ATP Depletion, a Possible Role in the Pathogenesis of Hyperuricemia in Glycogen Storage Disease Type I

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ABSTRACT Other investigators have shown that fructose infusion in normal man and rats acutely depletes hepatic ATP and P_i and increases the rate of uric acid formation by the degradation of preformed nucleotides. We postulated that a similar mechanism of ATP depletion might be present in patients with glucose-6-phosphatase deficiency (GSD-I) as a result of ATP consumption during glycogenolysis and resulting excess glycolysis. The postulate was tested by measurement of: (*a*) hepatic content of ATP, glycogen, phosphorylated sugars, and phosphorylase activities before and after increasing glycolysis by glucagon infusion and (*b*) plasma urate levels and urate excretion before and after therapy designed to maintain blood glucose levels above 70 mg/dl and thus prevent excess glycogenolysis and glycolysis.

Glucagon infusion in seven patients with GSD-I caused a decrease in hepatic ATP from 2.25±0.09 to 0.73±0.06 μmol/g liver ($P < 0.01$), within 5 min, persisting in one patient to 20 min (1.3 μmol/g). Three patients with GSD other than GSD-I (controls), and 10 normal rats, showed no change in ATP levels after glucagon infusion. Glucagon caused an increase in hepatic phosphorylase activity from 163±21 to 311±17 μmol/min per g protein ($P < 0.01$), and a decrease in glycogen content from 8.96±0.51 to 6.68±0.38% weight ($P < 0.01$). Hepatic content of phosphorylated hexoses measured in two patients, showed the following mean increases in response to glucagon; glucose-6-phosphate (from 0.25 to 0.98 μmol/g liver), fructose-6-phosphate (from 0.17 to 0.45 μmol/g liver), and fructose-1,6-diphosphate (from 0.09 to 1.28 μmol/g) within 5 min. These changes, except for glucose-6-phosphate, returned toward preinfusion levels within 20 min.

Treatment consisted of continuous intragastric feedings of a high glucose dietary mixture. Such treatment increased blood glucose from a mean level of 62 (range 28–96) to 86 (range 71–143) mg/dl ($P < 0.02$), decreased plasma glucagon from a mean of 190 (range 171–208) to 56 (range 30–70) pg/ml ($P < 0.01$), but caused no significant change in insulin levels. Urate output measured in three patients showed an initial increase, coinciding with a decrease in plasma lactate and triglyceride levels, then decreased to normal within 3 days after treatment. Normalization of urate excretion was associated with normalization of serum uric acid.

We suggest that the maintenance of blood glucose levels above 70 mg/dl is effective in reducing serum urate levels and that transient and recurrent depletion of hepatic ATP due to glycogenolysis is contributory in the genesis of hyperuricemia in untreated patients with GSD-I.

INTRODUCTION

The hyperuricemia associated with glycogen storage disease type I (GSD-I)[1] or glucose-6-phosphatase deficiency, is often of such severity that it produces gout and its ensuing complications (1, 2). Although several investigators have demonstrated an excess production of uric acid from increased synthesis of purines *de novo* (2–7), the precise mechanism giving rise to this anomaly has not been elucidated.

Roe and Kogut (6) recently showed that within 30 min after an injection of glucagon, patients with GSD-I were unique in showing a significant increase in plasma uric acid levels and a two to threefold increase in excretion of urate. This observation suggested that such a rapid increase in urate levels resulted primarily from enhanced nucleotide catabolism rather than from a

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Received for publication 15 July 1977 and in revised form 3 April 1978.

[1] *Abbreviations used in this paper*: G-6-P, glucose-6-phosphate; GSD-I, glycogen storage disease type I.

direct effect on the rate of purine synthesis as originally suggested by Howell (7). Thus, the observed increase in *de novo* synthesis of purines in GSD-I would appear to be secondary to the increased rate of purine catabolism. Such a sequence has been shown to occur after fructose infusions in normal man (8–10) and rats (11–13). Although the metabolic sequences are complex, a series of publications has shown that phosphorylation of large amounts of fructose leads to acute depletion of hepatic ATP and P_i levels, which in turn favor degradation of preformed AMP to uric acid (8–13).

The similarity between the blood chemistries of patients with GSD-I and subjects infused with fructose (hypertriglyceridemia lacticacidemia, hypophosphatemia, and hyperuricemia), prompted the hypothesis that the mechanism for hyperuricemia in these two conditions might be analogous (6, 14). For example, patients with GSD-I can degrade glycogen to glucose-6-phosphate (G-6-P) but because of the enzyme defect, hydrolysis to glucose and P_i is impaired. Frequent periods of hypoglycemia should therefore stimulate the formation of large amounts of G-6-P which escapes primarily through the Embden-Meyerhof pathway to be further phosphorylated utilizing equimolar amounts of ATP (15) (see Fig. 1). Thus, both fructose infusion in normal man and hypoglycemia in patients with GSD-I should result in an increase in phosphorylated sugars and a decrease in ATP and P_i in the liver.

Demonstration that patients with GSD-I have low hepatic levels of ATP and P_i and high levels of phosphorylated sugars, during periods of hypoglycemia, would support this postulate. Since it is not medically feasible to obtain hepatic tissue samples during periods of hypoglycemia, we have used glucagon administration to simulate some of the biochemical changes resulting from hypoglycemia: e.g., activation of phosphorylase, and reduction in hepatic glycogen. In addition to the glucagon-induced changes, glucose therapy given in quantities to prevent frequent and recurrent episodes of low blood glucose concentrations, should decrease the elevated serum uric acid levels (16). This report presents the results of glucagon-induced changes in glycogen content, phosphorylase activity, and ATP concentration in seven patients with GSD-I, two of which also had measurements of G-6-P, fructose-6-phosphate, and fructose-1, 6-diphosphate. In addition, the effect of continuous glucose therapy on uric acid excretion and plasma urate levels was measured. The findings provide support for the hypothesis that increased degradation of preformed purines is important in the genesis of hyperuricemia in GSD-I.

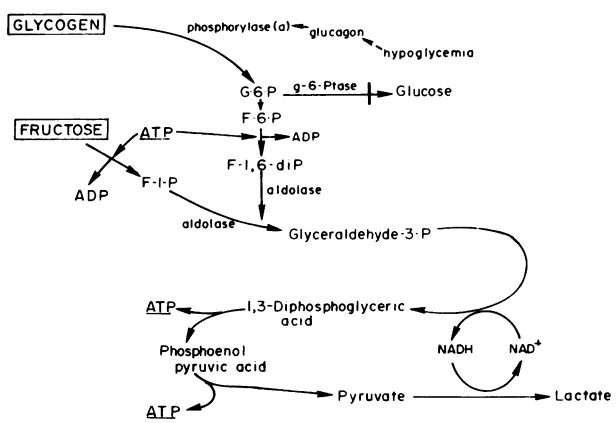

FIGURE 1 A loading dose of fructose has been shown to deplete hepatic ATP through rapid phosphorylation to fructose-1-phosphate (F-1-P). By analogy, ATP depletion may occur in patients with glucose-6-Ptase deficiency as a result of rapid glycogenolysis and secondary glycolysis. By this mechanism ATP depletion could occur during the conversion of F-6-P to F-1,6-diP if sufficiently large amounts of G-6-P are released from glycogen.

METHODS

Patient studies. The patient group consisted of 10 patients (ages 18 mo to 19 yr) with hepatic glycogen accumulation. Seven patients had the clinical and laboratory features of GSD-I and deficient glucose-6-phosphatase activity (group I). Of the remaining three patients, one had debrancher enzyme (amylo-1-4,1-6-glucosidase) deficiency (type III-GSD) and the other two had excessive hepatic glycogen content without a detectable defect in glycogenolytic enzyme activities (group II). These latter patients showed the expected glycemic response to glucagon. All patients were admitted to the Clinical Research Center of Vanderbilt University Hospital for definitive diagnosis and treatment of GSD. They were maintained on a diet consisting of 60% starch or glucose and <50 mg purine/100 g of food daily (17). Daily calorie-protein intake varied between patients because of age differences; the range was between 45 and 65 kcal/kg body wt. During the initial 7-day hospitalization (control period) each patient consumed the diet ad lib. as far as total caloric intake was concerned. The diet (high in carbohydrate but low in fructose and galactose) utilized during the 2 mo before admission was continued in the hospital. The mean daily caloric intake during the first 7 days was maintained throughout the remainder of the study. Drugs known to effect production or excretion of uric acid were not administered.

To determine the effects of continuous glucose treatment, patients were monitored for blood uric acid levels and uric acid excretion during the 7-day control period. During period II (treatment) all patients received continuous nasogastric infusions of a high glucose diet (Vivonex, Eaton Laboratories, Norwich, N. Y.) for 24 h a day for 14 days (16), followed by 1 wk of frequent oral feedings during the day and continuous nocturnal feedings of Vivonex as described previously (14). During the final 24 h of the control and treatment periods, blood samples were obtained for lipids, lactate, glucose, glucagon, and insulin levels. All liver biopsies were performed either for the purpose of confirming the diagnosis of GSD (18) or to evaluate the effect of long-term treatment. Studies were approved by the Committee on Human Experimentation and informed patient and/or parental consent was obtained.

Liver biopsies were performed with a Menghini liver biopsy needle (1.4 mm) in the eight younger patients during general anesthesia. The two older patients were sedated with

diazepam and the liver biopsies were performed using local anesthesia. 6 h before biopsy, the patients were given nothing by mouth and were maintained on a continuous glucose infusion of 0.25 g/kg per h. After an initial biopsy, a 20-μg/kg continuous infusion of glucagon was administered intravenously for 1 min, and a second biopsy was obtained 4 min after completion of the glucagon infusion (18, 19). Biopsy specimens were frozen within 3–5 s by immersion in liquid nitrogen for later assay of glycogen and ATP content, as well as phosphorylase, α-1-4, 1-6-glucosidase, and glucose-6-phosphatase activities.

One patient (E. R.) required exploratory surgery for a rapidly expanding hepatic nodule as did a second patient (D. T.) for an enlarging abdominal mass. In these patients, surgical liver biopsies were obtained before and after the glucagon infusion as illustrated in Fig. 3. Samples were sufficiently large in these instances to measure G-6-P, fructose-6-phosphate, and fructose-1,6-diphosphate. Insufficient tissue was available for measurement of other substrates.

Animal studies. Animal studies were performed on Sprague-Dawley rats weighing 230–260 g. 10 rats were infused with 50 μg/kg of glucagon (treatment group) into the inferior vena cava (20). Eight rats were infused with an equivalent volume of glucagon diluent (control group). Liver specimens were obtained before and 5, 10, and 20 min after the infusions in both groups. Liver specimens were obtained by freeze clamping and immersion immediately in liquid nitrogen (21).

Biochemical assays. Uric acid in serum and urine was determined spectrophotometrically by the uricase method which showed no demonstrable interference by elevated lipid levels (22). Blood glucose was measured by the glucose oxidase method (Glucostat, Worthington Biochemical Corp., Freehold, N. J.).

Insulin, and glucagon levels were measured by radio-immunoassay (23). Liver protein was determined by the method of Lowry et al. (24). Measurement of glycogen content was made by a microtechnique using a modification of the diazyme method in which a 1% liver homogenate is hydrolyzed with amyloglucosidase and then assayed for liberated glucose with the commercial Glucostat reagent (25). Measurements of phosphorylase, α-1-4,1-6-glucosidase (26), glucose-6-phosphatase activities (27), G-6-P (28), fructose-6-phosphate (29), and fructose-1,6-diphosphate (30), content were made by standard assay procedures. ATP levels were determined by the firefly luciferase method (31). Analysis of data was made by Student's *t* test (32).

RESULTS

Blood and urine. All children showed elevated serum urate levels. Although there was some variation in the degree of elevation between patients (range 11.8–18.6 mg/dl) the degree of elevation was not a function of age but more a function of frequency and severity of hypoglycemia, acidosis, and hypophosphatemia. Within 10 days of treatment, all patients showed uric acid levels of <7 mg/dl and lactate levels <3 meq/liter. Because of inherent problems of accurate urine collection in children under 4 yr of age, uric acid excretion was measured in only the three older patients (ages 12, 16, and 19 yr).

Fig. 2 presents the changes in uric acid excretion and plasma uric acid levels during the control and treatment periods. Uric acid excretion increased during the first 3 days of the treatment period. This coincided with a progressive decrease in serum urate levels toward normal and was preceded by a decrease in blood lactate levels from a mean of 10.4 (range 9.1–16.3) to <2.5 meq/liter (normal <3 meq/liter) and a decrease in triglyceride concentrations from 3,612±123 to 1,216±89 mg/dl (normal <200 mg/dl). The decrease in lactate, triglyceride, and urate levels was preceded by an increase in blood glucose from a mean of 62

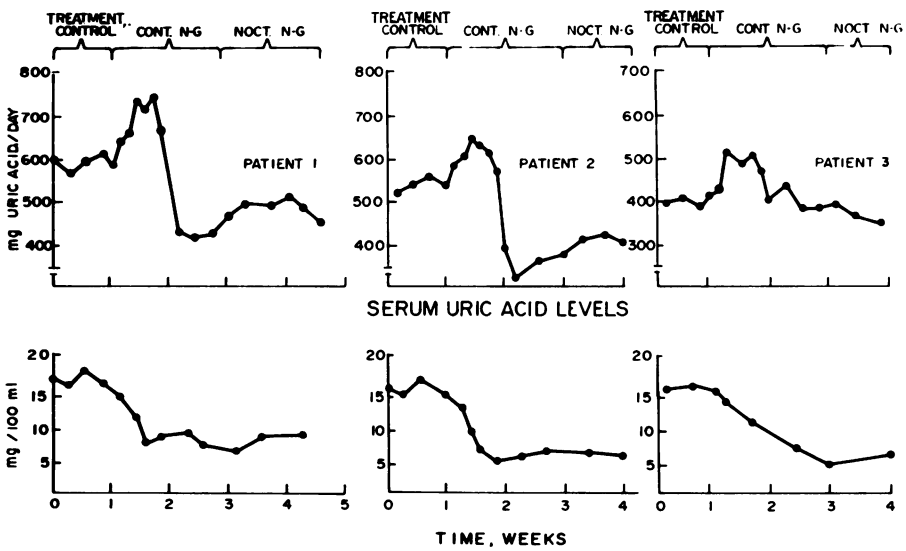

FIGURE 2 Comparison of urate excretion vs. serum urate concentration before and after treatment in three patients. Cont. N-G = treatment begun with continuous (24 h) nasogastric infusion of Vivonex formula. Noct. N-G = frequent (every 3 h) feedings of high starch diet while awake, plus nocturnal nasogastric feedings of Vivonex.

(range 28–96) to 86 (range 71–143) mg/dl ($P < 0.02$) and by a concomitant decrease in plasma glucagon from a mean of 190 (range 171–208) to 56 (range 30–70) pg/ml ($P < 0.01$). Insulin levels increased from a mean of 25 (range 7.5–50) to 44 (range 26–53) μU/ml but this apparent increase was not significant ($P > 0.05$). During the last 2 wk of treatment, uric acid excretion decreased to normal values and was significantly less than that seen during the control period ($P < 0.01$).

Hepatic tissue determinations in patients. Table I lists the results of hepatic tissue measurements before and after the glucagon infusion in two groups of patients. All patients showed a substantial increase in phosphorylase activity after the glucagon infusion. In the patients with GSD-I, glucagon caused a mean decrease in glycogen of 2.28% ($P < 0.01$). The patient with type III GSD showed little change in glycogen content after glucagon whereas the patients with hepatomegaly, elevated glycogen and glycemic response to glucagon showed a substantial decrease in glycogen after glucagon administration.

Initial ATP levels were normal in all patients (33). However, glucagon caused a significant decrease in mean levels of ATP from 2.25 to 0.72 μmol/g tissue ($P < 0.01$) in the patients with GSD-I. No significant change in ATP levels resulted from glucagon in any of the patients in group II. Fig. 3 illustrates the temporal changes in phosphorylase activity, glycogen, ATP, G-6-P, fructose-6-phosphate, and fructose-1,6-diphosphate levels after a glucagon infusion in one patient (E. R.). Results from one additional patient showed similar changes in G-6-P and fructose-6-phosphate but insufficient tissue was available to measure fructose-1,6-diphosphate. The changes in phosphorylase activity, glycogen and ATP content are similar to those in other patients with GSD-I. Initial levels of the phosphorylated sugars were slightly higher than those from fasted rats and may reflect the continuous glucose infusion for 6 h before biopsy. Each of the phosphorylated sugars showed a substantial increase after glucagon. Fructose-1,6-diphosphate levels were maximal at 5 min and were about 30 times those of fasting rats (11). By 20 min,

TABLE I
Hepatic Tissue Measurements before and after Glucagon Infusion

Patient	Age	Phosphorylase		Glycogen		ATP	
		Before*	After*	Before	After	Before	After
	yr	*μmol/min/g protein*		*% of wt*		*μmol/g tissue*	
Group I (GSD-I)							
D. T.	19	110	304	9.90	7.68	2.54	0.70
P. J.	11	291	370	8.88	6.13	2.23	0.56
E. R.	9	121	219	9.90	7.23	2.53	0.60
H. A.‡	3	175	360	8.63	6.18	2.38	0.93
		136	288	5.92	4.94	1.93	0.86
G. S.	3	175	289	9.31	6.68	2.33	0.68
C. A.	2	191	309	8.39	6.91	2.13	0.71
A. C.	3	100	350	10.74	8.46	1.89	0.79
Mean±SEM		163±21	311±17	8.96±0.51	6.68±0.38	2.25±0.09	0.73±0.06
		($P < 0.01$)		($P < 0.01$)		($P < 0.01$)	
Group II							
(GSD-III) M. S.‡	4	52	168	9.45	9.37	1.90	1.92
		69	193	8.66	8.44	—	1.78
(GSD-?) R. D.§	4	40	258	11.83	9.23	2.10	1.84
(GSD-?) M. R.§	18 mo	202	320	7.31	5.83	1.81	1.76

* "Before" refers to tissue measurements before glucagon infusion and "after" refers to measurements 5 min after glucagon infusion.

‡ Patients who had two biopsy procedures. The initial values were obtained before treatment and the second value after treatment.

§ These patients had elevated hepatic glycogen but normal glycogenolytic enzyme and G-6-P activities and the glycemic response to glucagon was normal.

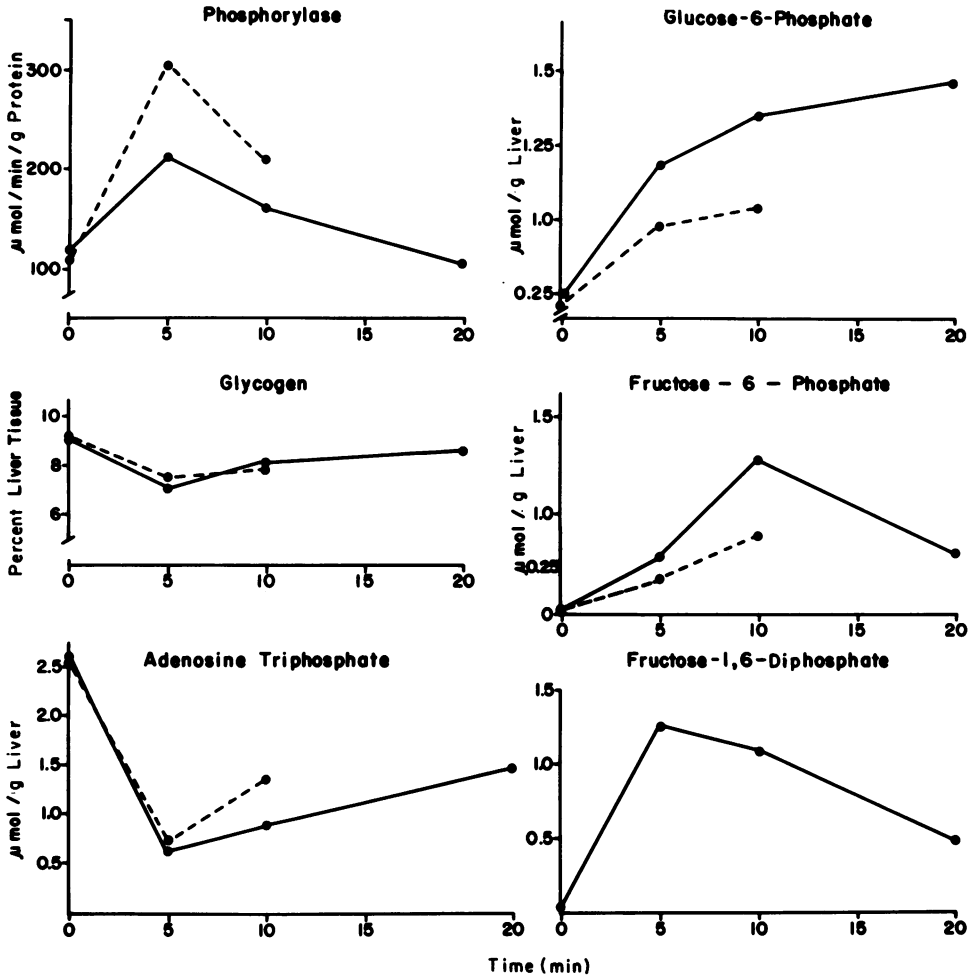

FIGURE 3 Results of hepatic phosphorylase activities and hepatic substrate changes after intravenous glucagon administration (20 μg/kg) in two patients (E. R., D. T.) who required abdominal surgery.

phosphorylase activity had almost returned to preinfusion levels, and the concentration of all substrates except G-6-P had begun to return to preinfusion levels.

Animal studies. Because comparative studies with glucagon infusion could not be performed in normal man, ATP measurements were performed in a series of rats. Fig. 4 illustrates the changes in ATP levels in the two groups of animals. No statistical changes in hepatic ATP levels were produced by the glucagon infusion.

DISCUSSION

Published studies on the mechanism of fructose-induced hyperuricemia suggested that an analogous mechanism may exist in patients with GSD-I as a result of the increased glycolytic rate induced by recurrent hypoglycemia and glucagon release (4–7). For this analogy to be appropriate, at least three test conditions should be met. First, glucagon infusion should decrease hepatic ATP levels in patients with GSD-I. Indeed, glucagon infusion caused a marked decrease in hepatic ATP levels from a mean of 2.3 to 0.76 μmol/g of liver, a change analogous to that demonstrated to induce excess urate formation after fructose infusion in normal man (from 1.91 to 1.05 μmol/g), (8). No such changes in ATP levels were seen in the two patients with normal glucose-6-phosphatase activity and neither patient had elevated serum uric acid levels. In addition, no statistical change in ATP levels occurred in normal rat liver after glucagon administration. Therefore, glucagon-induced ATP depletion would appear to be unique to patients with GSD-I.

Second, glucagon infusion should activate hepatic phosphorylase causing hydrolysis of a sufficient quantity of glycogen to give rise to large amounts of phosphorylated sugars, particularly fructose-1,6-diphos-

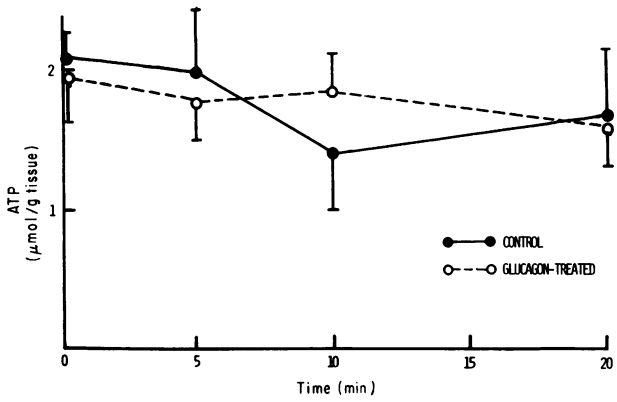

FIGURE 4 Hepatic ATP levels in rats after injection of either glucagon (50 μg/kg) or diluent into the inferior vena cava. There was no significant difference in any points ($P > 0.05$).

phate (see Fig. 1). Glucagon increased the activity of phosphorylase and decreased hepatic glycogen levels in all patients (mean of 2.2%) except the one with type III glycogenosis. Such changes would give rise to ~22 g of glucose as G-6-P in 1 kg of liver within 5 min. This amount of G-6-P is equivalent to the fructose load that caused hyperuricemia in normal man when infused over a 15-min period (8–10). Measurement of phosphorylated sugars in two patients during surgery further supports the concept that marked increases in G-6-P result in its rapid phosphorylation to fructose-1,6-diphosphate and represents a possible cause for the glucagon-induced ATP depletion.

The third test condition to be met is that prevention of hypoglycemia should reduce hyperuricemia. Improvement in serum uric acid levels occurred within the 1st wk of treatment and was associated, first with an increase in urinary excretion of uric acid, and second by decreased excretion to levels less than those present during pretreatment. The phase of increased urate excretion was associated with a decrease in the levels of blood lactate (a known inhibitor of urate excretion) (34). The correction of hyperlacticacidemia could therefore contribute to the increased excretion of urate during the early phase of treatment (3, 4), although Fine et al. have shown that glucose infusion increases renal urate excretion before lactate levels fall (35). Normalization of serum urate, followed by an overall decrease in uric acid excretion after 2 wk of treatment suggests that urate production was decreased by treatment. This therapy prevented the frequent and often prolonged periods of low blood glucose (levels <70 mg/dl) and was associated with a statistically significant decrease in plasma glucagon levels. This observation suggests that the continuous maintenance of normal blood glucose levels would be helpful in the proper management of GSD-I and implicates glucagon in the pathogenesis of hyperuricemia.

Hyperuricemia in GSD-I has been shown previously to result from at least two mechanisms: (*a*) decreased urinary excretion due to chronic lacticacidemia and ketonemia (34) and (*b*) increased production of uric acid (2–7). The second mechanism has not been clearly defined although an increase in purine synthesis *de novo* is suggested by an increase in labeled glycine incorporation into urinary uric acids (3–7). Howell (7) has suggested that deficient activity of glucose-6-phosphatase diverts more G-6-P into the hexose monophosphate shunt than in normal man. This would lead secondarily to increased formation of phosphoribosylpyrophosphate, the substrate for the first irreversible step in *de novo* synthesis of purines. This is a plausible explanation for the observed increase in the rate of purine synthesis since intracellular concentrations of phosphoribosylpyrophosphate (36, 37) and glutamine (38, 39) have both been implicated in the regulatory control of purine biosynthesis in mammalian cells. However, such a sequence alone would not account for the increase in urate excretion and concomitant increase in serum uric acid levels which resulted within 30 min after a glucagon infusion (6). We are suggesting then that there are two important phases leading to the increased production of uric acid in GSD-I: first, a phase of rapid degradation of preformed AMP as also proposed by Roe and Kogut (6) and further supported by the present studies; and a second, compensatory phase of increased purine synthesis as suggested by Howell (7).

The mechanism of the first phase is supported by the known mechanism for fructose-induced hyperuricemia in normal liver. This mechanism has been studied extensively by a number of investigators (8–13) who demonstrated that rapid phosphorylation of large amounts of fructose (see Fig. 1) caused secondary depletion of hepatic ATP and P_i. Depletion of ATP and P_i favored degradation of AMP to IMP which was further degraded to uric acid (8–13). By contrast, glucose infusion given under similar test conditions did not cause ATP depletion. This difference was explained on the basis that glucose was phosphorylated only one-fifth to one-third as rapidly as fructose (40, 41). Thus, even with large amounts of glucose, the rate of glucose phosphorylation to G-6-P is sufficiently slow to prevent depletion of ATP in normal man. In addition, hydrolysis of glycogen would not be expected to deplete ATP in normal liver since most G-6-P should be hydrolyzed to glucose and P_i by the action of glucose-6-phosphatase. However, in the absence of glucose-6-phosphatase, hydrolysis of large amounts of glycogen should yield sufficient substrate as G-6-P and fructose-6-phosphate for utilization of ATP during formation of fructose-1,6-diphosphate (see Fig. 1). Such an increase in phosphorylated hexoses was seen after glucagon infusion in two patients. In addition, ATP levels were

consistently depleted by glucagon in all patients with GSD-I. In fact, the observed glucagon-induced decrease in hepatic glycogen content was sufficient to produce amounts of G-6-P which were quantitatively similar to the dose of fructose which repeatedly caused ATP depletion in normal liver (8–10).

Other hormones such as catecholamines may also play a contributory role in the genesis of hyperuricemia. It would be expected that the role of catecholamines is minor since patients with a functioning portacaval shunt continue to have periods of hypoglycemia but normal uric acid levels (42).

Insufficient tissue was available to substantiate the effect of glucagon on the second proposed phase of increased uric acid production (e.g., increased purine synthesis). In addition, to the substrate control of purine synthesis, feedback control also appears important (43, 44). Phosphoribosylpyrophosphate amidotransferase, the first enzyme unique to the pathway of purine biosynthesis, is inhibited by the purine-6-ribonucleotides produced by the synthetic pathway (45). Thus a reduction in hepatic ATP and subsequent removal of AMP by degradation should tend to release the feedback inhibition of purine synthesis and thereby permit more rapid synthesis of IMP. Such a mechanism has been proposed for the marked stimulation in the rate of purine synthesis observed with fructose infusions in normal liver or in patients with hereditary fructose intolerance (46, 47).

A concomitant of ATP depletion seen with fructose infusion is depletion of tissue P_i. P_i levels were not determined in our patients. However, the inability to release P_i from G-6-P, coupled with the observed increase in phosphorylated sugars, suggests that the glucagon also lowered the intracellular P_i levels. Such a depression of hepatic P_i might not only promote degradation of preformed AMP to urate (6) but also be rate-limiting toward restoration of normal levels of ATP (5, 8).

It must be emphasized that, in our study and that of Roe and Kogut (6), pharmacological doses of glucagon were used and the marked changes demonstrated after glucagon injection may not necessarily occur under physiologic conditions. However, the observed decrease in serum uric acid levels and a concomitant decrease in plasma glucagon levels after treatment suggests that repeated episodes of hypoglycemia and hyperglucagonemia may provide sufficient stimulus for excess degradation of ATP in untreated patients with GSD-I.

ACKNOWLEDGMENTS

The authors express appreciation to Sandra Bennett and L. Lockie Treanor for their laboratory assistance and to Martha Hoyle for her assistance in preparation of this manuscript.

This study was supported in part by a grant in aid from the U. S. Public Health Service 5-MO1-RR95 and March of Dimes Genetics Services grant C-116.

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