No toxic substance was demonstrated in the milk of nursing females on the pea diet. The feeding procedure employing pregnant female rats proved to be a reasonably accurate and sensitive method for the toxicity assay of aminonitriles. Aminoacetonitrile was the most toxic of the compounds tested.

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Received August 29, 1955. P.S.E.B.M., 1955, v90.

Effect of Diet on Betaine-Homocysteine Transmethylase of Rat Liver.* III. B-Vitamins Other Than Vitamin B_{12}. (22014)

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In the rat, a dietary deficiency of vit. B_{12} causes a decrease in the betaine-homocysteine transmethylase activity of the liver(1-4). The addition of a purified "coenzyme" preparation of the transmethylase, to liver homogenates prepared from rats fed a diet deficient in vit. B_{12}, has recently been shown to restore the transmethylase activity(4). To ascertain whether this effect is specific for vit. B_{12} the effect of a deficiency of each of the other B-vitamins on the transmethylase activity of rat liver has been investigated.

Methods. Albino rats of the Sprague-Dawley strain were used throughout the investigation. The rats were kept in individual cages and were fed *ad libitum*, unless otherwise stated. The complete vitamin mixture used for the various basal diets described below provided in mg per 100 g of ration: thiamine hydrochloride, 0.5; riboflavin, 0.5; niacin, 1; calcium pantothenate, 2; pyridoxine, 0.25; folic acid, 0.02; biotin, 0.01; vit. B_{12}, 0.002; inositol, 10; and choline chloride, 150. Each week each rat was given orally 2 drops of halibut liver oil fortified with vit. E and K (5). For the deficient diets the vitamin under study was omitted and in some cases succinyl sulfathiazole ("sulfasuxidine") or the corresponding antivitamin was added to the diet as described in the tables of results. All diets contained 4% of salts 4(6) and 5% of corn oil. The basal diets contained the following percentages of protein, sucrose and supplementary amino acids: Diet I. Casein 25, sucrose 65.8. Diet II. Casein 25, DL-methionine 0.2, sucrose 65.6. Diet III. Casein 12, DL-methionine 0.2, DL-threonine 0.36, sucrose 78.2. Diet IV. Egg white 24, sucrose 66.8, the biotin content of the complete diet was increased to 0.1 mg per 100 g of ration. Diet V. Casein 9, gelatin 12, sucrose 69.8.

At the end of the experimental period the rats were killed by decapitation and were bled, and their livers were removed immediately and chilled in ice. Pooled homogenates

* Published with approval of the Director of the Wisconsin Agric. Exp. Station. Supported in part by grants from the Nutrition Foundation, New York, the National Livestock and Meat Board, Chicago, Ill. The crystalline vitamins were kindly provided by Merck and Co., Rahway, N. J., and the Sulfasuxidine by Sharp and Dohme, Philadelphia, Pa.

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TABLE I. Effect of Vitamin Deficiencies on Activity of Betaine-Homocysteine Transmethylase of Rat Liver.

	Exp. I					Exp. II				
		Duration of exp.,	Rate of gain		Transmethylase		Duration of exp.,	Rate of gain		Transmethylase
Vitamin	Diet*	days	Control, g/wk	Deficient, g/wk	Control, Deficient, μg μg	Diet*	days	Control, g/wk	Deficient, g/wk	Control, Deficient, μg μg
Thiamine	I	21	41.3	9.8	410 450	II	10	33.6	24.5	445 595
Riboflavin	I	14	35.0	19.5	320 460	II	10	33.6	24.2	445 550
Niacin	I	21	38.8	37.1	360 310	III	9	23.3	10.1	400 445
Pyridoxine	I†	14	52.0	5.0	405 535	II	10	33.6	14.0	445 595
Pantothenic acid	I	18	38.8	17.8	220 270	II	10	33.6	24.5	445 550
Biotin	I‡	21	40.0	35.0	360 380	IV	24	30.1	23.3	635 490
Choline	V§	14	21.0	15.0	575 535	V	9	21.0	9.1	385 365

* Diets are described in the experimental section. Weanling rats were used throughout unless otherwise noted.
† This diet contained 3 mg % desoxypyridoxine. Initial wt of rats was approximately 85 g.
‡ This diet contained 2% sulfasuxidine.
§ Initial wt of rats was approximately 150 g.

were prepared from the livers of 5 or 6 rats from each experimental group and the transmethylase activity was determined as described previously(4). The transmethylase activity is expressed as μg of methionine formed per g of fresh liver during a 3 hour incubation period.

Results. The effect of a deficiency of each of the following: thiamine, riboflavin, niacin, pyridoxine, pantothenic acid, biotin and choline, on the activity of the betaine-homocysteine transmethylase of rat liver is shown in Table I. It can be seen that the transmethylase activity of the livers of the rats fed the deficient diets was generally *higher* than that of the control group. Only in the case of a biotin (Exp. II) or a choline deficiency was the transmethylase activity found to be lower than that of the corresponding control group. The effect of the biotin deficiency on the transmethylase was evident only when the deficiency was severe enough to retard the growth of the rats significantly (compare rate of gain and transmethylase activity in Exp. I and II) and even then the absolute value for the deficient group was quite high. Omission of choline from the (high protein) diet used in these experiments lowered the transmethylase activity only slightly; however, the results are in agreement with previous findings with low protein diets(7).

The results of 5 experiments with rats made deficient in folic acid in various ways are given in Table II. The transmethylase activity of livers of deficient rats was in each case higher than in the corresponding control group. This is particularly noticeable in experiments in which no antivitamin (Aminopterin, Lederle Laboratories, Pearl River, N. Y.) was administered. Experiments with pair-fed rats (Exp. II) demonstrated that high transmethylase activity of deficient animals could not be attributed to an effect of starvation(7). Livers from rats fed diet deficient in both folic acid and vit. B_{12} (Exp. III) also had a higher transmethylase activity than livers of rats fed the complete diet. As the diet in this case contained casein, and growth was retarded by development of a folic acid deficiency, it is doubtful whether a deficiency of vit. B_{12} developed. Dinning *et al.*(8) have

TABLE II. Effect of Folic Acid Deficiency on Activity of Betaine-Homocysteine Transmethylase of Rat Liver.

Exp. No.	Treatment	Duration of exp., days	Initial wt of rats, g	Rate of gain		Transmethylase	
				Control, g/wk	Deficient, g/wk	Control, μg	Deficient, μg
1	Diet I + 2% sulfasuxidine	21	45	40.0	25.0	360	685
2	" I + 2% sulfa (pair fed)	21	45	25.2	18.5	380	800
3	" I + 2% sulfa—B_{12}	21	45	35.6	21.0	365	840
4	" I + 4 mg/kg aminopterin	7	150	−23.0	−23.0	445	505
5	" II + 1 " "	7	210	20.0	15.0	475	485

reported that, in chicks, a dietary deficiency of folic acid causes a decrease in betaine homocysteine transmethylase activity of the liver. Williams(9) found liver transmethylase activity of rats fed a complete diet containing aminopterin (4 mg/kg of diet) and showing very severe symptoms, to be lower than that of comparable group receiving complete diet without aminopterin. It is difficult to compare our results with these results since in our investigation, comparison was made between a group that received a complete diet and one that received a folic acid-deficient diet, both of which received aminopterin. It is of interest that Kring and Williams(10) found the activity of liver tyrosine oxidase system, higher in rats receiving diets deficient in thiamine than in control rats receiving an adequate diet, and that Beaten and Ozawa(11) found the activity of glutaminase I to be increased in vit. B_6-deficient rats. There are also several reports (see reference 10) that folic acid deficiency causes increased xanthine oxidase activity of rat liver.

A comparison of values for growth and for transmethylase activity of livers of rats fed Diet I (containing 25% casein) and Diet II (Diet I, supplemented with 0.2% DL-methionine) shows that a supplement of sulfur amino acids stimulated synthesis of betaine-homocysteine transmethylase although no growth response was observed.

The experiments do not provide any clue as to the nature of the "coenzyme" of betaine-homocysteine transmethylase. However, the data presented here and elsewhere(4,7), give some insight into the extremely complex relationships that apparently exist between dietary factors and activity of this enzyme.

Summary. When a diet deficient in one of the following vitamins: thiamine, riboflavin, niacin, pyridoxine, pantothenic acid, or folic acid was fed to rats, the activity of the betaine-homocysteine transmethylase of their livers was found to be higher than that of the livers of rats fed a complete diet. Deficiencies of biotin or choline, on the other hand, caused a slight decrease in the transmethylase activity.

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Received September 1, 1955. P.S.E.B.M., 1955, v90.