Clinica Chimica Acta, 71 (1976) 477—484

CCA 8041

D-GLYCERIC ACIDEMIA IN A PATIENT WITH CHRONIC METABOLIC ACIDOSIS

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(Received May 6th, 1976)

Summary

A patient is described with glyceric acidemia and glyceric aciduria.

The main clinical problems in infancy were severe metabolic acidosis and failure to thrive. The patient needs permanent treatment with bicarbonate. Hyperglycinemia, as described in the first case discovered elsewhere, was not present. The glyceric acid was found to have the D-configuration, as analyzed by capillary gas chromatography of its di-*O*-acetyl-l-menthyl ester.

The abnormality may result from a defect in serine metabolism.

Introduction

Recently, Brandt et al. described hyper-D-glyceric acidemia together with hyperglycinemia in a Serbian boy with many neurological abnormalities and severe mental retardation [1,2]. The child never had any manifest acidotic episodes. The clinical picture resembled that of non-ketotic hyperglycinemia.

Excretion of L-glyceric acid has been described in some patients with hyperoxaluria [3] as a result of D-glyceric acid dehydrogenase deficiency.

In this paper we present a second patient excreting D-glyceric acid in excess. The clinical picture, however, was different from that of the patient of Brandt c.s.

Some attempts were made to localize the underlying enzyme defect.

* With support of Het Praeventiefonds.

** Supported by a grant of the Landesamt für Forschung des Ministeriums für Wissenschaft und Forschung des Landes Nordrhein-Westfalen.

Case report

The patient is the third child of apparently healthy Afghanese parents. Consanguinity in the family is denied. The two elder brothers of the patient are reported to be normal.

Our patient was born by caesarean section because of intrauterine asphyxia; weight 2730 g, length 50 cm. Until the 13th day of life the child appeared quite well, but he then stopped gaining weight and refused oral feedings. Three days later he developed tachypnoea, tachycardia and a severe metabolic acidosis (blood pH 6.96, pCO_2 17.5 mmHg, standard bicarbonate 3.7 mequiv./l, base excess −27 mequiv./l), which led to his admission to the intensive care unit of the University Children's Hospital at Düsseldorf.

Parenteral administration of buffer, electrolyte, and glucose solutions resulted in an almost immediate clinical improvement. The subsequent administration of a commercially available formula (Pomila®: 1.6 g protein, 3.7 g fat, 8.0 g carbohydrate, and 74 calories per 100 ml) in slowly increasing amounts was well tolerated up to an amount of 2.9 g protein/kg body weight/day on the 24th day of life. But there was a constant compensated metabolic acidosis (base excess up to −10 mequiv./l), which responded well to oral administration of sodium bicarbonate. A renal origin of this acidosis was improbable as the urinary pH was always below 6. Urinary calcium, phosphorus, and oxalate * excretions were normal.

There was never any hypoglycemia. Cholesterol, triglycerides and uric acid in plasma were normal, as were blood ammonia, lactate and pyruvate. The intravenous pyelogram, the eye fundi and the electroencephalogram did not reveal abnormalities. Bone age at 3 months was slightly retarded. The patient developed satisfactorily. Intercurrent otitis media and gastroenteritis were adequately treated. At the age of $4\frac{1}{2}$ months the patient was discharged and unfortunately lost for follow-up observation. His weight at that time was 4850 g, he still required 30 mequiv. bicarbonate administered per day. He smiled readily and reached out for objects and was neurologically normal.

He was again seen at another hospital at the age of 16 months because of bronchopneumonia; weight then 8.5 kg and length 77 cm. He was able to sit without support, but could not walk and seemed moderately mentally retarded. A urine sample for gas chromatography was obtained from the hospital, but the parents could not be prevailed upon to come for regular checks of the acidosis and the patient's development.

Methods

Organic acids in urine

For routine screening the acids were extracted with ethylacetate according to our routine procedure [4]. For glyceric acid the extraction efficiency is as low as 6%. Therefore, a more intensive extraction procedure was used for this compound: the aqueous phase was kept at exactly 5 ml and extracted five times with 20 ml ethylacetate, giving a recovery of 25%. The reproducibility

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was sufficient (variation coefficient 8%). Also an isolation procedure using a 6 ml Sephadex DEAE A-25 anion exchange column was used [5]. However, elution with 15 ml 1.5 M pyridine/acetate buffer proved to be insufficient for glyceric acid, and the eluant volume had to be increased to 25 ml. Both extraction and ion-exchange methods gave reasonably comparable results.

The acids were analyzed by gas chromatography of their trimethylsilyl derivatives mainly as described previously [4]. However, the temperature programming was modified: 75°C for 10 min followed by an increase of 2°C per min up to 220°C and finally 10 min at 220°C. Packing material was 5% GE SE-52 on Chromosorb W-AW-DMCS 100-120 mesh (high performance).

Organic acids in serum

Organic acids in serum were extracted according to the same routine procedure as used for urine [4]. Insufficient material was available for repeating the determinations with the intensive extraction method, which was developed later.

Gas-liquid chromatography — mass spectrometry

The 75 eV mass spectra were recorded on a Jeol JGC-20 KP/JMS-D 100/W-JMA system combination at an ion source temperature of 150°C, an accelerating voltage of 3 kV and an ionizing current of 300 μA. The gas-liquid chromatography conditions were the same as described above.

Configuration determination of glyceric acid

Glyceric acid was isolated from urine, either by the ethylacetate extraction procedure followed by preparative two-dimensional paper chromatography (Whatman 3 MM; solvents isopropanol/ammonia 10%/water (8 : 1 : 1, by vol.) and butanol/acetic acid/water (4 : 1 : 1, by vol.)) or directly by the anion exchange method on Sephadex DEAE A-25.

For the determination of the absolute configuration, the isolated glyceric acid was esterified under anhydrous conditions with natural 1-menthol/HCl for 2 h at 105°C. The excess of menthol and HCl was removed by a stream of nitrogen at 100°C [6]. Subsequently the ester was acetylated with pyridine/acetic anhydride (1 : 1, v/v) for 30 min at 100°C. The reagents were evaporated with absolute ethanol in vacuo and the residue was dissolved in chloroform.

The $CHCl_3$ solution was investigated by capillary gas-liquid chromatography on a Varian Aerograph 2740-30-01 gas chromatograph with a flame ionization detector and an SP-1000 coated capillary glass column (LKB; 25 m × 0.3 mm I.D.). The carrier gas N_2-flow rate was 1 ml/min, the oven temperature 200°C, the make-up gas N_2-flow rate 30 ml/min. Injection port 200°C; detector 220°C. The menthyl ester of di-*O*-acetyl-D,L-glyceric acid, giving separated peaks for the D- and L-form on SP-1000, was used as a reference substance. The free acid was generated by filtration through a column of Dowex 50 X8 (H^+). D- and L-glyceric acids were synthesized according to Wohl and Schellenberg [7].

For the determination of the configuration of glyceric acid in blood serum this compound was isolated from a 0.6 ml sample by the Sephadex anion exchange method after deproteinization with 9 volumes of ethanol.

Amino acids

Quantitative amino acid analysis was done by automated ion exchange column chromatography (Technicon TSM1 apparatus; standard method for physiological fluids).

Results

Gas chromatographic analysis of the organic acids in serum and urine showed high concentrations of glyceric acid, a compound which is normally not detected (Fig. 1). Its identity could easily be proven by mass spectrometry. In addition only minor abnormalities were seen: in some serum samples lactic acid was increased up to 5 mM; however, the excess of lactic acid may be artificially produced during the clotting process. Urinary lactate was normal. Ketosis (β-hydroxybutyric aciduria) did not occur. Glycolic acid was normal both in serum and urine, and no oxalic acid was detected.

The determination of the optical configuration showed that the urinary as well as the serum glyceric acid had the D-configuration. The two peaks of the D- and L-glyceric acid derivatives are well separated, the L-compound being the faster.

In Fig. 2a the gas chromatogram of urinary glyceric acid, as its di-*O*-acetyl menthyl ester derivative in patient B.B. is given. Fig. 2b shows the D,L-glyceric acid derivative and in Fig. 2c the result of the co-chromatography experiment is given.

In Table I the urinary concentrations of glyceric acid are shown. The highest urinary concentration measured was 116 mM, the patient being on a non-restricted diet. The serum concentrations were less than 4 mM.

A few loading tests were performed in order to investigate whether a direct precursor of D-glyceric acid could be traced.

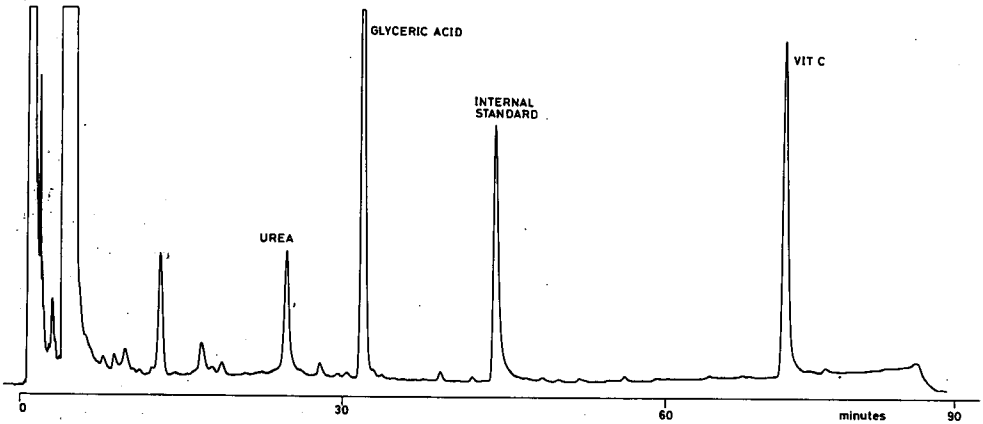
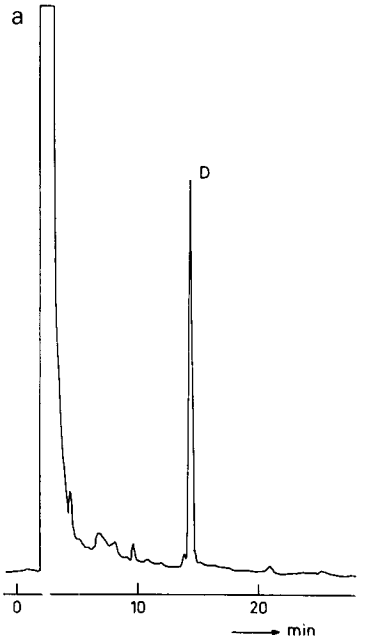
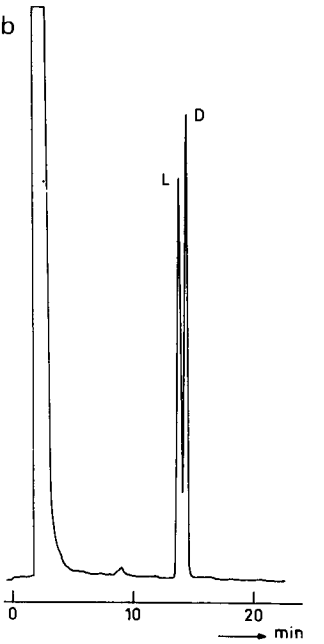


Fig. 1. Gas chromatography of urinary organic acids (TMS-derivatives) in patient B.B. Internal standard, 4-phenylbutyric acid.





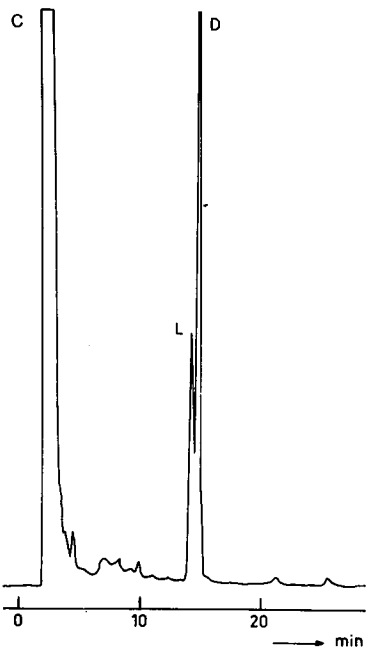


Fig. 2. Gas-liquid chromatography of glyceric acid (di-*O*-acetylated menthyl esters) a, urine from patient B.B.; b, authentic D,L-glyceric acid; c, co-chromatography of urine B.B. and authentic D,L-glyceric acid.

TABLE I

D-GLYCERIC ACID IN THE URINE OF PATIENT B.B.

	Volume (ml)	Creatinine (g/l)	Glyceric acid		
			mmol/l	mmol/g creatinine	mmol
18th day: parenteral glucose + electrolytes, some milk	230	0.125	13.9	111	3.2
55th day: 2.9 g protein/day 6-h sample, 3—9 a.m.	50	0.185	28.4	153.5	1.4
55th day: 2.9 g protein/day 6-h sample, 9 a.m.—15 p.m. after fructose, 1 g/kg	100	0.115	17.2	149.5	1.7
58th day: 2.9 g protein/day 5,5-h sample, 15.30 p.m.—21 p.m.	90	0.140	26.6	190.0	2.4
113th day: 2.5 g protein/day 6-h sample, 3—9 a.m.	95	0.120	19.3	160.8	1.8
113th day: 2.5 g protein/day 6-h sample, 9 a.m.—15 p.m. after serine 200 mg/kg = 9.3 mmol total	70	0.155	44	286	3.1
1 year 4 months: free diet. Admitted for bronchitis elsewhere. 24-h sample.	120	1.235	116	93.9	13.9

An oral fructose test performed at the age of 8 weeks (1 g fructose/kg) showed a normal increase of blood glucose and no aggravation of acidosis. Urinary glyceric acid did not respond.

Three days later an oral loading test with L-serine (100 mg/kg) was done. Serum serine increased little, from 0.15 mM to 0.22 mM after 2 h. Urine was collected in 6-h samples before and after loading. Unfortunately the first sample after loading was not collected completely, which caused the absolute increase of glyceric acid excretion not to be measured. Expressed as mol per g creatinine we obtained 0.16 for the 6-h sample before loading, and 0.21 and 0.18 respectively for the two subsequent samples after loading.

Two months later a second oral L-serine loading test was done; this time 200 mg/kg was given. Serum serine increased from 0.21 mM to 0.75 mM after 2 h, a much higher value than in the first loading test. The urinary 6-h excretion of glyceric acid increased from 1.8 mmol to 3.1 mmol, the difference corresponding to 14% of the serine load.

Also after loading with L-serine only D-glyceric acid was excreted. No oxalic acid was found and the gas chromatogram showed the same low glycolic acid excretion before and after loading, 0.18 mmol per 6 h. Special attention was paid to hydroxypyruvic acid, which could not be detected. (Method used: chromatography of keto acid dinitrophenyl hydrazones and of their corresponding amino acids obtained by catalytic reduction).

Clinically the patient did not show aggravation of the acidosis during the serine loading tests; the negative base excess remained practically constant.

For comparison a control patient was loaded with L-serine (200 mg/kg). Only in the night sample (21 p.m. — 3 a.m.) a trace of glyceric acid was found (less than 0.01 mmol/l).

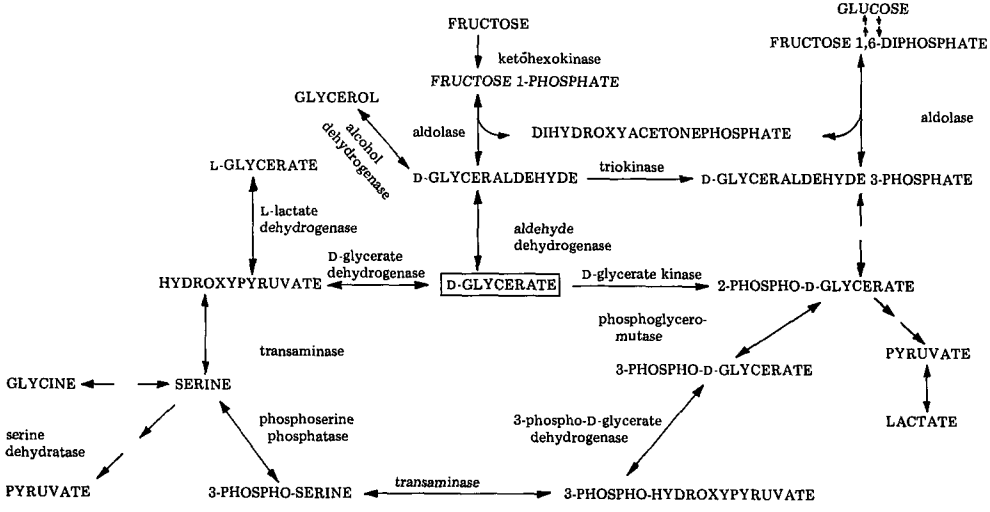
Amino acid analyses did not reveal essential abnormalities. Notably glycine was normal in serum (less than 361 μmol/l) and in urine (<2 mmol/l). Also serine was normal. Initially there was an increased amino aciduria of a transient type. Most increased were cysteine and lysine. Loading with serine did not influence the other amino acids; serum and urinary glycine remained practically unaltered.

Discussion

From the results given it becomes clear that our patient with D-glyceric aciduria shows a considerable difference from the first patient described with this biochemical abnormality. The latter had practically normal acid-base parameters and no signs of severe metabolic acidosis in the neonatal period. In our patient metabolic acidosis and failure to thrive were the main problems. However, it has to be questioned whether the amount of base deficit may be accounted for by D-glyceric acid only. No serum from periods with severe acidosis was available for analysis. The rather low serum values obtained when acidosis was less severe, together with high urinary excretions, point to a low renal threshold for D-glyceric acid.

In the patient of Brandt c.s. plasma and urinary glycine were highly elevated and a clinical syndrome of non-ketotic hyperglycinemia (severe mental retardation, pronounced hypotonia and seizures) was present. Our patient did not show these abnormalities. Apparently overflow of D-glyceric acid is not necessarily connected with hyperglycinemia. Similar differences were observed in patients with α-methyl-β-ketobutyric aciduria [8,9].

No conclusive statements about the underlying defect can be made with the data obtained so far.



Scheme 1. Metabolism of D-glycerate.

A lack of response after loading with fructose revealed that this carbohydrate is not likely to be metabolized via D-glycerate. More probably fructose is degraded via glyceraldehyde-3-phosphate. See Scheme 1. D-Glycerate dehydrogenase does not seem to be defective, as is the case in hyperoxaluria II described by Williams and Smith [3]. They assumed that normally hydroxypyruvic acid is converted into D-glycerate in humans. In the absence of D-glycerate dehydrogenase hydroxypyruvate is reduced to L-glycerate with L-lactate dehydrogenase. Administration of hydroxy[^{14}C]pyruvate to one of their patients resulted in the urinary excretion of [^{14}C]glycerate. The administration of 10 g of serine, however, did not result in an increased glycerate excretion. Despite this the authors assumed that degradation of serine via hydroxypyruvate is operative.

We assume that a partial transamination of serine to hydroxypyruvate is possible in certain conditions. In human liver the presence of hydroxypyruvate: L-alanine transaminase has been demonstrated [10]. Our loading experiment with L-serine could indicate that in our patient the degradation of serine is disturbed (e.g. at the level of serine dehydratase), leading to an increased transamination. The activity of D-glycerate kinase is low in human liver [11] and even absent in the kidney [12] and intestine [13]. At the moment it is not known whether it plays any role in the conversion of D-glycerate into 2-phospho-D-glycerate, but we have to consider the possibility of a D-glycerate kinase deficiency.

Even in the case of normal D-glycerate kinase activity, increased transamination of serine could lead to accumulation of D-glycerate, D-glycerate dehydrogenase being normal.

Further investigations into the serine metabolism will be necessary to locate the enzymatic defect in our patient.

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