A Derangement in B_{12} Metabolism Leading to Homocystinemia, Cystathioninemia and Methylmalonic Aciduria

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In mammals, vitamin B_{12} derivatives are known to function as coenzymes for two reactions: (a) methionine formation from 5-methylfolate-H_4 and homocysteine and (b) isomerization of methylmalonyl-CoA to succinyl-CoA (Weissbach and Dickerman, 1967; Hogenkamp, 1968). We have recently investigated an infant with abnormalities of both the sulfur amino acids and methylmalonic acid. This paper summarizes the results of our investigation which indicate the occurrence of a hitherto unrecognized metabolic abnormality, a defective ability to accumulate the coenzymatically active derivatives of B_{12}. As far as we are aware, this is the first proven instance of deranged vitamin metabolism in a human.

Experimental: The child was a patient at the Massachusetts General Hospital from 4 weeks of age until his death at 7-1/2 weeks. Tissues were obtained at postmortem examination and stored frozen. Quantitative analysis of amino acids was performed by ion-exchange chromatography (Efron, 1966). Methylmalonic acid in urine was assayed colorimetrically (Giorgio and Plaut, 1965). Serum folate and serum B_{12} bioassays were kindly performed by Dr. Roy Kisliuk and William Beck using respectively L. casei and L. leishmanii. Total tissue B_{12} was measured by Euglena gracilis bioassay (Lear, et al., 1954) and by a radioisotope method (Lau, et al., 1965). The two methods agreed within 15 percent. Deoxyadenosyl-B_{12} was measured as described (Abeles, et al., 1966). Betaine-homocysteine methyltransferase (betaine: L-homocysteine S-methyltransferase, EC 2.1.1.5) and methionine-activating enzyme (ATP-L-methionine-S-adenosyltransferase, EC 2.5.1.6 were assayed according to Finkelstein and Mudd (1967), cystathionine synthase and cystathionase (EC 4.2.1.15) according to Mudd, et al. (1965), and methylfolate-H_4 methyltransferase by a modification of the method of Weissbach, et al. (1963).

Results: Abnormalities of sulfur amino acids. The urine and plasma of the patient consistently contained abnormal elevations of homocystine, cystathionine, and the mixed disulfide of homocysteine and cysteine (Table I). Methionine was extremely low in plasma and not detected in urine. Other amino acids were not correspondingly decreased.

The data of Table I show also that methionine concentrations of frontal and occipital lobes of the brain were 10 percent or less of values for control tissues. The methionine of liver was low, but within the control range.

Cystathionine concentrations were high in both brain and liver.

Table I

Sulfur-containing amino acids in plasma, urine, and tissues

	Methionine	Homocystine	Cystathionine	Homocysteine-cysteine mixed disulfide
Plasma (day 12)	0.07 (0.4-0.6)	1.92 (0)	0.20 (0)	1.61 (<0.5)
Urine (days 11-12)	0 (<1.0)	5.10 (<1.0)	2.04 (<0.5)	0.83 (0)
Urine (days 16-17)	0	2.89	3.73	0.21
Brain, frontal lobe	0.1 (1.0-2.3)	0 (0)	34.4 (1.7-12.3)	1.6 (0)
Brain, occipital lobe	0.2 (2.1-2.6)	0 (0)	32.0 (1.8-9.1)	0 (0)
Liver	1.9 (1.0-8.0)	0 (0)	3.9 (0.7-2.9)	0 (0)

Plasma values are expressed as mg/100 ml; urine, as mg/24 hours; and tissue, as mg/100 g wet tissue. The values for the patient are shown, followed by control values in parentheses. The homocystine and cystathionine were isolated from the urine by column chromatography and further identified by paper chromatography, high voltage electrophoresis, and characteristic staining reactions. Ranges for free amino acids of liver and brain were obtained by analysis of tissues from four control infants. The infants from whom tissues were obtained postmortem for use as controls in the studies reported in this paper ranged in age from 7 days to 17 months. They had died of diseases without known vitamin or amino acid abnormalities.

The following enzymes were assayed in extracts prepared from liver and kidney: betaine-homocysteine methyltransferase, methylfolate-H_4 methyltransferase, cystathionine synthase, cystathionase, and methionine-activating enzyme. Of these enzymes, only methylfolate-H_4 methyltransferase was consistently decreased (Table II). In both liver and kidney a slight activity of methylfolate-H_4 methyltransferase was present, amounting to 4 percent and 23 percent of the mean control value in kidney and liver respectively. In each case the decrease was statistically significant ($p < 0.05$ by the "one-tailed" probability test (Fisher, 1948)). Betaine methyltransferase was not decreased in liver, but was slightly lower in kidney from the patient than in kidney from any control subject. The value was 39 percent of the mean control value. Although this decrease is statistically significant, the decrease is unlikely to have been sufficient to contribute markedly to the patient's difficulties, especially in view of the normal concentration of this enzyme in his liver.

Table II

Enzyme specific activities in extracts of tissues from the patient and control subjects

Enzyme and source	Patient	Control subjects	
		Mean ± SE*	Range
Methylfolate-H_4 methyltransferase			
liver	0.25	1.10 ± 0.14	0.52-1.62
kidney	0.10	2.34 ± 0.46	0.80-4.56
Betaine methyltransferase			
liver	4.66	6.22 ± 0.98	2.1 -12.5
kidney	4.05	10.4 ± 1.1	7.33-15.4

*Mean ± standard error.

For the control series, each enzyme was assayed in an extract of liver from each of 10 control subjects. Similar studies were performed in extracts of kidneys from 8 control subjects (not necessarily the same subjects that were used for the studies of liver). Specific activities, all per mg protein, are given in the following units: methylfolate-H_4 methyltransferase, mμmoles methionine/60 minutes; betaine-homocysteine methyltransferase, mμmoles methionine/45 minutes.

The possibility that a lack of 5-methylfolate-H_4 contributed to the deficient methionine synthesis was eliminated by investigation of the folate derivatives in a specimen of serum obtained from the patient on hospital day 20 five days after the administration of folic acid. The total concentration, measured by the growth response of L. casei, was 20 mμg/ml (calculated as 5-methylfolate-H_4), a high value. No growth response of either P. cerevisiae or S. faecalis could be demonstrated. These findings, while short of absolute chemical identification, strongly suggest that the dominant folate derivative in the patient's plasma was 5-methylfolate-H_4 (Stokstad and Koch, 1967) and thus are in accord with the evidence that there was a deficient activity of the enzyme which utilizes this compound.

Methylmalonic aciduria: Elevated amounts of methylmalonic acid were found in the urine of our patient. The compound was identified by thin layer and paper chromatography. For unequivocal identification the methylmalonic acid was isolated from urine (days 20-21) by chromatography on Dowex 2. The eluate was extracted into ether and crystallized from toluene-acetic acid. The crystalline material had properties typical of authentic methylmalonic acid during IR spectroscopy and gas-liquid chromatography. Quantitatively, the patient

excreted 17.6-19.3 mg/24 hours before the administration of cyano-B_{12}, 22.0 mg on hospital day 20, five days after receiving 50 µg cyano-B_{12} parenterally and 16.4 mg on hospital day 23, two days after receiving an additional 25 µg cyano-B_{12}.

B_{12} derivatives in serum and tissues (Table III): A serum sample, obtained on day 20, contained a high concentration of B_{12}. Total B_{12} in liver of the patient was within the control range. In contrast, deoxyadenosyl-B_{12} in the patient's liver was reduced to less than ten percent of the mean control value. This decrease was highly significant statistically ($p<0.01$). No deoxyadenosyl-B_{12} was detected in the patient's kidney.

Table III

B_{12} derivatives in serum and liver of the patient and control subjects

	Patient	Control subjects	
		Mean ± SE	Range
Serum, total B_{12} (day 20), mµg/ml	1.75	—	0.2-0.5
Liver, mµg/g wet tissue			
total B_{12}	56	104 ± 22	52-150
deoxyadenosyl-B_{12}	13	160 ± 22	67-240
Kidney, mµg/g wet tissue			
deoxyadenosyl-B_{12}	<2	—	9-14

Total B_{12} was measured in livers from four control subjects; deoxyadenosyl-B_{12}, in liver from seven and kidney from two control subjects. The patient's tissues were frozen within one hour of death and assayed for deoxyadenosyl-B_{12} six months later. Control tissues were obtained more than one hour after death and assayed after storage from three months to three years. The values for liver are discordant in that the content of deoxyadenosyl-B_{12} is apparently higher than total B_{12}. That this discrepancy results from a systematic difference in the two assay systems employed, rather than random error, is indicated by the fact that for the control patients the two sets of values correlate well ($r = 0.96$, $p<0.05$).

Discussion: The combination of homocystinemia (-uria), cystathioninemia (-uria) and a specific decrease of methionine in a desperately ill infant led us to postulate a difficulty in remethylation of homocysteine to methionine. This postulate was confirmed postmortem by demonstration of a specific deficiency in methylfolate-H_4 methyltransferase activity. Betaine methyltransferase activity was not commensurately decreased. The normal activities of cystathionine synthase and cystathionase rule out the enzyme deficiences which are known alternative causes of homocystinuria (Mudd, et al., 1964; Mudd, et al., 1966) and

cystathioninuria (Frimpter, 1965; Finkelstein, et al., 1966). Together, the findings indicate the physiological importance of methylfolate-H_4 methyltransferase.

The methylmalonic aciduria (discovered postmortem) indicated a second area of abnormality, most probably a deficient activity of methylmalonyl-CoA isomerase. Since methylfolate-H_4 methyltransferase and methylmalonyl-CoA isomerase are the only two mammalian enzymes known to be dependent upon B_{12} derivatives as coenzymes, an inability to accumulate coenzymatically active derivatives of B_{12} was postulated. This postulate was confirmed by demonstration that the tissues contained very little deoxyadenosyl-B_{12}, although total B_{12} was within the control range. The normal concentration of total B_{12} in the patient's liver suggests that his defect lay not in failure to transport B_{12} into cells, but rather in an abnormal metabolism of B_{12}. The abnormality could have been a failure to transform vitamin B_{12} into coenzymatically active derivatives. A less likely alternative is excessive breakdown or loss of such derivatives. The precise location of the metabolic block and the relationship of this patient to others recently described who share one or more abnormalities with him are currently under investigation.

Future cases of the disease described here might benefit from methionine treatment and/or large doses of betaine or choline. Possibly, massive doses of vitamin B_{12} (Rosenberg, et al., 1968) or hydroxy-B_{12} will be useful. Attempts should certainly be made to circumvent the presumptive metabolic block by administration of methyl-B_{12}, deoxyadenosyl-B_{12} or another modified form of B_{12}.

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