$ 2.87, K_2HPO_4 1.442, $CaCO_3$ .903, $MgSO_4 \cdot 7H_2O$ .840, NaCl .70, Fe Citrate .182, $MnSO_4 \cdot 4H_2O$ .035, $ZnCO_3$ .014, $CuSO_4 \cdot 5H_2O$ .007, and KI .007. Vitamin supplements, mg/kg diet, were as follows: thiamine 3, pyridoxine 4, riboflavin 6, calcium pantothenate 20, niacin 50, inositol 1000, folic acid 1, biotin .25, alpha tocopherol 25, alpha tocopherol acetate 25, and menadione 5. The diets were mixed at 10-day intervals and stored at 1°-5°C. The basal diet furnished a maximum of .007% choline and .3% methionine. Preliminary studies had established that supplementation of this basal diet with .6% choline chloride was necessary for maximum growth.

A summary of four experiments is given in Table I. Concentrates of vitamin B_{12} were added to the basal diet at the expense of sucrose and the choline supplement was reduced in the amount that was furnished by the concentrates. Choline was determined by the procedure of Engel,[4] modified by washing the choline reineckate precipitate with cold N-propyl alcohol in place of methyl alcohol. Vitamin B_{12} concentrates Nos. 1 and 3 were a charcoal and fullers earth adsorbate, respectively. The crystalline vitamin B_{12} was in the form of a solution containing 25 μg/ml.

When the basal diet was supplemented with .6% choline chloride, the addition of vitamin B_{12} concentrates Nos. 1 or 3 at a level to supply 30 μg vitamin B_{12} per kg diet caused an average increase in weight gain of 24 to 28 g in 2 weeks, or 59 to 66 g in 4 weeks. The feeding of the concentrates to supply 15 μg vitamin B_{12} per kg diet resulted in a weight increment of 52 to 62 g in 4 weeks.

When the choline supplement was reduced to .2%, the addition of vitamin B_{12} concentrate caused an increase in weight gain at 2 weeks of 32 g and 100 g in 4 weeks. At the .1% level of choline chloride supplement the greatest increase in weight gain due to the vitamin B_{12} concentrate was observed. The weight increment was 63 g at 2 weeks and 186 g at 4 weks. The total weight of these birds was as good as that obtained when the

* Published with the approval of the Director, Alabama Agricultural Experiment Station. This study was supported in part by a grant from the American Cancer Society upon recommendation of the Committee on Growth, National Research Council. It was aided by donations of vitamin B_{12} concentrates, crystalline vitamin B_{12}, and other vitamins by Merck & Company, and of folic acid by Lederle Laboratories. These data were presented in part at the Detroit meeting, Fed. Am. Soc. Exp. Biol., April 20, 1949.

1 Schaefer, A. E., Salmon, W. D., and Strength, D. R., PROC. SOC. EXP. BIOL. AND MED., 1949, **71**, 193.

2 Engel, R. W., *J. Nutrition*, 1948, **36**, 739.

3 Salmon, W. D., *J. Nutrition*, 1947, **33**, 155.

4 Engel, R. W., *J. Biol. Chem.*, 1942, **144**, 701.

TABLE I.

Exp. No.	Dietary supplement: Choline chloride %	Dietary supplement: Source of vitamin B_{12} ≅ μg B_{12}/kg diet	No. of chicks	Avg wt gain g/chick: 2 wk	Avg wt gain g/chick: 4 wk
4	.05	0	11	20	46
3, 4	.1	0	23	27	60
2	.2	0	12	31	95
1, 2, 3, 4	.6	0	47	54	154
4	.05	B_{12} conc. No. 1 ≅ 15	11	41	150
3	.1	" " " 1 ≅ 30	12	90	246
4	.1	" " " 1 ≅ 15	11	72	262
1, 2, 3	.2	" " " 1 ≅ 30	34	63	195
1, 2, 3, 4	.6	" " " 1 and 3 ≅ 30	59	79	218
1, 3	.6	" " " 1 and 3 ≅ 15	22	78	210

diet was supplemented with .6% choline chloride plus vitamin B_{12} concentrate (Fig. 1).

When the choline supplement was .05%, the increase in weight resulting from the addition of vitamin B_{12} concentrate was less than that observed in the groups receiving .1% and .2% choline chloride. This indicates that this level of vitamin B_{12} cannot replace the entire dietary choline required by the chick but that a minimum level of choline must be present.

That vitamin B_{12} was the active principle in the concentrates is indicated by the data in Table II. Pure vitamin B_{12} fed at a level of 15 μg per kg of diet caused an average increase in weight gain of 14 g at 1 week and 40 g in 2 weeks as compared to 14 g at 1 week and 45 g in 2 weeks for the group receiving the vitamin B_{12} concentrate.

Discussion. The choline requirement for normal growth of chicks fed the basal diet without a source of vitamin B_{12} was .6% choline chloride as compared to .1% when vitamin B_{12} was added to the diet. This interrelationship of vitamin B_{12} and choline for the chick is in agreement with the results obtained with rats.[1]

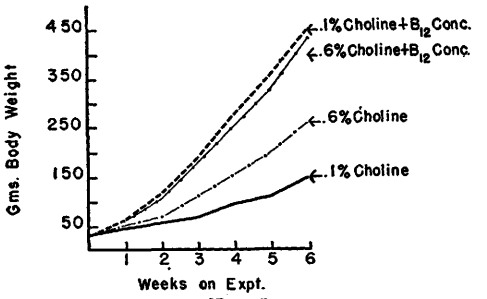

FIG. 1.

Sparing action of vitamin B_{12} concentrate on the choline requirement of chicks. (12 chicks per group).

The chicks used in these experiments were progeny of hens from a strain that had been bred for high livability. The breeding flock is on commercial breeding ration, which supports good egg production. The progeny of these hens have shown a very uniform growth response to the addition of vitamin B_{12}. Not only was this growth increment uniform but it was also much greater than that reported by other investigators using other types of diets. For example, when the basal diet was supplemented with .1% choline chloride plus a source of vitamin B_{12} ≅ 30 μg per kg of diet, the growth increment was 63 g at 2 weeks and 186 g at 4 weeks. This increment is considerably greater than that reported by the following workers: Lillie, *et al.*,[5] 34 to 60.3 g increase in body weight from the second to the fourth week; Nichol, *et al.*,[6] 79 to 88 g for a similar period, and Ott, *et al.*[7] of 20 to 46 g at 17 days.

These observations of the influence of choline level on the vitamin B_{12} requirement may be useful when procedures for a chick assay of vitamin B_{12} are being standardized. The data presented in Fig. 1 indicate that vitamin B_{12} can be assayed more effectively if

[5] Lillie, R. J., Denton, C. A., and Bird, H. R., *J. Biol. Chem.*, 1948, **176**, 1477.

[6] Nichol, C. A., Dietrich, L. S., Cravens, W. W., and Elvehjem, C. A., PROC. SOC. EXP. BIOL. AND MED., 1949, **70**, 40.

[7] Ott, W. H., Rickes, E. L., and Wood, T. R., *J. Biol. Chem.*, 1948, **174**, 1047.

TABLE II.
Crystalline Vitamin B_{12} Compared with Vitamin B_{12} Concentrate.

Dietary supplement		No. of chicks	Avg wt gain g/chick	
Choline chloride %	Source of vitamin B_{12} $\cong$ μg per kg diet		7 days	14 days
.1	None	11	16	27
.1	Crystalline vit. $B_{12} \cong 15$	7	30	67
.1	B_{12} conc. No. 1 $\cong$ 15	11	30	72

the basal diet is supplemented with .1% choline chloride than when it is supplemented with .6%. It would be of interest to determine whether the feeding of a low-choline diet to hens would increase the requirement for the "animal protein factor" in the progeny, similar to that noted when the hens were restricted to a diet low in the "animal protein factor".[5,6]

The interesting interrelationship of vitamin B_{12}, choline, and methionine is being studied further in this laboratory.

Summary. 1. Vit. B_{12} was fed to Leghorn chicks receiving a choline-low basal diet supplemented with .6%, .2%, .1%, and .05% choline chloride. The 14-day increment in weight gain over the controls that did not receive vitamin B_{12} was 26 g, 32 g, 63 g, and 21 g, respectively.

2. Crystalline vit. B_{12} fed at a level of 15 μg per kg of diet and at a choline level of .1% was as effective as vit. B_{12} concentrate in a 2-week feeding period.

3. The choline requirement of chicks under the conditions of these experiments was markedly reduced by the supplementation of the diet with vit. B_{12}.

4. It appears that dietary choline has a significant sparing action on vit. B_{12}.

Received April 19, 1949. P.S.E.B.M., 1949, **71**.

17134. An Oxygenator with Increased Capacity: Multiple Vertical Revolving Cylinders.*

KARL E. KARLSON, CLARENCE DENNIS, DAVID SANDERSON, AND CHARLES U. CULMER.
From the Department of Surgery, University of Minnesota, Minneapolis, Minn.

The vertical revolving cylinder has been shown to be an effective oxygenator for blood.[1,2] Its efficiency as an oxygenator is probably increased by making the cylinder shorter in relation to its diameter.[3] These data were applied in the construction of another oxygenator with approximately eight times the capacity of the oxygenator previously studied.

Experimental. Eight concentric cylinders (Fig. 1, C) 21 cm in height, ranging from 27.5 cm to 17.0 cm in diameter are mounted so that the lower edges of the cylinders are approximately one centimeter above the inner surface of a cone (f). The surface area is 14,500 cm^2. Blood is delivered through individual spouts (b) onto the inner surface of each cylinder, drops off of its lower edge onto the inner surface of the cone, flows into the vertical shaft (t), runs down the inner surface of the shaft, and collected in the stationary cup (h) beneath. A 94% O_2 and

* Aided by a grant from the United States Public Health Service, and by a research grant from the Graduate School, University of Minnesota.

1 Gibbon, J. H., Jr., *J. Lab. and Clin. Med.*, 1939, **24**, 1192.

2 Gibbon, J. H., Jr., and Kraul, C. W., *J. Lab. and Clin. Med.*, 1941, **26**, 1803.

3 Karlson, K. E., Dennis, C., and Westover, D. E., PROC. SOC. EXP. BIOL. AND MED., 1949, **70**, 225.