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Hepatic phosphorylase deficiency

Its differentiation from other hepatic glycogenoses

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Fernandes, J., Koster, J. F., Grose, W. F. A., and Sorgedrager, N. (1974). *Archives of Disease in Childhood*, **49**, 186. **Hepatic phosphorylase deficiency: its differentiation from other hepatic glycogenoses.** Two brothers with glycogen storage disease of the liver are reported. The clinical symptoms were hepatomegaly and a slight muscle hypotonia; hypoglycaemic symptoms seldom occurred. There was deficient activity of phosphorylase and normal phosphorylase b kinase activity in the leucocytes and in the liver.

Three aspects of carbohydrate metabolism were investigated. Glycogenolysis was studied by glucagon tests, either performed after an overnight fast or postprandially; the ensuing glucose curves were flat or almost flat. Glycolysis was investigated by oral tolerance tests with glucose, galactose, or fructose; the ensuing blood lactate curves showed a significant lactate increase. Gluconeogenesis was investigated by tolerance tests with L-alanine or glycerol administered intravenously, and dihydroxyacetone administered orally; the ensuing plasma glucose curves were characterized by a rapid conspicuous glucose increase.

A screening method is described to diagnose tentatively a phosphorylase deficiency on the basis of hexose and glucagon tolerance tests.

Deficiency of the phosphorylase system (type VI) is one of the three most common types of glycogen storage disease of the liver (Van Hoof *et al.*, 1972; Fernandes *et al.*, unpublished data). Deficiencies of the phosphorylase system are a heterogeneous group because several enzymes are involved. Fig. 1 illustrates the cascade of enzymes, which under the stimulus of hormones, especially glucagon, ultimately activate phosphorylase. The latter enzyme splits the 1–4 glucosidic linkages of glycogen, glucose-1-phosphate is liberated and is further degraded to glucose or to pyruvate and lactate. In the past 15 years we have observed 43 children with hepatic glycogenosis caused by deficiency of the phosphorylase system, phosphorylase b kinase deficiency being involved in most cases.

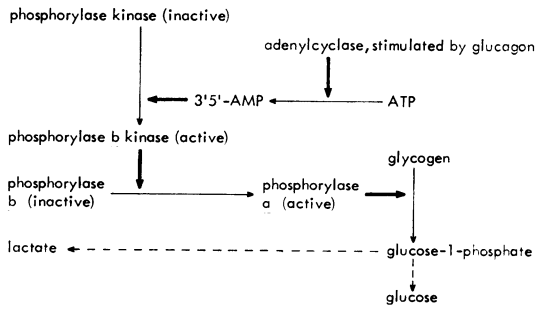

FIG. 1.—*The phosphorylase activating system (activation is indicated by thick arrows).*

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Recently, two children came to our attention who appeared to have a decreased activity of the phosphorylase system in the leucocytes due to a deficiency of phosphorylase itself. We also had the opportunity to investigate a liver biopsy. We found no demonstrable phosphorylase activity and no activation thereof by 5'AMP together with normal activities of phosphorylase b kinase and debranching enzyme (see the Table).

Phosphorylase deficiency has already been described by others (Hug and Schubert, 1970; Drummond, Hardwick, and Israels, 1970; Guibaud and Mathieu, 1972). We report our patients for the following reasons.

TABLE

Enzyme assays and glycogen determination in leucocytes and liver of the two patients

	Case 2		Case 1		Normal range	
	Leucocytes	Liver	Leucocytes	Liver	Leucocytes	Liver
Phosphorylase (nmol/min per mg protein)						
−AMP	5·6	0·0	7·9	0·0	15·8–47·9	4·4–25·1
+AMP	6·1	0·0	9·8	0·0	18·0–50·1	4·3–29·1
Debranching enzyme (nmol glucose/min per mg protein)	2·18	6·96	2·63	—	2·07–5·04	2·13–9·09
Phosphorylase b kinase (units phosphorylase b activated/min per mg protein at a phosphorylase b concentration of 20 units/ml)	0·29	0·61	0·65	0·45	0·37–0·65	0·27–0·98
Glucose-6-phosphatase (nmol p_i/min per mg protein)	—	26·2	—	32·9	—	2·40–93·0
Glycogen (μg/mg protein)	120	1156	134	1358	52–163	480–780

Firstly, the laboratory data obtained from the screening procedure by means of tolerance tests (Fernandes, Huijing, and van de Kamer, 1969) had pointed to a debranching enzyme deficiency, but for the first time this tentative diagnosis was not confirmed by the enzyme assay. Secondly, we wish to report the results of our investigation into some aspects of the carbohydrate metabolism of the two patients. We studied glycogenolysis by means of glucagon tests under varied conditions, glycolysis by loading the patients with different hexoses, and gluconeogenesis by tolerance tests with L-alanine, glycerol, and dihydroxyacetone. Finally, we propose a slightly modified screening procedure for a tentative differentiation between phosphorylase, phosphorylase b kinase, and debranching enzyme deficiency before the enzyme assay.

Case reports

Two boys, aged 2 and 3 years at the time of the study, are the only children of healthy parents who are first cousins. The psychomotor development of the children was normal. The parents had observed a protuberant abdomen in both children at the age of 6 to 12 months. Hypoglycaemic attacks had not occurred. Hepatomegaly was discovered at a routine investigation at the respective ages of 1 and 2 years. Physical examination showed that both boys had short stature according to Dutch standards, but these do not apply to this family of Turkish origin. There was a slight hypotonia of the muscles of the extremities. The livers of both children were enlarged to 10 cm below the right costal margin, were not tender, and were smooth with a rounded edge. The younger child, Case 1, had a left inguinal hernia. The elder child, Case 2, had diarrhoea at the age of 2·5 years with oedema of the legs and hypoproteinaemia. No evidence of malabsorption was found as the excretion of fat, nitrogen, and lactic acid in the faeces was normal. Overt symptoms of hypoglycaemia were not manifest during this period of malnutrition, but the fasting blood glucose levels were low (see Fig. 3, glucagon test 1). The diarrhoea subsided after a period of gradual realimentation. The only persistent abnormal laboratory findings were increased levels of SGOT and SGPT, which amounted to 100 units/l. Serum cholesterol levels, initially slightly raised, were normal later, as was the lipoprotein electrophoretic pattern on cellulose acetate. Even after prolonged fasting, normal blood glucose levels and only a mild ketosis were observed. Serum uric acid levels were normal, serum lactate was normal in the fasting state, and lactate excretion in the urine was within the normal range. Aminoaciduria was not present. Histological examination of liver tissue obtained from both children revealed a distinct glycogen accumulation, no fibrosis, and a normal liver cell architecture. The data of the enzyme assays are presented in the Table.

Methods

The methods for the assay of hepatic phosphorylase (Hülsmann, Oei, and Van Creveld, 1961), phosphorylase b kinase (Huijing, 1967), debranching enzyme (Huijing, 1964), glucose-6-phosphatase (Harper, 1962), and glycogen content (Huijing, 1970) have been described previously. Glucose levels were determined in plasma with a Beckman glucose analyser in order to follow glucose changes as closely as possible. Lactate levels were estimated in blood according to Hohorst (1970).

The doses of all substances administered in the tolerance tests are given in the legends to Fig. 2–5. The dose of glucagon administered intramuscularly in each test was 0·5 mg, amounting to approximately 30 μg/kg body weight.

Results

Oral hexose tolerance tests with blood lactate estimations and glucagon tests with plasma glucose estimations were performed in order to screen both children with hepatomegaly for the existence of one of the enzyme deficiencies occurring most frequently

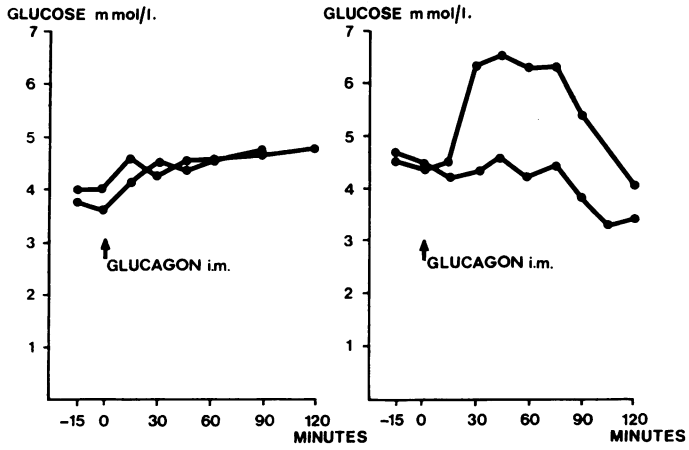

FIG. 2.—*Case 1.* (*a*) *Tolerance tests with 0·5 mg glucagon i.m. after 11 or 15 hours' fasting.* (*b*) *Tolerance tests with 0·5 mg glucagon i.m. 3 hours after a normal breakfast.*

in liver glycogenosis (Fernandes *et al.*, 1969). The results are presented in Fig. 2–4 and described below. The failing hyperglycaemic response to glucagon (Fig. 2) and the considerable lactate rise after hexose feeding (Fig. 4) were indicative of a debranching enzyme deficiency. After this procedure, enzyme assays of the leucocytes and of liver tissue obtained by needle biopsy were performed. The results are given in the Table. They have been described elsewhere (Koster *et al.*, 1973). Both children were diagnosed as having a phosphorylase-deficient liver glycogenosis. Subsequently, we investigated three pathways of carbohydrate metabolism, namely glycogenolysis, glycolysis, and gluconeogenesis.

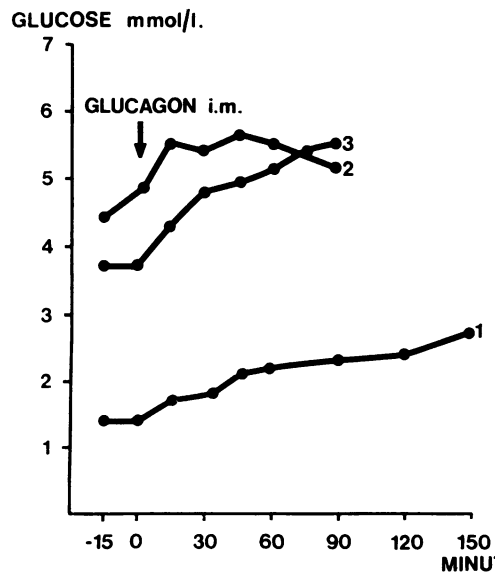

FIG. 3.—*Case 2. Tolerance tests with 0·5 mg glucagon i.m. after 5 hours' fasting (1) or 15 hours' fasting (2); cyclic AMP was determined in the urine before and after glucagon administration of test 3, which was performed after a 9-hour fast.*

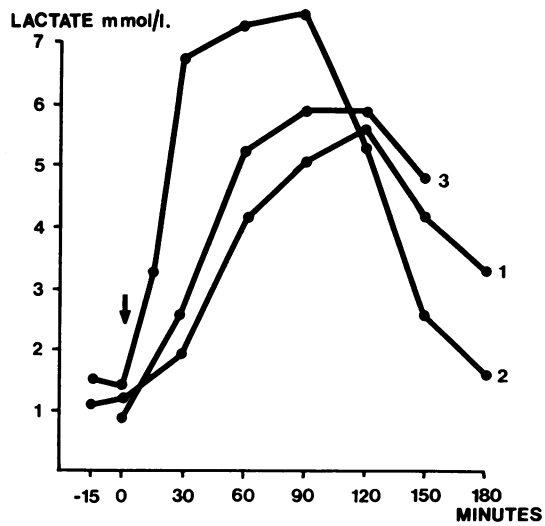

FIG. 4.—*Case 2. Blood lactate curves after glucose (1), galactose (2), or fructose (3) orally. The sugars were administered as 10% solutions in water in doses of 2 g/kg body weight. Time of administration is indicated by arrow.*

Glycogenolysis. Intramuscular glucagon tests were performed in both children. Glucagon was administered in Case 1 after a normal overnight fast (Fig. 2a) and 3 hours after breakfast (Fig. 2b). A delayed increase of plasma glucose and plateau formation occurred during only one test, performed postprandially; the other glucose curves were flat, whether made after a normal overnight fast or postprandially. (Glucagon tests are normally characterized by an increase of the blood glucose concentration within 15 minutes after the glucagon injection; peak glucose levels occur usually within 30 minutes and amount to approximately 4 mmol/l. above fasting levels.)

The results of 3 glucagon tests performed in Case 2 are given in Fig. 3. Test 1, performed after 5 hours' fasting, showed a plasma glucose curve increasing slowly from 1·4 to 2·7 mmol/l. The patient was malnourished at that time. Tests 2 and 3 were performed after 15 and 9 hours' fasting, respectively, when the patient was in good condition. For a better interpretation of the small glucose increases, cyclic 3'5'AMP was measured in two 4-hour urine samples before and after glucagon administration during test 3. The data revealed a normal stimulation of cyclic 3'5'AMP production by glucagon as compared with two normal children.

Glycolysis. Oral hexose tolerance tests were performed in both children with comparable results. The lactate curves after oral administration of glucose, fructose, or galactose to Case 2 are presented in Fig. 4. The lactate levels, which were normal in the fasting state, increased conspicuously, exceeding the upper limit of 3·5 mmol/l. observed in normal children. The glucose increase was substantial after the administration of all three hexoses, whereas the fructose increase after fructose administration and the galactose increase after galactose administration was only slight, as might be expected.

Gluconeogenesis. Tolerance tests with intravenous L-alanine or glycerol and oral dihydroxyacetone were performed in Case 1 (see Fig. 5). Only the glucose curves are presented, the lactate curves having been omitted for the sake of clarity. During all three tests there was an immediate substantial increase of the blood glucose concentration. Blood lactate increases were slight in the alanine and glycerol tests, moderate in the dihydroxyacetone test.

Discussion

Since our patients with a deficiency of phosphorylase b kinase without exception had shown a normal glucose rise during the glucagon test, the failure of glucose release after glucagon administration during the screening procedure that preceded the enzyme assay had led us to expect the diagnosis to be a debranching enzyme deficiency. The impaired glycogenolysis could not be due to an insufficient activity of adenylate cyclase because the cyclic 3'5'AMP excretion in the urine showed the normal increase during the test. Strikingly, however, the glucose curves were not affected by shortening the fasting period before the test. In this respect phosphorylase-deficient patients clearly differ from debranching enzyme-deficients, in whom the glucagon test is normal if performed postprandially (Hug *et al.*, 1963). Thus, in their reaction to the glucagon test the phosphorylase-deficient patients differed from those who were deficient in phosphorylase b kinase under all circumstances, but they only differed from patients deficient in debranching enzyme when the test was performed postprandially. Apparently, glucagon does stimulate the formation of phosphorylase a in patients with a phosphorylase b kinase or a debranching enzyme deficiency, but not in patients with a phosphorylase deficiency. (As for debranch-

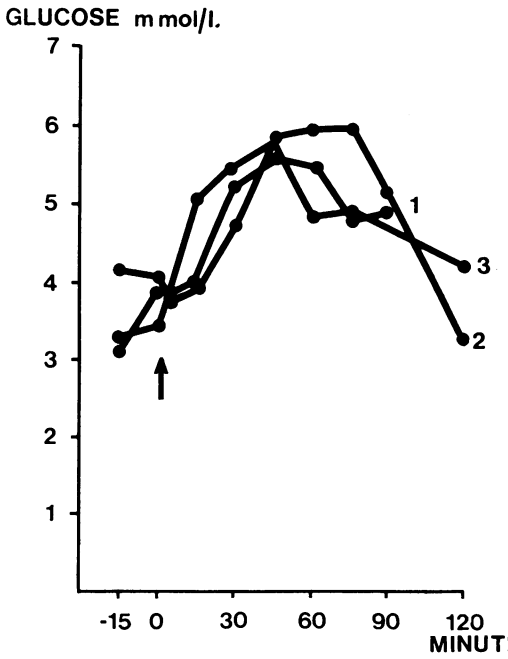

FIG. 5.—*Case 1. Tolerance tests with L-alanine i.v. (1), glycerol i.v. (2), or dihydroxyacetone orally (3). The doses were 0·5, 0·35, and 1 g/kg body weight, respectively. Time of administration is indicated by arrow.*

ing enzyme deficiency, the effect of glucagon is restricted to the postprandial phase when the outer branches of glycogen are still present.)

Regarding the carbohydrate metabolism of the two phosphorylase-deficient patients, *glycogenolysis* has already been discussed above. *Glycolysis* appeared to be enhanced because hexose feeding to the patients resulted in much higher blood lactate levels than found in normal children. This 'overflow' phenomenon, previously described by us in patients deficient in debranching enzyme or phosphorylase kinase (Fernandes *et al.*, 1969), is possibly due to an insufficient activity of glycogen synthetase. Indeed, it has been shown that glycogen inhibits the activation of glycogen synthetase phosphatase in rats (Hers, De Wulf, and Stalmans, 1970). It is therefore conceivable that the excess glycogen in the liver of the patients inhibits further glycogen synthesis. As a result, hexose administration causes an enhanced lactate formation instead of enhanced glycogen synthesis.

Gluconeogenesis was normal or even increased as the administration of a substrate for gluconeogenesis such as L-alanine, glycerol, or dihydroxyacetone resulted in a conspicuous increase of blood glucose, even after prolonged fasting. Adequate gluconeogenesis combined with a glycogenolysis which, though impaired, is not entirely absent, apparently protects the patients against hypoglycaemia except under circumstances of prolonged protein deficiency.

We now consider the question whether a differentiation between patients with a debranching enzyme deficiency and those with a deficiency of the phosphorylase system is feasible. We think it is, if our screening procedure as described earlier (Fernandes *et al.*, 1969) is slightly modified. It then might even serve to differentiate tentatively a phosphorylase b kinase deficiency from a phosphorylase deficiency.

Fig. 6 illustrates our modified screening procedure. The first steps, a glucose and galactose tolerance test with determination of the blood lactate levels, are the same as before (Fernandes *et al.*, 1969). If blood lactate increases in one or both tests from $\leqslant 1 \cdot 5$ mmol/l. to $\geqslant 5$ mmol/l., a debranching enzyme deficiency or a deficiency of the phosphorylase system is probable. A glucagon test after prolonged fasting (at least 12 hours) is indicated. A normal glucose curve after glucagon injection indicates a deficiency of phosphorylase b kinase. A flat glucose curve is indicative of either a debranching enzyme deficiency or a phosphorylase deficiency. Now a second glucagon test is performed 2 hours after a high carbohydrate breakfast. A significant glucose increase indicates a debranching enzyme deficiency, and a flat glucose curve a deficiency of phosphorylase proper. It must be stressed that this screening procedure is based on the data of many patients with a debranching enzyme or a phosphorylase b kinase deficiency, but on the data of only two patients with a phosphorylase deficiency. Data of some investigators regarding patients deficient in one of the enzymes of the phosphorylase system are in agreement with our findings (Drummond *et al.*, 1970; Hug, Schubert, and Chuck, 1970), though data of other investigators are not (Hug and Schubert, 1970). The reason for the discrepancies is not clear at present, but might be that the underlying enzyme defect has not always been established unequivocally (Schwartz *et al.*, 1970) and the glucagon tests might therefore have been misinterpreted.

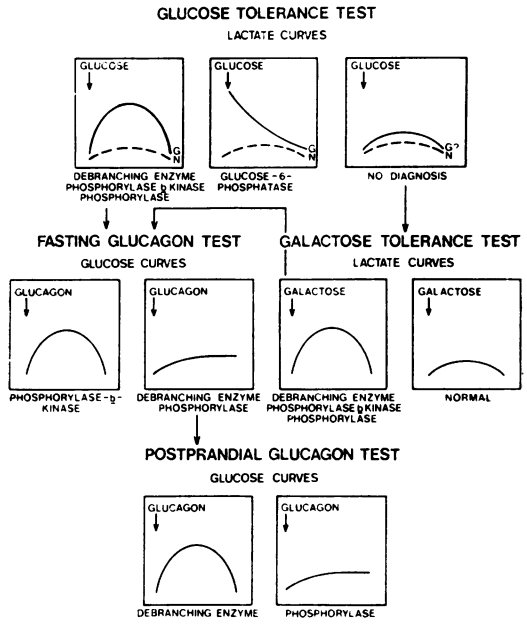

FIG. 6.—*Screening procedure for differentiating patients with glycogen storage disease (G) and normal children (N). The deficient enzymes are indicated below the diagrams.*

It should be realized, of course, that our screening procedure can only yield a tentative diagnosis that needs confirmation by an enzyme assay. It does, however, establish which enzyme assay should be performed first and which tissue should be used, namely leucocytes (debranching enzyme or phosphorylase b kinase) or liver (glucose-6-phosphatase or phosphorylase).

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