Methylmalonic acidemia and nonketotic hyperglycinaemia

Clinical and biochemical aspects

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Corbeel, L., Tada, K., Colombo, J. P., Eeckels, R., Eggermont, E., Jaeken, J., Den Tandt, W., Harvengt, L., Delhaye, J., and Deloecker, W. (1975). *Archives of Disease in Childhood*, 50, 103. **Methylmalonic acidemia and nonketotic hyperglycinaemia: clinical and biochemical aspects.** The clinical and metabolic data of 2 cases of methylmalonic acidemia with propionic acidemia are reported together with those of 3 other patients with nonketotic hyperglycinaemia.

Liver enzymatic studies showed decreased activity *in vitro* of the glycine cleavage enzyme in one patient with methylmalonic acidemia as well as in 2 unrelated patients with nonketotic hyperglycinaemia, while the activity of the serine hydroxymethylase enzyme was normal.

Hyperammonaemia was substantiated in one patient with methylmalonic acidemia and also in one child with nonketotic hyperglycinaemia. The activity of the enzymes of the urea cycle, determined in the liver of this nonketotic child, was normal except for a decrease of the carbamyl phosphate synthetase enzyme to 15% of normal.

Severe episodes of ketoacidosis in early life, first described as ketotic hyperglycinaemia by Childs *et al.* (1961), are associated with four distinct conditions: isovaleric acidemia (Tanaka *et al.*, 1966), methylmalonic acidemia (Oberholzer *et al.*, 1967), propionic acidemia (Hommes *et al.*, 1968), and a defect in isoleucine metabolism (Keating *et al.*, 1972). Hyperglycinaemia is variable in these cases of organic aciduria and is often accompanied by an increase in several other plasma amino acids. Neutropenia, anaemia, thrombocytopenia, and hypoproteinaemia are frequently observed. In nonketotic hyperglycinaemia (Mabry and Karam, 1963; Schreier and Müller, 1964) hypotonia, lethargy, and convulsions develop soon after birth. There is no ketoacidosis, no neutropenia, no thrombocytopenia, and no increase in propionic, isovaleric, or methylmalonic acid in this condition. It was shown by Ando *et al.* (1968) and Baumgartner, Ando, and Nyhan

(1969), and *in vitro* by means of enzymatic liver studies by Tada *et al.* (1969) and Yoshida and Kikuchi (1969), that patients with nonketotic hyperglycinaemia have a defect of the glycine cleavage enzyme necessary for the first step in the conversion of glycine into serine, while the serine hydroxymethylase enzyme governing the second step is intact (Fig. 1).

This study concerns the clinical and biochemical data of 2 cases of methylmalonic acidemia and 3 patients with nonketotic hyperglycinaemia.

Methods

Analytical procedures. Amino acids were analysed with a Technicon amino acid analyser using the technique of Efron (1965) except for the determination of serine during glycine loading tests because of the overlapping of serine and asparagine. Serine was measured with a Beckman amino acid analyser according to the technique of Spackman, Stein, and Moore (1958). Gas chromatography of the volatile fatty acid was performed with a Varian Aerograph instrument

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FIG. 1.—Metabolic interconversion of glycine and serine.

equipped with a Porapak X column according to Mahadevan and Zieve (1969). Methylmalonic acid was identified in the urine by thin layer chromatography, using the technique of Gutteridge and Wright (1970). Urinary oxalates were determined according to Archer *et al.* (1957), blood ammonia with the microdiffusion method of Seligson and Hirahara (1957).

Biopsy specimens for enzyme studies. Liver specimens of Case 4 were obtained by needle biopsy, those of Cases 2 and 5 at necropsy performed within 15 minutes of death. The tissue specimens were kept frozen at -20° until analysis. Liver specimens of 3 other patients were obtained in the same conditions and served as controls: one had trisomy 13 and died at the age of 4 months, one died 3 days after birth from congenital heart disease, and the third, aged 6 days, died from pneumonia. The enzymes of the urea cycle were measured as described by Colombo and Richterich, (1968). The glycine cleavage and serine hydroxymethylase activities were measured as previously described (Tada *et al.*, 1969). As shown in Fig. 1, $^{14}\text{CO}_2$ formation from the oxidation of glycine-1- ^{14}C is normally much higher compared with $^{14}\text{CO}_2$ formation from glycine-2- ^{14}C . When the glycine cleavage reaction does not proceed normally the amount $^{14}\text{CO}_2$ formed is about equal for the two components glycine-1- ^{14}C and glycine-2- ^{14}C . The incorporation of ^{14}C into serine will then also be much lower as will be the ratio of ^{14}C incorporation glycine-2- ^{14}C /glycine-1- ^{14}C into serine.

Patients

Case 1 (methylmalonic acidemia). A female was the fourth child of healthy unrelated parents. A

sib died at the age of 10 days after an episode of vomiting. The child started vomiting at the age of 6 days and was referred to our department at the age of 5 weeks. She was dehydrated and weighed only 2700 g. Consciousness and muscle tone were diminished. The liver was felt 4 cm below the costal ridge. In spite of intravenous fluid therapy, low protein diet, and administration of vitamin B₁₂, she died at the age of 7 weeks. Laboratory data are shown in Table I.

TABLE I
Laboratory data of 2 patients with methylmalonic acidemia

	Case 1 (at 5 w)	Case 2 (at 2½ m)
Blood		
Hb (g/dl)	7.2	14.8(*)
Red cells (N/mm ³)	2 540 000	4 100 000(*)
White cells (N/mm ³)	1 000	2 700
Thrombocytes (N/mm ³)	24 000	318 000
pH	7.31	7.06
HCO ₃ (mEq/l)	14.5	9.5
PCO ₂ (mmHg)	23	29
PO ₂ (mmHg)	—	46
Total proteins (g/100 ml)	5.3	5.95
Ammonia (μg/100 ml)	454	—
Lactic acid (mg/100 ml)	36.1	104
Propionic acid (mg/100 ml)	2.696	43.076
Urine		
Acetest	++	+++
Ketoacids	++	++
Methylmalonic acid	+++	+++

*After transfusion.

Case 2 (methylmalonic acidemia). A girl was the first child of healthy unrelated parents. She vomited from the first week of life and was hospitalized at the age of 2½ months. She was comatose, dehydrated, and underweight (3700 g). She was also hypertonic and cyanotic. The liver was enlarged to 5 cm below the costal margin. She died 48 hours after admission in spite of intravenous fluid therapy, vitamin B₁₂, and peritoneal dialysis. Laboratory data shown are in Table I.

Case 3 (nonketotic hyperglycinaemia). The second child of healthy, unrelated parents with 5 girls. The first sib died on the fourth day of life after an episode of lethargy, convulsions, and cyanosis. The third child died 2 days after birth in identical conditions. The fourth child is still alive and normal. The fifth child is also affected by the same condition and is reported as Case 4.

On admission at the age of five days she had been comatose and hypotonic since the third day of life and had convulsed. She developed microcephaly and severe mental retardation and died at the age of 7 years.

Case 4 (nonketotic hyperglycinaemia). The sib of Case 3 had blood levels of glycine up to 6 mg/100 ml on the third day of life but the parents refused hospitalization up to the age of 3 months. At that time she was already retarded and had convulsive seizures. Low protein diet, pyridoxine up to 1000 mg/day, folic acid up to 20 mg/day, and nicotinamide up to 100 mg/day

did not prevent mental retardation. She is still alive and is now 6 years of age.

Case 5 (nonketotic hyperglycinaemia). A female born of healthy unrelated parents was normal up to the fourth day of life, when she was found comatose and hypotonic. On arrival in hospital she was unconscious. Examinations of blood and urine were normal except for glycine (17.55 mg/100 ml), serum GOT (306 IU; normal up to 32 IU), glucose (152 mg/100 ml), and blood ammonia (295 µg/100 ml). In spite of symptomatic treatment, the child died 24 hours after admission.

Results

Methylmalonic acidemia (Cases 1 and 2).

Blood and urine amino acids. Plasma glycine was increased up to 8.2–12.7 mg/100 ml in Case 1 but only to 4.97 mg/100 ml in Case 2 (Table II). In both patients increased levels of glutamic acid, valine, isoleucine, leucine, and lysine were also found. Plasma alanine was high only in Case 1. The changes of plasma amino acids could explain the observed aminoaciduria. In Case 1 the 24-hour urinary excretion of glycine was 218 mg (normal 72±30), of glutamine and glutamic acid 327 mg (normal 90±50), of valine 15 mg (normal 5±2), of leucine 12.5 mg (normal 5±2), of lysine 55 mg (normal 34±24), and of alanine

TABLE II
Plasma amino acids (mg/100 ml) in methylmalonic and nonketotic hyperglycinaemia

	Methylmalonic acidemia*				Nonketotic form			Normal values
	Case 1		Case 2		Case 3	Case 4	Case 5	
	A	B	A	B				
Taurine	1.7	0.4	0.62	2.15	0.61	0.51	7.1	0.9–2.7
Threonine	0.81	1.7	0.62	0.95	1.64	0.85	0.45	1.36–3.9
Serine	1.32	2.5	1.15	1.13	1.89	1.55	0.62	0.99–2.55
Glutamine	5.24	6.8	2.46	0.11	1.28	1.0	5.85	7.86–14.0
Glutamic acid†	5.32	14.4	4.55	4.7	3.5	2.6	14.4	0.3–1.5
Citrulline	0.3	0.4	0.29	0.53	0.53	0.5	0.63	0.13–0.50
Glycine	8.2	12.7	3.47	4.97	7.71	6.81	17.55	1.6–3.8
Alanine	4.58	7.86	1.87	1.83	3.17	3.97	18.0	2.10–3.6
α-Aminobutyric acid	0.24	0.3	0.26	0.12	0.08	0.24	?	0.06–0.3
Valine	2.87	6.12	5.16	2.41	2.24	1.39	3.85	0.94–2.8
Cystine	0.33	0.4	0.18	0.04	0.02	0.2	0.18	0.83–2.0
Methionine	0.21	0.5	0.28	0.29	0.42	0.15	0.17	0.13–0.61
Isoleucine	0.67	2.55	2.66	1.06	0.95	0.68	1.55	0.35–0.69
Leucine	2.6	7.4	5.95	2.32	1.84	1.38	1.1	0.61–1.4
Tyrosine	0.65	1.44	1.02	1.26	1.57	0.78	2.4	0.76–1.8
Phenylalanine	0.92	1.8	0.8	1.41	1.39	0.83	4.1	0.69–1.82
Ornithine	0.38	0.65	0.59	1.28	1.47	0.82	1.7	0.65–2.0
Lysine	2.3	6	5.07	4.36	3.8	2.73	7.8	1.67–3.93
Histidine	0.8	5.6	1.19	1.32	2.0	1.2	2.2	1.76–1.77
Arginine	0.49	0.67	0.51	0.42	0.8	0.6	0.1	0.38–1.53

*Plasma amino acids measured on admission (A) and just before death (B).

†High values are due to degradation of glutamine in glutamic acid during storage of the plasma.

TABLE III
Plasma organic acids in methylmalonic acidemia

Acids ($\mu\text{g}/100\text{ ml}$)	Cases			Parents of Case 2		Normal values†
	(1)	(1)*	(2)	Father	Mother	
Propionic	2 696	7 600	4 307	185	330	0-160
Isobutyric	76	—	96	197	245	0- 53
Isovaleric	114	—	240	—	—	0- 60
Methylmalonic	—	12 100	—	—	—	
β -OH-isovaleric	—	4 100	—	—	—	

*Measurements made by Dr. O. Stokke, Oslo.

†Normal values from present study.

62 mg (normal 16 ± 6). In Case 2, the aminoaciduria was within normal limits.

Organic acids. Propionic acid was conspicuously increased in the plasma of the 2 patients and slightly raised in the parents of Case 2 (Table III). There were also slightly increased levels of isobutyric and isovaleric acids. Increased plasma levels of methylmalonic acid and β -hydroxyvaleric acid could also be shown*. Blood lactate was 36 mg/100 ml in Case 1, while blood glucose was down to 34 mg/100 ml and blood bicarbonate to 14.5 mEq/l. In Case 2 blood lactate was 104 mg/100 ml while blood glucose and bicarbonate were 37 mg/100 ml and 9.5 mEq/l, respectively. However, the patient was cyanotic and the capillary Po_2 was 56 mmHg while breathing 100% oxygen. In Case 2 plasma free fatty acids were as high as 873 $\mu\text{Eq/l}$, but had a normal pattern and contained no compounds with an odd number of carbons. In the urine, methylmalonic acid was found in the 2 cases

and amounted to about 300 mg/100 ml in Case 1 and 500 mg/100 ml in Case 2 (normal values $<0.3\text{ mg}/100\text{ ml}$).

Blood ammonia, urea, and transaminases. In Case 1 blood ammonia was 454 $\mu\text{g}/100\text{ ml}$ (normal $<80\text{ }\mu\text{g}/100\text{ ml}$), blood urea 37 mg/100 ml, SGOT 76 IU (normal 7-35 IU), and SGPT 47 IU (normal 6-32 IU).

Enzyme studies in Case 2. The activity of the glycine cleavage enzyme (Fig. 1) as determined by the formation of $^{14}\text{CO}_2$ formed from glycine-1- ^{14}C and the incorporation of carbon 2 from glycine-2- ^{14}C into serine was about 20% lower than that of the controls, while the activity of the serine hydroxymethylase enzyme was normal (Tables IV and V).

Nonketotic hyperglycinaemia (Cases 3, 4, and 5).

Amino acid metabolism. The plasma amino acid pattern of Cases 3 and 4 showed only an increase

TABLE IV
*Glycine cleavage reaction in the liver**

	$^{14}\text{CO}_2$ Formation		Incorporation of ^{14}C into serine		
	From glycine-1- ^{14}C (cpm/10 mg protein)	From glycine-2- ^{14}C (cpm/10 mg protein)	From glycine-1- ^{14}C (cpm/10 mg protein)	From glycine-2- ^{14}C (cpm/10 mg protein)	Rate of incorporation glycine-2- ^{14}C / glycine-1- ^{14}C
Controls					
1	3 668	883	4 470	7 739	1.73
2	2 886	858	2 580	4 160	1.67
3	3 320	751	2 617	5 430	2.07
Hyperglycinaemia					
Case 2 methylmalonic acid	728	874	818	1 014	1.24
Case 4 nonketotic	548	615	782	855	1.09
Case 5 nonketotic	476	423	649	795	1.22

*Determinations by K.T.

*Kindly determined by Dr. O. Stokke, Oslo.

TABLE V

Serine hydroxymethylase activity in the liver

Subjects	Serine hydroxymethylase ($\mu\text{mol/mg protein per h}$)
Controls	
1	0.83
2	1.09
3	0.66
Case 2 (methylmalonic acid)	0.65
Case 5 (nonketotic hyperglycinaemia)	0.72

of glycine (Table II). Case 5 had a conspicuous increase of glycine and also of alanine and taurine. Oral administration of 100–200 mg glycine/kg body weight resulted in coma and convulsions lasting from 2 to 4 days in both patients. During the loads of glycine the electroencephalogram deteriorated diffusely and no biochemical alterations except the hyperglycinaemia itself and a decrease of most amino acids, serine and taurine included, could be detected. Even pyridoxine, administered in a dose of 2000 mg/day for 2 weeks before glycine loading, was without effect. Normal children tolerate very well similar amounts of glycine. An oral load of 200 mg/kg body weight serine to Case 4 resulted in an increase of plasma glycine from 6.2 to 12 mg/100 ml. When 100 mg/kg L-serine was administered orally to Case 3, plasma glycine increased only from 5.7 to 6.5 mg/100 ml. The rise of glycine did not result in clinical symptoms. Furthermore, oral administration of 100–200 mg/kg L-leucine, L-valine, L-methionine, or L-threonine was clinically well tolerated.

Organic acids. Blood propionic acid and urinary methylmalonic acid were normal in the 3 patients with nonketotic hyperglycinaemia. Even a load of 100 mg/kg glycine did not result in abnormal levels of propionic, isobutyric, or isovaleric acids. Oral administration of 200 mg propionate/kg was without effect on plasma glycine.

Oxalates. Urinary excretion of oxalates was normal and varied between 0.24 and 1.95 mg/kg per day in Cases 3 and 4. During glycine loading the oxalate excretion increased from 1.5 mg to 7.7 mg/kg per day.

Enzyme studies in Cases 4 and 5. The activity of the cleavage enzyme as measured by the formation of $^{14}\text{CO}_2$ from glycine-1- ^{14}C was reduced to about 15% of the control values. The incorpora-

tion of carbon 2 from radioactive glycine into serine was about 20% of the activity measured in control livers. On the other hand, serine hydroxymethylase enzyme activity was normal in Case 5 (Table IV and V). As shown in Table VI, the liver

TABLE VI

Liver enzymes of the urea cycle ($\mu\text{mol/h}$ and g of wet weight)

	Case 5	Control
Carbamyl phosphate synthetase	46	296
Ornithine carbamyl transferase	95	99
Argininosuccinate synthetase	12	15
Argininosuccinate cleavage enzyme	44	65
Arginase	7 210	7 553

enzyme activities of the urea cycle were normal except for carbamyl phosphate synthetase which was 15% of the control value.

Necropsy findings in Case 5. There was slight fatty degeneration of the hepatocytes without sign of hepatitis or liver necrosis. In the brain, myelin was totally absent. There was also a spongy degeneration of the white matter and to a lesser extent of the atrophic cortical neurones.

Discussion

Although we were unable to measure methylmalonyl-CoA mutase and racemase, the biochemical findings in Cases 1 and 2 suggest a primary block at this level (Oberholzer *et al.*, 1967; Stokke *et al.*, 1967; Rosenberg, Lilljeqvist, and Hsia, 1968; Ando *et al.*, 1971; Kang, Snodgrass, and Gerald, 1972). This could explain the simultaneous accumulation of propionic acid, and has also been found by Ando *et al.*, (1971). An increase of isobutyric acid and isovaleric acid was similarly noticed in propionic acidaemia (Ando *et al.*, 1971). However, it is clear from Fig. 2 that neither the accumulation of ketones nor the increased levels of glycine, lactate, and leucine, together with their degradation products isovaleric and β -OH-isovaleric acid, can be explained only by a block of methylmalonyl-CoA mutase or racemase. Gompertz *et al.* (1970) suggested that some of the biochemical lesions seen in propionic acidaemia, such as the defective oxidation of valine and isoleucine and the production of long chain ketones, could be due to a relative lack of free coenzyme A, secondary to the accumulation of propionyl-CoA. The recent finding of Ando *et al.* (1972b) of an appreciable excretion of methylcitrate in patients

FIG. 2.—Metabolic disturbances in ketoacidosis of infancy.

with defective propionic acid metabolism is interesting. Methylcitrate could result from the condensation of propionyl-CoA with oxaloacetate, similar to the condensation of acetyl-CoA and oxaloacetate to citrate. The accumulated methylcitrate could thus interfere with the normal function of the citric acid cycle and this could explain many of the biochemical features. The finding of a decreased activity *in vitro* of the liver glycine cleavage enzyme in Case 2 with methylmalonic acidemia was also observed *in vivo* in 2 patients with propionic acidemia by Ando *et al.* (1972a). Since there is no doubt that the primary block of methylmalonic acidemia is located in methylmalonyl-CoA mutase or racemase, it is reasonable to presume that the decreased activity of the glycine cleavage enzyme reaction may be a secondary metabolic disturbance.

Nonketotic hyperglycinaemia. The cause of the deleterious effect of administration of glycine could be glycine itself, since no disturbances of amino acid metabolism besides the increase of glycine and the decrease of the other amino acids, no increase in blood ammonia, no abnormality of carbohydrate and electrolyte metabolism, and no ketoacidosis could be detected. According to Snyder *et al.* (1973), glycine is a potent post-synaptic inhibitory neurotransmitter in the brainstem and spinal cord and possibly in the cerebral cortex too. In normal conditions the central nervous system is protected from abnormally high levels of glycine by regulatory mechanisms at the blood brain barrier (Shank and Aprison, 1970). In nonketotic hyperglycinaemia, however, CSF gly-

cine levels are up to 10 times higher than normal and therefore are able to inhibit neurotransmission. The neurological symptoms appearing after the administration of glycine could satisfactorily be explained by this mechanism. The absence of myelination, as observed in Case 5, could be another factor contributing to the severe mental retardation in these patients.

Hyperammonaemia can occur in methylmalonic acidemia, as in Case 1, as well as in nonketotic hyperglycinaemia, as in Case 5. The hyperammonaemia can explain a secondary increase of glutamine and is also found in cases of hyperammonaemia due to a defect in ureogenesis (Corbeel *et al.*, 1969). Kirkman and Kiesel (1969) found a decreased activity of carbamyl phosphate synthetase in a case of methylmalonic aciduria with ammonia intoxication. This was also observed by Freeman *et al.* (1970) in a ketotic patient but no propionic acid values were reported. Since this enzyme is very labile the decreased activity may be an artefact. Wada *et al.* (1972) found normal activities of the carbamyl phosphate synthetase as well as of the other enzyme of the urea cycle in a case of nonketotic hyperglycinaemia with hyperammonaemia.

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