

more complex process than excessive postsynaptic inhibition. Such a process would certainly not explain the dysmyelination seen pathologically. In NKH, in which a defect exists in the conversion of glycine to serine, the normal balance of amino acids available for the synthesis of proteins in the developing brain may be altered, and dysmyelination could ensue.

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## *Homocystinuria caused by 5,10-methylenetetrahydrofolate reductase deficiency: A case in an infant responding to methionine, folinic acid, pyridoxine, and vitamin B<sub>12</sub> therapy*

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DEFICIENCY of 5,10-methylenetetrahydrofolate reductase (EC 1.1.1.68) has been described by Mudd et al<sup>1</sup>; the clinical manifestations of this disorder varied greatly, depending on the age of onset. When the onset has occurred in infancy, 5,10-MTHF reductase deficiency has been lethal. We describe the clinical features in an infant who showed good response to therapy with methionine, vitamins B<sub>6</sub>, B<sub>12</sub>, and folinic acid.

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## CASE REPORT

A 7-month-old black girl was admitted to hospital because of progressive deterioration of mental development. There was no consanguinity in her family. She was born after an uneventful gestation and delivery (birth weight 3,140 gm, height 50 cm, head circumference 33 cm). She was breast fed and had been doing

### Abbreviations used

5,10-MTHF:	5,10-methylenetetrahydrofolate
HC:	head circumference
EEG:	electroencephalogram
5, methyl THF:	5, methyltetrahydrofolate

well until the age of 5 months, at which time she began to regress. At 7 months of age she was microcephalic (HC 40.5 cm) and lethargic. Rapidly she became comatose, with frequent apnea and hypothermia of 35.1°C. Initial laboratory evaluation, including cerebrospinal fluid analysis, was unremarkable. Radiographs

Gnosis Ex. 1026

**Table I.** Concentration of amino acids\* and serum folate before and during therapy

Amino acids and folate	Normal range	Day of therapy			
		0	13	32	106
Homocysteine	0 (0)	233 (667)	0 (196)	0 (0)	0 (0)
Cystine	50-110 (40-160)	25	30	30 (48)	120 (26)
Methionine	10-50 (20-95)	ND <sup>+</sup> (27)	39 (32)	79 (43)	40 (13)
Glycine	200-300 (160-1420)	297 (561)	342 (1350)	257 (378)	255 (88)
Serine	140-240 (150-540)	350 (116)	223 (9)	256 (79)	278 (9)
Folate (ng/ml)	6-15	1.3		19	
Treatment					
Folinic acid (im mg/day)		50	25	25	15
Methionine (by mouth mg/day)		900	900	900	900
Vitamin B <sub>6</sub> (by mouth mg/day)		100	100	100	100
Vitamin B <sub>12</sub> (im mg/day)		1	0.5	0.5	0.5

\*Plasma values micromoles per milliliter and urine values in parenthesis micromoles per day.

<sup>+</sup>Not detectable.

of the skull showed narrowed sutures and fontanelles. Computed tomographic scan revealed mild cerebral atrophy without abnormality of the brain substance. EEG showed slowing of the baseline tracing occurring at the same time as the apnea. Nerve conduction velocity was normal. Cyanide nitroprusside reaction on urine was positive. Ion exchange chromatography<sup>2</sup> of urine amino acids showed the presence of homocystine: 667  $\mu\text{mol}/24$  hours (normal = 0). Methylmalonic acid was not detected by *p*-nitroaniline.<sup>3</sup> Serum amino acid ion exchange chromatography showed a homocystinemia of 233  $\mu\text{mol}/\text{L}$  (normal = 0); methionine was not detectable (normal = 10 to 50  $\mu\text{mol}/\text{L}$ ), and no other amino acid abnormality was detected in blood or urine. Serum folate concentration was 1.3 ng/ml, *Lactobacillus casei*, normal 6 to 15<sup>4</sup> (SI units 2.9 nanomol/L, normal 13.6 to 27.5). Serum vitamin B<sub>12</sub> level was 880 pg/ml, *Lactobacillus leishmanii*, normal 200 to 400<sup>4</sup> (SI units 590 picomol/L, normal 145 to 370). Thus the diagnosis of 5,10-MTHF reductase deficiency was highly probable. At the age of 7½ months, treatment was started with methionine and pyridoxine orally, and with folinic acid and hydroxocobalamin intramuscularly (Table I). The degree of emergency did not allow us to try each drug separately. The response to therapy was dramatic. Rapidly the patient became more alert, with disappearance of apnea and hypothermia. One week later she could eat again. The neurologic follow-up was satisfactory. At 16 months of age she had an HC of 46 cm. Results of an EEG were normal. Repeated ophthalmologic examination showed no abnormality in her lenses or fundi.

## SPECIAL INVESTIGATIONS

The 5,10-MTHF reductase activity in cultured skin fibroblasts, obtained after parents' informed consent, was measured by methods previously described.<sup>5,6</sup> Folate distribution in cultured skin fibroblasts was assayed by previously described methods.<sup>7</sup>

## RESULTS

The 5,10-MTHF reductase activity, expressed in n.mol CHO/mg protein/hour, was 8.7 for the control (MCH 23) and 0.18 for the Patient (735), i.e., 2% of control value.

Data obtained from study of folate distribution in cultured skin fibroblasts were as follows (Table II): Normal cells grown under these conditions have 60 to 80% of intracellular folate as 5-methyltetrahydrofolate.<sup>7</sup> In our patient's cultured fibroblasts, 5-methyl THF was estimated to represent 18% of total cell folate when assayed by the differential growth of *L. casei* and *Pediococcus cerevisiae*, and only 1.4% of total intracellular folate was estimated to represent 5-methyl THF when the extracts were analyzed by DEAE Sephadex chromatography followed by microbiologic assay. This finding is consistent with severe deficiency of 5,10 MTHF reductase.

## DISCUSSION

Since the first report of three cases of 5,10 MTHF reductase deficiency by Mudd et al<sup>1</sup> in 1972, nine other patients have been described (for listing of published cases see reference<sup>7</sup>). The clinical severity and enzyme deficiency have varied greatly, depending on the age of the patient; an infantile form of this disorder can be identified. In infants, after the disease has become clinically apparent, there is rapid neurologic deterioration and almost certain death. The biologic abnormalities of 5,10-MTHF reductase deficiency include homocystinuria, homocystinemia, hypomethioninemia, and low serum folate concentration. Low serum folate values result from a lack of 5-methyl THF, which is the main serum (and CSF) form of folate. This substance is also lacking in cells, in which it is normally the main fraction of folate.<sup>7</sup> 5-methyl THF deficiency prevents the formation of methionine and S-adenosyl-methionine, the important methyl donor for cerebral amine synthesis.<sup>8</sup> It therefore seems reasonable to supplement the diet of patients with 5,10-MTHF reductase deficiency with folate and methionine. In 5,10-MTHF reductase deficiency, as in classical homocystinuria caused by cystathionine- $\beta$ -synthase deficiency (E.C. 4.2.1.22), vascular lesions are present but the difference between the extent of vascular lesions and the severity of demyelination observed<sup>9</sup> (S. Berlow: Personal communication) makes it likely that homocystine itself is toxic. Therefore, lowering blood

Table II. Folate distribution in fibroblasts

Microbiologic determination*		L. casei		P. cerevisiae		% 5-CH <sub>3</sub> H <sub>4</sub> PteGlu
		ng folate/10 <sup>6</sup> cells				
Control cells (n = 10, $\bar{x} \pm$ S.D.)		3.31 $\pm$ 1.57		0.56 $\pm$ 0.51		0.83 $\pm$ 0.10
Patient		4.69		3.80		0.18

DEAE Sephadex†	Fraction number					
	I	II	III	IV	V	Total
	ng folate/10 <sup>6</sup> cells					
Controls (n = 2, $\bar{x}$ )	0.45	0.12	0.73	3.71	0.24	5.24
Patient	0.43	0.12	0.26	0.03	0.05	2.14

\*Folate levels in fibroblast extracts were determined directly by microbiological assay as previously described.<sup>7</sup>  
†Fibroblast extracts were fractionated on DEAE-Sephadex as previously described.<sup>7</sup> The presumed identity of the folate fractions eluted sequentially: I, 10-formyltetrahydrofolate; II, 10-formyldihydrofolate; III, 5-formyltetrahydrofolate; IV, 5-methyltetrahydrofolate; V, tetrahydrofolate.

homocystine concentration is a rational goal of treatment, in addition to folate and methionine supplementation.

Among the cases of 5,10-MTHF reductase deficiency that have occurred in infants, various treatments have been tried without clinical improvement; these include folic acid alone or in association with thiamine, pyridoxine, vitamin B<sub>12</sub>, and choline.<sup>6, 7</sup> However, in the patient of Mudd and Freeman<sup>7</sup> (15 years of age) there was a good response to folic acid therapy. In our patient the response to therapy was dramatic (methionine and pyridoxine by mouth, folic acid and vitamin B<sub>12</sub> intramuscularly). The variation of response to therapy seems roughly related to the degree of enzyme deficiency. If some enzyme activity persists, giving pharmacologic doses of folinic or folic acid will allow the formation of 5, methyl THF and thus methionine, with a decrease of homocystinemia.<sup>1</sup> If the enzyme activity is very low or almost absent, the low level of tetrahydrofolate will be normalized but only negligible production of 5,methyl THF may be expected. Although 5,methyl THF would be desirable for use in therapy, it is probably too unstable. Furthermore, fibroblasts from patients with a deficiency of 5,10-MTHF reductase cannot grow in tissue culture medium in which homocysteine replaces methionine<sup>1</sup> even when supplied with exogenous 5,methyl THF (Hoffman RM, Rosenblatt DS, and Erbe RW: Unpublished data). This finding might explain the requirement for exogenous methionine in our patient. Vitamin B<sub>12</sub> supplementation may enhance methionine formation but only in the presence of 5,methyl THF; in our case every attempt to lower the dose of vitamin B<sub>12</sub> resulted in reappearance of lethargy. Pyridoxine therapy may increase cystathionine-β-synthase activity, thus decreasing homocystinemia. Thus, it seems that in patients with 5,10-MTHF reductase deficiency of infancy,

the replacement therapy should be: methionine, folinic or folic acid, and vitamins B<sub>12</sub> and B<sub>6</sub>. Indeed, we have no proof that these four drugs were necessary in our patient, but the degree of emergency did not allow us to try each drug one after the other. In a future case, beginning therapy with methionine alone could be tried, and followed by folinic or folic acid.

The rapidity of neurologic deterioration in infants with 5,10-MTHF reductase deficiency leads us to recommend starting the treatment as early as possible. Urine screening of newborn infants with cyanide, or preferably with silver nitroprusside, seems the best means of early detection of this condition. In families at risk, antenatal diagnosis of 5,10-MTHF reductase deficiency, an autosomal recessive condition, is possible by study of cultured amniotic fluid cells.<sup>6, 10</sup>

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## *The vagus nerve and sudden infant death syndrome: A morphometric study*

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ABNORMAL NEURAL BREATHING CONTROL has been implicated in the sudden infant death syndrome. Since the vagus nerve is important in tidal volume, breathing rate, and respiratory reflexes, especially in infants, we examined the structure of the vagus nerve in SIDS victims and similarly aged infants who had died of other causes.

### SUBJECTS AND METHODS

Our subjects were infants on whom autopsies were performed in the Department of Pathology during 1976 to June, 1979: group A consisted of approximately three-quarters of all infants who had died of SIDS and had autopsies performed; group B consisted of infants of similar postconceptual age (PCA: gestation plus postnatal age) at death who had died of other causes (acute infection, acyanotic congenital heart disease, trauma).

From each subject, we removed 2 cm of left cervical vagus nerve, proximal to the origin of the left recurrent laryngeal nerve, within 24 hours of death. The nerve was removed by one person at the same site. Specimens were fixed by immersion at 4°C for 12 hours in 3% glutaraldehyde dissolved in 0.1M sodium phosphate buffer (pH 7.4). After a half-hour wash in the same buffer, each nerve was dehydrated in a series of increasingly concentrated ethyl-alcohol solutions, infiltrated with propylene oxide,

embedded in Epon with Araldite,<sup>1</sup> sliced into 1  $\mu$ m thick transverse sections, and stained with 1% toluidine blue.<sup>2</sup>

A low-power photomicrograph (10 $\times$ ) was made of the entire cross-section of each specimen, and enlarged to 100 $\times$ . We traced the perimeter of the endoneurium of each fascicle within the nerve cross-section with a digitizer connected to a programmed calculator (Hewlett-Packard 9810). Then, the sum of the areas of the fascicles within each nerve was divided by the square of the final magnification of the entire nerve cross-section, to obtain the transverse fascicular area.

#### Abbreviations used

SIDS:	sudden infant death syndrome
TFA:	transverse fascicular area
MVF:	myelinated vagus fibers
PCA:	postconceptual age

We tallied the myelinated vagus fibers in alternate fields (photomicrographs of final magnification 1,000 $\times$ , Fig. 1) within the TFA; we included fibers touching the left and lower boundaries of the field and excluded those touching the right and upper boundaries. Then we measured the MVF diameters (axon + myelin) with a Zeiss TGZ3 particle-size analyzer; we approximated the light-spot to the area of circular fibers, the central area of scalloped fibers, the minor axis of elliptical, and the base of triangular ones. We did not measure irregular-shaped fibers. The analyzer was set in the "regular range," in exponential mode, and for frequency distribution.

The computer calculated estimated density of MVF/mm<sup>2</sup> of TFA, and per cross-section of vagus nerve, and plotted size-distribution histograms.<sup>3</sup> We then plotted the

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