Proceedings

of the

Society

for

Experimental Biology and Medicine

Vol. 129 November 1968 No. 2

Homocystinuria as Affected by Pyridoxine, Folic Acid, and Vitamin B_{12}* (33314)

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Homocystinuria resulting from an enzymatic defect of metabolism was simultaneously discovered in 1962 by Carson and Neill (1) in North Ireland and by Gerritson, Vaughn, and Waisman (2) in the United States. More than 60 cases have since been reported (3), and it is now thought to be the second most common metabolic disease responsible for mental retardation (4).

Mudd and co-workers (5) failed to detect cystathionine synthetase in liver biopsy samples from a child with homocystinuria. The enzyme, which has a pyridoxal phosphate prosthetic group, catalyzes the condensation of homocystine with serine to form cystathionine. Deficiency of the enzyme thus could explain the accumulation of homocystine and methionine found in this disorder. The same investgators (6) later found the enzyme to be absent from liver of another child with homocystinuria. The mother, father, and a paternal cousin had liver enzyme levels that were 40% of normal control values, presumably representing the heterozygous state.

In a study of inorganic sulfur excretion after methionine administration, Laster *et al.* (7) concluded that the principal pathway of degradation of methionine sulfur to inorganic sulfur was through cystathionine since patients with homocystinuria having a deficiency of cystathionine synthetase showed an impaired capacity to metabolize the sulfur administered as methionine, but not when administered as cystine. These observations suggested to the authors that cystathionine formation was an obligatory step in human catabolism of methionine to inorganic sulfate. Using methionine-^{35}S, Brenton and Cusworth (8) observed that in two patients with homo-

* This investigation was supported in part by U. S. Public Hhealth Service Research Grant HD-00353 from the National Institute of Child Health and Human Development and Mo-1 FR61 from the National Institutes of Health.

cystinuria, a much smaller proportion of the excreted urine radioactivity was in the form of inorganic and ethereal sulfate compared to normal subjects.

In a recent study, Perry *et al.* (9) isolated an unusual homocystine-containing compound, 5-amino-4-imidazolecarboxamide-5′-S homocysteinylriboside, from the urine of a child with homocystinuria and detected this compound in the urine of six additional patients with homocystinuria. This suggested the possibility of an unknown alternate pathway for the metabolism of methionine and of purines.

The same group of investigators (10) by chromatographic procedures isolated small amounts of homolanthionine from the urine of patients with homocystinuria. They postulated that this compound was derived from homoserine and homocysteine. They also added S-adenosylhomocysteine and two unidentified disulfide compounds (11) to the list of compounds excreted in homocystinuria. Other compounds excreted include methionine, methionine sulfoxide, homocystine, and the mixed disulfide of cysteine and homocysteine.

Kennedy and associates (12) administered 200 mg of pyridoxine, a cofactor for cystathionine synthetase, as the hydrochloride to two patients with homocystinuria, but found no change in homocystine excretion. The pyridoxine was discontinued after 1 day because of severe headaches that ceased on withdrawal of the vitamin and recurred when the vitamin was again administered.

Barber and Spaeth (13) found that large doses of pyridoxine administered to three patients with homocystinuria reduced to normal the raised plasma levels and urinary excretion of homocystine and methionine. Hooft *et al.* (14) observed less striking changes in two children given large doses of pyridoxine. Turner (15) from similar studies with two patients concluded that adequate protein intake, possibly with cystine supplementation were necessary to obtain this effect. However, the characteristic features of the disease, homocystinuria, are apparently not attributable solely to toxic effects of the high levels of homocystine and methionine since administration of these amino acids in the diet of experimental animals (16,17) produce deleterious effects but do not duplicate the conditions of the disease.

We inaugurated the investigation reported here after observing a marked change in the excretion of homocystine when the diet of two children with homocystinuria was improved. The two Negro girls studied, now aged 15 and 18, are from a family with 11 children of which 4 were found to have homocystinuria. The two younger affected sibs are now aged 11 and 9 years. A report of the four cases and the ocular changes and ocular pathology of the two oldest girls has been published (18) and a full report of metabolic studies is in preparation. Amino acid excretion figures are included in the present report.

Materials and Methods. The two older girls studied were fed an adequate weighed diet (diet I) similar to, but better than, the usual home diet, which consisted of corn meal, "fat back" (salted fat pork), with oleomargarine and occasional meat, eggs, cheese, greens, and other foods. This home diet was estimated to provide pyridoxine at borderline levels with reference to the requirements for a growing child.

Diet I fed during the metabolic studies in 1964 supplied 1764 calories and 67 g of protein. The diet (diet II) fed in 1966 was similar but lower in methionine.

Amino acid excretion was determined by use of the Beckman 120 B amino acid analyzer as described by Spackman, Stein, and Moore (19). The amino acid nitrogen figures shown in Table II were calculated from these values. Because some of the urine collections made at home did not represent exactly 24 hr, excretion values are expressed as per gram of creatinine. Serum glutamic-oxalacetic and serum glutamic-pyruvic transaminases were determined using the Sigma-Frankel[1] method. Xanthurenic acid excretion studies were done as described by Babcock *et al.* (20) before and after a loading dose of 4 g of tryptophane (about 0.1 g/kg of body weight). Cystathionine synthetase activity of

[1] Sigma Chemical Co., 3500 DeKalb St., St. Louis, Mo. 63118.

TABLE I. Effect of Pyridoxine on Excretion of Amino Acids by Four Siblings with Homocystinuria.[a]

Patient	M.J.		B.J.		R.J.		D.J.	
Age/sex (1964)	15♀		11♀		8♂		7♀	
Weight (kg) (1964)	42.2		41.1		22.3		18.0	
Height (cm) (1964)	161		161		133		114	
Diet	Diet I	Home	Diet I	Home	Hospital	Home	Hospital	Home
Date	11/6/64	1/8/68	11/6/64	1/8/68	5/16/65	1/8/68	5/16/65	1/8/68
Creatinine	1.148		1.041		0.631		0.558	
Amino acids	Before B_6	After B_6	Before B_6	After B_6	Before B_6	After B_6	Before B_6	After B_6
Threonine	1.37	0.247	0.622	0.250	0.481	0.144	0.330	0.116
Serine	2.09	0.712	1.083	0.543	0.705	0.401	0.614	0.433
Glutamine	2.14	0.860	0.992	0.710	1.062	0.625	0.945	0.584
Glycine	5.61	2.44	5.657	2.117	1.788	0.612	2.434	0.578
Alanine	0.654	0.333	0.687	0.304	0.344	0.232	0.507	0.244
Valine	0.118	0.042	0.084	0.034	0.080	0.031	0.121	0.035
Half cystine	0.081	0.064	0.058	0.083	0.036	0.073	0.044	0.092
Cystathionine	0.103		0.052	0.034	0.060	0.035	0.062	
Methionine	0.165	0.056	0.131	0.076	0.199	0.069	0.272	0.067
Isoleucine	0.160		0.985		0.096		0.141	
Leucine	0.168	0.096	1.022	0.095	0.127	0.091	0.149	0.106
Tyrosine	0.232	0.086	0.213	0.088	0.146	0.098	0.136	0.063
Phenylalanine	0.118	0.062	0.121	0.051	0.066	0.066	0.080	0.057
β-Aminoisobutyric acid	0.657	0.238	0.186	0.035	0.192	0.028	0.305	0.025
Half homocystine	4.28	0.236	3.461	0.207	0.906	0.066	0.517	0.015
Half cystine–homocystine[b]	0.708	0.179[c]	0.486	0.165[c]	0.267	0.079[c]	0.290	0.060[c]
Ethanolamine		0.233		0.235		0.021		
Lysine		0.251		0.239		0.104		
Histidine		1.09		0.946		0.878		
Neutral + acidic amino acid N (-homocystine)	18.60	6.94	14.77	6.25	8.840	4.613	10.74	5.134
Basic amino acid N		2.06		1.83		1.424		
Total amino acid N		9.00		8.08		6.037		

[a] Mmoles per gram creatinine. Pyridoxine administered = 100 mg per day for 2 months.

[b] The "cystine-homocystine disulfide" is the mixed disulfide formed from cysteine and homocysteine calculated by using the average analytical constant of the two amino acids.

[c] In these runs these values may include isoleucine.

liver biopsy tissue was determined essentially by the method of Mudd and co-workers (5) using serine 3-^{14}C. The reaction mixture was chromatographed on a paper strip and then the relative activity of the separated serine and cystathionine was measured with a Tracerlab 4 π filter paper scanner. Urinary inorganic sulfates were determined by Folin's gravimetric method (21).

Results and Discussion. Table I shows the excretion values for a number of amino acids before and after the four siblings had been given at least 100 mg of pyridoxine per day for 2 months. It is presumed that the ablation of the generalized though slightly increased amino acid excretion found on admission by pyridoxine administration was due to effects of pyridoxine in the kidney, since plasma amino acid levels were normal both before and after administering the vitamin. The figures shown in the table were selected as representing the initial and the recent and most nearly comparable data on the four siblings. The amino acid excretion values for January 8, 1968 were almost identical to the values obtained a few weeks after pyridoxine

Effect of Pyridoxine, Folic Acid, and Vitamin B_{12} on Excretion of Sulfur Compounds and Amino Acid N by Four Siblings with Ho tinuria.[a]

, age (1964)	Date	Diet	Vitamin supplement	Half homo-cystine	Half cystine	Half cystine–homocystine[b]	Inorganic SO_4	Amino (-homoc
	1964							
15 years	10/19	Hosp. diet	None	5.29	Trace	0.424		18.(
	11/6	Spec. diet I	None	4.28	Trace	0.708	7.80	18.(
	11/7	Spec. diet I	None	2.10	Trace	0.230	10.0	8.8
	11/13	Spec. diet I	None				9.7	
	11/22	Spec. diet I	B_6 6 days				19.3	
	11/23	Spec. diet I	B_6 7 days	0.245	0.112	0.260		9.8
	11/28	Spec. diet I	B_6 12 days	0.108	0.109	0.069		5.1
	11/29	Spec. diet I	B_6 13 days				19.3	
	1965							
	5/7	Home diet	No B_6 for 6 months	2.50	Trace	0.391		10.7
	5/14	Home diet	B_{12} + folic acid 7 days	1.40	Trace	0.397		6.8
	10/11	Home diet	B_6 + B_{12} + folic acid 1 month	0.131	0.132	0.161		12.9
	1966							
	3/9	Home diet	No B_6, B_{12} + folic acid 7 days	1.40	Trace	0.280		10.7
	3/16	Spec. diet II	B_6 6 days	0.290	0.130	0.184		11.8
	1968							
	1/8	Home diet	100 mg B_6 7 weeks	0.236	0.064	0.179		6.9
	1964							
11 years	10/5	Hosp. diet	None	3.21	Trace	0.337		9.6
	11/6	Spec. diet I	None	3.46	Trace	0.486	10.6	14.7
	11/7	Spec. diet I	None	3.40	Trace	0.483	13.7	14.7
	11/13	Spec. diet I	None				10.0	
	11/21	Spec. diet I	B_6, 5 days & 5 days after methionine load	1.11	0.121	0.468	22.8	13.9
	11/23	Spec. diet I	B_6 7 days	0.440			23.4	
	11/29	Spec. diet I	B_6, 13 days & 5 days after methionine + serine load				23.7	

TABLE II (continued)

age (1964)	Date	Diet	Vitamin supplement	Half homocystine	Half cystine	Half cystine–homocystine[b]	Inorganic SO_4	Amino a (-homocy
	1965							
	5/7	Home diet	None for 6 months	2.87	Trace	0.536		18.5
	5/14	Home diet	B_{12} + folic acid 7 days	1.36	Trace	0.402		12.8
	10/11	Home diet	B_6 + B_{12} + folic acid 1 month	0.76	Trace	0.133		7.9
	1966							
	3/9	Home diet	No B_6, B_{12} + folic acid 7 days	1.84	Trace	0.256		8.3
	3/16	Spec. diet II	B_6 6 days	0.142	0.260	0.226		10.8
	1968							
	1/8	Home diet	100 mg B_6 for 7 weeks	0.207	0.083	0.165		6.0
	1965							
8 years	5/16	Hosp. diet	None	2.26	Trace	0.267		8.8
	10/11	Home diet	B_6 + B_{12} + folic acid 1 month	0.023	0.125	0.086		4.5
	12/3	Hosp. diet	No vitamins 5 weeks	1.12	Trace	0.260		10.0
	1968							
	1/8	Home diet	B_6, 100 mg 7 weeks	0.066	0.073	0.079		4.6
	1965							
7 years	5/16	Hosp. diet	None	1.63	Trace	0.290		10.7
	10/11	Home diet	B_6 + B_{12} + folic acid 1 month	0.063	0.117	0.132		6.5
	12/3	Hosp. diet	None for 5 weeks	0.509	Trace	0.160		7.4
	1968							
	1/8	Home diet	B_6, 100 mg 7 weeks	0.015	0.092	0.060		5.1

xpressed as mmoles per gram of creatinine. Pyridoxine administered, 25 mg/day except that before 1/8/68, 100 mg/day was given.
ay, B_{12}, 50 μg/day for periods shown.
stine–homocystine'' is the mixed disulfide formed from cysteine and homocysteine, calculated by using the average of the analytical co
ino acids.

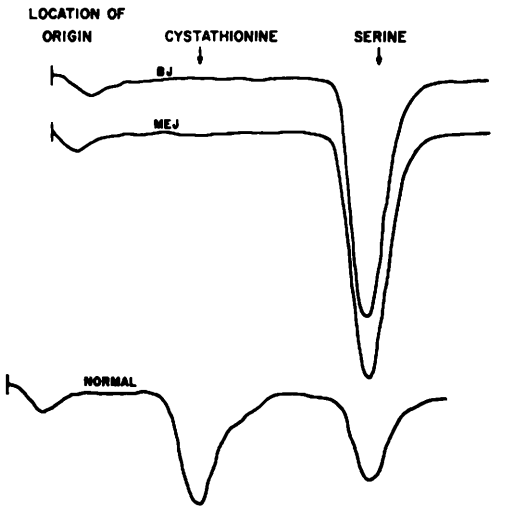

FIG. 1. Cystathionine synthetase activity of liver biopsies from two homocystinuric girls and one normal control. Shown are tracings of records of radioactivity in strips of filter paper on which the ^{14}C-labeled serine and cystathionine were separated from the cystathionine synthetase enzyme activity assay mixture by chromatography. On the chart records made by this instrument a dip in the record line indicates increased radioactivity at that point on the filter paper strip.

was first given in amounts of 25 mg per day.

As shown in Fig. 1 the liver biopsy samples showed no significant cystathionine synthetase activity while liver from normal subjects exhibited activity of the order obtained by Mudd and co-workers (5,6). Since cystathionine synthetase is an enzyme with a pyridoxal phosphate prosthetic group, 25 mg per day of pyridoxine was administered for 13 days before the biopsy specimen was taken and an excess of pyridoxal phosphate was added to the enzyme assay mixture. In view of the lack of cystathionine synthetase activity, it is unlikely in these two sibs that there was a partial enzyme block of this enzyme as suggested by Gerritsen and Waisman (22) or a failure of apoenzyme binding of pyridoxal phosphate as in cystathioninuria (23).

The urinary excretion of xanthurenic acid after tryptophane loading for the two older sisters was reduced after the pyridoxine administration (B.J., 24.6 reduced to 20.0 and M.J., 60.1 reduced to 30.3). The activity of another pyridoxal phosphate–containing enzyme, serum oxalacetate-glutamate transaminase, was increased (B.J., 10.5 to 19.5 Sigma-Frankel units and M.J., 2.3 to 48.5 units). While there was no definite indication of pyridoxine deficiency, especially in the younger sib, B.J., the administration of the vitamin improved the functional activity of these enzymes requiring pyridoxine as a coenzyme.

In Table II are shown the effects of the diets and vitamin administration on excretion of homocystine, cystine, the cystine-homocystine disulfide, inorganic sulfate, and amino acid nitrogen. The diets fed in the hospital were better than the home diet and resulted in a decrease in homocystine excretion in M.J. although no such effect was noted in B.J. who had a lower homocystine excretion.

Administration of 25 mg per day of pyridoxine produced a striking decrease in excretion of homocystine in all four children and a marked increase in inorganic sulfate excretion. In terms of milligrams per gram of creatinine, the change in homocystine excretion represented a reduction of from 449 to 705 mg to levels of between 59.9 and 14.1 mg. Excretion values for homocystine after administration of 100 mg per day of pyridoxine were similar to the values found on administration of 25 mg per day. The decrease in homocystine excretion reflected a similar reduction in plasma resulting from administration of the vitamin. These data will be reported in detail in a subsequent paper.

The administration of 10 mg per day of folic acid and of 50 μg per day of B_{12}[2] resulted in a consistent and significant decrease in homocystine excretion, though not as marked as the decrease resulting from pyridoxine administration.

Since the liver assay procedure showed no cystathionine synthetase activity, it seems likely that the decrease in homocystine and increase in inorganic sulfate excretion after pyridoxine must represent an increased metabolism of methionine through an alternate pathway such as that involving desulfhydra-

[2] The folic acid used was Folvite (Lederle) and Folica (Upjohn) and the B^{12} was Rhodavite (The Viterine Co.).

tion and transamination to α ketobutyric acid. The enzymes for this pathway also require pyridoxal phosphate as a prosthetic group (24).

The N^5-methyl-folate-H_4 with a cobamide-dependent methyl transferase has been shown to be a methyl donor for methionine synthesis from homocysteine in animal liver (25), hence it is probable that the reduction in homocystine after folic acid and vitamin B_{12} administration is due to increased resynthesis of homocysteine to methionine.

In general the results reported here with pyridoxine administration are similar to those obtained by Barber and Spaeth (13), Hooft *et al.* (4), and by Turner (15), although the smaller doses (25 and 100 mg) used did not result in complete elimination of homocystine excretion as did the 250–500 mg doses used by Barber and Spaeth as cited above.

In general, as observed by Turner (15), the better diet and pyridoxine administration seemed to produce favorable changes in the metabolic pattern involving a reduction in the abnormal excretion of metabolites.

Summary and Conclusions. Administration of pyridoxine to the four children with homocystinuria who had been on a diet low in pyridoxine resulted in ablation of a general but slight aminoaciduria and a striking reduction in homocystine excretion. Further studies on two of the children showed increases in serum oxalacetic-glutamic transaminase and reduction in xanthurenic acid excretion after tryptophane loading. The marked decrease in homocystine excretion after pyridoxine administration was correlated with increased excretion of inorganic sulfate. The total lack of activity for cystathionine synthetase in liver biopsy tissue after pyridoxine administration to the children even when pyridoxal phosphate was added to the enzyme assay system, lead to the conclusion that the metabolism of methionine sulfur to inorganic sulfate must have been increased through one of the alternate pathways of metabolism. Administration of vitamin B_{12} and folic acid resulted in a decrease of excretion of homocystine though not as marked as the effects of pyridoxine.

Thanks are due to Theresa Skidmore and Martha Hickman for technical assistance.

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Received March 8, 1968. P.S.E.B.M., 1968, Vol. 129.