LOSS OF CYCLIC 3'5'-AMP DEPENDENT KINASE AND REDUCTION OF PHOSPHORYLASE KINASE IN SKELETAL MUSCLE OF A GIRL WITH DEACTIVATED PHOSPHORYLASE AND GLYCOGENOSIS OF LIVER AND MUSCLE.

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SUMMARY

A girl with hepatomegaly had increased glycogen and deactivated phosphorylase in liver and muscle. Her muscle homogenate did not activate either its own phosphorylase or rabbit muscle phosphorylase b except at 10 to 20 % of normal rate under conditions where phosphorylase kinase is active without prior phosphorylation by cyclic 3'5'-AMP dependent kinase. The latter enzyme's activity was restored to the girl's muscle homogenate by mouse muscle lacking phosphorylase kinase activity. We conclude that the patient's muscle had (1) no detectable activity of cyclic 3'5'-AMP dependent kinase and (2) reduced activity of phosphorylase kinase. We speculate that (1) might lead to (2) if phosphorylase kinase was less stable in its non-phosphorylated than in its phosphorylated form.

INTRODUCTION

The syndrome of glycogen storage disease can be divided into nine types (1) of which four involve the system of glycogen phosphorylase. Type V designates deficient muscle phosphorylase (2). Type VI designates deficient liver phosphorylase (3). Type VIII is characterized by degenerative brain disease (4,5) and by lack of proper activation of the hepatic phosphorylase system whose enzymatic components appear intact (5). Finally, type IX denotes deficient phosphorylase kinase in liver; skeletal muscle is normal (6,7). We now report an additional type of glycogen storage disease involving the phosphorylase system of liver and of muscle. To our knowledge, the condition has not been described except in the form of an abstract (8).

PATIENT AND METHODS

The patient was a white girl with lifelong hepatomegaly. At age five years, the edge of the liver was palpable 8 cms. below the right costal margin. Otherwise, the child was clinically healthy. Function and contour of the skeletal muscle were normal. No rise in blood sugar occurred after intravenous administration of glucagon, even when the drug was given two hours after a meal. However, there never was clinical or biochemical evidence for hypoglycemia.

The liver was biopsied twice with a Menghini needle and the gastrocnemius muscle was biopsied four times with a Vim Silverman needle. Specimens were analyzed for glycogen concentration (7) and for activities of hepatic glucose-6-phosphatase (9), lysosomal α-glucosidase (10), amylo-1,6-glucosidase (11), muscle phosphorylase a and muscle phosphorylase b (12), and active as well as total liver phosphorylase (7). For phosphorylase determination homogenization of the specimens in ice cold 0.1 M NaF began between 10 and 120 seconds after the biopsy.

We examined whether the patient's muscle homogenate could activate either its own phosphorylase or rabbit muscle phosphorylase b. Both types of experiments were conducted at pH 6.8 and at pH 8.6, with and without the addition of exogenous 3'5'-AMP. The detailed compositions of the homogenates are listed in Figures 1 and 2. Phosphorylase b was prepared from rabbit muscle (13) and re-crystallized four times when it was found free of detectable kinase activity. Phosphorylase kinase was prepared from rabbit muscle (13). Dr. J.B. Lyons, Jr. kindly supplied the mouse with deficient activity of muscle phosphorylase kinase (14). The nucleotides and substrates were purchased from Sigma Chemical Co., St. Louis, Mo.

RESULTS

The concentration of glycogen was increased in liver and muscle (Table 1). The activities of glucose-6-phosphatase, amylo-1,6-glucosidase, and lysosomal acid α-glucosidase were normal, as was <u>total</u> activity of phosphorylase. How-

TABLE I

Activities of active hepatic phosphorylase and of muscle phosphorylase a were obtained without the addition of 5'-AMP to the substrate solution. Total hepatic phosphorylase activity was obtained in the normals: after the inclusion of ATP, $MgCl_2$ and cyclic 3'5'-AMP to the homogenate (7); and in the patient: after the additional inclusion of active phosphorylase kinase to the homogenate (7). Total muscle phosphorylase activity (phosphorylase a and phosphorylase b) was obtained with the addition of 10^{-3} M 5'-AMP to the substrate solution.

* Mean activity ± 1 S.D.

**Figures in parenthesis indicate: in the case of the normals, the number of different individuals; and in the case of the patients, the number of different biopsy specimens.

	Liver		Muscle	
	Normal	Patient	Normal	Patient
Phosphorylase (μ moles phosphate per gm per min.)				
Total	44.3 ± 9.6*(9)**	38.2 (1)	78.0 ± 21.1 (13)	54.1(2)
Active	25.1 ± 6.5 (42)	0.1 (2)	47.7 ± 13.2 (13)	0 (2)
Percent activation	57	<1	61	0
Glycogen concentration (% weight of wet tissue)	<6.0	11.5 (2)	<1.0	2.1(2)
Acid α-glucosidase (μ moles glucose per gm per min.)	0.258 ± 0.093(43)	0.292 (1)	0.035 ± 0.011 (21)	0.044(1)
Glucose-6-phosphatase (μ moles phosphate per gm per min.)	4.7 ± 1.9 (35)	6.85 (1)		
Amylo-1,6-glucosidase (glucose-^{14}C incorporation into -1,6-branch points of glycogen. Qualitative assay.)		Normal		Normal

ever, the phosphorylase was deactivated in the two liver specimens and in the three different muscle biopsy specimens in which it was measured. In the liver specimens, active phosphorylase was less than 1% of total activity. In the muscle specimens, there was no detectable phosphorylase a (i.e. no active phosphorylase).

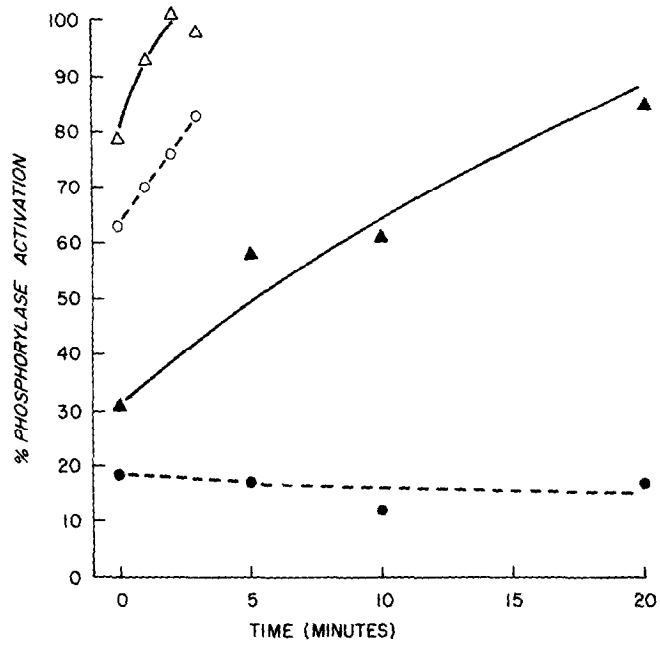

Figure 1. Conversion of phosphorylase b to phosphorylase a in human muscle homogenate. Activation of human muscle phosphorylase in 0.82 cc final volume of the reaction mixture containing gastrocnemius muscle 10 mg, glycogen 4 mg; and (in micromoles): Tris-sodium β-glycerophosphate 37.5, ATP 10, $MgCl_2$ 12.8, NaF 20, at pH 8.6. At pH 6.8 additional inclusions were: 3'5'-AMP 0.14, theophyllin 1. During preparation of the reaction mixture, 19% phosphorylase activation occurred as an artifact in the patient's muscle homogenate prior to its adjustment to pH 6.8. At the time intervals indicated, phosphorylase activity was determined in aliquots of the reaction mixture with and without the addition of 10^{-3} M 5'-AMP to the substrate solution.

Activation in normal human muscle:
at pH 8.6 without cyclic 3'5'-AMP: △ —— △
at pH 6.8 with cyclic 3'5'-AMP: ○ - - - ○
Activation in the patient's muscle:
at pH 8.6 without cyclic 3'5'-AMP: ▲ —— ▲
at pH 6.8 with cyclic 3'5'-AMP: ● - - - ●

For lack of liver tissue, further experiments were restricted to skeletal muscle (Figures 1,2). The patient's muscle homogenate activated its own phosphorylase as well as rabbit muscle phosphorylase b at pH 8.6 without exogenous 3'5'-AMP, but only at 10 to 20% of the rate that occurred with normal human muscle homogenate under the same conditions. The patient's muscle homogenate did not activate either its own phosphorylase or rabbit muscle phosphorylase b at pH 6.8 even in the presence of exogenous cyclic 3'5'-AMP while normal human muscle homogenate accomplished both types of activation under these conditions.

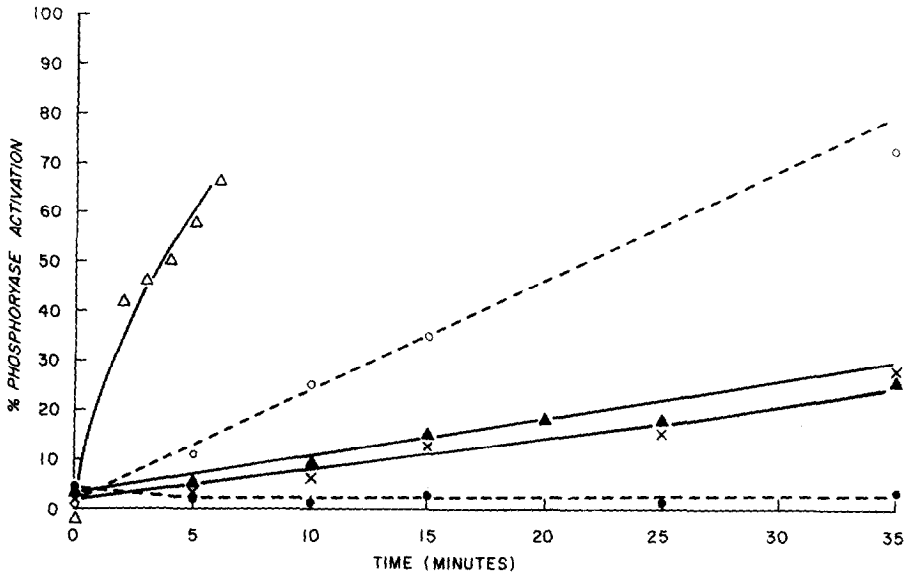

Figure 2. Conversion of rabbit muscle phosphorylase b to phosphorylase a by human muscle homogenate. For this experiment, 0.3 mg of rabbit muscle phosphorylase b (specific activity: 48.4 μMP/min./mg.) has been added to the reaction mixture listed in Figure 1. At the time intervals indicated, 0.05 cc aliquots of the reaction mixture were diluted in 1.5 cc of ice cold 0.03 M cysteine - 0.04 M sodium β-glycerophosphate buffer, pH 6.0 in which phosphorylase activity was determined with and without the addition of 5'-AMP to the substrate solution.

Activation by normal human muscle homogenate:
at pH 8.6 without cyclic 3'5'-AMP: △——△
at pH 6.8 with cyclic 3'5'-AMP: ○- - -○
Activation by the patient's muscle:
at pH 8.6 without cyclic 3'5'-AMP: ▲——▲
at pH 6.8 with cyclic 3'5'-AMP: ●- - -●
at pH 6.8 with cyclic 3'5'-AMP and 5 mg of phosphorylase kinase deficient mouse muscle added to the homogenate: ×——×

The patient's homogenate was then fortified with mouse muscle that had no detectable phosphorylase kinase activity, i.e. that did not activate its own phosphorylase or rabbit muscle phosphorylase b in the presence of cyclic 3'5'-AMP either at pH 8.6 or 6.8. The patient's homogenate thus fortified activated rabbit muscle phosphorylase b at pH 6.8 in the presence of cyclic 3'5'-AMP at the same rate that had occurred at pH 8.6 without added mouse muscle and without added cyclic 3'5'-AMP.

DISCUSSION

In the patient's liver and muscle, total activity of phosphorylase was

normal but the enzyme was in the inactive form. Under the present conditions of biopsy and assay, 60% of total activity is normally in the active form ((7) and Table 1). Hence, the patient had a defect of phosphorylase activation in liver and muscle. Lyon and Porter studied mice with deactivated phosphorylase in muscle but not in liver. In the muscle of the adult animals they found no activity of phosphorylase kinase (14). Our patient had phosphorylase kinase but at 20% of normal activity since her muscle homogenate at pH 8.6 activated phosphorylase but at the reduced rate.

At pH 6.8, the girl's muscle homogenate did not activate phosphorylase despite the inclusion of cyclic 3'5'-AMP. This defect was rectified when phosphorylase kinase deficient mouse muscle was also added to the homogenate.

The results can be explained by deficient activity of cyclic 3'5'-AMP dependent kinase, an enzyme recently described by Walsh, Perkins and Krebs in rabbit muscle (15). This enzyme phosphorylates phosphorylase kinase. Without this phosphorylation, phosphorylase kinase is practically inactive at pH 6.8 and thus does not activate phosphorylase at this pH. At pH 8.6, however, phosphorylase kinase can activate phosphorylase without having first been activated by cyclic 3'5'-AMP dependent kinase (16). We could not assay cyclic 3'5'-AMP dependent kinase with the method of Walsh, et al in the small needle biopsy samples (10 to 15 mgs). However, we suggest this enzymatic activity to be the correcting factor that was added to the patient's homogenate by the phosphorylase kinase deficient mouse muscle.

In a purely speculative manner, the reduction of phosphorylase kinase activity in the child's muscle could be explained as the consequence of the cyclic 3'5'-AMP dependent kinase deficiency if phosphorylase kinase was less stable in its non-phosphorylated than in its phosphorylated form.

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