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Effect of Dietary Vitamin B_{12} on Methionine Biosynthesis by Chick Liver Homogenates.* (26816)

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It was observed several years ago that dietary vitamin B_{12} would spare the methyl requirements for animals(1). More recently, considerable evidence has accumulated to implicate Vit. B_{12} in methionine biosynthesis in bacterial systems(2,3). Arnstein(4) has demonstrated in *in vivo* experiments with rats that Vit. B_{12} stimulates the conversion of formate to the methyl groups of choline and methionine. We have reported(5,6) that Vit. B_{12} stimulates the conversion of formate to the 5-methyl group of thymine by chick bone marrow cells but does not stimulate conversion of formaldehyde to the 5-methyl group of thymine. These results suggest that Vit. B_{12} functions in reduction of one-carbon compounds between the formate and formaldehyde levels of oxidation. In the present experiments we have studied the effect of Vit. B_{12} deficiency in chicks on formation of the methyl group of methionine by liver homogenates and the results suggest that B_{12} stimulates the conversion of formate to methionine methyl but does not increase conversion of formaldehyde to methionine methyl, a situation analogous to our previously reported results in thymine formation by chick bone marrow cells.

Experimental. Day-old White Leghorn chicks were given the Vit. B_{12} and methionine-deficient basal diet described by Spivey-Fox *et al.*(7). When present, methionine was added at a concentration of 6% of the diet and Vit. B_{12} was given by weekly injections at a dosage of 3 μg per chick the first week and 9 μg per chick thereafter. After 3 to 4 weeks on the various diets, the chicks were taken for experiment. Peripheral blood counts were made using standard hematological procedures.

Methionine formation from homocysteine and formate-C^{14} or formaldehyde-C^{14} was determined in liver homogenates. The livers were homogenized in an all-glass homogenizer with 2 volumes of Robinson's buffer(8). The incubation mixture consisted of 2 ml of homogenate, 3 mg of DL-homocysteine, and either 0.27 μmole of formate-C^{14} (142,000 c.p.m.) or 2 μmoles of formaldehyde-C^{14} (140,000 c.p.m.). The final volume was 2.5 ml. After an hour of incubation at 37° under air in the Dubnoff shaker, the reaction was stopped by addition of 0.5 ml of 50% trichloroacetic acid and the contents of the incubation beakers were transferred to 12 ml conical centrifuge tubes and centrifuged. 1.5

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TABLE I. Effects of Dietary Supplements of Methionine and Vitamin B_{12} on Growth and Hemogram of Chicks.

Supplement	Weight gain, g/4 wk	Erythrocytes, millions/μl	Hemoglobin, g/100 ml	Hematocrit, %
None	154	2.83	9.6	29
Methionine	241	2.70	9.1	29
B_{12}	237	2.83	9.5	29
Methionine + B_{12}	250	3.04	9.7	31

ml of the supernatant solution was taken and 200 mg of carrier L-methionine added and dissolved with heating. The methionine was precipitated on cooling with addition of 95% ethanol and recrystallized in this fashion a total of 6 times. The methionine was then plated on aluminum planchets and counted at infinite thickness with an end-window Geiger counter. The results are reported as counts per minute of the infinite thickness samples. To correct for any non-enzymatic reaction between formaldehyde, formate and homocysteine zero time samples were taken and the counts obtained subtracted from the counts obtained in the incubated samples. Five chicks from each group were used in these experiments.

Results and discussion. Supplements of either B_{12} or methionine stimulated growth of chicks as indicated by the data in Table I. In the presence of adequate methionine B_{12} produced no significant effect on the growth rate. These data again point out the well-known effect of Vit. B_{12} in sparing the methyl requirements of animals. The hemogram of the chicks was not significantly influenced by B_{12} deprivation indicating the mildness of the B_{12} deficiency.

TABLE II. Influence of Dietary Vitamin B_{12} and Methionine on Formation of Methionine Methyl by Chick Liver Homogenates. Results reflect total C^{14} incorporated into methionine. See text for details of calculation.

Supplement	C^{14} substrate employed: Formate	Formaldehyde
None	239*	43†
B_{12}	511	12
Methionine	237	44
Methionine + B_{12}	353	25

* B_{12} effect statistically significant. $P < .01$. Methionine effect not statistically significant.
† B_{12} effect statistically significant. $P < .05$. Methionine effect not statistically significant.
Statistically treated by analysis of variance.

Vit. B_{12} stimulated the conversion of formate to methionine methyl but appeared to inhibit conversion of formaldehyde to methionine methyl as indicated by the data in Table II. This observation is in agreement with studies of the influence of Vit. B_{12} on biosynthesis of the methyl group of thymine (5,6). Methionine supplementation did not exert a statistically significant effect on methionine biosynthesis by liver homogenates.

These results again demonstrate the involvement of Vit. B_{12} in methyl synthesis by animals and substantiate our earlier suggestions concerning the site of action of B_{12}. The results indicate that Vit. B_{12} functions between the formate and formaldehyde levels of oxidation, a reaction catalyzed by the enzyme methylene tetrahydrofolic dehydrogenase. We have previously reported a reduction in activity of this enzyme in tissues from B_{12} deficient animals(9).

Summary. Liver homogenates from B_{12}-deficient chicks converted formate to methionine methyl at a reduced rate and converted formaldehyde to methionine methyl at an accelerated rate. These results suggest the involvement of Vit. B_{12} in reduction of formate to formaldehyde, a reaction mediated by methylene tetrahydrofolic dehydrogenase.

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