Interrelationships of Methionine and Vitamin B_6 in Human Subjects.* (22031)

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(Introduced by C. A. Elvehjem.)

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That vit. B_6 is involved with the metabolism of methionine has been shown by various investigators. Miller and Baumann(1) and Cerecedo and Foy(2) found that high protein diets increased the vit. B_6 requirement. Later work indicated that the adverse effects of high protein diets are due in part to the methionine content of the protein. Methionine in excess of the normal requirement resulted in depressed growth(3-5), an effect particularly evident when the diet was low in vit. B_6(6,7). Linkswiler *et al.*(8) added only 0.4% methionine to an assay diet containing 18% casein and observed that the symptoms of deficiency of the vitamin were aggravated sufficiently that the rat assay could be conducted without a preliminary depletion period. It was observed by DeBey *et al.*(9) that while vit. B_6 counteracted the effects of moderate amounts of methionine, with 2.5% methionine, high levels of the vitamin failed to restore growth. The role of B_6 and methionine in the formation of N^1-methylnicotinamide (NMeN) from nicotinamide has been suggested by other workers. Handler and Dann(10) suggested that nicotinamide is methylated *in vivo* by methionine and excreted in the urine as NMeN. In studies on the urinary excretion of NMeN, Rosen *et al.*(11) noted that B_6-deficient rats did not excrete NMeN. When pyridoxine was administered to these animals, there was a very gradual reappearance of NMeN. Perlzweig *et al.*(12) demonstrated that NMeN is formed *in vitro* by rat liver slices from nicotinamide but not from nicotinic acid. Ellinger (13) confirmed these results and showed that the particular enzyme involved was specific for nicotinamide but not for nicotinic acid. These workers(12,13) observed that the extent of transformation was increased by the addition of methionine to the system. No report on the study of the effect of both pyridoxine and methionine in NMeN formation in man has been found in the literature.

The present investigation was designed to study the effects of pyridoxine and methionine on NMeN formation in man and the effect of a high intake of methionine on the urinary excretion of B_6 and its metabolites by subjects on a diet low in vit. B_6.

Methods. Four normal adult women participated in each experiment, 3 of them taking part in both experiments. This study consisted of 2 experiments of 21 days each, with 3 months intervening between them. Each experiment was divided as follows: a 9-day period during which the subjects received only the basal diet followed by two 6-day periods in one of which the basal diet was supplemented by 8 g of dl-methionine and in the other by 8 g dl-methionine and 10 mg of pyridoxine hydrochloride. The methionine was

TABLE IA. Basal Diet Low in Vit B_6.

Food	Wt, g	Vit B_6 (analytical value), mg	Methionine (calculated value), mg
Orange juice, frozen	100	.021	3.0
Peaches, canned	100	.018	2.0
Apple sauce	100	.001	3.0
Lettuce	10	.003	
Carrots, raw	30	.008	
Celery	5	.017	
Onion	5	.004	
Cream of wheat, dry	25	.024	58.0
Rice, uncooked	50	.045	53.8
Cottage cheese, dry	30		142.5
Gelatin	5		36.0
Biscuits*		.026	
Minerals*			
Vitamin supplements*			
Totals		.167	528.7

* Composition given in Table IB.

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TABLE IB. Basal Diet—Continued.

	g
Biscuits:	
Cake flour	100
Baking powder	8
Crisco	34
Vitamin-free casein	40
Water	70
Salt mixture:	
$CaCO_3$	1.2
$KHCO_3$	1.88
$Mg\ SO_4\ 7H_2O$	.52
$Fe_2\ (C_6H_5O_7)_3\ 3H_2O$	.14
$Mn\ SO_4 \cdot 4H_2O$	Trace
$Cu\ SO_4 \cdot 5H_2O$	"
KI	"
Vitamins:	
Thiamine hydrochloride	1.2
Riboflavin	1.5
Niacin or nicotinamide	10.0

divided into 3 portions, one portion being taken with each meal. Pyridoxine was taken with breakfast during the last period. A supplement of 10 mg niacin was given during the first experiment and 10 mg of nicotinamide, during the second. The *basal diet* for the entire experiment is given in Table I. This diet was found by analysis to furnish 0.167 mg of vit. B_6 per day and 58 g of protein. Forty grams of the protein came from "vitamin-free" casein which was incorporated in biscuits. By computation, the diet was found to contain 428 mg of methionine and 2.69 mg of niacin. Cookies consisting of butter, sugar and cornstarch furnished additional calories to meet the energy requirement of each individual. The intake for each subject remained constant throughout the experiment. After a 3-day preliminary period in each experiment, 24-hour urine collections were made and preserved with toluene and 5 ml of glacial acetic acid. These were pooled into 3-day periods and samples were stored in a refrigerator for analyses. The B_6 content of the food and the urine was determined using the method of Atkin *et al.*(14). The 4-pyridoxic acid in the urine was determined by the method of Huff and Perlzweig as modified by Sarett(15); NMeN was determined according to the acetone-fluorometric method of Huff and Perlzweig(16).

Results. Effect of pyridoxine and methionine on N^1-methylnicotinamide excretion. The data on the urinary excretion of NMeN are presented in Table II. The fourth subject differed in the 2 studies but the values are shown consecutively for comparison. From these data it may be concluded that the methionine supplement and the later addition of pyridoxine hydrochloride to the diet low in B_6 had no effect on the urinary excretion of NMeN when the diet was supplemented with nicotinic acid. This was observed in all 4 subjects. During the low B_6 period, when nicotinamide was the supplement, the excretion of NMeN was approximately the same as when nicotinic acid was taken. The addition of methionine alone had little effect on the excretion. However, when pyridoxine was added in the presence of methionine the NMeN excretion rose markedly for each subject. For 3 subjects the excretion during this period was equal to more than 100% of the nicotinamide supplement and for the fourth subject, it was equal to approximately 65% of the supplement.

If nicotinic acid were readily converted to nicotinamide in the body, one would expect the same effect from both supplements. The increased excretion of NMeN observed only when the supplement was in the form of nicotinamide but not when it was nicotinic acid demonstrates the difficulty in the formation of NMeN from nicotinic acid in man. These results agree with the findings of Perlzweig and his coworkers(12) and Ellinger(13), using rat liver preparations.

The role of B_6 in NMeN formation is shown

TABLE II. Average Daily Urinary Excretion of N^1-methylnicotinamide.

Subject	Basal diet, mg	Basal + 8 g dl-methionine, mg	Basal + 8 g dl-methionine + 10 mg pyridoxine HCl, mg
Period I—Nicotinic acid supplement			
RZ	5.20	4.24	4.02
LM	4.76	5.10	5.10
KC	3.18	4.42	4.32
RB	3.56	5.57	4.74
Period II—Nicotinamide supplement			
RZ	8.95	7.20	11.95
LM	3.75	4.50	12.75
KC	5.85	6.15	14.20
AW	5.40	5.40	6.85

TABLE III. Average Daily Urinary Excretion of Vitamin B_6 and 4-Pyridoxic Acid by Human Subjects on a Diet Low in B_6 and Supplemented with dl-Methionine and with dl-Methionine and Pyridoxine HCl.

	Subject RZ		Subject LM		Subject KC		Subject RB	
	Vit B_6	4-P.A.	Vit B_6	4-P.A.	Vit B_6	4-P.A.	Vit B_6	4-P.A.
Period 1	mg							
Basal diet*	.06	2.48	.04	1.51	.02	2.74	.04	2.72
Basal diet + 8 g dl-methionine	.04	2.69	.03	2.35	.06	2.40	.04	3.46
Basal diet + 8 g dl-methionine + 10 mg pyridoxine hydrochloride	.47	8.10	.39	6.82	.59	5.68	.60	8.17
Period 2							Subject AW	
Basal diet	.06	2.38	.05	1.98	.06	2.57	.06	2.39
Basal diet + 8 g dl-methionine	.06	2.15	.06	2.63	.06	2.60	.07	3.11
Basal diet + 8 g dl-methionine + 10 mg pyridoxine hydrochloride	.53	8.37	.47	6.99	.63	6.62	.63	6.51

* Basal diet furnished 0.16 mg B_6.

by the increased excretion by human subjects of NMeN in the presence of both pyridoxine and methionine but not of methionine alone. A more complete picture of the role of B_6 in the methylation of nicotinamide might have been obtained had there been included an additional period in which pyridoxine without methionine was the supplement.

Urinary excretion of B_6 and metabolites. Data on the urinary excretion of B_6 and 4-pyridoxic acid are presented in Table III. The 4-pyridoxic acid values have been converted to pyridoxine by using the factor 0.92 based on the molecular weights of the 2 compounds. From these data, it appears that a high intake of methionine had no effect on the urinary excretion of vit. B_6 and 4-pyridoxic acid. Similar results were obtained in both experiments. De Bey(9) reported that dietary methionine had no effect on the concentration of vit. B_6 in the blood or liver of rats.

It may be noted from Table III that the 4-pyridoxic acid excretion exceeded the intake of vit. B_6 during the first 2 periods of the study. The amount of B_6 in the diet was 0.167 mg per day, whereas the excretion of 4-pyridoxic acid and B_6 ranged from 1.55 to 3.55 mg per day. Linkswiler *et al.*(17) reported a similar elimination of B_6 and metabolites in excess of intakes of 0.78 mg and 2.78 mg per day.

The fact that the diet used in the current study was almost a synthetic one and excluded foods reported by Sarett(15) to contribute interfering fluorescent substances in the urine, makes it likely that the fluorescent substance measured was actually 4-pyridoxic acid. The 10-mg supplement given the subjects during the last period of the study accounts for the high excretion of B_6 and 4-pyridoxic acid during this period.

Summary. 1. A study of the interrelationship of methionine and pyridoxine was made in adult human subjects maintained on a semi-synthetic diet, low in vit. B_6. 2. No effect of a high dosage of methionine on the urinary excretion of B_6 and its metabolites was observed in the study. 3. The role of vit. B_6 in the methylation of nicotinamide is shown by the increased urinary excretion of N^1-methylnicotinamide by human subjects, when given methionine plus pyridoxine but not when given only methionine.

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Effect of Meningococcal Endotoxin on the Immune Response.* (22032)

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Administration of sublethal quantities of various preparations of Gram negative bacterial endotoxin produce fever, leucocytosis preceded by leucopenia, depletion of liver glycogen, alterations in blood sugar levels, vasomotor disturbances and hemorrhagic necrosis of rapidly growing tumor tissues(1,2). In addition to these systemic reactions endotoxins produce profound effects on the host's natural defense mechanisms(3-5). An intravenous injection of suitable endotoxin establishes a state of "preparation" for the generalized Shwartzman phenomenon(6), so that a subsequent intravenous injection of endotoxin results in widespread hemorrhagic necrosis in the visceral organs of rabbits. The rabbit in this "prepared state" exhibits impaired activity of the reticuloendothelial system, as measured by rate of clearance of colloidal gold or chromic phosphate from the systemic circulation(3,5). Moreover, rabbits receiving a single intravenous injection of a bacterial free meningococcal culture filtrate prior to production of a pneumococcal skin infection show a marked decrease in resistance to the infection (4). The demonstration that phagocytic activity and resistance to experimental infections can be significantly altered by Gram negative bacterial endotoxins suggested that these compounds might alter the immune response. Our study was undertaken to determine the effect of various doses of meningococcal endotoxin on antibody response of rabbits to a single, purified foreign serum protein, crystallized bovine serum albumin.

Materials and methods. Hybrid albino rabbits of both sexes, obtained from a single breeding stock and weighing 2 kilos were used. Rabbits were maintained on diet of Purina rabbit pellets and were free of disease. The endotoxin employed was derived from a strain of meningococcus (44-B) supplied by Dr. Gregory Shwartzman, Mount Sinai Hospital, New York. The method for preparing the toxin has been described in an earlier communication(7). The antigen used was crystallized (BSA) bovine plasma albumin, lot #67009, obtained from Armour and Co. All toxin dilutions and antigen solutions were made up in pyrogen free saline just prior to use. Solutions for injection were prepared so that the final desired dilution of toxin plus the antigen, 15 mg BSA, were contained in total volume of 2 cc. Control animals received 15 mg of BSA in 2 cc volume of pyrogen free saline. Injections were given by way of marginal ear vein. Eleven days after antigenic stimulation, at a time when circulating antibody was presumed to be at its peak level

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