Deficiency of Glycerol Kinase (EC 2.7.1.30)

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We describe the case of a 10-year-old boy who had been admitted on several occasions with a diagnosis of gastroenteritis. He had been severely ill, and on one occasion lost consciousness. He had a metabolic acidosis on these occasions. Examination of the urine by gas chromatography–mass spectrometry showed a large peak, identified as glycerol. The concentration of glycerol in the urine was 40–280 mmol/L and the concentration in plasma about 2 mmol/L. He was subjected to a fast of 21 h, at the end of which he expressed feelings of discomfort and nausea, began vomiting, and became somnolent. During this period the blood glucose concentration was only slightly decreased, the plasma glycerol concentration increased to 4.9 mmol/L, and the plasma lactate concentration increased to 3.8 mmol/L. During work on a bicycle ergometer for 35 min (40 W) he complained of muscle pain and became nauseated, but there was no significant increase in the concentration of plasma glycerol. The activity of glycerol kinase (EC 2.7.1.30) in leukocytes and cultured fibroblasts was less than 1% of the value for healthy subjects.

Two groups (1–4) have described cases with a deficiency of glycerol kinase (ATP:glycerol 3-phosphotransferase, EC 2.7.1.30) but the clinical picture in their cases was entirely different. This report describes studies on a case of glycerol kinase deficiency in a 10-year-old boy.

Case History

The boy is the only child of healthy unrelated parents of Swedish origin. A maternal granduncle suffered from epilepsy. There are no known cases of psychomotor or mental retardation among the relatives. The pregnancy was uneventful, and the patient was born in the thirty-eighth week with a birth weight of 4.08 kg. His physical and mental development has been normal and he entered school at the normal age. He has had one episode of otitis media, a few viral respiratory infections, morbilli, and rubella, all without any complications.

The child has been hospitalized at the Boden General Hospital, on five occasions: at age 4, 6, 7, 8.5, and 9 years. On all occasions he has developed a clinical picture interpreted as viral gastroenteritis, and hospitalization has been necessary because of excessive vomiting and rapidly deteriorating general condition. During the first episode he was hospitalized after two days of fever, diarrhea, and vomiting. On admission he was pale, somnolent, hyperventilating, and complaining of severe abdominal pain. An intestinal invagination was suspected, but ruled out by normal roentgenographic findings after a barium enema. He had a metabolic acidosis, pH 7.31, pCO2 4.1 kPa, base excess −10.5 mmol/L, and standard bicarbonate 17.1 mmol/L. Sodium and potassium concentrations in serum were within the normal reference interval. The acidosis was rapidly corrected by therapy with intravenous fluid. The two following episodes were essentially similar, but his general condition was less affected and dehydration was corrected by therapy with oral fluid. No blood analyses of acid–base parameters or electrolytes were performed.

The hitherto most severe episode occurred at the age of 8.5 years, when he was hospitalized after 36 h of moderate diarrhea and vomiting. On admission he was tired, pale, and hardly capable of answering questions. Within a few hours of admission his condition deteriorated and he became unconscious. There was a metabolic acidosis, pH 7.24, pCO2 4.3 kPa, standard bicarbonate 13.9 mmol/L, and base excess −11.5 mmol/L. The activities of aspartate aminotransferase, alanine aminotransferase, and alkaline phosphatase in serum were all slightly above the normal reference interval. An acute encephalitis was suspected, but results of examination of cerebrospinal fluid, including the electrophoretic pattern of proteins, were entirely normal. An electroencephalogram (EEG) showed a general but unspecific abnormality. A screening for rising viral titers gave negative results. The metabolic acidosis was normalized within one day by intravenous fluids containing bicarbonate and glucose, and the patient had recovered completely within one week. The last episode was preceded by two days of excessive physical activity; vomiting started in the evening of the second day, and on admission the next morning he was pale and tired, but not unconscious. Again, there was a metabolic acidosis, pH 7.2, pCO2 4.6 kPa, base excess −12.9 mmol/L, standard bicarbonate 14.6 mmol/L. After therapy with intravenous fluid he recovered within one day.

At the age of eight years—a few months before the most severe episode—the patient had two epileptic seizures of the grand mal type. The EEG recording revealed epileptic activity (Rolandiic spikes) in the central and parietal parts of the right hemisphere. Phenytoin (200 mg/day) was started and has been continued ever since. He has remained free of seizures and the last EEG recording at age 8.5 years showed a normal pattern. At present, at the age of 11 years, a general clinical and neurological examination has revealed no abnormality. The activity of creatine kinase (EC 2.7.3.2) has been 1.85, 1.32, and 2.40 μkat/L with the activity of the B subunit <0.15 μkat/L. These values are within the reference interval used in our laboratory. Results of a standard intravenous corticotropin-stimulation test were normal. A urine sample, examined for the presence of abnormal compounds by the procedure used in screening for
organic acidurias, gas chromatography–mass spectrometry, revealed the presence of an increased amount of glycerol.

Materials

All the materials we used are commercially available. [1-14C]Glycerol (2.039 GBq/mmol) was obtained from New England Nuclear, Boston, MA.

Methods

Organic acids in urine were determined by gas chromatography–mass spectrometry. Urine was stored at −20 °C immediately after collection until worked up. Organic acids were extracted with ethyl acetate from acidified urine, and the extract was evaporated and the residue derivatized with methoxylamine hydrochloride followed by trimethylsilylation reagent (BSTFA), according to the procedure detailed elsewhere (5).

The organic acids were separated by capillary gas chromatography on a Varian Model 3700 equipped with a 25 m (0.5 mm i.d.) open tubular Pyrex column statically coated with SE-54 according to Grob (6) to yield a film thickness of 1.2 μm. The carrier gas was helium with a flow rate of 3 mL/min at 200 °C. The oven temperature was programmed from 50 °C to 280 °C at a rate of 10 °C/min. Peak identities were established by mass spectrometry with a Varian MAT 44S quadrupole instrument (Finnigan MAT, Bremen, F.R.G.) operating in the electron-impact mode at ion-source temperature of 220 °C and an electron voltage of 70 eV. The mass spectrometer was interfaced to the capillary gas chromatograph through an open split coupling. The quadrupole analyzer was mass calibrated with bis(perfluoroheptyl)-S-triazine up to m/z 1066 to yield relative peak intensities comparable to the mass spectrum obtained from a sector field instrument.

The concentrations of lactate, 3-hydroxybutyrate, acetoacetate, free fatty acids, triglycerides, and glucose were determined according to standard techniques (7–11). The activity of creatine kinase was determined according to the Scandinavian Committee on Enzymes (12).

The radiochemical assay for glycerol was that developed by Newsholme and Taylor (13). The assay is based on the glycerol kinase-catalyzed conversion of [1-14C]glycerol to [1-14C]glycerol 1-phosphate. Per liter, the assay mixture contained 83 mmol of Tris HCl buffer, pH 7.6, 1.7 mmol of EDTA, 16.6 mmol of NaF, 4.1 mmol of MgSO4, 16.6 mmol of mercaptoethanol, and 4.1 mmol of ATP, plus 40 ng of glycerol kinase in a total reaction-mixture volume of 200 μL. Assay temperature was 25 °C. The concentration of labeled glycerol in the assay was 45.8 μmol/L, and we used 50 μL of serum, plasma, or urine. The labeled glycerol and glycerol 1-phosphate were separated by use of the ability of diethylaminoethyl-paper to bind negatively charged groups. The radioactivity incorporated into glycerol 1-phosphate was determined in a liquid scintillation spectrometer (Packard Tri-Carb Model 3320). The total amount of 14C incorporated into glycerol 1-phosphate (C0) was first determined and then the incorporation of 14C in the presence of different amounts of unlabeled glycerol (C1). We then plotted a standard curve of C0/C1 vs C1.

Glycerol kinase activity was assayed in homogenates prepared from isolated leukocytes and fibroblasts. Leukocytes were prepared from EDTA-anticoagulated blood by dextran sedimentation and freed of contaminating erythrocytes by lysis in cold NaCl, 40 mmol/L, for 90 s. Skin fibroblasts were grown in McCoy's medium supplemented with 18 mL of fetal calf serum per deciliter. When confluent, monolayers of fibroblasts were washed twice with phosphate-buffered isotonic saline and trypsinized. The harvested fibroblasts were washed twice before use in enzyme assays. The cells were homogenized in Tris HCl buffer (50 mmol/L, pH 7.4) containing 2 g of bovine serum albumin per liter, with a Potter-Elvehjem homogenizer. Total protein in the homogenate was measured by the method of Lowry et al. (14).

The assay mixture was that described above for the glycerol determination. Per liter, it contained 0.72–137 μmol of [1-14C]glycerol, 67 μmol to 10.6 mmol of glycerol, and 0.1–1.0 mg of homogenate. The incubations were terminated after 10–60 min by adding ethanol. The formed [1-14C]glycerol 1-phosphate was then determined as described above.

Results

Glycerol in Urine and Blood Plasma

Figure 1 shows the result of gas chromatography–mass spectrometry of a urine sample. The insert in this figure shows the mass spectrum, which is characteristic of glycerol (15). Because the extraction efficiency for glycerol is rather low, we used the enzymatic method for measuring its excretion in the urine. For nine urine samples collected over a period of six months, it ranged from 43 to 280 mmol/L, whereas in control samples it was <0.2 mmol/L (Table 1). The concentration of glycerol in the urine from the parents was also <0.2 mmol/L. The plasma glycerol concentration was about 2 mmol/L in the boy, and fell within the normal reference interval in the parents (Table 1).

Activity of Glycerol Kinase

The activity of glycerol kinase was determined in homoge-
Table 1. Concentrations of Glycerol in Plasma and Urine

<table>
<thead>
<tr>
<th></th>
<th>P-Glycerol (mmol/L)</th>
<th>U-Glycerol (mmol/L)</th>
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<tbody>
<tr>
<td>Our case</td>
<td>2.1</td>
<td>43–280</td>
</tr>
<tr>
<td>Mother</td>
<td>0.029</td>
<td>0.092</td>
</tr>
<tr>
<td>Father</td>
<td>0.041</td>
<td>0.053</td>
</tr>
<tr>
<td>Controls (n = 20)</td>
<td>0.02–0.25</td>
<td>0.05–0.2</td>
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Table 2. Glycerol Kinase Activity in Leukocytes and in Fibroblasts

<table>
<thead>
<tr>
<th></th>
<th>Leukocytes (pmol/min per mg protein)</th>
<th>Fibroblasts (pmol/min per mg protein)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Our case</td>
<td>0.24</td>
<td>0.1</td>
</tr>
<tr>
<td>Mother</td>
<td>56.7</td>
<td>8.3</td>
</tr>
<tr>
<td>Father</td>
<td>56.5</td>
<td>1.8, 2.3</td>
</tr>
<tr>
<td>Controls</td>
<td>35–62 (n = 10)</td>
<td>5.9–13.0 (n = 4)</td>
</tr>
</tbody>
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nates of leukocytes from the patient, his parents, and five reference subjects (Table 2). The activity in the patient was 0.5% of that in normals, whereas the activity of glycerol kinase in leukocytes from the parents did not differ from that in the reference subjects. In fibroblasts (Table 2), the activity of the enzyme in the patient was about 1% of that in normal controls.

Effect of Fasting

To study the effect of fasting we withheld food, beginning at 16:00 hours. The following morning the boy appeared completely normal and active, but drowsiness gradually developed; he became irritable and went to bed. About 21 h after the start of the experiment he complained of nausea and began to vomit. The clinical picture then resembled what had been noted when he had previously been hospitalized for “gastroenteritis.” Food was given and he recovered quickly to a normal state.

Blood samples were analyzed according to standard techniques for free fatty acids, triglycerides, 3-hydroxybutyrate, acetoacetate, lactate, and glucose (Figure 2). The changes in concentration of glucose, acetoacetate, 3-hydroxybutyrate, and free fatty acids were those expected in a normal subject. The lactate concentration rose to 3.8 mmol/L. The glycerol concentration, initially 1.94 mmol/L, increased to a peak value of 4.9 mmol/L.

Urine samples were analyzed for glycerol and organic acids (Figure 3). The glycerol concentration increased to 42 mol/mol of creatinine. The pattern of organic acids showed ketocidosis.

The parents of the patient were voluntarily fasted for 16 h. During this period the urine was sampled and analyzed for glycerol, and after 16 h of fasting a blood sample was analyzed for glycerol. There was no increase in the concentration of glycerol in urine and the concentration of glycerol in plasma fell within the normal range. The parents felt well during the whole fasting period.

Effect of Physical Exercise

The boy was subjected to work on a bicycle ergometer (40 W) for 35 min. After about 15 min he complained of pain in the leg muscles. Immediately after the work period he complained of nausea and began to vomit. When glucose was given orally he immediately felt better. Figure 4 shows the plasma concentrations of glycerol, and free fatty acids, and the concentrations of lactate and glucose in blood.

Discussion

In 1977, McCabe et al. (2, 3) reported on two brothers, two and five years old, with a concentration of glycerol in serum of about 0.5–1.0 mmol/L. The urinary excretion of glycerol was not reported. The children had poor somatic growth, psychomotor retardation, spasticity, nonparalytic esotropia, and pathological bone fractures. They had had no epileptic seizures. Both had osteoporosis and an unspecific myopathy with a very high concentration of serum creatine kinase. At six years of age, the elder brother was hospitalized for an episode of gastroenteritis. He was lethargic and dehydrated, and had hyponatremia and hyperkalemia. Addisonian pigmentation was noted and an endocrinological workup gave results characteristic of MB Addison. In the younger brother a surgical procedure for correction of strabismus was performed at age 33 months. Two days later, at home, he became very ill and died within 12 h.
Our case had none of the multiple symptoms characteristic of the two American children, but the clinical picture during his repeated hospitalizations to some extent resembles that of the episode described for the elder American brother, which was also ascribed to gastroenteritis. In the American case, laboratory data characteristic of an Addisonian state were obtained at the time of hospitalization; our case showed a normal response to a standard intravenous corticotropin-stimulation test.

In 1969, Pitkänen and Palo (16) found increased excretion of glycerol in four of 900 patients with mental deficiency. Its 24-h urinary excretion in these cases was about 2–3 mmol (reference value 0.007–0.033 mmol) in spite of a normal plasma glycerol. From results of loading tests with glycerol and fructose, Palo et al. (17) concluded that the glyceroluria was due to a disturbed reabsorption capacity of the kidney tubuli rather than to a metabolic defect in the tubular cells. Besides, Palo et al. (17) reported that the disturbance also had been noted in an apparently healthy female with normal intelligence.

Both McCabe et al. (1–3) and Rose and Haines (4) determined the activity of glycerol kinase in leukocytes and found values <2% of normal. McCabe et al. (1–3) also measured the activity of the enzyme in several tissues of the child who died, and confirmed that it was very low also in (e.g.) liver and kidney, which contain the major part of the enzyme in the body. We have measured the enzyme activity in leukocytes and fibroblasts and found values <1% of normal in both. Our case and that of Rose and Haines (4) would indicate that the enzyme deficiency does not necessarily result in the severe clinical symptoms described by McCabe et al. The Finnish group did not measure glycerol kinase but the low excretion of glycerol, 200–300 mg/day, makes it unlikely that the patients had a glycerol kinase deficiency as did our case and those discussed above.

The overall balance sheet of glycerol metabolism gives no support for a quantitative importance of glycerol to either gluconeogenesis or oxidation. Studies with infusion of labeled glycerol in healthy volunteers (18) have shown that during an overnight fast 38% of the glycerol turnover is used for synthesis of glucose. This represents about 3% of the total synthesis of glucose. About 60% of the glycerol was oxidized, which amounts to about 2% of the total production of carbon dioxide.

Our patient does not tolerate situations with an increased fat mobilization—i.e., fasting or physical exercise. One may ask then if the increase of plasma glycerol concentration to 5 mmol/L, or possibly above, would cause severe symptoms. Infusion of 50 g of glycerol has been used in the treatment of cerebral infarction (19) without causing any ill effects. Guisado et al. (20) published an extensive experimental study in dogs. After 6 h with a plasma glycerol concentration of 12 mmol/L, there was no significant change in brain water content or brain glycerol concentration. After 12 h of high glycerol infusion, resulting in a concentration in plasma of about 40 mmol/L, there was a significant dehydration of white but not of grey matter of the brain. Other reports of experimental studies have indicated that an acute change of plasma osmolality by at least 30 mosmol/kg is needed to induce a movement of water from brain to blood (21). These data would indicate that brain dehydration is not the cause of the rather dramatic condition observed after a short fasting period or of the syndrome observed at hospitalization. The clinical observation during the hospitalizations as well as the available laboratory data—i.e., hemoglobin concentration and packed cell volume—also did not indicate a dehydration of any significance.

There are no other indications that hyperglycerolemia in

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**Fig. 3.** Changes in the concentration of urine constituents during a 21-h fasting period. The arrow indicates the time of the last meal.

**Fig. 4.** Changes in the concentration of blood constituents during work on a bicycle ergometer (40 W) for 35 min.

Rose and Haines (4) studied a 70-year-old mildly diabetic man in which a high plasma glycerol concentration was accidentally discovered as an interference in the determination of serum triglyceride concentration. The concentration of glycerol in plasma was about 1 mmol/L and the excretion of free glycerol in urine about 13 g/day (140 mmol/day). The patient was not mentally retarded, had no epilepsy, and had lived an essentially normal life. He was subjected to a fasting experiment, during which the excretion of glycerol in urine increased to about 20 g/day without any concomitant increase in the concentration of serum glycerol. He did not react in any abnormal way during the fasting experiment.
patients with a normal metabolism of glycerol causes symptoms resembling those seen in our case.

Maclaren et al. (22) described an undergrown boy with glycerol intolerance and intermittent hypoglycemia. He was investigated for numerous episodes of fatigue, irritability, pallor, and sweating, which began at 11 months of age when he had an episode of symptomatic hypoglycemia with ketonuria. The concentration of glycerol in blood and urine was within the normal reference interval. He had euphoria, mental confusion, drowsiness, nausea, and vomiting 2–3 h after oral administration of 0.5 g of glycerol per kilogram body mass. Orally administered medium-chain triglycerides (1 g/kg) caused similar effects. The glycerol was well absorbed, with a maximal concentration in serum within 15 min indicating a normal absorption rate (23). The half-life of glycerol elimination was not increased (18). A higher dose of oral glycerol (1 g/kg) caused semiconsciousness and a rapid decline in blood glucose. Despite adequate therapy with glucose and normalization of blood glucose, the symptoms of vomiting, diarrhea, lethargy, and confusion persisted up to 5 h. The laboratory results included a serum glycerol of 16.7 mmol/L after 60 min, which could indicate a defect in the metabolism of glycerol, either delayed absorption or slow elimination. The above-cited glycerol tolerance tests were preceded by a 9–10 h fast. On another occasion the same amount of oral glycerol (1 g/kg), given postprandially, did not cause hypoglycemia but did induce a disturbance of the central nervous system. No values for serum glycerol were reported from this experiment. After intravenously administered glycerol there was a rapid loss of consciousness but no decline in blood glucose concentration. The peak glycerol value was recorded at 8 min, with a fall to basal values at 30 min at the time of clinical recovery; i.e., the elimination of glycerol was normal. Studies of glycerol metabolism in leukocytes from the boy reported by Maclaren et al. (22) did not reveal any enzyme deficiency. Restriction of dietary intake of fat was associated with improvement in physical and mental activities.

The symptoms of our patient and of the Maclaren case were quite similar. A low blood glucose concentration occurred in one experiment by Maclaren but on other occasions this was not the case, and in our patient the symptoms occurred without significant decrease in blood glucose concentration. This symptomatology is very similar to what has been called neuroglycopenia (24).

A connecting link between the two cases could be that, in both, high glycerol concentration interferes with cellular utilization of glucose. In our case the cause of the high glycerol concentration was defined; in the Maclaren case an abnormality in the glycerol metabolism was not established. The relation between an impaired cellular utilization of glucose and impaired cellular metabolism of glycerol remains to be elucidated.

The first sample sent to the laboratory was reported to contain "trace of glycerol"; in a later sample no glycerol was found, but finally it was realized that the excretion of glycerol was significantly increased. As pointed out by McCabe et al. (2, 3), procedures for studying "organic acids" in urine are likely to overlook neutral compounds such as glycerol. Thus, without correction for extraction efficiency and detector response differences, the gas-chromatographic method gives an estimated excretion of about 0.5 mmol/day—still well above normal—whereas the enzymatic method yielded a value of 250 mmol/day. It is important therefore that cases which show slight increases of glycerol in urine should be more closely studied.

This study was supported by a grant (13X-585) from the Swedish Medical Research Council.

Note added in proof: A healthy 76-year-old Frenchman had a glycerol concentration of 4.3 mmol/L in plasma and a urinary excretion rate of 20 g (240 mmol)/24 h. The activity of glycerol kinase in his leukocytes was 11% of that in controls (Chau B, Bakir R, Gousault Y, et al. Psuedo hypertriglyceridemia due à hyperglycérolémie par déficit en glycérol-kinase. Nouv Presse Med 11: 1498–1499, 1982).

References