Acute Neonatal Nonketotic Hyperglycinemia: Normal Propionate and Methylmalonate Metabolism

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Extract

Propionyl-CoA carboxylase and combined methylmalonyl-CoA (MMA-CoA) racemase and -mutase activities were studied in liver and fibroblasts of two patients with the acute neonatal form of nonketotic hyperglycinemia. In all experiments, these enzyme activities studied in tissues of the patients were within the range of healthy control subjects, whereas no propionyl-CoA carboxylase activity was measurable in the fibroblasts of a patient with propionic acidemia. Subcellular fractionation of liver and fibroblasts indicated that the normal amounts of MMA-CoA found after incubation of whole tissue homogenate were formed by propionyl-CoA carboxylase, a mitochondrial enzyme, and not by acetyl-CoA carboxylase, which theoretically could also be involved in the carboxylation of propionyl-CoA.

From the above data as well as from clinical and biochemical observations in three patients, it was concluded that there exists a true nonketotic hyperglycinemia which is not related etiologically to the different disorders of the ketotic hyperglycinemia syndrome. True nonketotic hyperglycinemia is not associated with ketoacidosis even after loading with propionate- and MMA precursors. It must be distinguished by exclusion from mild forms of the ketotic hyperglycinemia syndrome which may present clinically as hyperglycinemia without ketosis.

Speculation

The metabolism of propionate, methylmalonate, and branched chain amino acids is normal in true nonketotic hyperglycinemia. Further investigations will have to show whether the observed block in the glycine cleavage reaction to CO_2 , NH_3 , and a single-carbon tetrahydrofolate derivative represents the primary enzymatic defect. In view of the four enzymatic steps involved in this complex reaction, it may well be that true nonketotic hyperglycinemia also represents a heterogeneous syndrome. The elevated glycine concentrations in the central nervous system (CNS) may play a significant role in the development of the neurologic symptoms.

Hyperglycinemia is found in nonketotic hyperglycinemia and in disorders of propionate, methylmalonate (MMA), and branched chain amino acid intermediate metabolism (ketotic hyperglycinemia syndrome) (29). Mild forms of the ketotic hyperglycinemia syndrome, in which mental retardation and elevated concentrations of glycine are present without episodes of ketoacidosis, may be impossible to distinguish from nonketotic hyperglycinemia on the basis of clinical symptoms alone (24, 27, 34).

In nonketotic hyperglycinemia a defective oxidation of C_1 of glycine to CO_2 combined with a defective conversion of C_2 of glycine to C_3 of serine have been demonstrated *in vivo* (2, 8) and *in vitro* (10, 33). Recently, however, a similar defect has also been found in patients with the ketotic hyperglycinemia syndrome (four patients with propionic acidemia (1, 27, 34) and one patient with methylmalonic acidemia (33)); i.e., in all five patients a coexistence of defective activity of the glycine cleavage reaction and propionyl-CoA carboxylase or methylmalonyl-CoA mutase, respectively,

was demonstrated. In line with these data, Hillman *et al.* (15) found that isoleucine inhibited the oxidation of glycine to CO_2 in fibroblasts of a patient with a β -ketothiolase deficiency and ketotic hyperglycinemia. These observations suggest that the defect in the glycine cleavage reaction found in nonketotic hyperglycinemia may also represent a secondary phenomenon and not the primary enzymatic defect. It was even proposed that all forms of hyperglycinemia might be associated or even caused by a defect in the metabolism of propionate or branched chain amino acids (24, 31).

We have recently observed three unrelated neonates with a fatal course of nonketotic hyperglycinemia (5, 8, 30). The severe neurologic symptoms suggesting depression of CNS functions and the absence of ketoacidosis implied that the disease present in these infants was biochemically different from the conditions which may lead to ketotic hyperglycinemia syndrome. This paper presents indirect biochemical evidence for the existence of a true nonketotic hyperglycinemia (37) in which propionate and branched chain amino acid metabolism is normal *in vitro* and *in vivo*.

CASE REPORTS

DA was the patient reported previously by Bachmann *et al.* (5). No abnormal amounts of short chain fatty acids, other organic acids, their glycine conjugates, or of aldehydes and ketones were detected in her plasma and urine by gas chromatography-mass spectrometry (4).

MN was the second child born to healthy, nonconsanguineous parents by cesarean section at the 39th week of gestation (30). Hyperglycinemia (843–1,213 μM) and hyperglycinuria were first demonstrated on the 2nd day of life. Metabolic acidosis and ketosis were consistently absent. Excretion of MMA was not elevated. The clinical course was complicated by hyaline membrane disease and concomitant intraventricular hemorrhage. Muscular hypotonia and progressive loss of reflexes were predominant features. The patient died at the age of 5 days in spite of artificial respiration and two exchange transfusions.

The older brother of this child, *MD* (4 years old), survived acute neonatal manifestations of nonketotic hyperglycinemia but has developed severe mental retardation.

Details for *patient HR* have been reported previously and a defective glycine cleavage reaction *in vivo* was demonstrated (8). Oral loading tests with leucine, isoleucine, valine, methionine, and threonine had no clinical effect and caused neither metabolic acidosis nor ketosis. Plasma propionate and urinary MMA were not elevated (3, 6).

MATERIALS AND METHODS

Enzyme assays were performed on liver or fibroblast homogenates and subcellular fractions, respectively.

LIVER PREPARATION

The livers of *patients DA* and *MN* (38) and six control subjects were obtained and frozen at -18° within 14 hr after death (Table 2). All subsequent steps were carried out at $0-4^\circ$.

Table 1. Conversion of propionyl-CoA to methylmalonyl-CoA and succinyl-CoA by liver homogenate from patient DA and control subjects¹

Subject	n	Methylmalonate ²		Succinate ²	
		$\bar{x}$	Range	$\bar{x}$	Range
Patient DA	2	11.5	11–12	55	32–78
Control subjects					
HT	3	173	72–272	27	6–57
SY	2	44	42–46	58	51–64
AS	10	21	8–36	29	38–61
Assay controls					
HT, no propionyl-CoA	1	0		0	
HT, boiled liver	1	0		0	

¹ Assay system I: incubation time: 30 min. n: number of assays. For the source of liver specimens see Table 2.

² Counts per minute per milligram of protein per 30 min.

Liver Homogenate. Samples from the frozen livers were homogenized in 3 volumes of phosphate-buffered saline (PBS) in a motor-driven Thomas Teflon homogenizer. After 3–5 up and down strokes at 1,400 rpm, the homogenate was further disintegrated ultrasonically (150-watt disintegrator (39)) for four 15-sec intervals (samples were allowed to cool for 15 sec between sonication).

Subcellular Fractions. Fresh liver. A mitochondrial preparation made according to the procedure of Hogeboom (16) was isolated from the liver of patient MN and control subject JR. The mitochondrial pellet was resuspended in PBS and treated five times by rapid alternating freezing and thawing.

Frozen liver. From patient DA only frozen liver was available. As it was thus impossible to isolate intact mitochondria, a 100,000 × g (1 hr) sediment was obtained after removal of the nuclear fraction. The resuspended 100,000 × g sediment was frozen and thawed three times and used for the assay. This preparation allowed a clear-cut separation of all particular cell components from the soluble cell extracts.

FIBROBLASTS

Cell lines were derived from skin biopsies of patient DA, eight healthy control individuals (40), and from CE, a patient with propionic acidemia (3, 41). The skin explants were grown and subcultured in F-10 medium (42) with added 20% fetal calf serum and antibiotic-antimycotic mixture (43). Fibroblasts used for the assay were obtained after two to eight passages. Monolayers grown to confluence in plastic Falcon flasks (7,500 mm²) were harvested with 0.1% Versene-trypsin, and washed twice with PBS. The pellet of the intact fibroblasts was resuspended in a small volume of PBS and treated by freezing and thawing five times; this was termed "whole cell extract."

Subcellular Fractions from Fibroblasts of Patient DA and Control Subject SJ. The pellet of intact, washed fibroblasts (5–10 × 10⁶ cells) was homogenized in buffered 0.25 M sucrose 1:10 with 5 strokes of a Teflon homogenizer. After removing the nuclear fraction, a 100,000 × g (1 hr) sediment was obtained and treated as frozen liver.

ENZYMATIC ASSAY

Propionyl-CoA carboxylase (EC 6.4.1.3) and combined methylmalonyl-CoA racemase (EC 5.1.99.1) and mutase (EC 5.4.99.2) activity were assayed by a modification of the methods described by Hsia *et al.* (17) and Flavin and Ochoa (11). Liver or fibroblast preparations were incubated with propionyl-CoA (44) and sodium [¹⁴C]bicarbonate and the radioactive MMA-CoA and succinyl-CoA formed were measured after hydrolysis of the thioesters.

Assay System I. This was used for the first assays with liver and fibroblasts from patient DA and corresponding control samples. The final volume of 1.0 ml contained (in micromoles): Tris-HCl buffer (pH 7.4), 200; propionyl-CoA, 0.6; NaH¹⁴CO₃, 10 (5 μ Ci); ATP, 10; Mg⁺⁺, 10; glutathione (reduced), 10. The reaction was started with 0.4 ml enzyme preparation (liver: 0.5–22 mg protein, fibroblasts: 0.8–9 mg protein).

Assay System II. This was used for patient MN, corresponding control subjects, and for all assays with subcellular fractions. The following components differed from assay system I: Tris-HCl buffer (pH 8.0), NaH¹⁴CO₃, 20 μ mol (5 μ Ci); KCl, 200 μ mol. This assay system resulted in at least a fourfold increase of enzyme activity.

After incubation in a metabolic shaker at 30° for 30–60 min, the reaction was terminated by addition of 0.5 ml 1 N KOH. After hydrolysis of the thioesters (room temperature, 15 min), the proteins were precipitated by the addition of 0.5 ml 5 N HCl. The mixture was then centrifuged and the supernatant heated in a boiling water bath for 20 min to remove all ¹⁴CO₂. Fifty microliters of the aqueous phase were removed for counting (= total ¹⁴CO₂ fixation). The remaining supernatant was extracted four times with 15-ml portions of diethyl ether. The combined ether extracts were evaporated to dryness after alcalinization with 0.2 ml 0.5 N KOH in methanol. The residue was dissolved in 1.7 ml methanol: 0.1 ml was removed for counting ("ether extract") and 1.3 ml were transferred to a 2-ml test tube and again evaporated to dryness. The residue was taken up in 50 μ l aqueous solution containing the following unlabeled carriers: MMA, succinic, and 2-ketoglutaric acids. Aliquots of 20 μ l were subjected to high voltage electrophoresis (HVE): Schleicher Schüll paper, SS 2043 B, in pyridine buffer, pH 3.9 (pyridine-acetate-H₂O 15:55:930); 25 min, 2,200 v.

The samples incubated in assay system II were further separated in a second dimension by ascending paper chromatography (2-methyl-1-butanol saturated with 4 N formic acid for 4 hr). Additional unlabeled organic acids, mainly Krebs cycle intermediates, were added as reference acids (7).

The reference acids were located by staining the chromatograms with bromphenol blue (0.04% in ethanol at pH 6.7) and afterwards with fast blue B reagent (14). The spots were cut out, placed in vials containing 10 ml of 0.8% butyl-PBD (45) in methanol-toluol (250:750) and counted in a Packard Tri-Carb liquid scintillation counter.

The recovery of authentic MMA- and succinate when treated by the same procedure was (in percentage; $\bar{x} \pm$ SD): in ether extract: MMA ($n = 19$), 84 $\pm$ 12.3; succinate ($n = 19$) 80.6 $\pm$ 9.4; after HVE: MMA ($n = 6$) 40.3 $\pm$ 12.9; succinate ($n = 8$) 60.7 $\pm$ 6.0. After combined HVE and paper chromatography: MMA ($n = 20$): 37.9 $\pm$ 7.7; succinate ($n = 21$): 53.4 $\pm$ 11.1.

Protein was determined according to the method of Lowry *et al.* (23).

RESULTS

The activities of propionyl-CoA carboxylase, and of combined MMA-CoA racemase and mutase were estimated by the measurement of the amount of MMA-CoA and succinyl-CoA formed during the assay. The enzyme activities found in homogenates of frozen liver samples markedly decreased with time of storage and frequency of thawing.

Table 1 shows that enzyme assays with liver homogenate from patient DA and three control subjects gave similar activities. The liver samples used for this experiment were matched for storage time and frequency of thawing (Table 2). Similarly, experiments with cultured skin fibroblasts (whole cell extract) from patient DA indicated that enzyme activity was present in the range of seven control individuals (Table 3). In contrast, no MMA-CoA was formed by the fibroblasts of a patient with propionic acidemia (CE)

The liver of the second patient, MN, and that of control subjects

Table 2. Source of liver samples¹

Patient	Age at death	Cause of death	Time until frozen, hr	Time frozen before assay	No. of times thawed before assay
DA	3 weeks	NKHG	3	18 mo 32 mo ²	1-2 3-4 ²
HT	3 days	IRDS, premature, intracranial hemorrhage	3	3 mo 17 mo ²	2 3-4 ²
SY	12 days	Congenital nephrotic syndrome	1	6 mo	4
AS	6.5 yr	Brain damage after severe hypoglycemia	3	2 mo	2-3
MN	5 days	NKHG IRDS, intracranial hemorrhage	4 ³	3 days 24 hr on ice ³	1
JR	5 days	Congenital heart disease	14 ³	2 days 36 hr on ice ³	0
FJ	10 mo	Accident, intracranial hemorrhage	7	3 weeks	0

¹ NKHG: nonketotic hyperglycinemia; IRDS: idiopathic respiratory distress syndrome.² Sample used for the preparation of a 100,000 × g sediment.³ Sample used for the mitochondrial preparation.Table 3. Conversion of propionyl-CoA to methylmalonyl (MMA)-CoA and succinyl-CoA by cultured skin fibroblasts: whole cell extract (patient DA, control subjects, and a patient, CE, with propionic acidemia)¹

Subject	n	Propionyl-CoA, mM/liter	Methylmalonate + succinate ²		Succinate, % ³
			$\bar{x}$	Range	
Patient DA	2	0.6	133	130-136	0-30
Patient DA	5	1.8	115	64-140	25-50
Control subjects ⁴					
1, 2, 3, 4	4	0.6	108	86-130	1-6
5, 6, 7	5	1.8	60	45-74	12-20
Patient CE ⁵	2	0.6	0		0

¹ Incubation was carried out for 30 min in assay system I with 0.9-9 mg fibroblast protein/assay; n: number of assays.² Sum of radioactive MMA and succinate found in counts per minute per milligram of protein per 30 min.³ Percentage of the sum of MMA and succinate.⁴ Fibroblasts from healthy control subjects, 1-7, derived from seven different individuals (40).⁵ CE was a patient with propionic acidemia (3, 41).

JR and FJ were assayed with assay system II which yielded at least 4 times higher enzyme activity than assay system I. Table 4 shows that a frozen liver sample from patient MN converted propionyl-CoA to MMA-CoA and succinyl-CoA to an extent similar to the livers from the two controls. Addition of glycine (1.5 mM) to the incubation mixture did not inhibit enzyme activity.

It seemed desirable to rule out the possibility that the radioactive MMA-CoA found was formed by acetyl-CoA carboxylase, since it is known that propionyl-CoA is carboxylated not only by propionyl-CoA carboxylase, a mitochondrial enzyme, (18) with a pH optimum at pH 8.0 (12), but also by acetyl-CoA carboxylase, a soluble enzyme with a pH optimum between pH 6.5 and 7.5 (25, 35). This problem could be solved easily on the basis of the different properties of the two enzymes, as follows.

pH Optimum. Incubation of liver homogenate from patients and control subjects at pH 8.0 resulted in a threefold yield of product as

Table 4. Formation of methylmalonyl-CoA, succinyl-CoA, and other organic acids by liver homogenate obtained from frozen liver of patient MN and control subjects¹

Subject	n	Methylmalonate ²		Succinate ²		Other organic acids ³	
		$\bar{x}$	Range	$\bar{x}$	Range	$\bar{x}$	Range
Patient MN	2	423	327-518	1,783	1,754-1,812	234	176-293
Control subjects							
HR	1	704		3,487		1,677	
FJ	3	2,039	848-3,445	1,671	1,386-1,876	260	200-355
Assay control MN ⁴	1	0		4		5 ⁵	

¹ Incubation for 30 min in assay system II, with 9-19 mg protein/assay. Source of liver sample: see Table 2. n: number of assays.² Counts per minute per milligram of protein per 30 min.³ Counts mainly found in spots corresponding to intermediates of the citric acid cycle as well as to lactic acid and an unidentified spot.⁴ For the assay control liver homogenate from MN was incubated in assay system II with propionyl-CoA omitted.⁵ Citric, malic and lactic acids.

compared with incubation at pH 7.4. Even less enzyme activity was found at pH 6.5.

Subcellular Fractionation. Estimation of propionyl-CoA carboxylase activity and MMA metabolism in subcellular liver fractions of patient MN and a matched control sample (JR) indicated that the highest specific activity was associated with the mitochondrial fraction (Table 5). These preparations readily converted propionyl-CoA to MMA-CoA and succinyl-CoA. The latter was further metabolized by the tricarboxylic acid cycle. In two-dimensional pherograms, the spots corresponding to fumaric acid and malic acid regularly gave the highest counts. Less radioactivity was associated with citric, isocitric, cis-aconitic, 2-ketoglutaric and also lactic acids and an unidentified spot.

The results obtained with the subcellular fractions from liver and fibroblasts of patient DA and control subjects are summarized in

Table 5. Metabolism of propionyl-CoA in subcellular fractions of liver from patient MN and control subject JR¹

Subject	Cell fraction	n	Methylmalonate ²		Succinate ²		Other organic acids ³	
			$\bar{x}$	Range	$\bar{x}$	Range	$\bar{x}$	Range
Patient MN	Mitochondrial fraction	3	3,336	2,247-4,425	6,255	5,867-6,642	1,023	578-1,461
	Supernatant from mitochondrial fraction	1	5,426		79		85	
Control, JR	Mitochondrial fraction	2	7,449	6,938-7,961	8,410	8,231-8,590	1,817	1,685-1,949
	Supernatant from mitochondrial fraction	1	5,616		50		309	
Assay controls (Patient MN) ⁴	Mitochondrial fraction	3	0		13		37 ⁵	
	Supernatant from mitochondrial fraction	4	0		0		0	

¹ Incubation for 30 min in assay system II, with liver kept on ice for 24 hr (MN) and 36 hr (JR) after death. n: number of assays.

² Counts per minute per milligram of protein per 30 min.

³ Counts found in spots corresponding to intermediates of the citric acid cycle and lactic acid and an unidentified spot.

⁴ The assay controls were liver fractions from patient MN incubated in complete assay system II with propionyl-CoA omitted.

⁵ Fumaric, citric, malic, 2-ketoglutaric acids.

Table 6. Ability of subcellular fractions of liver and fibroblasts to metabolize propionyl-CoA to methylmalonyl (MMA)-CoA and succinyl-CoA (patient DA and control subjects)¹

Subject and source	Cell fraction	n	Methylmalonate + succinate ²	
			$\bar{x}$	Range
Patient DA, liver	100,000 × g (1 hr) sediment	1	608	
	Supernatant (100,000 × g)	2	118	109-127
Control subject, HT, liver	100,000 × g (1 hr) sediment	2	472	n.d.
	Supernatant (100,000 × g)	1	1,254	
Patient DA, fibroblasts	100,000 × g (1 hr) sediment	1	659	
	Supernatant (100,000 × g)	2	245	173-313
Control subject, SJ, fibroblasts	100,000 × g (1 hr) sediment	3	643	428-991
	Supernatant (100,000 × g)	2	152	127-176

¹ Incubation in assay system II for 60 min. Source of liver sample: see Table 2. n: number of assays; n.d.: Further separation was carried out only in one assay. Assay controls (no propionyl-CoA) were carried out with each experiment and gave consistently no labeled methylmalonate or succinate.

² Counts per minute per milligram of protein per 60 min, Sum of MMA + succinate.

Table 6. The high rate of MMA-CoA formation in the 100,000 × g sediments indicates the presence of an intact propionyl-CoA carboxylase in the tissues of DA.

The radioactive products formed during the incubation of whole liver homogenate, fibroblast extract, and all subcellular fractions were further analyzed by autoradiography of the two-dimensional pherograms. No additional radioactive spots could be detected in the specimens of the patients as well as in the control subjects. Thus, there was no evidence for an alternate pathway of propionate metabolism in our experiments.

DISCUSSION

Experiments with crude liver homogenate from both our patients with nonketotic hyperglycinemia (MN and DA) and with whole cell extract of fibroblasts from DA demonstrate a normal conversion of propionyl-CoA to MMA-CoA and succinyl-CoA. Any major contribution of acetyl-CoA carboxylase activity to the formation of MMA-CoA was excluded by experiments done at different pH levels and with subcellular fractions. We conclude, therefore, that *in vitro* the enzyme activities of propionyl-CoA carboxylase as well as of MMA-CoA racemase and mutase were normal. Absence of clinical symptoms and of ketoacidosis after loading HR (the third patient) with propionate and MMA precursor amino acids and lack of accumulation of intermediary metabolites in patient DA are evidence that *in vivo* (1) propionate metabolism is not affected, (2) the metabolism of branched chain amino acids is not grossly abnormal in nonketotic hyperglycinemia.

So far, only a few reports on propionate metabolism in nonketotic hyperglycinemia have been published. Ando *et al.* (1) found evidence for normal propionate metabolism in the fibroblasts of the index patient (SF) with nonketotic hyperglycinemia by demonstration of normal oxidation of [1-¹⁴C]propionate to ¹⁴CO₂. Koepp *et al.* (20) observed no elevation of propionate in their case of nonketotic hyperglycinemia. Both results are in agreement with our findings.

On the other hand, Mahoney *et al.* (24) discussed propionyl-CoA carboxylase deficiency as a possible cause for nonketotic hyperglycinemia. They reported significant impairment of propionyl-CoA carboxylase activity in the fibroblasts of a 1-year-old hyperglycinemic patient who had never shown clinical attacks of ketoacidosis and, therefore, had been considered to suffer from nonketotic hyperglycinemia. Oral loads of propionyl-CoA precursors, however, led to the classic ketotic syndrome and thus established the diagnosis of propionic acidemia *in vivo*. A similar clinical course could be anticipated for other mild variants of defects which, in the severe form, are known to lead to the ketotic hyperglycinemia syndrome. In fact, we have recently seen a neonate with methylmalonic acidemia whose disease presented clinically as a mild form of nonketotic hyperglycinemia (6).

The coexistence of a defective activity of propionyl-CoA carboxylase (or MMA-CoA mutase, respectively) and of the glycine cleavage reaction to CO₂, NH₃, and a C₁ unit has been demonstrated both *in vivo* and *in vitro* in some other patients with propionic acidemia and methylmalonic acidemia (1, 27, 33). Keating *et al.* (19) described recently a patient in whom a ketotic hyperglycinemia syndrome was associated with a defect in the

isoleucine degradation pathway (presumably at the β -ketothiolase site). Glycine oxidation in the cultured fibroblasts of this patient was inhibited by isoleucine (15). The authors concluded that hyperglycinemia seen in the ketotic hyperglycinemia syndrome would appear to be secondary to accumulation of products of isoleucine catabolism. A similar mechanism might also explain propionic acidemia and other metabolic defects leading to the ketotic hyperglycinemia syndrome.

In nonketotic hyperglycinemia, however, a defective glycine cleavage reaction is the only enzymatic defect so far known. In one of our three patients (HR) with the acute neonatal form of nonketotic hyperglycinemia, such a defect has been demonstrated *in vivo* (8). Defects belonging to the ketotic hyperglycinemia syndrome have been clearly excluded by *in vivo* and *in vitro* studies in all three patients presented in this paper. As expected from biochemical studies *in vivo*, it was found that added glycine did not inhibit either propionate or methylmalonate metabolism in the liver of patients and control subjects. It has been postulated by several investigators that the defective glycine cleavage reaction represents the primary enzymatic defect of this clinically well defined disease (10, 28). If this is so, further genetic heterogeneity is to be expected, as four different enzyme systems seem to be involved in this overall reaction (26, 28).

Until the enzymatic basis of this disease has been clarified, we suggest that the term "true nonketotic hyperglycinemia" be used to separate it clearly from mild forms of the "ketotic hyperglycinemia syndrome."

The extremely high glycine concentrations found in the CNS of patients with true nonketotic hyperglycinemia in contrast to the only moderate elevations found in ketotic hyperglycinemia (5, 8, 21) suggest that the defect in glycine metabolism is directly involved in the pathogenesis of true nonketotic hyperglycinemia. Glycine is known to be the major inhibitory transmitter in the brain stem and spinal cord (36). Accordingly, the severe neurologic manifestations of nonketotic hyperglycinemia, which differ markedly from those in ketotic hyperglycinemia, may well be a direct consequence of the glycine accumulation in the CNS.

SUMMARY

1. Propionyl-CoA carboxylase and MMA-CoA mutase (combined with MMA racemase) activities were studied in liver and fibroblasts of two patients with a lethal neonatal form of nonketotic hyperglycinemia. The enzyme activities in the tissues of the patients were found to be within the range of healthy control subjects.

2. An assay of increased sensitivity was developed to measure propionyl-CoA carboxylase activity.

3. The contribution of acetyl-CoA carboxylase in the formation of the normal amounts of MMA-CoA was excluded by assays at various pH levels and in different subcellular fractions of liver and fibroblasts.

4. No alternate pathway of propionate in the tissues of the patients could be detected by analysis of the radioactive products formed during the assay.

5. It is concluded that true nonketotic hyperglycinemia is a distinct disease.

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- A preliminary report of the results obtained with tissues of one of these patients was published earlier (9).

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39. MSE, London, England.
40. The skin biopsies were obtained with the informed consent of the parents of the patient and control subjects. We are grateful to Dr. Erika Bühler for the cultivation of the fibroblasts and to Dr. Ulrich Wiesmann, Bern, for providing us with additional control fibroblasts.
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42. Difco, Detroit, Mich.
43. Gibco, Grand Island, N.Y.
44. Propionyl-CoA was prepared from propionic anhydride and coenzyme A (11). Its

purity was confirmed by ascending chromatography of its hydroxamic acid derivative in water-saturated butanol (32). The concentration of propionyl-CoA was measured by its reaction with hydroxylamine (22) and by titration of the —SH groups before and after hydrolysis by the nitroprusside method of Grunert and Phillips (13).

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Growth neonate
metabolic acidosis sodium bicarbonate

Growth and Mineral Metabolism in Very Low Birth Weight Infants. I. Comparison of the Effects of Two Modes of NaHCO_3 Treatment of Late Metabolic Acidosis

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Extract

Twenty-six infants weighing less than 1,300 g at birth were divided into pairs according to birth weight (900–1,100 and 1,101–1,300 g) and gestational age ("appropriate" (AGA) = mean 31 weeks; and "small" (SGA) = mean 34 weeks). One member of the pairs was then allocated randomly to one of two treatment regimens with oral sodium bicarbonate. *Group A* was treated whenever base excess was greater than -8 mEq/liter as detected on twice weekly testing and/or when suspected to be acidotic from failure to gain weight. In *group B*, base excess was maintained within 1 SD of normal (-3.2 ± 1.7 mEq/liter). The infants received Enfalac 200 ml/kg/24 hr, at 67 cal/100 ml, with vitamin D 400 IU/24 hr added from age 2 weeks.

The following measurements were made: daily weight, weekly length, skinfold thickness, head circumference, twice weekly blood pH, PaCO_2 , base excess, and weekly plasma total calcium, ionic calcium, total magnesium, inorganic phosphorus, and total protein.

There were six pairs each of AGA and SGA infants and two unpaired *group A* infants. Weekly weight gains did not differ between *group A* and *group B* or between AGA and SGA. Length increment was greater in AGA than in SGA babies (0.94 ± 0.02 vs 0.85 ± 0.04 cm/week) but not significantly so ($P < 0.1$), and in *group B* babies compared to *group A* babies (0.973 ± 0.029 vs 0.83 ± 0.037 cm/week) ($P < 0.01$).

Plasma pH was lower in *group A* (7.23 ± 0.02) than in *group B* (7.30 ± 0.02) and calcium ion activity higher (*group A* 2.72 ± 0.04 ; *group B* 2.51 ± 0.06 mEq/liter) between ages 20 and 29 days. Plasma magnesium was higher in *group A* (1.77 ± 0.04 mEq/liter) than in *group B* (1.56 ± 0.06 mEq/liter) from age 20 to 39 days. Inorganic phosphorus concentrations were consistently higher in *group A* than in *group B*, but the differences did not reach significance. Mean total protein concentrations did not rise above 4.5 g/100 ml and tended to be higher in babies of *group A* than of *group B*.

Bone age was retarded in all babies. Radiographs available for 7 of 13 SGA infants were normal, whereas 6 of 11 radiographs of AGA babies showed some osteoporotic changes.

Speculation

Careful control of late metabolic acidosis in infants < 1.3 kg birth weight results in less ionic hypercalcemia and improved growth in length. In the more immature AGA infants, radiologic osteoporosis was seen. Mineral and protein content of formula currently used for feeding infants of less than 1,300 g birth weight is inappropriate to their requirements for growth.

Late metabolic acidosis as defined by Kildeberg (12) is common in very low birth weight (VLBW) (< 1.3 kg) infants (22), and the condition may require intermittent correction for several months (18).

Unless corrected, one of the adverse consequences of late metabolic acidosis is said to be failure to gain weight (12). Acidosis may inhibit bone mineralization (4, 14), lead to hypercalcemia (ionic fraction), hypercalciuria (23), and may even be associated with reduced rates of skeletal growth (5). These potential complications might be aggravated by routine administration of vitamin D in doses unrelated to body weight as was recently recommended for the prevention of rickets in these infants (15).

Since the survival rate of VLBW infants is increasing, we consider it important to study the effects of acidosis on their growth and mineral homeostasis. The purpose of this initial study was to compare the influence of two treatment regimens for late metabolic acidosis in infants pair-matched for birth weight and gestational age. We assessed the influence of the regimens on body and skeletal growth and on mineral homeostasis. We hoped to favor bone mineralization by strict maintenance of normal acid-base balance.