

# EFFECTS OF A VITAMIN B<sub>12</sub> DEFICIENCY ON LIVER ENZYMES IN THE RAT\*

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Certain general functions of vitamin B<sub>12</sub> and folic acid in intermediary metabolism are now well recognized. For example, folic acid appears to be involved in purine and formate metabolism and vitamin B<sub>12</sub> in the utilization of methyl groups. However, while there have been several reports concerning the influence *in vivo* and *in vitro* of folic acid and its antagonist, aminopterin, on specific enzyme systems such as xanthine oxidase (1-4), choline oxidase (5-8), and transmethylase (9, 10), there is scant information on the effects of dietary vitamin B<sub>12</sub> on these or other enzyme systems.

In the course of work on the biological action in the rat of a reported specific antagonist to vitamin B<sub>12</sub> for *Lactobacillus leichmannii* 4797 (11), the authors have undertaken a study of the effects of this antagonist as well as of simple vitamin B<sub>12</sub> deficiency on certain enzyme systems in the rat. The liver enzymes chosen for study, transmethylase, xanthine oxidase, endogenous respiration, and choline oxidase, have been implicated indirectly by various workers with vitamin B<sub>12</sub> metabolism. These investigations should throw some light on the involvement of vitamin B<sub>12</sub> with specific enzyme systems. In addition, the effects of the *L. leichmannii* antagonist can be compared with a simple vitamin B<sub>12</sub> deficiency in the rat.

## EXPERIMENTAL

Weanling male rats of the Sprague-Dawley strain weighing 40 to 45 gm. were employed as experimental animals. The rats were separated at random into four groups, all of which were fed the basal vitamin B<sub>12</sub>-deficient corn-soy meal ration of Lewis *et al.* (12) with the omission of iodinated casein or desiccated thyroid. Group I served as the negative control and received only the basal ration without supplement. The animals of Group II were injected with an "antivitamin B<sub>12</sub>" preparation equivalent

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to 20  $\gamma$  of vitamin B<sub>12</sub> per rat per day. The rats of Group III received an intraperitoneal injection of 2  $\gamma$  of crystalline vitamin B<sub>12</sub> in solution per rat per day. The "antivitamin" was prepared fresh each day by treatment of the vitamin in strong acid solution with hydrogen peroxide according to the procedure of Beiler *et al.* (11). Group IV was also given the vitamin B<sub>12</sub> oxidation product, followed a few minutes later by vitamin B<sub>12</sub> as in Group III. Individual cages with raised bottoms were used for housing the animals, and ration and water were given *ad libitum*. The animals of all four groups were maintained on their respective rations for 5 weeks before being used in the enzyme studies. Weights were recorded at intervals during that period.

When used in the enzyme experiments, the animals were decapitated and bled. The livers were excised immediately, chilled in ice, and blotted free of moisture. A weighed portion of each liver was homogenized with 5 volumes of ice-cold 0.039 M sodium potassium phosphate buffer of pH 7.4 and strained through gauze. The xanthine oxidase activity of the homogenate was determined manometrically according to the procedure of Axelrod and Elvehjem (13). The choline oxidase activity of the homogenates was also followed manometrically by the method of Williams, Litwack, and Elvehjem (14). The first 10 minute oxygen uptake before the substrate was added in the xanthine oxidase determination was taken to represent the endogenous respiration of the respective livers. Transmethylease activity was followed by a modification of the method outlined by Dubnoff and Borsook (15). The modified procedure finally adopted was as follows: 2 ml. of the 16.7 per cent homogenate were incubated at 38° in evacuated Thunberg tubes with 0.5 ml. each of 0.5 per cent betaine hydrochloride (neutralized) and 1 per cent DL-homocysteine prepared in 0.039 M sodium potassium phosphate buffer. Control tubes were run simultaneously without the betaine hydrochloride. At the end of 3 hours, the samples were treated with 0.5 ml. of 30 per cent trichloroacetic acid and 2 ml. of water, mixed, and filtered. To 2 ml. of the filtrate was added 0.2 ml. of 5 N sodium hydroxide plus 0.3 ml. of 1 per cent freshly prepared sodium nitroprusside solution. Methionine standards were run concurrently by mixing 0 to 1 ml. of 110 mg. per cent of methionine, 0.5 ml. each of 1 per cent homocysteine, 30 per cent trichloroacetic acid, and water to give a final volume of 5.5 ml. Aliquots (2 ml.) of these standards corresponding to 0 to 400  $\gamma$  of methionine with several intermediate values were taken for reaction with the alkali and nitroprusside solutions. Samples and standards were allowed to stand at room temperature for 15 minutes, or until no pink color was seen on shaking the tubes. The tubes were then treated with 4 ml. of water and 0.3 ml. of a mixture of 9 volumes of concentrated hydrochloric acid and 1 volume of 85 per cent phosphoric acid.

After a 10 minute incubation period at room temperature, color intensities were read in an Evelyn colorimeter with a green filter.

## RESULTS AND DISCUSSION

In order to show the effects of a vitamin B<sub>12</sub> deficiency in the presence and absence of the vitamin B<sub>12</sub> oxidation product, the weights of the animals of the various groups after a 5 week feeding period are presented in Table I. From these results it can be seen that a simple vitamin B<sub>12</sub> deficiency (Group I) produces significantly less growth than occurred in the normal controls receiving vitamin B<sub>12</sub> (Group III). Moreover, the vitamin B<sub>12</sub> "antagonist" has no effect on growth of the rats either in the pres-

TABLE I  
*Effect of Vitamin B<sub>12</sub> and Oxidized Vitamin B<sub>12</sub> (L. leichmannii Antagonist) on Growth of Rats*

Group No.	Supplement	No. of rats	Weight after 5 wks.
			<i>gms.</i>
I	None	6	176 (153-198)*
II	Oxidized vitamin B <sub>12</sub> †	12	177 (152-211)
III	Vitamin B <sub>12</sub> ‡	12	238 (205-254)
IV	Oxidized vitamin B <sub>12</sub> + vitamin B <sub>12</sub>	6	241 (198-268)

\* Range of individual values.

† The oxidized vitamin was injected at a level equivalent to 20  $\gamma$  of vitamin B<sub>12</sub> per rat per day.

‡ The vitamin B<sub>12</sub> was injected at a level of 2  $\gamma$  per rat per day.

ence or absence of vitamin B<sub>12</sub>. In unpublished work from this laboratory the vitamin B<sub>12</sub> oxidation product has occasionally shown an antivitamin B<sub>12</sub> action on the growth of rats. As shown in the present experiments, however, this effect cannot always be repeated and may be due to other effects besides an antivitamin B<sub>12</sub> action.

The results of the enzyme experiments are reported in Table II. The values represent in each case the average results obtained from five to six different animals. From Table II it can be observed that the oxidation product of vitamin B<sub>12</sub>, which appears to be an antivitamin for *L. leichmannii* (11), has little or no effect on the enzyme activities determined in these experiments. On the other hand, a simple vitamin B<sub>12</sub> deficiency produces marked changes in most of the enzymes studied. These enzymes are not further affected to any great extent by the "antivitamin." Thus, in a vitamin B<sub>12</sub> deficiency there is a decrease in the endogenous oxidative activity of the liver. These results may be related to the observations of

earlier workers on the effect of vitamin B<sub>12</sub> on hyper- and hypothyroid conditions in experimental animals (16-19). Vitamin B<sub>12</sub> also markedly stimulates liver xanthine oxidase activity and is, therefore, in this respect dissimilar to folic acid. An opposing effect of folic acid and vitamin B<sub>12</sub> has also been recorded on D-amino acid oxidase activity of chick liver (1). In unpublished work from this laboratory, Feigelson, Williams, and Elvehjem have observed that dietary vitamin B<sub>12</sub> is essential for maintaining normal xanthine oxidase activity in rat liver.

TABLE II

*Effect of Vitamin B<sub>12</sub> and Oxidized Vitamin B<sub>12</sub> (L. leichmannii Antagonist) on Certain Enzyme Systems in Rat Liver*

Group No.	Supplement	No. of rats	Liver enzyme determined			
			Endogenous respiration, $\mu$ l. O <sub>2</sub> per hr. per gm. liver	Xanthine oxidase, $\mu$ l. O <sub>2</sub> per hr. per gm. liver	Choline oxidase, $\mu$ l. O <sub>2</sub> per hr. per mg. liver	Trans-methylase, $\gamma$ methionine formed per hr. per gm. liver
I	None	6	710 $\pm$ 95*	114 $\pm$ 13	3.31 $\pm$ 0.14	157 $\pm$ 29
II	Oxidized vitamin B <sub>12</sub> †	5	800 $\pm$ 95	163 $\pm$ 10	3.13 $\pm$ 0.28	166 $\pm$ 30
III	Vitamin B <sub>12</sub> ‡	6	1080 $\pm$ 170	280 $\pm$ 16	2.56 $\pm$ 0.23	360 $\pm$ 36
IV	Oxidized vitamin B <sub>12</sub> † + vitamin B <sub>12</sub> ‡	6	1100 $\pm$ 132	281 $\pm$ 20	2.70 $\pm$ 0.16	320 $\pm$ 50

\* Standard error of the mean.

† The oxidized vitamin was injected at a level equivalent to 20  $\gamma$  of vitamin B<sub>12</sub> per rat per day.

‡ The vitamin B<sub>12</sub> was injected at a level of 2  $\gamma$  per rat per day.

While a relationship of vitamin B<sub>12</sub> to choline oxidase and transmethylase activities might be predictable on the basis of the probable mediation of this vitamin in methyl group metabolism, the observed decrease in choline oxidase activity as a result of vitamin B<sub>12</sub> supplementation could not apparently account for the increased transmethylase activity in the vitamin B<sub>12</sub>-fed group, since transformation *in vivo* of choline to methionine reportedly parallels choline oxidase activity (20). To some extent the depression in choline oxidase activity may possibly be attributed to the increased endogenous respiration brought about by vitamin B<sub>12</sub>, resulting in competition between choline and endogenous substrate oxidation for common hydrogen transport systems.

Therefore, the observations in these experiments indicate that vitamin B<sub>12</sub> does not influence the oxidation of choline to betaine but exerts its effect on the actual transmethylation from betaine to homocysteine. That

vitamin B<sub>12</sub> is necessary for the utilization of the methyl groups of betaine has been shown *in vivo* for the rat by Day *et al.* (21). Jukes *et al.* have also presented evidence which suggests that chicks deficient in vitamin B<sub>12</sub> are unable to utilize homocysteine and betaine for the synthesis of methionine (22). Further work is needed to show more specifically whether the influence of vitamin B<sub>12</sub> on transmethylation *in vivo* is on betaine utilization rather than on choline oxidation, which is influenced more directly by folic acid and the *Leuconostoc citrovorum* factor (5, 6). It should also be ascertained whether vitamin B<sub>12</sub> might exert its influence on choline metabolism through increased potentiation of folic acid activity (23-25).

## SUMMARY

1. A vitamin B<sub>12</sub> oxidation product, reported to be antagonistic to the vitamin for the growth of vitamin B<sub>12</sub>-requiring microorganisms, has been shown to exhibit no antivitamin B<sub>12</sub> properties in the rat as measured either by growth or its effects on liver enzyme systems.

2. A simple vitamin B<sub>12</sub> deficiency has been shown to decrease liver endogenous respiration, betaine-homocysteine transmethylase, and xanthine oxidase activity markedly. Liver choline oxidase activity is somewhat increased by the vitamin deficiency, which might partially be explained on the basis of the increased endogenous respiration. These observations are discussed with respect to other known facts concerning vitamin B<sub>12</sub> metabolism.

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