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Methionine Requirement in Children with Cystinosis*

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Cystinosis is a recessively inherited disease characterized by widespread deposition of cystine in many organs. Clinically, renal tubular dysfunction and progressive glomerular damage result in severe disability and death before late childhood. The basic biochemical abnormality leading to accumulation of cystine is not known.

Because of the severity and progression of symptoms, various therapeutic approaches have been attempted. Large doses of Vitamin D and sodium and potassium citrate have been used in the treatment of of the accompanying rickets, dehydration and metabolic acidosis. These agents give only temporary relief and fail to alter the progressive decrease in glomerular function.

Penicillamine, a synthetic thiol compound, has been used because it reacts with cystine, converting it to a soluble mixed disulfide, penicillamine-cysteine.[1, 2, 3, 4] Despite the reduction of plasma cystine concentration, no clinical improvement has been observed.[3, 4]

Renal transplantation has been performed in children with cystinosis using heterozygous donors as well as non-related donors.[5, 6, 7] Repeated biopsies of the transplanted kidneys revealed crystal accumulation in the interstitial tissues in four patients[7] and in the tubular epithelium in one patient.[5]

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Dietary restriction of methionine and cystine has also been used. Contradictory results have been reported. In one patient, Bauer and Antener reported improvement in both tubular and glomerular function.[8] M. Seip *et al.* observed improvement in a patient on a diet low in protein, methionine, and cystine with reduction of fluid intake and diuresis. No improvement in the rate of growth[9] was observed, however. In another patient, clinical improvement was observed with healing of the rickets and normal growth.[10] In other patients, dietary restriction of methionine and cystine proved to be a very efficient way of depressing the plasma cystine concentration.[4, 9] After more than a year on this diet, however, no reduction in intracellular cystine crystal accumulation was observed.[3, 4]

At the present time the prevention of further deposition of crystals by dietary limitation of methionine and cystine would appear to be a most rational means of delaying the progressive loss of glomerular function. To do this adequately the requirement for methionine in these patients must be established in order to set appropriate guidelines for long-term management.

Excessive urinary loss of amino acids and altered rates of catabolism may affect the requirement for sulfur-containing amino acids in these patients. Because of these possibilities, this study was carried out to determine the requirement for methionine in children with cystinosis.

Methods and Materials

Subjects: Four children with cystinosis, two of whom were siblings, were studied. The diagnosis of cystinosis was established in all on the basis of the presence of multiple renal tubular dysfunction and cystine crystals in the bone marrow and cornea. All were below the third percentile in height and weight and had advanced reduction of glomerular function. Each patient had been admitted many times before to the hospital for vomiting, dehydration, and metabolic acidosis. Three of the four patients had been on a methionine restricted diet (approximately 45 mg. methionine/kg./day) for over a year.

Dietary Study: An isonitrogenous, isocaloric diet was offered throughout the study. A synthetic amino acid mixture was prepared using commercially available L-amino acids in proportions similar to human milk, except for methionine and cystine which were excluded. Differences in methionine intake for each period were compensated by the addition of glycine to assure comparable nitrogen intake for each period. Low protein foods were also offered. The methionine intake was increased

periodically either through natural foods or by the use of synthetic L-methionine. Table I shows the composition of the amino acid mixture. The nitrogen, methionine, cystine, and caloric intake of each subject for the various periods is summarized in Table II.

TABLE I

COMPOSITION OF SYNTHETIC L-AMINO ACID MIXTURE*

Isoleucine	129 mg.
Leucine	242 mg.
Lysine Monohydrochloride	119 mg.
Phenylalanine	96 mg.
Threonine	93 mg.
Tryptophan	33 mg.
Valine	135 mg.
Histidine	34 mg.
Arginine	77 mg.
Alanine	53 mg.
Aspartic Acid	174 mg.
Glutamic Acid	344 mg.
Glycine	90 mg.
Proline	120 mg.
Serine	104 mg.
Tyrosine	93 mg.

* 1.94 gms. of this amino acid mixture is equivalent to 0.240 gms. of nitrogen

The first three subjects had four separate studies at different levels of methionine intake and each period lasted at least four days. When the subjects vomited or refused the diet in significant amounts, the periods were extended until four consecutive days were satisfactory. Subject R.B. had a five-day baseline period on natural foods on approximately 45-50 mg. methionine/kg./day and 30 mg. cystine/kd./day followed by a six day period on the synthetic amino acid mixture offering 20 mg. methionine/kg./day.

Blood samples were drawn at the beginning and at the end of each period for hemoglobin, hematocrit, total protein, SGOT, amino acids, alpha-amino nitrogen, creatinine, and blood urea nitrogen. Two or three consecutive 24-hour urine collections were made at the end of each period. The following determinations were carried out on the urine samples: total nitrogen, amino acids, alpha-amino nitrogen, urea, and crea-creatinine. Pooled stool samples, a duplicate diet, and refusals and vomitus for each period were analyzed for total nitrogen content.

TABLE II

AVERAGE DAILY NITROGEN BALANCE IN CYSTINOSIS SUBJECTS

Subjects	Sex and Age	Duration of Period (Days)	Daily Caloric Intake	Nitrogen Intake (GM./DAY)	Methionine Intake (IN MG/KG/DAY)	Cystine Intake (IN MG/KG/DAY)	Total Nitrogen Excretion	Nitrogen Retention (GMS./DAY)	Urinary Urea Nitrogen Excretion (% of the Total Urinary Nitrogen Excreted/ Day)	Urinary Alpha-Amino Nitrogen Excretion (% of the Total Urinary Nitrogen Excreted/ Day)
R.F.	Male	8	1195	4.62	45	3	3.07	+ 1.55	38	9
	5 years	9	1253	4.69	10	4	4.03	+ 0.66	41	9
		4	1196	4.45	20	4	4.36	+ 0.10	39	7
		4	1256	5.00	30	3	3.56	+ 1.44	26	8
		4	1248	4.47	40	3	2 88	+ 1.59	57	10
J.G.	Male	6	1138	5.39	40	8	3.97	+ 1.42	50	3
	6 and 5/12	6	1187	5.58	30	7	3.91	+ 1.67	66	3
	years	10	1181	5.74	20	7	4.73	+ 1.01	62	4
		7	1081	4.50	10	6	4.50	0.00	65	7
D.G.	female	8	1112	4.83	40	7	3.11	+ 1.72	41	9
	3 and 5/12	6	1143	4.58	30	8	3.81	+ 0.78	43	6
	years	8	1229	4.50	20	6	3.98	+ 0.52	44	5
		8	1156	4.09	10	6	3.89	+ 0.21	32	8
R.B.	female	5	890	6.65	46	29	5.45	+ 1.19	—	5
	6 and 10/12	6	945	6.29	20	12	5.40	+ 0.89	—	7
	years									

Laboratory Methods: Total nitrogen determinations were made by the modified Folin-Farmer micro-Kjeldahl method.[11, 12] Total urinary alpha-amino nitrogen was determined by the method of Saifer *et al.*[13] Amino acids were analyzed quantitatively by a modification of the Piez-Morris method.[14, 15] Fasting venous blood was collected in EDTA, separated and the plasma proteins were precipitated immediately with 5-sulfosalicylic acid. For cysteine determinations, fasting blood was immediately added to buffered iodoacetate according to the method of Brigham, Stein, and Moore.[16] Cysteine was determined as s-carboxymethyl-cysteine by ion exchange chromatography. Both samples were stored at –4°C until analyzed.

Results

Average nitrogen retention values are shown in Table II for each patient at the various levels of methionine intake. All subjects had positive nitrogen retention at the various levels of intake of methionine except for J.G., who did not retain a significant amount of nitrogen on an intake of 10 mg. methionine/kg./day. With decreasing methionine intake, generally, there was decreasing nitrogen retention. There was no significant change in body weight during these periods.

Plasma amino acids, urinary amino acid excretion and tubular reabsorption of amino acids on two different levels of intake, 40 mg. and intakes of 10 mg. methionine/kg./day, are shown in Table III and IV. Except for R.F., the plasma amino acids were slightly lower than published amino acid values in normal children.[17, 18] Significantly lower levels were observed for the following amino acids: isoleucine, leucine, tyrosine, lysine, histidine, and arginine. Somewhat decreased plasma amino acid values have been reported previously for children with cystinosis.[4, 19] Except for the sulfur-containing amino acids, there were no constant or significant differences in plasma amino acid concentrations at these two levels of methionine intake. The excretion of nearly all of the amino acids was many times higher than the expected value for that age.[18, 20] This is apparently the result of markedly decreased tubular reabsorption of these amino acids.

As far as the sulfur-containing amino acids, methionine, cystine, and cysteine, are concerned (Table IV), R.F. and D.G. showed no significant change in plasma levels during the different periods. In J.G., a correlation between amount of ingestion of the sulfur-containing amino acids and plasma levels was evident. Plasma methionine, cystine, and cysteine levels gradually decreased with decreasing methionine intake.

TABLE IV

CONCENTRATIONS OF PLASMA SULFUR-CONTAINING AMINO ACIDS ON DIFFERENT LEVELS OF METHIONINE INTAKE

Concentration Amino Acid in MG. Per 100 Ml.	Baseline Period 45 MG. Methionine/KG/DAY			40 MG. Methionine/ KG/DAY		30 MG. Methionine/ KG/DAY	20. MG. Methionine/ KG/DAY			10 MG. Methionine/ KG/DAY	
	J.G	D.G	R.B.	J.G.	B.G.	D.G.	J.G.	D.G.	R.B.	J.G.	D.G.
Methionine	0.32	0.22	0.20	0.19	0.12	0.28	0.18	0.15	0.18	0.15	0.29
Cystine	1.64	0.93	1.62	1.04	0.68	0.94	0.79	0.80	1.14	0.70	1.56
Cysteine*	0.41	0.13	0.14	0.37	0.11	0.39	0.17	0.10	0.20	0.13	0.20
Ratio Cystine/Cysteine	3.9	7.2	12.0	2.8	6.1	2.4	4.7	7.7	5.6	5.4	8.01

* Measured as s-carboxymethyl cysteine

TABLE III

RENAL TUBULAR REABSORPTION OF AMINO ACIDS ON OF 10 TO 40 MG. METHIONINE/KG/DAY

Amino Acid	Subject R. F.						Subject J. G.					
	40 Mg. Methionine/Kg./Day			10 Mg. Methionine/Mg./Day			40 Mg. Methionine/Kg./Day			10 Mg. Methionine/Kg./Day		
	PAA* (Mg %)	UAA** µG/Min/1.73 M²	PTR***	PAA (Mg %)	UAA µG/Min/1.73 M²	PTR	PAA (Mg. %)	UAA µG/Min/1.73 M²	PTR	PAA (Mg. %)	UAA µG/Min/1.73 M²	PTR
Threonine, Serine, and Glutamine	7.22	174.96	97.79	12.93	572.47	90.07	1.56	438.90	57.62	1.93	91.19	91.55
Glutamic Acid	2.03	62.90	92.12	7.05	122.60	95.98	3.34	263.02	83.02	2.44	101.28	92.53
Proline	3.38	168.28	87.35	8.73	268.15	93.13	2.10	216.97	84.45	1.77	11.89	98.81
Citrulline	0.77	97.12	67.83	3.47	399.08	74.26	2.98	376.28	80.95	3.01	54.94	96.72
Clycine	3.10	172.61	85.86	3.71	161.44	90.24	3.10	305.63	50.21	1.30	104.16	85.64
Alanine	4.99	193.10	90.18	5.30	231.19	90.21	0.85	235.35	58.45	1.19	796.25	87.86
Valine	1.85	78.63	89.15	3.41	115.99	92.36	0.58	71.72	81.60	1.05	204.36	96.49
Cystine	*	*	—	*	*	—	1.04	82.53	87.97	0.70	145.61	96.27
Cysteine	*	*	—	*	*	—	0.37	+	—	0.13	+	—
Methionine	0.63	5.59	97.69	0.40	19.27	89.26	0.19	23.07	81.15	0.15	0.00	100.00
Isoleucine	0.75	27.13	90.89	0.78	60.16	82.76	0 19	67.04	68.69	0.37	8.29	96.03
Leucine	0.92	63.30	88.51	1.10	108.52	77.84	0 33	68.84	68.05	0.70	14.06	96.29
Tyrosine	0.66	55.16	78.77	0.66	99.48	66.15	0.21	65.60	54.13	0.34	16.58	91.23
Phenylalanine	0.99	35.77	90.85	0.65	54.72	81.14	0.32	57.70	82.09	0.46	12.25	95.23
Ornithine	0.81	74.31	76.85	0.81	94.88	73.75	0 94	34.24	94.53	x	x	x
Lysine	2.91	98.68	91.39	2.76	102.65	91.68	0.76	94.43	81.19	x	x	x
Histidine	1.18	85.67	81.54	0.61	76.28	71.89	0.43	102.35	63.97	x	x	x
Arginine	2.59	22.80	97.75	1.45	12.19	98 10	0.10	23.79	65.07	x	x	x

Amino Acid	Subject R. B.						Subject D. G.					
	40 Mg. Methionine/Kg./Day			10 Mg. Methionine/Kg./Day			40 Mg. Methionine/Kg./Day			20 Mg. Methionine/Kg./Day		
Threonine, Serino, and Glutamine	1.77	307.60	20.77	4.75	482.67	45.34	0 93	60 96	73 46	1.83	370.71	0
Glutamic Acid	1.89	118.68	71.35	1.87	404.82	88.34	1.06	57.25	76 06	2.55	70.44	85.16
Proline	2.22	302.75	37.63	1.88	149.81	57.21	0.74	44.07	75.77	0.90	86.91	48.24
Citrulline	3.65	67.82	91.49	2.50	91.34	80.28	0.67	126.04	24.30	3.48	80.32	87.56
Glycine	0.52	108.29	5.59	1.13	73.69	64.94	0.97	78 67	67.06	1.89	131.40	62.63
Alanine	0.68	123.52	17.34	1.34	161.24	35 33	0.70	91.03	47.97	1.87	189.89	45.46
Valine	1.22	69.89	73.84	0.62	34.25	62.82	0.51	67.55	46.44	1.42	84.85	67.74
Cystine	1.62	68.16	81.61	1.14	87.53	58.66	0.68	121.92	27.56	1.56	63.43	78.17
Cysteine	0.14	+	—	0.20	+	—	0.11	+	—	0.20	+	—
Methionine	0.20	8.69	79.88	0.18	6.22	82.19	0.12	24.30	89.42	0.29	4.94	90.77
Isoleucine	0.58	32.18	74.88	0.28	132.86	0.0	0.33	38.72	52.82	0.95	30.48	82.65
Leucine	0.89	53.98	72.31	0.32	26.98	54.52	0.50	48.60	61.06	1.14	67.55	68.33
Tyrosine	0.22	22.14	54.45	0.32	55.01	7.50	0 37	57.25	37.18	0.37	44.89	34.70
Phenylalanine	0.38	25.95	68.98	0.34	64.01	0	0 39	46.55	52.41	0.55	43.66	56.88
Ornithine	0.81	41.18	76.54	0.90	67.82	59.42	1 28	29.66	90.61	1.59	52.31	52.68
Lysine	0.84	50.86	72.36	0.89	124.21	24 67	1 20	46.13	84.34	0 80	68.28	54.21
Histidino	0.56	41.17	66.59	0.40	64.01	13.26	0.45	30.82	72.22	0.35	51 08	21.61
Arginino	0.74	16.26	90.10	0.61	55.01	51.10	0.68	15.24	90.84	0.44	18.95	76.19

* Plasma Amino Acids

** Urinary amino acid excretion

*** Percent tubular reabsorption of amino acid

x Technically lost

÷ Not determined

Plasma s-carboxymethyl-cysteine levels and the ratio of cystine to cysteine were generally in good agreement with reported values in cystinosis.[4, 16] In R.B. at baseline levels, this ratio was two times higher than reported values. However, on an intake of 20 mg. methionine/kg./day this ratio was approximately the same as that found in the other subjects studied. In J.G. these ratios were lower.

Methionine excretion was lower than the excretion of any other amino acid for almost every period. Generally, methionine excretion reflected the level of its intake (Table III). This trend was much clearer in the second patient, J.G., and in the last period this subject did not excrete any detectable methionine. The urinary excretion of cystine was usually high and there appeared to be no relation between methionine and cystine intake and urinary excretion of cystine.

No significant change in hemoglobin, hematocrit, electrolytes, urea and creatinine clearances was observed throughout the study in any of subjects.

Discussion

Methionine is principally metabolized via cystine in man. Until a more rational therapy directed at correction of the fundamental defect is defined, dietary restriction of methionine and cystine would appear to be a reasonable temporizing approach in cystinosis. Preliminary reports of dietary treatment have not been too encouraging. The prevention of further deposition of crystals by dietary limitation of methionine and cystine would not be expected to occur unless the methionine allowance were only sufficient for protein synthesis. The reported methionine requirement figures for normal children may not serve as satisfactory guidelines for these patients. Excessive urinary loss of amino acids and altered rates of catabolism may affect the requirement for sulfur-containing amino acids in cystinosis patients.

In normal children, a methionine intake of 10-20 mg./kg./day resulted in no nitrogen retention in three out of four subjects.[21] Without added cystine, 11 year old males required approximately 27 mg./kg./day to remain in positive nitrogen balance.[21] Snyderman *et al*[21] reported that a methionine intake between 32-69 mg/kg was compatible with optimal growth in infants.

The data presented here suggests that the requirement for methionine in children with cystinosis is lower than the reported values for normal children.

Retention of the non-protein nitrogenous substance, urea, could to a certain extent, result in an apparent increase in nitrogen retention, particularly in patients with reduced glomerular function. Throughout these studies urea nitrogen determinations were repeated in the blood. No significant changes were observed in any subject which would suggest that retention of urea was not significantly altered under these conditions unless clinically undetectable changes in the body's fluid compartments occurred.

In each of our subjects, the normality of the plasma methionine, cystine, and cysteine levels was considered as another criteria of adequacy of methionine intake. The relationship between the concentration of free amino acids in plasma and dietary amino acid intakes has been the subject of several recent studies in experimental animals and man.[23-26] These studies suggest that plasma amino acid levels reflect adequacy of intake. Plasma sulfur-containing amino acid levels in our subjects were generally within normal limits on various amounts of methionine intake (Table IV.)

Summary

Results of nitrogen balance studies conducted on four children with cystinosis at various levels of methionine intake suggest that their requirement for methionine is lower than the published values for normal children.

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