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Studies of the Glycine Metabolism in a Patient with D-Glyceric Acidemia and Hyperglycinemia

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Summary

A mentally retarded boy exhibiting both hyper-D-glyceric acidemia and hyperglycinemia and in whom a deficiency of D-glycerate dehydrogenase had previously been demonstrated was investigated to elucidate the ethiology of the glycine accumulation and its relationship to the D-glyceric acid accumulation. It was found that a positive correlation existed between excretion of D-glyceric acid and glycine (coefficient of correlation: $r = 0.62$, $P < 0.001$), that part of the IV injected [^{14}C]glycine was metabolized to D-glyceric acid whereas no [^{14}C]glyceric acid was metabolized to glycine, and that the *in vivo* degradation of IV injected [^{14}C]glycine to $^{14}CO_2$ was diminished. Measurement of glycine cleavage activity in autoptic liver tissue from the patient showed only 10% of normal activity. It is argued that this diminished activity could be caused by an endogenous inhibitor. D-glyceric acid is demonstrated not to possess such an inhibitory effect. Based on the finding of increased urinary excretion of both free and conjugated isobutyric acid, 2-methylbutyric acid, and isovaleric acid, it is hypothesized that the diminished glycine cleavage activity might be due, at least partially, to inhibition by 2-methylbutyryl-CoA and isobutyryl-CoA, two compounds that are known to inhibit the glycine cleavage system.

Speculation

The deranged branched-chain amino acid metabolism demonstrated in this patient is, like the hyperglycinemia, considered to be a secondary phenomenon, possibly caused by the D-glyceric acidemia.

In two previous communications (3, 13) we have described a child with a hitherto unknown inborn error of metabolism named D-glyceric acidemia.

Judged on the clinical picture and analysis of amino acids in serum, urine, and spinal fluid, this case was indistinguishable from the syndrome of nonketotic hyperglycinemia (17), a syndrome that has been shown to be due to a defect in the glycine cleavage system. However, when investigating the organic acids in serum and urine, a large accumulation of D-glyceric acid was demonstrated. Further studies revealed that leukocytes from the patient had diminished D-glycerate dehydrogenase activity. It was therefore proposed that the child suffered from an inborn deficiency of D-glycerate dehydrogenase and that the hyperglycinemia was a secondary phenomenon. The suggestion that the hyperglycinemia should be secondary would be analogous to the current concept on the ethiology of the syndrome ketotic hyperglycinemia. This syndrome includes the well-defined inborn errors of metabolism propionyl-CoA carboxylase deficiency (18), methylmalonyl-CoA mutase deficiency (15), and β-ketothiolase deficiency (10) in which severe hyperglycinemia has been found in addition to the primarily accumulated organic acids. Enzymatic studies have demonstrated that the hyperglycinemia seen in ketotic hyperglycinemia is due to diminished glycine cleavage activity (5, 16), although this decreased activity is considered to be a secondary phenomenon.

The present communication describes the investigations that we have performed to explain the hyperglycinemia seen in our patient. It is demonstrated that the hyperglycinemia is caused by diminished glycine cleavage activity, a decrease which might, at least partially, be due to inhibition by intramitochondrially accumulated isobutyryl-CoA and 2-methylbutyryl-CoA.

CASE REPORT

The patient, a boy, was a second child of nonconsanguineous emigrants from Serbia. Pregnancy and birth were uncomplicated. Birthweight was 3.350 g; length was 52 cm. He was hypotonic from birth to a degree that myasthenic paralysis had to be ruled out on the basis of lack of response of neostigmine injection.

At 8 wk of age, tonic fits with ocular crises developed. In periods, he was in a condition of agitation with persistent motor activity, sometimes described as athetoid movements with difficulties in swallowing. Later, intermittent choreiform movements were seen.

In the course of time, other abnormal paroxysms were noticed such as (1) myoclonic jerking with supraversion of eye balls; (2) cerebellar fits throwing him into opisthotonus for a few sec, (3) an almost classical "chorea minor" pattern quite unusual for his age; (4) universal grand mal seizures.

The hyperkinetic movements were partly controlled on phenobarbital or nitrazepam and later phenytoin combined with klonazepam. He never fixated and followed the light and never spontaneously tried to raise his head from the supine position. He was not able to grasp and could not coordinate any of his movements. He remained completely mentally extinguished and hypotonic and died at the age of $3\frac{1}{2}$ years from pneumonia.

The patient was fed normal diet and never had acidotic episodes, and growth and body weight remained normal for age.

MATERIALS AND METHODS

CHEMICALS

The following compounds were synthesized as previously described: D-glyceric acid (3), 3-hydroxy-isobutyric acid, 2-methyl-3-hydroxy-butyric acid, and 3-hydroxy-isovaleric acid (12). 2-Hydroxy-caproic acid was synthesized by reduction of 2-oxocaproic acid with sodium borohydride followed by purification of the product by silicic acid chromatography according to Kessner and Muntwyler (11). Tetrahydrofolic acid was synthesized as previously described (6). [1-^{14}C]Glycine was obtained from New England Nuclear Corp., West Germany. DL-[1-^{14}C]Glyceric acid was obtained from CEA, France. All other reagents used were of analytical grade.

GLYCINE AND GLYCERIC ACID IN URINE

Glycine concentrations in urine were measured on a Technicon amino acid analyzer. Glyceric acid concentrations in urine were measured gas chromatographically (Hewlett-Packard 5830 A) as trimethylsilyl derivatives after isolation of the organic acids by ion-exchange chromatography on Sephadex A-25 according to Chalmers and Watts (4). Pimilic acid was used as internal standard.

BRANCHED-CHAIN AMINO ACID METABOLITIES IN URINE

Urinary branched-chain amino acid metabolites were identified and quantified by a multiple ion detection system (MID). The instrument used was an AEI MS 30 mass spectrometer equipped with a 5-channel multiple ion detector and a Pye-Unicam gas chromatograph. Volatile monocarboxylic acids were analyzed before and after alkaline hydrolysis at 110°C for 24 hr. The acids were isolated by vacuum distillation according to Ando *et al.* (2) and separated on a Porapac (23) column at 170°C. MID-ions for the analysis were: propionic acid, isobutyric acid, and 2-methylbutyric acid: 73.0281; isovaleric acid, 73.0281 and 87.0417; methacrylic acid, 86.0344; tiglic acid, methyl-crotonic acid, and internal standard (4-pentenic acid), 100.0511. 2-Oxo-acids were analyzed after hydroxylamine treatment under alkaline conditions for 30 min at 60°C followed by ethylacetate extraction and trimethylsilyl derivatization by treatment with *N,O*-bis(trimethylsilyl)trifluoroacetamide containing 1% trimethylchlorosilane (21) for 10 min at 60°C. Separation was performed by programming the oven from 150°C with 10°C per min, with a dexsil 300 column. MID-ions: 2-oxo-iso-caproic acid, 2-oxo-3-methyl-valeric acid, and internal standard (2-oxo-caproic acid), 289.1529; 2-oxo-isovaleric acid, 275.1372. 3-Hydroxy-acids were analyzed after ethylacetate extraction and trimethylsilyl derivatization with *N,O*-bis-(trimethylsilyl)trifluoroacetamide containing 1% trimethylchlorosilane at 60°C for 10 min. Separation was performed on an OV-1 column by programming the oven from 100°C with 10°C per min. MID-ions: 3-hydroxypropionic acid and 3-hydroxyisobutyric acid, 177.0834; 2-methyl-3-hydroxy butyric acid, 219.0940; internal standard (2-hydroxycaproic acid), 261.1384. Measurement of 3-hydroxyisovaleric acid proved unsatisfactory in the MID-system and was measured gas chromatographically on a Hewlett-Packard 5830 A gas chromatograph with the same column and by programming the oven from 60° with 4°C/min.

[^{14}C]LABELED AMINO ACIDS AND ORGANIC ACIDS IN URINE

^{14}C-Labeled amino acids and organic acids in the urine were isolated and quantified in the following way. A urine sample was put onto a 2- x 12-cm column of Sephadex A-25. The column was washed with 20 ml water, and the total eluate was collected (fraction A). The column was then eluted with 30 ml 2.5 M pyridinium acetate (fraction B). Preliminary experiments with fraction A showed that the amino acids were not retained on the column and were eluted in the first 10 to 15 ml of this fraction, leaving the last 5 to 10 ml free of amino acids. Similarly, it has been ascertained both by us and by others (4) that when using 30 ml 2.5 M pyridinium acetate a very high recovery of organic acids is obtained in fraction B. This recovery was tested for glyceric acid and was found to be above 95%.

Fraction A was lyophilized, and redissolved in water, and the amino acids were separated on a Rank Hilger, Chromaspek amino acid analyzer, using fluorescence detection. The eluate was collected in fractions after detection, and transferred to counting vials containing Instagel (22) [^{14}C]activity was counted in a Packard liquid scintillation counter.

Fraction B was also lyophilized (in alkaline solution), dissolved in 0.05 N sulphuric acid (0°C), and subjected to silicic acid chromatography according to Kessner and Muntwyler (11). The eluate was collected in 10-ml fractions which were transferred to counting vials. The organic phase was evaporated (after addition of 100 μl 0.5 N sodium hydroxyde to each fraction). Instagel was added, and ^{14}C activity was counted. The identity and purity of ^{14}C-labeled compounds were further determined by radiogas chromatography of trimethylsilyl derivatives of the evaporated remnants of radioactive fractions. The radiogas chromatograph was a modified Hewlett-Packard 7620 A model where a splitter had been introduced between column and flame ionization detector. Thus, 15% of the helium flow reached the flame ionization detector, and 85% passed through a steel funnel to the outside of the oven. Here the organic acids separated on the column were trapped by placing Pasteur pipets containing glass wool soaked with Protozol (23) beneath the exit of the funnel. The Pasteur pipets were changed manually each 30 sec. The soaked glass wool was then transferred to counting vials, and after addition of scintillation fluid, ^{14}C activity was measured.

IN VIVO METABOLISM OF GLYCINE

To monitor the *in vivo* formation of $^{14}CO_2$ after IV injection of [1-^{14}C]glycine, expiration air was collected in meteorologic balloons during 5-min periods spread over a 2-hr interval immediately following injection. Total CO_2 was measured by titration with sodium hydroxyde after trapping in a mixture of ethanolamine:methylcellosolve (2:1) according to Fredrickson and Ono (7). $^{14}CO_2$ was measured by counting samples of the same solutions in a liquid scintillation counter. *In vivo* formation of $^{14}CO_2$ was expressed relatively as dpm/mmole CO_2 expired.

GLYCINE CLEAVAGE ACTIVITY IN LIVER TISSUE

Glycine cleavage activity in human liver tissue was measured by collecting $^{14}CO_2$ liberated from [1-^{14}C]glycine during incubation with liver homogenate as previously described (8, 12) [reaction mixture: 4.75 μmoles glycine (specific activity, 0.6 mCi/mmole) 0.5 μmole tetrahydrofolic acid, 0.8 μmole pyridoxal phosphate, 2.5 μmoles dithiotreitol, 0.8 μmole adenine-dinucleotide, 67.5 μmoles Tris-HCl (pH 8.1), 4 mg protein, total volume 1.0 ml].

Glycine cleavage activity was expressed in nmoles glycine metabolized per mg protein per hr. Protein was measured according to Lowry *et al.* (14).

INHIBITION OF THE GLYCINE CLEAVAGE SYSTEM

Inhibitory studies on the glycine cleavage system were performed on freshly prepared rat liver mitochondria and on acetone-dried rat liver mitochondrial powder as previously described (12). Reaction mixture for assays using fresh mitochondria was identical to the reaction mixture described above. Reaction mixture when using the solubilized system was: 4.75 μmoles glycine (specific activity, 1.5 mCi/mmole), 0.75 μmole tetrahydrofolic acid, 0.95 μmole pyridoxal phosphate, 4.0 μmoles dithiotreitol, 1.75 μmoles adenine dinucleotide, 67.5 μmoles Tris-HCl (pH 8.1), 6 mg protein, total volume, 1.35 ml. The effect of D-glyceric acid was investigated by addition of this compound in final concentrations: 0.3, 1.0, 3.0, and 10.0 mM.

RESULTS

IN VIVO CORRELATION BETWEEN GLYCINE AND GLYCERIC ACID

Correlation between the Urinary Excretions of Glycine and Glyceric Acid. Over a period of one year, 20 urine samples were collected, stored at −20°C and then measured for concentrations of glycine and glyceric acid. Corresponding values were plotted in a diagram (Fig. 1) to see if any correlation existed between the excretion of the two accumulated compounds. It was found that a weak positive correlation existed (coefficient of correlation, 0.62; $P < 0.001$). The best-fitted straight line had a slope of 0.22 corresponding to the excretion of 0.22 mole glycine per mole D-glyceric acid.

Metabolic Fate of In Vivo-Injected [1-^{14}C]Glycine and DL-[1-^{14}C]-Glyceric Acid. On two separate occasions when patient weighed 14.9 kg, he was administered ^{14}C-labeled compound IV. On the

first occasion, 50 μCi [1-^{14}C]glycine was given, and 11 days later, 50 μCi DL-[1-^{14}C]glyceric acid was given. In both cases, urine was collected the following 24 hr and examined for ^{14}C activity in amino acids and organic acids. When [1-^{14}C]glycine was administered, activity was found in both the organic acid fraction and in the amino acid fraction. In the amino acid fraction, the radioactivity was almost exclusively localized in glycine. In addition, trace amounts of ^{14}C activity was found in serine and cystathionine. The organic acid fraction showed only one ^{14}C-labeled peak on silicic acid chromatography with a retention time similar to glyceric acid. The identity and purity of this compound was further verified by radiogas chromatography, which also showed one peak with retention time similar to that of glyceric acid.

When administering DL-[1-^{14}C]glyceric acid, no activity was found in the amino acid fraction. In the organic acid fraction, one ^{14}C-labeled peak was found. This was identified as glyceric acid. The results are summarized in Table 1.

GLYCINE METABOLISM IN THE PATIENT

In Vivo Metabolism. The *in vivo* degradation of carbon atom 1 of the glycine molecule was investigated by injecting [1-^{14}C]glycine (2 μCi/kg body weight) IV and collecting $^{14}CO_2$ in expiration air at intervals over the following 120 min. The results are shown graphically in Figure 2. In this experiment, no control was included. Instead, the results found by earlier investigators (17) concerning the shape of this curve in normal persons and in patients with nonketotic hyperglycinemia are shown as *shaded areas*. It is quite evident that in this respect the patient closely resembles what has been found in patients with nonketotic hyperglycinemia.

Glycine Cleavage Activity in Liver Tissue. Autoptic liver tissue was obtained 6 hr post mortem from the patient and kept at −20°C until measurement of the glycine cleavage system was performed. As controls, two samples of autoptic liver tissue were used (obtained 7 and 10 hr post mortem) from two children who had died of congenital heart disease, and a surgical liver biopsy from a child with mild hepatic stasis was used. The results of the glycine cleavage measurements are shown in Table 2.

INHIBITORY STUDIES OF THE GLYCINE CLEAVAGE SYSTEM

To eliminate errors due to membrane transport, the inhibitory studies were performed on freshly prepared rat liver mitochondria and, additionally, on the solubilized glycine cleavage system. These investigations showed that D-glyceric acid in the concentration range 0.3 to 10.0 mmoles/liter had no significant effect on the glycine cleavage system in either fresh mitochondria or in the solubilized system.

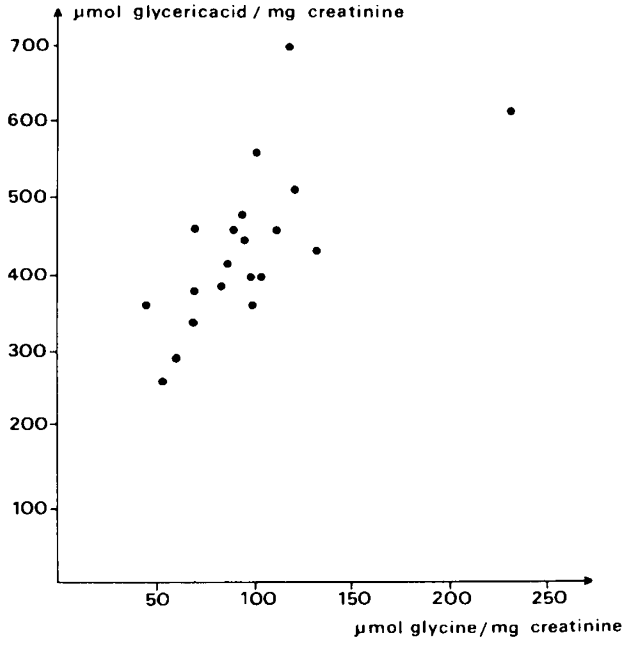

Fig. 1. Correlation between corresponding values of D-glyceric acid and glycine concentrations in urine samples from the patient. Correlation coefficient, 0.62; $P < 0.001$; slope of best fitted line, 0.22.

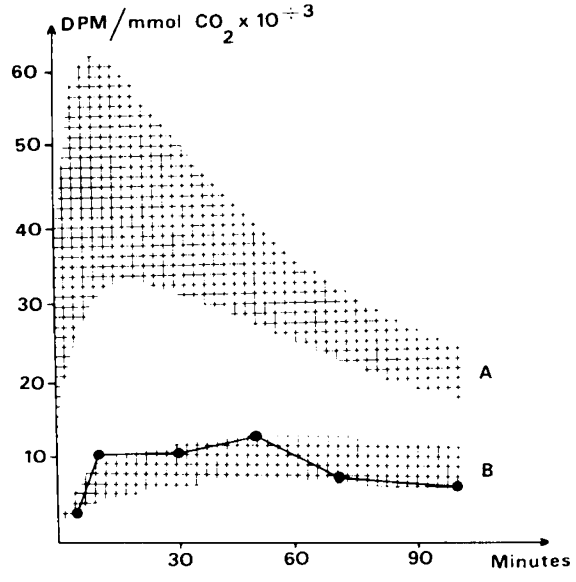

Fig. 2. Expiratory $^{14}CO_2$ formation from [1-^{14}C]glycine (3 μCi/kg administered IV at time zero). *Shaded areas*, pattern found in normal subjects (with and without artifically induced hyperglycinemia) (*A*) and in patients with nonketotic hyperglycinemia (*B*).

Table 1. *Urinary excretion of total and ^{14}C-labeled glycine and glyceric acid after IV administration of 50 μCi [^{14}C]glycine and 50 μCi DL-[1-^{14}C]glyceric acid*

	Glycine			Glyceric acid		
Compound administered	(μCi/mg) creatinine	(μmoles/mg) creatinine	Urinary specific activity (μCi/μmole)	(μCi/mg) creatinine	(μmoles/mg) creatinine	Urinary specific activity (μCi/μmol)
50 μCi[1-^{14}C] glycine IV	4.9×10^{-2} [1]	116	42.1×10^{-5}	0.4×10^{-2} [2]	472	0.8×10^{-5}
50 μCi DL-[1-^{14}C]glyceric acid IV	ND[4]	95	ND[4]	5.0×10^{-2} [5]	408	12.2×10^{-5}

[1] Estimated limit of detection, for amino acids, 0.1×10^{-2} μCi/mg creatinine.
[2] Estimated limit of detection for organic acids 0.05×10^{-2} μCi/mg creatinine.
[3] Estimated limit of detection, for total amino acid labelling 0.05×10^{-2} μCi/mg creatinine.
[4] ND, not detectable.
[5] Estimated limit of detection, for organic acids 0.05×10^{-2} μCi/mg creatinine.

EXCRETION OF BRANCHED-CHAIN AMINO ACID METABOLITES

Five 24-hr urine samples were collected from the patient over a period of 2 years. The patient was on all these occasions in his habitual condition, *i.e.*, without signs of illness apart from the severe cerebral damage. As controls, 24-hr urine samples from five normal children aged 2 to 6 years were used. Determination of branched-chain amino acid metabolites showed undetectable amounts of 2-oxo-acids (limit of detection, 1 μg/mg creatinine) and unsaturated short-chain fatty acids (limit of detection, 1 μg/mg creatinine for unhydrolyzed compounds) in both patient and controls. However, for the hydroxyacids and for both free and total short-chain fatty acids, significant elevation was demonstrated in all urine samples from the patient compared to the control children (Table 3).

DISCUSSION

The striking feature in this patient with a typical nonketotic hyperglycinemia syndrome is the accumulation of D-glyceric acid in addition to the glycine accumulation. Inasmuch as D-glyceric acid has never been found accumulated in any other cases of hyperglycinemia (17, 18), we believe that this new syndrome represents a separate entity.

In an earlier communication (13), we presented evidence for a diminished D-glycerate dehydrogenase activity in the patient, and we therefore proposed that the D-glyceric acid accumulation might be caused by an inborn deficiency of this enzyme (Fig. 3). The primary aim of the present communication is to investigate the ethiology of the glycine accumulation, and efforts were concentrated on demonstrating if a diminished enzyme activity could be demonstrated in the glycine degradation. Because our patient clinically resembled the syndrome nonketotic hyperglycinemia, we used the same principles of investigations as has been used in elucidating the ethiology of this disorder, namely *in vivo* and *in vitro* measurement of $^{14}CO_2$ formation from [1-^{14}C]glycine. The *in vivo* method has been used in both nonketotic and ketotic hyperglycinemia (17, 18), and it has been shown that although normal children have very rapid metabolism of [1-^{14}C]glycine and maximal excretion of $^{14}CO_2$ after about 15 min, at which time they excreted 20 to 30 nCi/mmole $CO_2 \times 10^{-4}$, the patients with nonketotic hyperglycinemia have very slow metabolism of [1-^{14}C]glycine, with a maximal excretion of about 10 nCi/mmole $\times 10^{-4}$. Patients with ketotic hyperglycinemia have been found generally to show intermediate values. These findings have been interpreted as an indication of diminished glycine oxidation in the patients. The main objection to this is that the large body pool of glycine will itself cause a slow rise and a low peak value due to dilution of the labeled glycine. Attempts to overcome this objection were made by Ando *et al.* (1) by infusing large amounts of glycine to control children to increase the body pool. They succeeded in raising the serum glycine concentration to the same level as seen in patients and keeping it here for about 60 min before the experiment was performed. Results from controls under these conditions still revealed peak excretion after 15 min and a rise to 20 to 30 nCi/mmole $\times 10^{-3}$. Whether this is conclusive is difficult to say because it is impossible to judge if this artificial glycine accumulation had spread to all compartments including the mitochondrial compartment containing enzymes for glycine degradation. It is therefore still debatable to what extent this type of *in vivo* experiment gives specific information about the degradation of glycine in hyperglycinemia patients. However, an experiment of this type was performed on our patient, and Figure 1 shows

Table 2. *Glycine cleavage activity in liver tissue*

	Diagnosis	Glycine cleavage activity (nmoles/mg protein/hr)
Patient	D-glyceric acidemia	0.070
Control A	Mild hepatic stasis (surgical biopsy)	0.570
Control B	Congenital heart disease	0.920
Control C	Congenital heart disease	1.050

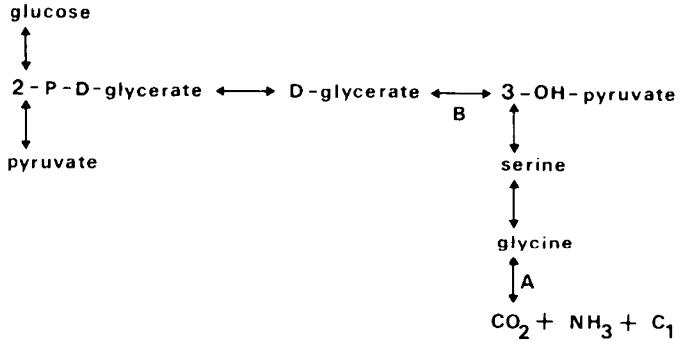

Fig. 3. The pathway between glucose intermediates and glycine, involving D-glyceric acid. *A*, the glycine cleavage system; *B*, D-glycerate dehydrogenase.

Table 3. *Urinary excretion of branched-chain short-chain fatty acids and hydroxyacids in five urine samples from the patient collected over a 2-year period compared with urine samples from five healthy children*

	Propionate	Isobutyrate	2-Methylbutyrate	Isovalerate	3-OH-Propionate	3-OH-Isobutyrate	2-Methyl-3-OH-Butyrate	3-OH-Isovalerate
Patient urine								
A	17 (1)[1]	12 (1)	13 (1)	6 (1)	63	84	10	64
B	20 (1)	9 (2)	9 (3)	3 (2)	22	36	7	48
C	19 (3)	11 (3)	11 (4)	3 (3)	29	37	10	22
D	8 (4)	5 (3)	6 (4)	4 (2)	19	32	7	32
E	10 (1)	7 (2)	6 (2)	3 (1)	79	101	10	96
Control urine								
A	16 (ND)[2]	2 (ND)	ND (ND)	ND (ND)	17	36	16	10
B	8 (ND)	ND (ND)	ND (ND)	ND (ND)	17	30	20	14
C	12 (ND)	2 (ND)	ND (ND)	ND (ND)	11	22	ND	13
D	11 (ND)	5 (ND)	ND (ND)	ND (ND)	13	24	ND	13
E	10 (ND)	2 (ND)	ND (ND)	ND (ND)	24	29	ND	13

[1] Short-chain fatty acids shown both as total acid and as free acid (numbers in parentheses) (μg/μg creatinine).
[2] ND, not detectable.

that the respiratory excretion pattern of $^{14}CO_2$ closely resembles what has been found in patients with nonketotic hyperglycinemia.

Attention was then focused on the glycine cleavage system, which has been shown to be diminished in varying degree in both ketotic and nonketotic hyperglycinemia. In our patient, we found activity about 10% of normal, which seems to correspond well with the fact that the clinical picture resembles classic nonketotic hyperglycinemia (19).

We are thus faced with a patient with what seems to be two distinct enzyme deficiencies and consequently two different accumulations. Because the probability of one child with two totally independent inborn errors of metabolism is extremely small, we are of the opinion that only one accumulation is due to a primary enzyme defect and that the other is a secondary phenomenon. The following points can be made in favor of the glycine accumulation being primary: (1) the clinical picture closely resembles the syndrome nonketotic hyperglycinemia where the current concept is that this disorder is due to an inborn error in the glycine cleavage system. The disproportionately high spinal fluid glycine concentration is considered especially typical for nonketotic hyperglycinemia (19); (2) the glycine cleavage system is diminished to a level which agrees with what has been found in patients with nonketotic hyperglycinemia.

The following points can be made against the hypothesis that the glycine accumulation is primary: (1) in no other patients with hyperglycinemia has there been demonstrated accumulation of D-glyceric acid, making it highly unlikely that his accumulation should be caused by the hyperglycinemia; (2) very substantial amounts of [1-^{14}C]glycine are converted to D-[^{14}C]glyceric acid *in vivo* (Table 1) suggesting only a partial deficiency of the glycine cleavage system and trapping of the degraded ^{14}C atoms in D-glyceric acid; (3) D-glycerate dehydrogenase was deficient in the patients' cells, and this deficiency was, in view of results from the mixing experiments, not due to inhibition (13); (4) secondary hyperglycinemia has repeatedly been described in connection with other organic acidurias, namely propionyl-CoA carboxylase deficiency, methylmalonyl-CoA mutase deficiency, and β-ketothiolase deficiency.

The following points can be made in favor of the D-glyceric acid accumulation being primary: (1) deficient D-glycerate dehydrogenase not due to inhibition; (2) partial support is given by the observation that only undetectable amounts of D-glyceric acid were converted to glycine *in vivo*, suggesting a much more extensive defect in the degradation of D-glyceric acid than in the degradation of glycine (Table 1). Because no observations have been obtained contradicting the hypothesis that the D-glyceric acid accumulation is primary, we are at present of the opinion that the child suffered from an inborn deficiency of D-glycerate dehydrogenase and that the hyperglycinemia was a secondary phenomenon.

The data in Figure 1 show that a positive correlation exists between the glycine accumulation and the D-glyceric acid accumulation. Because it can be ruled out that a part of the accumulated D-glyceric acid is converted to glycine (Table 1), this correlation seems to suggest that the glycine cleavage system is somehow influenced by the D-glycerate dehydrogenase deficiency. A likely mechanism for this could be that a compound accumulated because of the D-glycerate dehydrogenase deficiency inhibits either the activity or the synthesis of the glycine cleavage system. In this case, inhibition of activity seems most likely because the concomitant alterations in excretion of the two compounds can take place relatively fast. We have thus observed an almost 100% increase in excretion of both compounds within 2 wk (glycine, 53.7 μmoles/mg creatinine to 107.8 μmoles/mg creatinine; D-glyceric acid: 263.2 μmoles/mg creatinine to 459.4 μmoles/mg creatinine). The most likely candidate for such an inhibitor would of course be D-glyceric acid. It was therefore investigated whether D-glyceric acid might inhibit the glycine cleavage system, a mechanism that would correspond well with the current concept on the ethiology of ketotic hyperglycinemia (18). No inhibition could, however, be demonstrated, a result that is in accordance with the recent finding of a child with D-glyceric acidemia but without hyperglycinemia (20).

Bearing in mind the analogy to the ketotic hyperglycinemia syndrome, the possibility of other endogenous inhibitors of the glycine cleavage system was then considered. Efforts here were concentrated on metabolites of the branched-chain amino acids, firstly because the existence of the syndrome ketotic hyperglycinemia seems to indicate that deranged branched-chain amino acid metabolism can affect the glycine cleavage activity and secondly because in a previous communication, we have demonstrated that 2-methyl-butyryl-CoA and isobutyryl-CoA possess strong inhibitory action on the glycine cleavage system (12). The results in Table 3 indicate that the metabolism of the branched-chain amino acids is in fact severely deranged in the patient, with elevated excretion of a variety of metabolites. The elevated excretion of isobutyric acid, 2-methyl-butyric acid, and isovaleric acid after alkaline hydrolysis is striking. We consider these acids derived from conjugates, especially glycine conjugates because it is well documented, that accumulations of short-chain fatty acids in patients with a number of organic acidurias results in excretion of the corresponding glycine conjugates. In this context, it is interesting that these accumulations of branched-chain amino acid metabolites are constantly present and not, as described for glutaryl-CoA dehydrogenase deficiency, associated with severe intercurrent infections (9).

Although the ethiology of this deranged branched-chain amino acid metabolism is at present unknown, the constant occurrence of free and especially of conjugated isobutyric acid, 2-methyl-butyric acid, and isovaleric acid in urine seems to indicate that there exists an analogous intramitochondrial accumulation of the corresponding thiolesters, two of which, as mentioned, cause strong inhibition of the glycine cleavage system. It is therefore suggested that these accumulations play an ethiologic role in the diminished glycine cleavage activity found in liver tissue from the patient.

Finally, the possibility of inborn defect in a common factor for both enzymes must be considered. Both D-glycerate dehydrogenase and the glycine cleavage system are in principle NAD-requiring dehydrogenases, but nothing is known about any common structural element in the two enzymes. One thing might perhaps be counted in favor of this possibility, namely the fact that Wadman *et al.* (20) have described a child with D-glyceric acidemia, but with a clinical picture totally different from our patient and with no hyperglycinemia. In this child, no enzyme measurements were performed due to loss of contact with the patient. Assuming that this child also had an inborn deficiency of D-glycerate dehydrogenase, this might at least imply that more than one structural defect in this enzyme can exist. It can, however, also be speculated that the difference in hyperglycinemia between our patient and the one described by Wadman *et al.* could reflect the interindividual variation in tendency to hyperglycinemia known in patients with propionyl-CoA carboxylase deficiency, methylmalonyl-CoA mutase deficiency, and β-ketothiolase deficiency, thus suggesting that the two children suffered from the same inborn enzyme defect.

REFERENCES AND NOTES

1. Ando, T., Nyhan, W. L., Gerritsen, T., Gong, L., Heiner, D. C., and Bray, P. F.: Metabolism of glycine in the nonketotic form of hyperglycinemia. Pediatr. Res., *2*: 254 (1968).
2. Ando, T., Rasmussen, K., Nyhan, W. L., Donnell, G. N., and Barnes, N. D.: Propionic acidemia in patients with ketotic hyperglycinemia. J. Pediatr., *78*: 827 (1971).
3. Brandt, N. J., Rasmussen, K., Brandt, S., Kølvraa, S., and Schønheyder, F.: D-glyceric acidemia and nonketotic hyperglycinemia. Clinical and laboratory findings in a new syndrome. Acta Paediatr. Scand., *65*: 17 (1976).
4. Chalmers, R. A., and Watts, R. W. E.: The quantitative extraction and gas-liquid chromatographic determination of organic acids in urine. Analyst, *97*: 958 (1972).
5. Corbeel, L., Tada, K., Colombo, J. P., Eeckels, R., Eggermont, E., Jaeken, J., Den Tandt, W., Harvengt, L., Delhaye, J., and Deloecker, W.: Methylmalonic acidemia and the nonketotic hyperglycinemia. Arch. Dis. Child., *50*: 103 (1975).

6. Davis, L.: A simple method for the synthesis of tetrahydrofolic acid. Anal. Biochem., *26:* 459 (1968).
7. Fredrickson, D. A., and Ono, K.: An improved technique for assay of $^{14}CO_2$ in expired air using the liquid scintillation counter. J. Lab. Clin. Med., *51:* 147 (1958).
8. Gliemann, J.: Insulin-like activity of diluted human serum assayed by an isolated adipose cell method. Diabetes, *14:* 643 (1965).
9. Gregersen, N., and Brandt, N. J.: Ketotic episodes in a patient with glutaryl-CoA dehydrogenase deficiency. Pediatr. Res., *13:* 977 (1979).
10. Hillman, R. E., and Keating, J. P.: β-Ketothiolase deficiency as a cause of the ketotic hyperglycinemia syndrome. Pediatrics, *53:* 221 (1974).
11. Kesner, L., and Muntwyler, E.: Automatic determination of weak organic acids by partition column chromatography and indicator titration. Anal. Chem., *38:* 1164 (1966).
12. Kølvraa, S.: Inhibition of the glycine cleavage system by branched chain amino acid metabolites. Pediatr. Res., *13:* 889 (1979).
13. Kølvraa, S., Rasmussen, K., and Brandt, N. J.: D-glyceric acidemia. Biochemical studies in a new syndrome. Pediatr. Res., *10:* 825 (1976).
14. Lowry, O. H., Rosebrough, N. J., Farr, A. L., and Randall, R. J.: Protein measurement with the Folin phenol reagent. J. Biol. Chem., *193:* 265 (1951).
15. Morrow, G., Barness, L. A., Auerbach, V. H., DiGeorge, A. M., Ando, T., and Nyhan, W. L.: Observations on the coexistence of methylmalonic acidemia and glycinemia. J. Pediatrics, *74:* 680 (1969).
16. Nishimira, Y., Tada, K., and Arakawa, T.: Coexistense of defective activity in glycine cleavage reaction and propionyl-CoA carboxylase in the liver of a hyperglycinemic child. Tohuku J. Exp. Med., *113:* 267 (1974).
17. Nyhan, W. L.: Nonketotic hyperglycinemia. In: W. L. Nyhan: Hereditable Disorders in Amino Acid Metabolism. p. 309 (John Wiley & Sons, Inc., New York, 1974).
18. Nyhan, W. L.: Propionic acidemia and the ketotic hyperlgycinemia syndrome. In: W. L. Nyhan: Hereditable Disorders of Amino Acid Metabolism. p. 37 (John Wiley & Sons, Inc., New York, 1974).
19. Perry, T. C., Urquart, N., MacLean, J., Evans, M. E., Hansen, S., Davidson, A. G. F., Applegarth, D. A., MacLeod, P. J., and Lock, S. E.: Nonketotic hyperglycinemia. N. Engl. J. Med., *292:* 1269 (1975).
20. Wadman, S. K., Duran, M., Ketting, D., Bruinvis, L., De Bree, P. K., Kamerling, J. P., Gerwig, G. J., Vliegenthart, J. F. G., Przyrembel, H., Becker, K., and Bremer, H. J.: D-Glyceric acidemia in a patient with chronic metabolic acidosis. Clin. Chem. Acta, *71:* 477 (1976).
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