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THE EFFECTS OF GLUCAGON IN THREE FORMS OF GLYCOGEN STORAGE DISEASE *

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It now is clear that glycogen storage disease can be associated with a number of different abnormalities in glycolytic enzymes. The initial patients with this disorder in whom enzymes were studied showed hepatic glucose-6-phosphatase (G-6-Pase) deficiency, but similar clinical findings may result from lack of amylo-1 : 6 glucosidase, amylo-1 : 4,1 : 6 transglucosidase, or phosphorylase (1). Patients with more than one enzymic anomaly have been reported (2), and in at least one family two siblings had glycogen storage disease but each had a different biochemical abnormality (3).

It is apparent that therapeutic measures directed against such a complex group of disorders might well give variable results. Indeed, a variety of methods of treatment has been used, with inconstant benefit (4–6). Most recently, Lowe, Sokal, Doray and Sarcione have reported favorable results in a patient with G-6-Pase deficiency when glucagon was used as the sole therapeutic agent (7). In a later report from the same laboratory (2), distinct evidence of glycogenolysis was found when glucagon was given to six patients with varied types of hepatic glycogen storage disease. Four of these had proven or presumed G-6-Pase deficiency, one had combined phosphorylase and G-6-Pase deficiency and, in one, careful study failed to reveal the nature of the underlying biochemical abnormality. The similarity between the metabolic responses to glucagon exhibited by these patients in spite of the varied nature of their enzymic defects has been difficult to interpret.

We have had the opportunity to study the effects of the administration of glucagon to five patients with glycogen storage disease. Four were treated with glucagon, given intramuscularly, for 6 months. Two of these patients had proven or presumed amylo-1 : 6 glucosidase deficiency, two had proven or presumed G-6-Pase deficiency, and one patient was deficient in both G-6-Pase and amylo-1 : 6 glucosidase. The results of our studies suggest that the expected response to glucagon in glycogen storage disease does indeed depend upon the underlying biochemical abnormality. From a clinical point of view, the long-term treatment of these patients with glucagon was unsuccessful.

METHODS

Four of the five patients were studied on the metabolic ward of the Salt Lake County General Hospital. Patient 4 was seen in another hospital in Salt Lake City, and died before long-term therapy could be undertaken. Enzyme assays and glycogen estimations were performed on quick-frozen surgical biopsy specimens by Dr. Barbara Illingsworth Brown, from the Department of Biological Chemistry of Washington University in St. Louis. Leukocyte phosphorylase was measured in a frozen leukocyte button obtained from Patient 2 and from his mother by Drs. Hibbard Williams and James Field, National Institute of Arthritis and Metabolic Diseases. We are deeply indebted to Drs. Brown, Williams, and Field for their invaluable aid.

Epinephrine and glucagon tolerance tests were performed after a 14-hour overnight fast by giving 0.3 mg epinephrine subcutaneously or 1 mg glucagon [1] intramuscularly. Blood samples for glucose and lactic acid analyses were obtained at frequent intervals for 1 to 3 hours after injection. After control studies were completed, each patient received glucagon intramuscularly, 1 mg three times per day before meals, for 6 months. In Patient 3 nausea and vomiting made it necessary to reduce this dose to 1 mg twice daily after the first month of therapy. The blood studies were repeated after 1, 3, and 6 months of therapy in all four treated patients. Venous blood glucose was measured by the Nelson-Somogyi method (8), and lactic acid by the Barker-Summerson technique (9). Changes in liver size were documented

TABLE I

Diagnostic features of patients studied

Patients		Hypo-glycemia	Fasting blood lactic acid levels	Liver glycogen	Muscle glycogen	Liver G-6-Pase	Liver phosphory-lase	Glycogen split by purified phosphorylase		C^{14}-glucose incorp. into glycogen
No., Sex	Age							Liver	Muscle	
	yrs, mos			*g/100 g*		*μg P/100 mg/hr*	*μmoles P/ min/g*	*%*	*%*	*cpm/mg*
1 ♀	17	Mod.	Incr.	12.6	ND*	88	ND	33	ND	ND
2 ♂	14 9	Mod.	Incr.	Incr.	ND	ND	ND	ND	ND	ND
3 ♂	18	Severe	Normal	17.4	5.4	208	25.6	17	3.4	2.5†
4 ♂	25	Severe	Normal	Incr.	ND	ND	ND	ND ·	ND	ND
5 ♀	3 9	Severe	Normal	13.8	0.3	51, 62	23.2	16.9, 19	ND	4.1
Normal values				2-8‡	0.5-1.0‡	>500§	~22‖	35-40§	25-35	60-100§

* Not done.
† Addition of authentic amylo-1:6 glucosidase returned C^{14}-glucose incorporation to normal (15).
‡ Ref. 14.
§ Ref. 15.
‖ Ref. 13.

by serial photographs taken under standard conditions. A brief summary of each patient's history follows. The major diagnostic features are listed in Table I.

Case 1. D.A.G., female, age 17 months. This patient was referred to the hospital because of multiple severe upper respiratory infections, retarded growth, and progressive abdominal distention since birth. One 5-month old sibling was normal; a maternal grandmother had diabetes. Physical examination revealed a small child who sat but did not stand. Respirations were 50 per minute; hepatomegaly was marked; fasting hypoglycemia, ketonuria, and hyperlipemia were present; the epinephrine tolerance test was abnormal. Liver biopsy revealed marked deficiency of G-6-Pase; the liver glycogen concentration was 12.6 g per 100 g.

Case 2. J.C., male, age 14 years 9 months. This boy was studied elsewhere at age 2 years because of slow growth and a protuberant abdomen. Hepatomegaly and hyperlipemia were found, and a liver biopsy showed markedly increased content of lipid and glycogen (absolute values unavailable). He first came to the Salt Lake County General Hospital at age 6 because of frequent bruising and occasional epistaxes. At that time he was a small, alert child with marked hepatomegaly, and fasting hypoglycemia, ketonuria, and hyperlipemia. The blood glucose level failed to rise after the subcutaneous administration of epinephrine. The administration of galactose orally caused little or no rise in blood glucose level. The sulfobromophthalein test showed 36 per cent retention in 45 minutes; the thymol turbidity was 13 units. Bleeding time, coagulation time, and prothrombin time were prolonged. Although there was a paternal history of "bleeding tendency," careful study [2] of the patient's father failed to reveal any hemostatic abnormalities. Because of the patient's bleeding anomalies, which were ascribed to his hepatic dysfunction, liver biopsy was not performed. The patient's leukocyte phosphorylase activity was normal, as was that of his mother.

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[2] Kindly performed by Dr. Paul Didisheim.

Case 3. M.L., male, age 15 months. This patient, the younger brother of B.L. (Case 4) first was found to have grand mal seizures at the age of 4 months. These occurred irregularly at 1- to 3-week intervals. Subsequently, he grew slowly and was thought to be mentally retarded. Gradually he developed a protuberant abdomen, marked hepatomegaly, and severe fasting hypoglycemia and ketonuria. The blood glucose level did not rise in response to epinephrine. Liver biopsy showed marked deficiency of amylo-1:6 glucosidase as demonstrated by decreased splitting of the glycogen by purified phosphorylase and by reduced incorporation of C^{14}-glucose into the glycogen. After addition of authentic amylo-1:6 glucosidase, C^{14}-glucose incorporation was normal. A moderate reduction in G-6-Pase also was found (see Table I). The liver glycogen concentration was 17.4 g per 100 g.

Case 4. B.L., male, age 25 months. This boy was admitted to another hospital in Salt Lake City because of convulsions which began at age 1 month. At age 1 year hepatomegaly was found. He had marked mental retardation. Severe fasting hypoglycemia, ketonuria, and slight hyperlipemia were present. The blood glucose did not rise after subcutaneous injection of epinephrine. A liver biopsy showed increased glycogen content (absolute values not available). Shortly after discharge from the hospital the patient expired at home. Unfortunately, postmortem examination was not performed.

Case 5. C.B., female, age 27 months. This child was seen elsewhere at age 8 months because of hepatomegaly and failure to grow. Liver biopsy showed increased glycogen content (absolute values unavailable). When she first came to the Salt Lake County General Hospital, at age 27 months, she had had continued difficulty with frequent upper respiratory infections and vomiting. Physical examination showed marked hepatomegaly and dwarfism. Fasting hypoglycemia, ketonuria, and hyperlipemia all were present. The epinephrine tolerance test was abnormal. Liver biopsy showed marked deficiency of both amylo-1:6 glucosidase and G-6-Pase (Table I); liver glycogen concentration was 13.8 g per 100 g.

Thus, of the five cases available for study, Patient 1

TABLE II

Blood glucose and blood lactic acid levels in fasting subjects

Patient	Blood glucose			Blood lactic acid		
	Range	Average	No. of determinations	Range	Average	No. of determinations
	mg/100 ml			*mg/100 ml*		
1	36–57	47	10	55–171	96	10
2	32–55	45	13	10–127.5	59.8	6
3	15–21	20	10	9.8–19.8	14.1	6
4	25–28	27	2		13.8	1
5	24–39	32	13	10–27.3	18.7	9

was shown to have G-6-Pase deficiency, Patient 3 had amylo-1:6 glucosidase deficiency with moderately reduced G-6-Pase activity, and Patient 5 had marked deficiency of both enzymes. In Patients 2 and 4 the enzymic anomaly is unknown. The normal leukocyte phosphorylase in Patient 2, plus the similarity between the results of the studies in Patients 1 and 2, suggests that G-6-Pase deficiency was the underlying enzymic abnormality in both subjects. Likewise, the similar clinical findings and results obtained in the brothers, Patients 3 and 4, suggest that both had amylo-1:6 glucosidase deficiency. Based on these assumptions, the data from Patients 1 and 2 and from Patients 3 and 4 were combined in order to simplify the graphic presentation of the data. However, the actual data also are tabulated for each patient and all are identified throughout the text as having either "proven" or "presumed" enzymic deficiency.

RESULTS

Fasting blood glucose levels. All five patients exhibited fasting hypoglycemia (Table II). In Patients 1 and 2, with proven or presumed G-6-Pase deficiency, the hypoglycemia was definite but modest (average blood glucose levels, 47 and 45 mg per 100 ml, respectively). In contrast, Pa-

TABLE III

Blood glucose response (mg/100 ml) to glucagon, 1 mg i.m. and to epinephrine, 0.3 mg s.c.

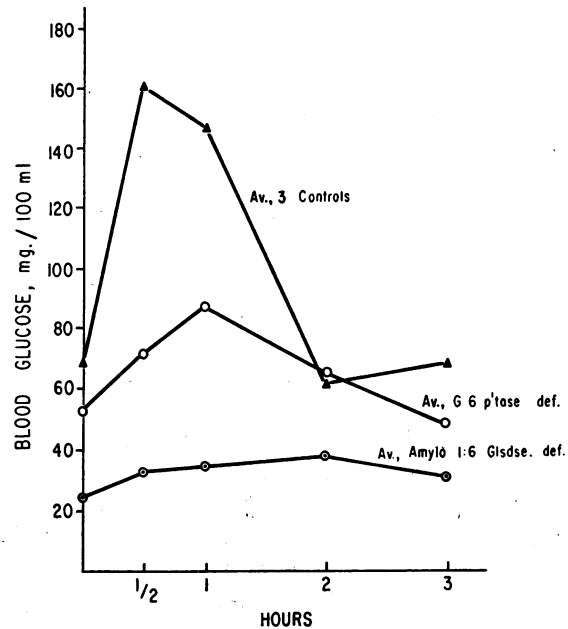| Patient	Glucagon Minutes after injection					Epinephrine Minutes after injection						
	0	30	60	120	180	0	30	45	60	90	120	180
1	57	85	86	63	49	45	55	53				
2	52	74	95	60	53	49	52		58		46	48
	52	57	78	71	46							
3		22	31	26	19	15	20			25		
	20	20	20	23	28							
4	25	40	44	47	51							
	28	38	34	46	41							
5	28	40	43	47	39	34	35		46		48	42

FIG. 1. AVERAGE BLOOD GLUCOSE RESPONSES TO GLUCAGON.

tients 3 and 4, with proven or presumed amylo-1:6 glucosidase deficiency, consistently showed severe fasting hypoglycemia (average blood glucose levels, 20 and 27 mg per 100 ml, respectively). Patient 5, with the combined enzymic anomalies, also had severe hypoglycemia (average blood glucose level, 32 mg per 100 ml).

Fasting blood lactic acid levels. Increased blood lactic acid levels were found only in Patients 1 and 2 (Table II). The subjects with proven or presumed amylo-1:6 glucosidase deficiency, either as the major defect or combined with severe G-6-Pase deficiency, had normal fasting blood lactic acid levels.

Response to epinephrine and glucagon. a) Blood glucose: In no patient did the subcutaneous

TABLE IV

Blood lactic acid response (mg/100 ml) to glucagon

Patient	Minutes after injection				
	0	30	60	120	180
1	141	126	109	100	91
2	51	90	90	51	49
3	10		13		11
4	19	17	17	17	15
	12	11	11	10	11
5	19		22		33

administration of epinephrine elicit more than a minimal rise in blood glucose level. On the other hand, after the administration of glucagon to the subjects with proven or presumed G-6-Pase deficiency, blood glucose levels rose (29, 43, and 26 mg per 100 ml). The patients with amylo-1 : 6 glucosidase deficiency showed less change in blood glucose level in response to glucagon administration (8, 26, 18, and 19 mg per 100 ml). These observations are recorded in Table III. In Figure 1 the average blood glucose response to glucagon in each group is compared with that of three normal children. b) Blood lactic acid levels: In Patients 2 and 5 epinephrine administration failed to produce significant changes in blood lactic acid. In Patients 1 and 2 glucagon administration resulted in definite increases in lactic acid levels (15 and 39 mg per 100 ml, respectively). In Patients 3, 4, and 5 the lactic acid levels did not change significantly (Table IV, Figure 2).

Changes with prolonged glucagon therapy. a) Clinical results: Glucagon therapy failed to produce significant clinical improvement in any of the four children treated. With the exception of Patient 1, who grew 2 inches during the period of study, height did not change and weight remained stable. It is worthy of note that even 2 inches' growth at age 17 months is subnormal. Normal growth at the age of the other patients varies from 0.7 to 3.6 inches, depending on age (10). In Patient 3, seizure frequency and pattern were unchanged. Of interest is the fact that in Patients 1, 2, and 5 liver size decreased during the first month of therapy. This change, shown in Figure 3, did not persist, and after 3 months of treatment liver size had returned to its previous magnitude.
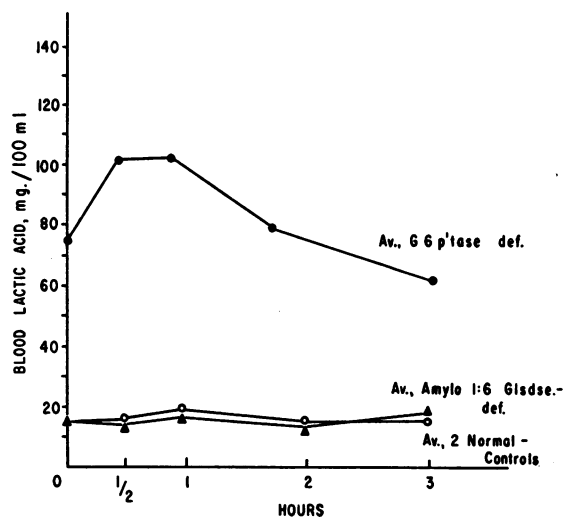

FIG. 2. AVERAGE BLOOD LACTIC ACID RESPONSES TO GLUCAGON.

In Patient 3, with amylo-1 : 6 glucosidase deficiency, the liver size increased steadily during the study period. b) Biochemical observations during therapy: Fasting hypoglycemia and increased blood lactic acid levels persisted throughout the treatment period in Patients 1 and 2. In these same patients the blood glucose response to glucagon, which was similar to pretreatment values at 1 and 3 months of therapy, decreased to insignificant levels by the sixth month (Figure 4). The lactic acid response to glucagon was unchanged throughout therapy in Patient 1, but was markedly decreased after 6 months' treatment in Patient 2. Patients 3 and 5, who showed little rise in blood glucose or lactic acid in response to glucagon before treatment, maintained this same pattern of response throughout therapy.
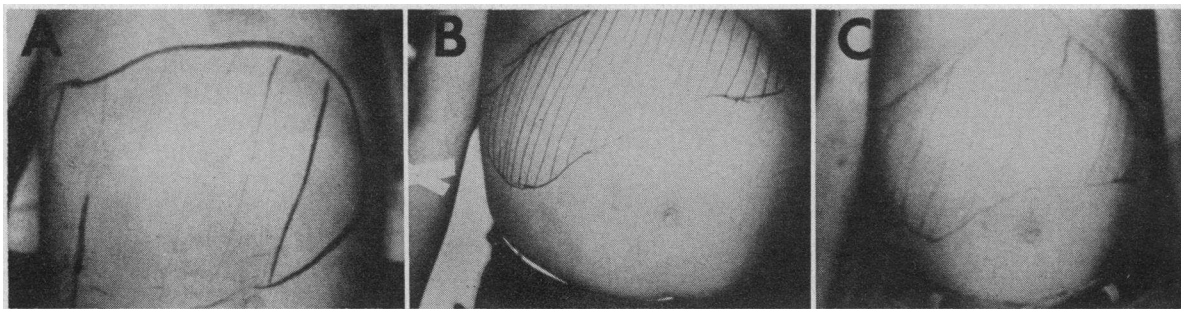

FIG. 3. PHOTOGRAPHS OF LIVER SIZE IN PATIENT 2. A, BEFORE TREATMENT; B, AFTER 1 MONTH OF TREATMENT; C, AFTER 6 MONTHS OF TREATMENT.
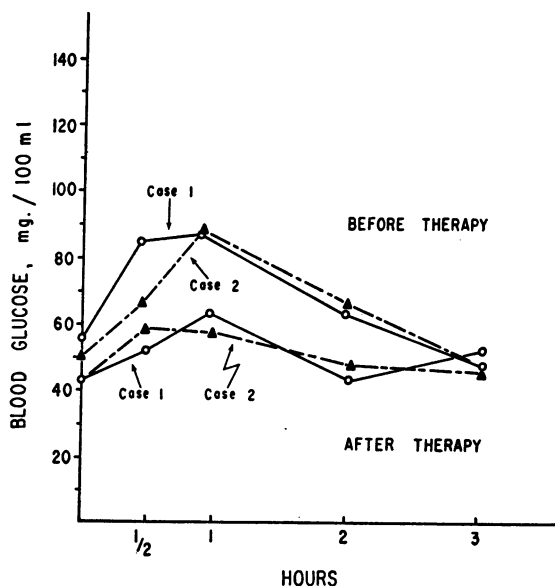
FIG. 4. REDUCED BLOOD GLUCOSE RESPONSES TO GLUCAGON AFTER 6 MONTHS OF TREATMENT.

DISCUSSION

Patients with different forms of glycogen storage disease now can be classified on the basis of the underlying enzymic anomaly responsible for the glycogenosis. In spite of the fact that these enzymic abnormalities are quite varied, some workers have viewed patients with glycogenosis of varied types as a homogeneous metabolic group (2), at least insofar as their response to glucagon is concerned. In the present study no discrepancies were noted between the responses to glucagon expected from the underlying enzymic anomalies and the responses observed in the patients. Within the limits of the patients available for study, the response to glucagon in a given type of glycogenosis could be predicted from consideration of the current concepts of glycogen metabolism (Figure 5).

In the synthesis of glycogen, long, non-branched chains of glucose in 1,4 linkage are formed from glucose-1-phosphate via uridine diphosphoglucose and uridine diphosphoglucose transferase. These chains then undergo branching under the influence of amylo-1:4,1:6 transglucosidase to form glycogen. Deficiency of this "branching enzyme" gives rise to a disorder called amylopectinosis, which is characterized by large deposits of an abnormal "glycogen" with fewer branch points than

has normal glycogen. In the initial steps of glycogenolysis, glycogen is acted upon in turn by phosphorylase a the 1,4 linkages and amylo-1 : 6 glucosidase ("debrancher") at the 1,6 linkages to yield glucose-1-phosphate and glucose, respectively.

Deficiency of debrancher leads to accumulation of a dextrin, which cannot be degraded further by phosphorylase, since phosphorylase cannot bypass the persisting 1,6 linkages. In the fasting state this type of abnormal glycogen has very short outer chains as a result of removal of the glucose units in 1,4 linkages by phosphorylase. It is this defect (limit dextrinosis) that was present in our Patient 3, presumed to be present in Patient 4, and that was combined with severe G-6-Pase deficiency in our Patient 5. Deficiency of phosphorylase leads to accumulation of normal glycogen, as does G-6-Pase deficiency.

Thus, in amylopectinosis the glucose 1,4 links should be susceptible to phosphorylase action and should yield glucose-1-phosphate when phosphorylase is activated by glucagon. Since normal levels of G-6-Pase are present in this condition, blood glucose should rise, and the blood lactic acid level should remain unchanged. Unfortunately, the response to gucagon has not been studied in this rare disorder. In limit dextrinosis, with
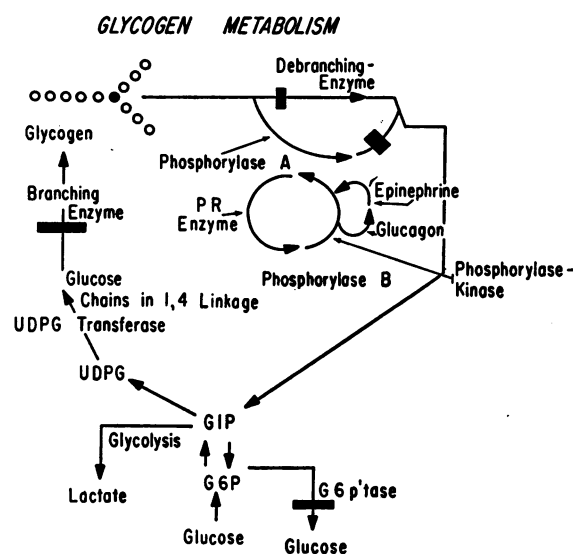

FIG. 5. SIMPLIFIED SCHEMATIC REPRESENTATION OF CURRENT CONCEPTS OF GLYCOGEN METABOLISM. The heavy solid bars indicate sites of enzymic abnormality in different forms of glycogen storage disease (see text).

short outer chains of glucose 1,4 units, activation of phosphorylase by glucagon should have little or no effect either on glucose or lactic acid, because phosphorylase acts only on 1,4 linkages and cannot continue glycogen degradation past the 1,6 linkages. Indeed, such was the case in our Patients 3, 4, and 5.

Phosphorylase deficiency is a more complex situation. Apparent deficiency of this enzyme theoretically could result from absolute loss of the enzyme, absence of phosphorylase kinase, or absence of some cofactor essential for activation of the kinase by glucagon or epinephrine. If absolute lack of phosphorylase were the abnormality, no response to glucagon would be expected. Sokal's Patient 2 (with combined phosphorylase and G-6-Pase deficiency) (2) did respond with increased lactic acid levels, suggesting that in this patient some abnormality was present in the phosphorylase-activating system which was corrected by glucagon. Hers's unsuccessful attempts to activate phosphorylase in the livers of his patients with adenosine triphosphate, Mg^{++}, and adenosine 3:5' (cyclic) monophosphate suggest that actual deficiency of enzyme may have been present in those cases. (11). In G-6-Pase deficiency glucagon would be expected to induce glycogenolysis but, without a means for splitting glucose-6-phosphate, increased lactic acid production would result. This was found in our Patients 1 and 2 and in cases 3, 4, 5, and 6 of Sokal and co-workers (2).

Several aspects of our results deserve further comment. First, the increased blood glucose levels which occurred in response to glucagon appeared to be greater in our patients with presumed or proven G-6-Pase deficiency than in the patients with proven or presumed amylo-1:6 glucosidase deficiency (limit dextrinosis). Even more marked were the differences in lactic acid response between these two types of glycogen storage disease. As Sokal and colleagues reported, glucagon administration resulted in an increase in the already elevated lactic acid levels in G-6-Pase deficiency; no change occurred in our patients with limit dextrinosis. These observations suggest that glucagon may be used to differentiate between limit dextrinosis and G-6-Pase deficiency before biopsy is performed. Thus, minimal blood glucose rise in response to glucagon or epinephrine, normal

fasting blood lactic acid levels, and no blood lactic acid rise after glucagon administration would suggest limit dextrinosis. Definite but subnormal blood glucose rise, increased fasting blood lactic acid levels, and increase in blood lactic acid in response to glucagon would suggest G-6-Pase deficiency. As demonstrated by our Patient 5, who had both limit dextrinosis and G-6-Pase deficiency, such a series of observations would not identify the second enzymic anomaly.

To our knowledge no one yet has commented on the curious but now well documented occurrence of glycogen storage disease with two enzymic defects. Both our Patient 5 and Patient 2 of Sokal and colleagues (2), with phosphorylase and G-6-Pase deficiency, demonstrated marked decrease in activity of two important glycolytic enzymes. Also, Forbes's patient (12) and our Patient 3 had amylo-1:6 glucosidase deficiency with an associated modest reduction in G-6-Pase activity. Finally, the family described by Calderbank and co-workers (3), in which one sibling had one type of glycogen storage disease while another sibling had a different type, is a genetic curiosity.

While a compensatory increase in the activity of various hepatic glycolytic enzymes has been noted in patients with G-6-Pase deficiency (13), the explanation for the occurrence of marked *deficiency* of two enzymes in one patient or in two patients from a single family is unknown. Similarly, the modest reduction in G-6-Pase activity in limit dextrinosis is unexplained. For two rare recessive genes to occur in the homozygous state in a single individual, or indeed in one family, is highly improbable. Rather, these isolated observations suggest that the enzymic abnormalities now used to classify the glycogenoses may not be specific for the primary genetic abnormality in these diseases.

Treatment of these patients with glucagon for 6 months was unrewarding. The lack of favorable results in limit dextrinosis was to be expected. However, the children with G-6-Pase deficiency showed definite evidence that glycogenolysis had occurred. In the follow-up studies of glucagon response conducted after 1 month of therapy, blood glucose again rose moderately, the lactic acid rose more obviously. The liver size had decreased dur-

ing the first month of therapy. After 6 months, however, glucagon administration caused little or no change. Diminished responsiveness to continued administration of a hormonal agent (tolerance) is a well known phenomenon in endocrinology (viz., gonadotrophins, parathyroid hormone) and may be the result of immunologic or other less well understood mechanisms. The mechanism underlying the diminished responsiveness to glucagon observed in our patients is unknown.

SUMMARY

1. The effect of glucagon in one patient each with glucose-6-phosphatase (G-6-Pase) deficiency, amylo-1 : 6 glucosidase deficiency (limit dextrinosis), and combined G-6-Pase and amylo-1 : 6 glucosidase deficiency, is reported. One additional patient was presumed to have G-6-Pase deficiency, and one was presumed to have amylo-1 : 6 glucosidase deficiency on the basis of the similarity between their biochemical response to glucagon and that observed in the subjects with known enzymic abnormalities.

2. The response to glucagon of the patients with different forms of glycogen storage disease was related to the nature of the underlying enzymic anomaly in each case.

3. In patients with limit dextrinosis reported here, fasting blood lactic acid levels were normal. In these subjects, glucagon did not induce either hyperglycemia or increased blood lactic acid levels. In the patients with G-6-Pase deficiency, increased fasting blood lactic acid levels were observed. A modest increase in blood glucose and a more marked increase in blood lactic acid occurred in response to glucagon.

4. Treatment of two patients with proven or presumed G-6-Pase deficiency, one patient with limit dextrinosis, and one patient with the combined defect of limit dextrinosis and G-6-Pase deficiency, failed to give lasting clinical benefit.

This failure may in part be the result of the development of some form of tolerance to glucagon.

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