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A Child with Lactacidemia and Fructose Diphosphatase Deficiency in the Liver

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Extract

A female infant with a progressive liver disorder associated with persistent lactacidemia and a tendency toward hypoglycemia was studied. The disorder appeared to be hereditary since the parents were consanguinous and there had been two siblings with the same disease. The existing fasting lactacidemia increased after administration of oral hexose or casein (Figs. 1 and 2). Levels of blood glucose declined to 26 mg/100 ml after casein challenge but the leucine tolerance test was normal. In a liver sample obtained postmortem at 6 months of age as well as in liver tissue from a control subject, pyruvate carboxylase, phosphoenolpyruvate carboxykinase, fructose-1,6-diphosphatase, and glucose 6-phosphatase activities were measured (Table I). Fructose-1,6-diphosphatase activity was found to be very low. Blocked gluconeogenesis due to a deficiency of fructose-1,6-diphosphatase is assumed to be the cause of this inborn error of metabolism.

Speculation

Some cases of congenital lactacidemia may be due to defective gluconeogenesis caused by a deficiency of one of the key enzymes of gluconeogenesis.

Introduction

An infant with a probable hereditary progressive liver disorder and muscular hypotonia, associated with a persistent severe lactacidemia, was studied. A deficiency of fructose-1,6-diphosphatase activity, which was demonstrated in tissue from a liver specimen taken after death, was considered the probable cause of this metabolic disorder.

Case Report

A female infant was referred for study at 6 weeks of age with symptoms similar to those manifested by two elder siblings who had died at 4 and 5 months, respectively. The other six siblings were healthy. She was the ninth child of healthy parents who had one great-grandmother in common. Two days after birth (birth weight 4.7 kg) she developed a moderate but persistent jaundice. In the hospital she had a poor appetite and vomited often; she appeared to have an aversion to food. Changes in formula neither corrected the feeding difficulties nor the failure to gain weight. She never exhibited hypoglycemic attacks. The liver, which on admission reached 2 cm below the costal margin, gradually became enlarged until it reached 4 cm below the costal margin. There was no splenomegaly. Muscles were hypotonic with weak tendon reflexes, but both motor and mental development were normal. The patient exhibited progressive wasting and when she was 6 months of age she died of supervening infection and severe acidosis. At necropsy the only abnormality found was the enlarged liver weighing 360 g

(normal weight 235 g); histologic examination revealed a change of the normal architecture; there were fat-laden liver cells and slight inflammation of the periportal areas. The bile canaliculi were patent and there were no signs of cirrhosis or fibrosis.

Laboratory examinations revealed grossly elevated blood lactate levels showing a minimum of 35 mg/100 ml in the fasting state, fluctuating between 40 and 70 mg/100 ml during the day, and rising to 206 mg/100 ml on the day before death. The lowest blood glucose values were 35 mg/100 ml after an 11-hr fast and 22 mg/100 ml at the end of a glucagon tolerance test. Glycogen storage disease of the liver was excluded because of normal activities of phosphorylase, debranching enzyme, branching enzyme, and acid maltase in the leukocytes, and by the normal glucose 6-phosphatase activity in the liver specimen obtained at autopsy. Galactose 1-phosphate uridyl transferase activity in erythrocytes of the patient was normal. Serum transaminases, lactate dehydrogenase isoenzyme V, and 5'-nucleotidase activities were elevated. Bilirubin levels reached 9.5 mg/100 ml. Other investigations, including intestinal, renal, and endocrine function tests and a histologic and histochemical examination of a muscle biopsy, revealed no abnormalities.

Methods

Samples of liver and of kidney were taken from the patient within 20 min after death. About 1 g of liver was obtained from a control subject by biopsy (*control I*), and, from another subject, autopsy material was obtained from liver and kidney 12 hr postmortem (*control II*). All specimens, except from *control II*, were immediately frozen in liquid nitrogen and kept at −80°. The material from *control II* was frozen and stored at −18°. Unless otherwise stated the tissues were slowly thawed in 9 volumes of isolation medium containing 0.25 M sucrose, 0.01 M Tris-HCl buffer (pH 7.4), and 0.005 M EDTA. The specimens were cut and homogenized in a Potter-Elvehjem homogenizer equipped with a Teflon pestle. Differential centrifugation yielded mitochondria (sedimenting between 5 min 750 × *g* and 10 min 12,000 × *g*), microsomes (sedimenting between 10 min 12,000 × *g* and 30 min 170,000 × *g*), and final supernatant. The mitochondrial fraction was suspended in isolation medium and diluted twofold with 0.15 M KCl containing 0.01 M mercaptoethanol, followed by sonification for 2 min at 4° at 20 kHz. The mitochondrial sonicates as well as the final supernatants were passed through columns of Sephadex G-25 equilibrated with 0.02 M Tris-HCl buffer (pH 7.4) containing 0.0002 M mercaptoethanol. The microsomal pellets were suspended in isolation medium.

Protein in the liver fractions was determined by the biuret reaction in the presence of deoxycholate [8]. Pyruvate carboxylase activity (EC. 6.4.1.1) [14] and phosphoenolpyruvate carboxykinase activity (EC. 2.7.1.40) [11] were determined in the mitochondrial sonicate and in the final supernatant. Fructose-1,6-diphosphatase activity (EC. 3.1.3.11) was determined in the final supernatant by two methods: *method A*, in which fructose 6-phosphate was measured [12] at pH 7.5 and 37° and *method B* in which formation of inorganic phosphate (at pH 9.5 and at 30°) from added fructose-1,6-diphosphate or fructose 6-phosphate was followed [10]. In *method B*, fructose-1,6-diphosphate hydrolysis was corrected for fructose 6-phosphate hydrolysis in order to correct for nonspecific phosphatase activity. The enzyme used in *method B* was obtained from frozen liver or kidney of the patient or from *control II*, homogenized in 0.05 M sodium borate buffer (pH 9.5), and centrifuged for 10 min at 27000 × *g* to remove the bulk of the particulate material. Glucose 6-phosphatase activity (EC. 3.1.3.9) was determined in the microsomal fraction [4].

Results

The child was challenged by oral administration of glucose, galactose, or fructose according to Fernandes, Huijing, and Van de Kamer [2] (Fig. 1). The elevated levels of lactate during fasting observed initially further increased in all tests and the reactive fall of glucose at the end of the glucose test was enhanced; insulin levels were normal. When casein was administered orally after a fast (Fig. 2*B*), the level of lactate increased further, but the glucose level gradually declined to hypoglycemic values; no insulin data were available. A leucine-induced hypoglycemic response was excluded (Fig. 2*A*). From the experiments it was tentatively deduced that glycolysis was intact and that impairment of gluconeogenesis probably existed.

Enzyme assays of liver tissue revealed that one of four enzymes essential for gluconeogenesis, *i.e.*, fructose-1,6-diphosphatase, was clearly deficient (Table I).

Discussion

A deficiency of fructose-1,6-diphosphatase activity can be expected to cause hypoglycemia. This condition can occur when the glycogen in the liver has been depleted

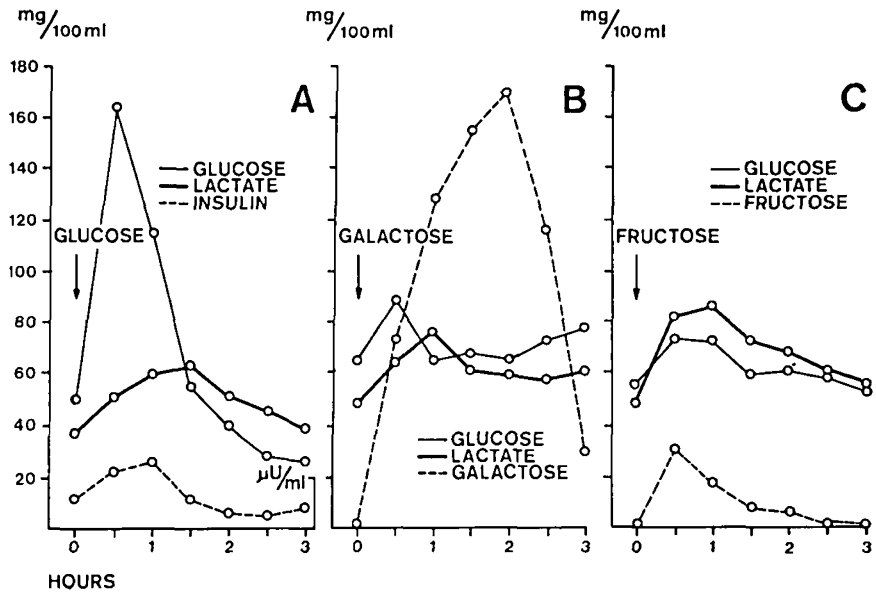

Fig. 1. The effect of oral administration of glucose (*A*), galactose (*B*), and fructose (*C*) to the patient. The dose used was 2 g/kg body weight in a 10% solution. All values are expressed in milligrams per 100 ml, except those of insulin, which are expressed in microunits per milliliter. Glucose, galactose, and lactate were estimated enzymatically with the reagent sets TC-M, TC-GA, and TC-B, respectively, of Boehringer and Soehne, Mannheim, Germany. Insulin was estimated by the dextran-coated charcoal technique of Herbert *et al.* [6].

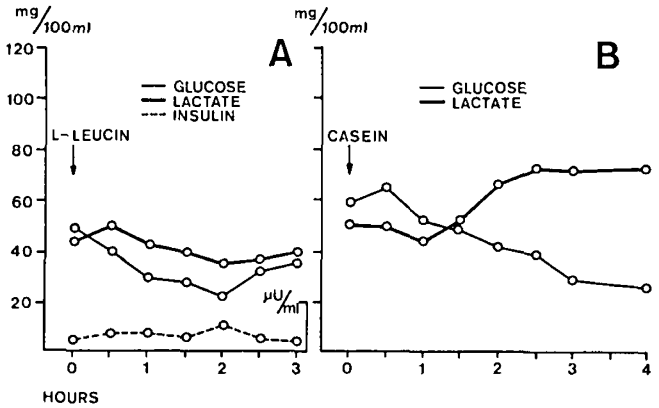

Fig. 2. The effect of oral administration of L-leucine (*A*) and casein (*B*) to the patient. The dose used was 150 mg/kg body weight for L-leucine and 1.5 g/kg body weight for casein. The values expressed are as described in the legend to Figure 1, in which the methods used are also indicated.

by a long fast, since gluconeogenesis from endogenous or exogenous amino acids (Fig. 1) is blocked; instead pyruvate and lactate will accumulate. Furthermore, accumulation of fructose-1,6-diphosphate should promote pyruvate formation from phosphoenolpyruvate since it has been shown that fructose-1,6-diphosphate activates pyruvate kinase allosterically [7, 13]. This may explain the further lactate increase after hexose or casein administration (Figs. 1 and 2).

The slight increase in blood glucose levels seen after fructose loading (Fig. 1) may be due to phosphorylation of fructose by hexokinase [1, 10] to fructose 6-phosphate, which is converted to glucose without the intervention of fructose-1,6-diphosphatase.

In addition to the lactacidemia and tendency toward hypoglycemia, our patient showed symptoms of liver disease. Liver disease often occurs in diseases in which phosphorylated hexoses accumulate in the liver, *e.g.*,

Table 1. Activities of four liver enzymes involved in gluconeogenesis

Enzyme	Patient		*Control I*	*Control II*	
	Liver	Kidney	Liver	Liver	Kidney
	μmoles/min/g fresh weight				
Pyruvate carboxylase	1.4		1.0		
Fructose-1,6-diphosphatase (*method A*)	0.6		5.5	5.0	
Fructose-1,6-diphosphatase (*method B*)	0.0	0.0		1.2[1]	1.7[1]
Phosphoenolpyruvate-carboxykinase	4.3		3.5		
Glucose 6-phosphatase	4.0		8.5		

[1] The activity was unchanged when patients' liver, respectively, kidney supernatant was also added. This indicates that in the patients' enzyme samples no materials were present that depressed fructose-1,6-diphosphatase activity.

in untreated fructose intolerance [3] and galactosemia; in galactosamine hepatitis there is an accumulation of galactosamine 1-phosphate [9]. Our patient and her two affected sisters had a persistent hyperbilirubinemia of fluctuating severity; at least two-thirds of the bilirubin found in the liver homogenate was conjugated (data not shown). This points to a properly functioning liver and rules out general impairment of hepatic function as a cause of impaired gluconeogenesis.

The finding that of the four enzymes typically involved in gluconeogenesis only fructose-1,6-diphosphatase was deficient indicates that the deficiency was primary rather than secondary. The low residual activity found in the sample from the patient when *method A* was used was probably due to nonspecific phosphatase activity, since with *method B*, which corrects for the latter activity, no fructose-1,6-diphosphatase activity was found. It is not very likely that the enzyme deficiency in the patient was caused by autolysis. In *control II*, where the enzyme was present in liver and kidney, autolysis was probably much more advanced, since here the sampling of liver and kidney occurred 12 hr after death. Moreover, in purification procedures an increase of specific enzyme activity is obtained by autolysis of liver homogenates at pH 4.5 for 8 hr at 37° [10]. In conclusion it can be said that a deficiency of fructose-1,6-diphosphatase activity due to a genetic defect is most probable.

Summary

This report concerns a child with deficient fructose-1,6-diphosphatase activity in the liver, probably as an inherited defect. Activity of three other enzymes important in gluconeogenesis, pyruvate carboxylase, phosphoenolpyruvate carboxykinase, and glucose 6-phosphatase, was normal. The main metabolic abnormalities observed in the patient were persistent lactacidemia, moderate hyperbilirubinemia, and a tendency toward hypoglycemia. Initially high fasting lactate levels in blood increased further after administration of hexoses or casein orally. Clinical symptoms were feeding difficulties with progressive wasting, moderate hepatomegaly, and muscular hypotonia. Death occurred at 6 months.

Addendum

After submission of this paper for publication in April 1970, Baker and Winegrad (Lancet, *ii:* 13 (1970)) described a patient with lactacidemia and fructose diphosphatase deficiency in the liver. There were two main differences between their case and the present case. Their patient had a relatively favorable clinical course when compared with our patient; however, a male sibling of their patient died at the age of 6 months with severe metabolic acidosis and hepatomegaly. The patient of Baker and Winegrad showed a decrease of the blood glucose level after oral administration of fructose whereas our patient showed no such decline, but rather a slight increase in level of blood glucose. The explanation of our result, given in the present paper, has recently been shown to apply also to rats loaded with fructose (H. B. Burch, P. Max, Jr., K. Chyu, and O. H. Lowry: Biochem. Biophys. Res. Commun., *34:* 619 (1969)). These investigators show that fructose loading results in the sequestration of inorganic phosphate, mainly as fructose 1-phosphate by ketohexokinase, because the former is more active than fructose 1-phosphate aldolase. Under these conditions the hexokinase route of fructose phosphorylation, leading directly to fructose 6-phosphate synthesis without the intervention of aldolase and fructose diphosphatase, probably predominates, so that glucose 6-phosphate and glucose may be formed, albeit slowly.

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