The Turkish Journal of Pediatrics

A Quarterly Publication

VOLUME : 5 OCTOBER 1963 NUMBER : 4

BIOCHEMICAL INVESTIGATIONS IN GLYCOGEN STORAGE DISEASE

Pınar Özand, M. D.,* Münire Onay, M. S.,**
Sevim Balcı, M. D., * and Sirus Amiri, M. D.*****

Introduction

The two different types of glycogen storage disease reported here are the first in Turkey to be biochemically proven cases. They are reported here in order to draw attention to some of the common errors in diagnosis and to emphasize the choice of laboratory tests necessary for correct diagnosis.

Glycogenoses may be divided into six categories, as follows :

1. Generalized glycogen storage disease (Pompe's disease).[1] This type involves all the organs, although cardiac tissue is the main site of involvement, and these cardiac complications lead to sudden death in early infancy. In contrast to the other types, the biochemical defect in this disease is not well-defined. The interested reader is referred to a review of the disease by Di Sant'Agnese.[2]

2. Hepatorenal glycogen storage disease (von Gierke's disease).[3] This type involves only the liver and kidneys. The absence of glucose 6-phosphatase in these organs leads to some of the clinical symptoms observed.[4]

From Ankara University, Hacettepe Medical Center and Hacettepe Children's Hospital.
* Biochemist
** Biochemist
*** Assistant in Pediatrics.

3. Hepatic phosphorylase deficiency (Hers' disease).[5] This type, described recently, presents a clinical picture similar to von Gierke's disease, although the enzymatic defect is clearly due to hepatic phosphorylase deficiency, and not to the absence of glucose 6-phosphatase.

4. Amylo 1,6 glucosidase deficiency (limit dextrinosis).[5, 6, 7, 8] There are no pathognomonic clinical symptoms in this form of the disease, which involves the liver, muscles, and heart, as well as other organs.

5. Amylo 1,4 - 1,6 transglucosidase deficiency (Brancher enzyme deficiency).[9] Only one case of this type has been published. The defect involves both the liver and the spleen. Clinical manifestations include biliary cirrhosis with a history of familial incidence.

6. Muscle phosphorylase deficiency (McArdle's disease).[10, 11, 12] Three cases have so far been reported in the world literature. Myopathy is the chief clinical finding.

Uridine disposphoglucose, a glycogen transferase deficiency (glycogen synthetase deficiency) has only recently been described.[13, 14] It is a disease in which the patient suffers from a hypoglycemia characterized by a lack of adrenal response. Although this does not lead to accumulation of glycogen, the disease can be considered with glycogenoses in general, as it is the result of a deficiency of one of the glycogen metabolism enzymes. Figure 1 illustrates the metabolism of glycogen and the defects which cause the various types of glycogenoses.

We report here a case of von Gierke's disease and a case diagnosed as generalized glycogen storage disease based on organ analysis. The approach to the diagnosis and the biochemical techniques will be discussed.

Case Reports

Case 1 : C. Ç., an 18 month old boy, was admitted to Hacettepe Children's Hospital with principle complaints of cyanosis, frequent upper respiratory tract infections, and failure to thrive. The child had developed an upper respiratory tract infection and diarrhea a few days prior to his admission to the Center. The cyanosis was first observed a few days after birth, and had been present intermittently since then. The parents stated that the baby had had many respiratory tract infections which had been considered bronchopneumonia, and had

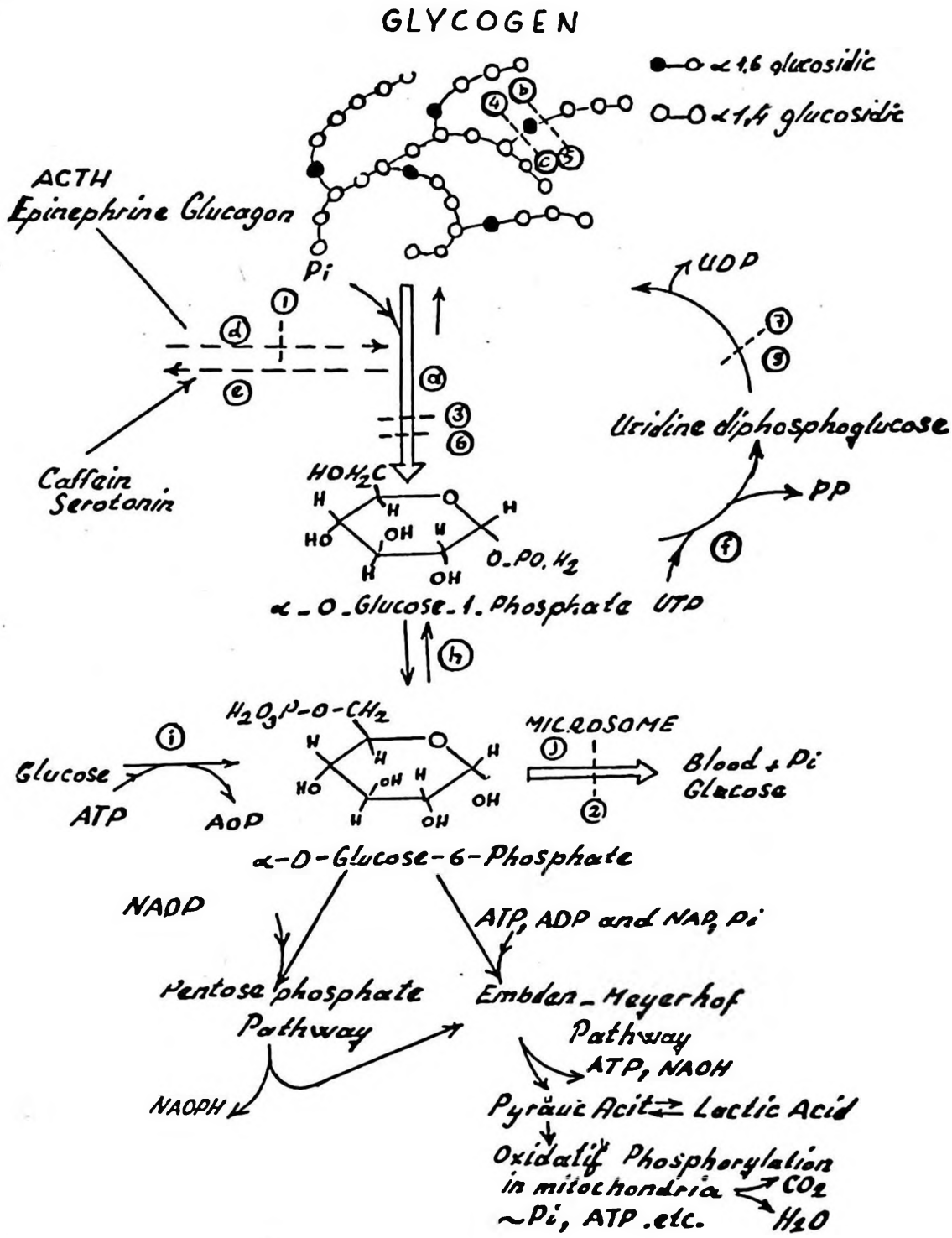

Figure 1. Abbreviated scheme of glucose and glycogen metabolism. The letters in circles indicate the enzyme catalyzing that step; the numbers in circles indicate the defect in glycogenosis: a. Phosphorylase (muscle, liver, etc.), b. Amylo 1,6 glucosidase (debrancher enzyme), c. Amylo 1,4-1,6 transglycosylase (brancher enzyme), d. Phosphorylase activating system, e. Phosphorylase inactivating system f. Uridine diphosphoglucose pyrophosphorylase, g. Uridine diphosphoglucose glycogen transglucosylase, h. Phosphoglucomutase, i. Hexokinase, j. Glucose 6-phosphatase. 1. Generalized glycogen storage disease (?), 2. Glucose 6-phosphatase deficiency, 3. Hepatic phosphorylase deficiency, 4. Amylo 1,6 glucosidase deficiency, 5. Brancher deficiency, 6. Muscle phosphorylase deficiency, 7. Glycogen synthetase deficiency.

been successfully treated as such. The baby never sat alone and did not toddle. The infection that led to the admission, and subsequently to the death of the child, developed a few days before entry to the hospital with cough and fever. Later he developed diarrhea characterized by watery stools and a foul smell. The patient's family was poor and the mother had had no obstetric care during her pregnancy. No history could be obtained relating to the pregnancy or birth of the child. The family history however was relevant: The patient had had five siblings, three of whom died following a similar clinical course with similar clinical findings, at the ages of 30, 18, and six months. They had all experienced cyanosis, and had had many upper respiratory tract infections. Presumably death came as a result of these conditions.

The physical examination at the time of admission showed a very lethargic baby in an advanced state of malnutrition, weighing only 6.9 Kg. He preferred to lie motionless, in the supine position with his head retracted. He had severe cyanosis of the lips and fingertips, and a generally cyanotic appearance. His fingers were clubbed and he had no teeth. The cardiac maximal apical pulse was located at the fifth intercostal space, to the left of the midclavicular line. Some examiners noted a first grade murmur during both the systole and diastole at the mesocardiac focus. The abdomen was protuberant and the liver margin could be felt seven to eight cm. below the right costal margin, to the right of the midclavicular line. The consistency of the liver on palpation was hard and the margin was blunt. The spleen could not be palpated. The muscles of the extremities were soft and gelatinous. There was no hypertrophy of the tongue.

The routine urine examination and blood count on admission were not relevant. The throat culture yielded pneumococcal growth. The cardiac series demonstrated the presence of biventricular and right atrial hypertrophy. The electrocardiogram indicated a right axis deviation and right ventricular hypertrophy.

Digitalis was administered and the child was observed during the following week. His general condition showed no improvement and the size of the liver remained the same. However, the infection responded to antibiotic treatment and he was soon afebrile. The clinical observations led to a diagnosis of generalized glycogen storage disease and a liver biopsy specimen was obtained. Using the periodic acid Schiff test (PAS), there was extensive staining of material in the parenchyma cells of the liver (figure 2). Further tests showed that the material was amylase digestible, and was therefore considered to be glycogen. This material was even found in the nuclei of the liver cells.

Despite continuous therapy, there was no fundamental improvement in his condition. The planned battery of laboratory tests consisting of glucose, fructose (or galactose) and epinephrine tolerance tests, could not be carried out because the child was in such poor condition. At the first opportunity an epinephrine tolerance test was performed, as this test yields more information about the nature of the disease than the others. Without previously maintaining a high carbohydrate diet, the patient was kept fasting for about four hours. He was then given, subcutaneously, 0.02 cc. of 1/1000 aqueous epinephrine. Femoral punctures were used to collect the blood samples, with care to avoid contamination of the samples. Serum lactic acid and blood sugar values were determined from these samples. The results are given in table 1.

Figure 2. Liver biopsy, stained with PAS stain in generalized glycogen storage disease.

TABLE 1

EPINEPHRINE TOLERANCE TEST IN GENERALIZED GLYCOGEN STORAGE DISEASE
(Details are given in the text)

Time in minutes after epinephrine administration	Blood glucose mg. %	Blood lactic acid mg. %
Initial level	90	29
15 minutes	100	20
30 minutes	104	13.5
60 minutes	90	15.5
120 minutes	82	9
240 minutes	110	12.8

The test clearly shows that the patient failed to break down liver and muscle glycogen. There was no apparent rise in blood sugar and lactic acid following the administration of the glycogenolytic drug. In fact, the blood lactic acid decreased from an initially high level. Blood sugar levels, determined enzymatically, were always within normal limits. This result was compatable with the diagnosis of glycogenosis: von Gierke's disease, hepatic phosphorylase deficiency, or generalized glycogen storage disease.

A muscle biopsy was planned, but unfortunately the patient developed fever and severe diarrhea and his clinical condition deteriorated rapidly, despite

intravenous electrolyte and supportive therapy. Repeated stool, throat and blood cultures failed to grow a pathogenic microorganism. The baby died seven days after the liver biopsy specimen was taken. The cause of the intercurrent infection was undetermined.

An autopsy was performed a few hours after death. The patient was found to have a greatly enlarged heart and liver. A sample of heart, liver, psoas muscle, kidney, stomach, thoracic aorta, pancreas, testicle, lung, and basal ganglia tissues were treated and fixed for PAS staining. Other samples were frozen immediately for future analysis. Glycogen and enzyme analyses were performed on all the tissue samples. Glycogen was determined by the conventional technique, and glycogen deposits were found in the heart and liver tissues only. The PAS staining was negative for all the other tissues. Histologic examination of the pancreas revealed no abnormalities. The results of organ analysis for glycogen are given in table 2.

TABLE 2

GLYCOGEN ANALYSIS IN GENERALIZED GLYCOGEN STORAGE DISEASE

Case Report

Patient's name and age	Diagnosis	Time interval, in hours, between death and autopsy	Glycogen per cent		
			Heart	Muscle	Liver
C.Ç., 1 ½ yrs.	Generalized glycogen storage disease	4	1.9	0.15	1.45
Controls					
F.B., 2 yrs.	Chicken pox encephalitis	2	0.16	0.02	0.15
K.Ş., 2 ½ yrs.	Pathogen staphylococcus aureus sepsis and pneumonia	7	0	0	0.62
E.B., 10 days	Neonatal tetanus	4	0.11	0.05	0.02
From the literature					
Villar-Palasi, C., and Larner, J.: Arch. Biochem. 86: 270, 1960.	Rat	Fed	0.6	0.4	2.0
		Fasted	0.18	0.1	0.14
Antopol, W., et al Am. J. Med. Sci. 188: 354, 1934.	Normal baby	Autopsy	0.06	0.01	0.1

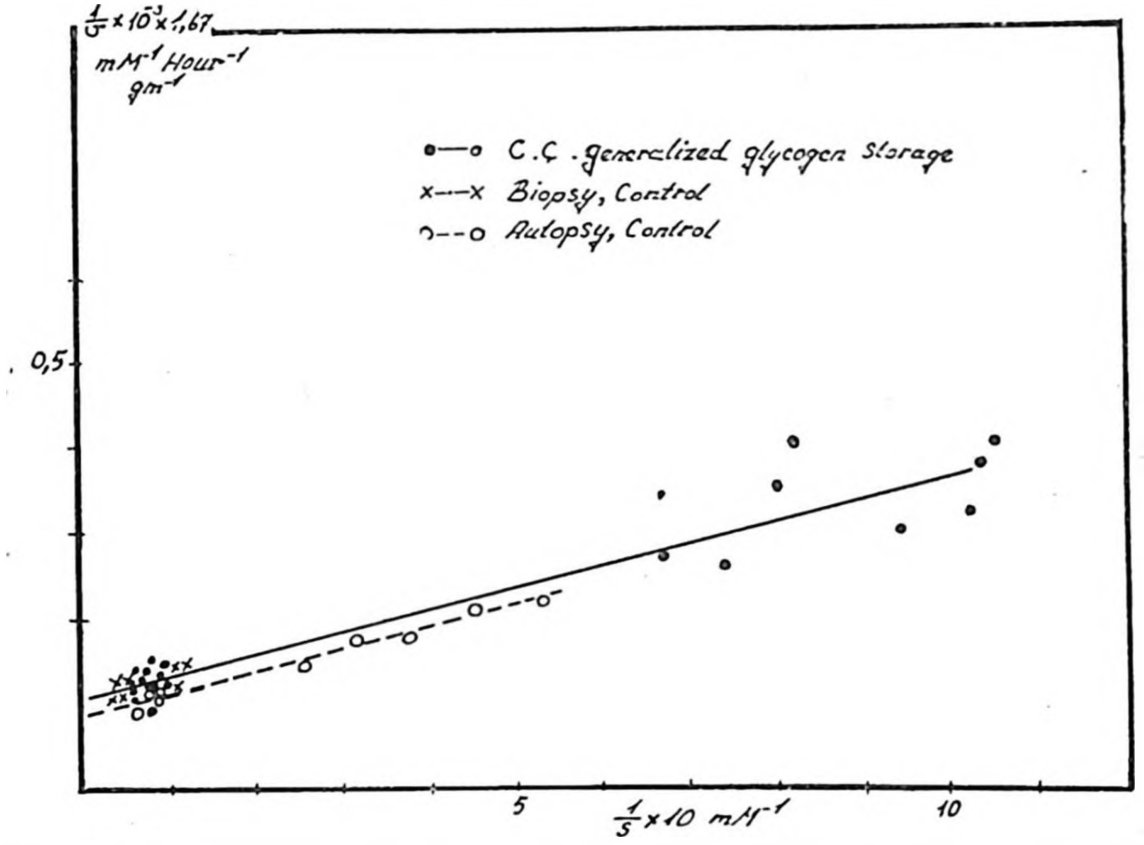

Figure 3. Lineweaver-Burke representation of liver glucose 6-phosphatase activity. The assay was carried out at 37.5 C. The biopsy and autopsy samples of liver were obtained from babies of approximately the same age as C. Ç. The calculations were made by the method of least squares.

Glucose 6-phosphatase was assayed according to a modified Swanson's method.[15] The pH of the reaction was carefully controlled during the assay procedure so that linear results in relation to time, and stoichiometric values in relation to the homogenate volume could be obtained, and a Lineweaver-Burke plot could be constructed. The results of this test are given in figure 3, together with those carried out on an autopsy liver specimen of similar age, and a normal biopsy liver sample.

From the data presented in figure 3, the apparent Michaelis-Menton coefficient (K'_s) and apparent maximal velocity(V_{Max}) of glucose 6-phosphatase for glucose 6-phosphate were calculated by the least squares method. The results are given in table 3.

The K'_s and V_{Max} values obtained in our laboratory are consistent with published reports and support our conclusion that there was no abnormal glucose 6-phosphatase activity of the liver in this case of generalized glycogen storage disease.

Our patient clearly had the cardiac type of glycogen storage disease. The etiology of this particular type is not clear at all, despite the fact that it is the most common form of the disease first described about 40 years ago.[1] No defect in liver glucose 6-phosphatase, phosphorylase, or uridine diphosphoglucose glycogen trans-

TABLE 3

THE KINETIC CHARACTERISTICS OF LIVER GLUCOSE 6-PHOSPHATASE ACTIVITY

(The values for C.Ç. taken from figure 3.)

Patient's name	Disease	K'_s value * for glucose 6-phosphate	V_{Max} value ** for glucose 6-phosphate
C.Ç.	Generalized glycogen storage	2.1×10^{-3} M	53.0
F.B.	Chicken pox encephalitis	2.5×10^{-3} M	62.0
I.D.	Hamman-Rich syndrome (?)	2.7×10^{-3} M	56.0
O.M.	von Gierke's disease	2.2×10^{-3} M	16.2

From the literature

Segal, H.: J. Biol. Chem. 234: 1937, 1959.	Normal rat	$1.2 - 2.3 \times 10^{-3}$ M	48 - 96
Swanson, M.A.: . Methods in Enzymology, p. 541	Normal rat	—	$\sim$ 51 ***
Crane, R. C.: Biochem. Biophys. Acta 17 : 443, 1955.	Normal rat	2×10^{-3} M	—

* The apparent Michaelis-Menton coefficient.
** The apparent maximal velocity expressed as millimols of inorganic phosphate liberated from glucose 6-phosphate at 37.5 C. during one hour of incubation by 100 grams of liver.
*** Converted from a different unit expression to the terms listed above.

glycosylase could be demonstrated.[16] The glycogen content in tissues of patients with this type of glycogen storage disease can be broken down when homogenized properly in vitro, in contrast to the in vivo findings. This is demonstrated in Hug's experiments.[17] We did not repeat these experiments with our patient, as a detailed investigation of phosphorylase metabolism was planned. However, the liver was unable to respond to a glycogenolytic stimulus (epinephrine in this case), as evidenced by lack of a rise in the glucose level of the peripheral blood stream. This indicated that glycogen was not broken down in vivo. The defect may have been in the activa-

tion or deactivation mechanism of phosphorylase as illustrated in figure 4.

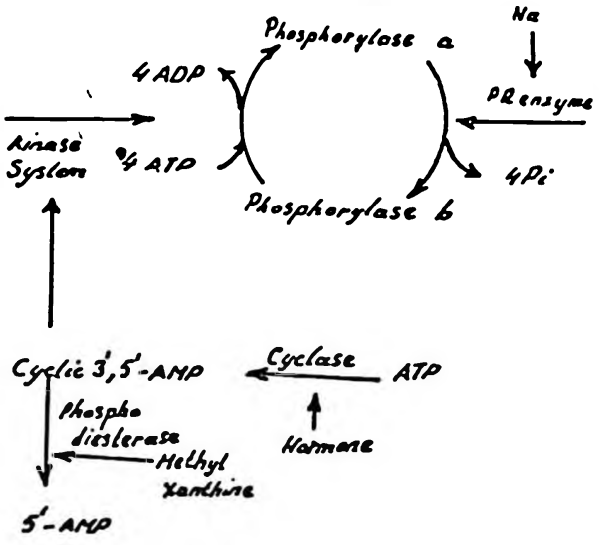

Figure 4. The metabolism of phosphorylase; the interconversion of active and inactive phosphorylases.

The majority of generalized glycogen storage disease patients die within the first year of life as a result of severe cardiac involvement. Our case was a milder one. The glycogen deposit, though abnormal in amount, was not excessive and the patient lived until 18 months of age. Glycogen was detected in only the liver and heart, and muscle involvement was not clear.

Case 2: O.M., a three and a half year old boy, whose chief symptom was an enlarged abdomen, was admitted to Hacettepe Children's Hospital. The parents noticed that the child had developed a prominent abdomen during the last year, but thought it might have started earlier. He wanted to eat frequently and had a decreased tolerance for fasting. The parents did not mention episodes of heavy breathing or frequent upper respiratory tract infections. He had had febrile diseases of an unknown nature, accompanied by convulsions. Although the parents claimed that he had always had polyuria and polydypsia, this could not be verified during hospitalization. Lymphangitis had developed on his leg shortly before admission. The family's economic status was low, the patient had never had well-baby care, nor had he been taken to a doctor. His past medical history and family history were not relevent otherwise.

On physical examination it was observed that the child had a protuberant abdomen and was malnourished, with thin extrementies. He had hepatomegaly with both lobes of the liver enlarged. The right lobe extended to the pelvis, and the left lobe was large enough to be palpable. The liver was hard, with the edge blunt and linear. The kidneys could not be palpated.

Blood count and urinalysis were normal on admission. Acetone level in fasting urine was 2+. The results of a battery of liver function tests, blood cholesterol and total lipids were all within normal limits. An intravenous pyelogram did not reveal enlarged kidneys. Various fasting blood sugar samples indicated hypoglycemia.

A clinical diagnosis of von Gierke's disease was supported by laboratory tests. However, other diagnostic tests were made to differentiate the disease from Hers' disease and, also, to find out whether the patient had hyperlactacidemia.

First, a liver biopsy was performed and the specimen stained with PAS. The staining indicated extensive deposits of material in the liver cells, though not in the nuclei of these cells. The glycogen content, determined chemically, was 14.4 per cent, well above the normal value for liver cells (table 2). Liver glucose 6-phosphatase activity was assayed as previously described. The results are given in figure 5.

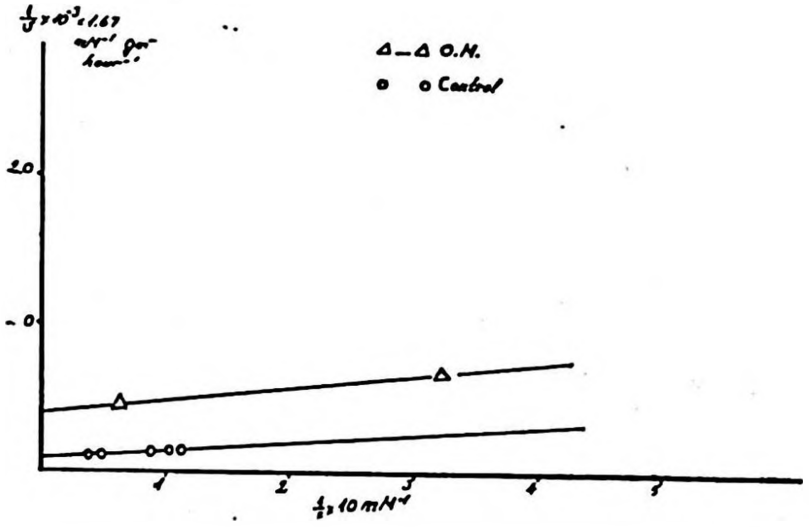

Figure 5. Lineweaver-Burke plot for glucose 6-phosphatase activity in liver in von Gierke's disease. The control value is obtained from Figure 3, by averaging.

When this result is compared with those of figure 3 and table 3, it is immediately apparent that this patient had an abnormal quantity of enzyme. The apparent Michaelis constant was normal, so the enzyme had a normal affinity for glucose 6-phosphate, but the V_{Max} was very low, indicating that the amount of enzyme was low indeed. The liver biopsy results clearly proved that the child had an hepatorenal type of glycogenolysis.

Patients with von Gierke's disease at times manifest severe lactacidemia, and as a result of this, severe acidosis. Because of this, the disease is divided into two sub-groups: one characterized by lactacidemia, the other by normal lactic acid metabolism. A glucose tolerance test was given in order to investigate lactic acid metabolism in our patient (table 4).

Obvious lactacidemia developed during the absorption of glucose from the intestinal tract. In normal subjects, the blood sugar level rises as the result of glucose given orally. In our patient, the blood glucose level increased only slightly, but the blood lactic acid level was very high. This hyperlactacidemia did not lead to any

TABLE 4

GLUCOSE TOLERANCE TEST RESULTS

1.75 grams of glucose per kilogram of body weight per os, administered after 12 hours of fasting.

Time in minutes after glucose administration	Blood glucose mg. %	Blood lactic acid mg. %
Initial level	38	11
30 minutes	43	14
60 minutes	73	37
120 minutes	89	46
180 minutes	97	79

untoward clinical symptoms, such as Kussmaul respiration or tetany. The results of the glucose tolerance test were verified by use of another substrate, fructose. This monosaccharide, after oral administration, is metabolized in the same way as glucose. It does not lead to high lactic acid levels in the blood if the liver biochemical cytoarchitecture is not altered. The results of our test are given in table 5.

TABLE 5

FRUCTOSE TOLERANCE TEST RESULTS

1.50 grams of fructose per kilogram of body weight per os.

Time in minutes after fructose administration	Blood glucose mg. %	Blood lactic acid mg. %	Blood fructose mg. %
Initial level	39.9	6 6	1.2
45 minutes	79.4	35.2	2.8
90 minutes	55.6	71.5	10.6
180 minutes	85.9	82.7	9.2
240 minutes	56.4	49.8	9.9

Blood fructose did not reach high levels during the test. This is probably due to the conversion of this monosaccharide to glucose in the intestinal epithelium, and efficient trapping in the liver of the circulating fructose. This test sugar led to some increase in blood sugar, and to hyperlactacidemia, as would be expected from this particular type of von Gierke's glycogenosis.

Both the glucose and the fructose tolerance tests clearly place our case in the hyperlactacidemia subgroup of von Gierke's disease. Had a galactose tolerance test been performed, it too might have

led to hyperlactacidemia. The explanation of this phenomenon is not clear. The liver is the site of the extra lactic acid produced in these cases of glucose 6-phosphatase deficiency. The liver's function however is not to produce lactic acid, but to remove that lactic acid produced by muscle contraction and peripheral tissues and to convert it into glycogen (the Cori cycle). The biochemical pathway in the liver parenchyma cell is so designed that it does not require the production of oxidized niacinadenine dinucleotide (NAD) via lactic acid dehydrogenase, as happens in the muscle. The liver mitochondria fully perform this task by oxidative phosphorylation, and under normal conditions the liver does not inject lactic acid into the peripheral blood stream. The lactic acid is removed from the blood, converted into pyruvic acid, and is either converted into glycogen or metabolized as pyruvic acid in the liver. However, in some patients with von Gierke's disease the reverse process takes place, and these patients convert the pyruvic acid produced in the Embden-Meyerhoff pathway into lactic acid, and inject it into the peripheral blood stream. The reason for this reversal and dissociation of the NADH produced in the Embden-Meyerhoff pathway from the oxidation step in the mitochondria is not known. It is tempting to speculate that this is due to deeply deranged liver cell structure, causing the liver cells to act like muscle cells in this regard.

An interesting observation was made in the case of a patient who had von Gierke's disease with McArdle syndrome: similar test sugars were used with similar test results. Here, too, blood lactic acid was high after administration of any one of the monosaccharides, and as the peripheral bulk of muscle tissue is unable to produce this extra lactic acid, the liver, then, must be its source. Fortunately, the patient did not manifest the severe symptoms caused by increased blood lactic acid, i.e., tetany and severe acidosis.

Our patient was also given an epinephrine tolerance test, using 0.02 cc. of aqueous epinephrine subcutaneously after 12 hours of fasting. Tests to determine the blood sugar and lactic acid levels were performed with results as shown in table 6.

There was no rise in blood glucose or lactic acid levels. The reason for this is obscure, but we obtained similar results in another case of von Gierke's disease with a high liver lactic acid output. Under normal conditions, there is a rise in blood glucose, but little change in blood lactic acid. Since we have already postulated that in our case any substrate converted into an Embden - Meyerhoff intermediate in the liver would lead to increased production of lactic

TABLE 6

EPINEPHRINE TEST RESULTS

Epinephrine administered after 12 hours of fasting.

Time in minutes after epinephrine administered	Blood glucose mg. %	Blood lactic acid mg. %
Initial level	11.7	9.2
30 minutes	23.8	10.2
60 minutes	37.0	9.7
120 minutes	36.5	7.3

acid, this observation is rather surprising. Theoretically, there should be no difference in the fate of glucose 6 - phosphate formed from intestinal glucose, fructose or galactose, and that formed from the breakdown of liver glycogen. The explanation may be one of the following :

1. Hepatic phosphorylase activity may be deficient. Tests to verify this possibility were not made on this patient. It is a possible explanation, though a remote one, as it would mean that our patient and the other mentioned above would have had both Hers' disease and von Gierke's disease at the same time.

2. The injection of epinephrine may lead to increased absorption of lactic acid by the peripheral tissues.

At present we are unable to determine which of these two possible explanations is the correct one. A slight rise in blood sugar levels is evidence of the fact that some glycogenolysis is occurring. Our patient definitely had hypoglycemic blood sugar values at the beginning of the glucose, fructose and epinephrine tolerance tests, although no clinical manifestations of this condition were observed. The reason for this is not clear, as the brain's fuel is glucose supplied by the blood, and it would seem that lack of this fuel would be clinically evident. However, patients with von Gierke's disease, despite low blood sugar values, are known to be able to tolerate hypoglycemia better than normal individuals and fail to convulse.

These two cases illustrate how complicated the interpretation of both the clinical and biochemical findings can be in the various types of glycogenoses. A complete series of tolerance tests and enzyme assays must be performed before a final diagnosis can be given with any assurance.

Summary

Two cases of glycogenosis are reported and the clinical and biochemical aspects of the disease are discussed.

REFERENCES

1. Pompe, J. C.: Hypertrophie idiopathique du coeur, Ann. d'Anat. Path. **10**: 23, 1933.
2. Sant'Agnese, P. A.: Diseases of glycogen storage with special reference to the cardiac type of generalized glycogenosis, Ann. N. Y. Acad. Sci. **72**: 439, 1959.
3. von Gierke, E.: Hepato-nephromegalia glykogenica (Glykogenspeicher krankheit der leber und der nieren), Beitr. z. path. anat. u. z. allg. Path. **82**: 467, 1929.
4. Cori, G. T., and Cori, C. F.: Glucose 6-phosphatase of liver in glycogen storage disease, J. Biol. Chem. **199**: 661, 1952.
5. Hers, H. G.: Études enzymatiques sur fragments hépatiques; application à la classification des glycogénoses, (Enzymatic studies of hepatic fragments; application to the classification of glycogenoses), Rev. Int. d'Hepat. **9**: 35, 1959.
6. Sidbury, J. B. Jr., Cornblath, M., Fisher, J., and House, E.: Glycogen in erythrocytes of patients with glycogen storage disease, Pediatrics **27**: 103, 1961.
7. Illingworth, B., Cori, G. T., and Cori, C. F.: Amylo-1,6 glucosidase in muscle tissue in generalized glycogen storage disease, J. Biol. Chem. **218**: 123, 1956.
8. Illingworth, B.: Glycogen storage disease, Amer. J. Clin. Nutr. **9**: 683, 1961.
9. Anderson, D. H.: in Symposium on the Clinical and Biochemical Aspects of Carbohydrate Utilization in Health and Disease, Najjar, V. A., editor, Baltimore, Johns Hopkins Press, 1952, p. 28.
10. McArdle, B.: Myopathy due to defect in muscle glycogen breakdown, Clin. Sci. **10**: 13, 1951.
11. Pearson, C. M., Rimer, D. G., and Mommaerts, W. F.: A metabolic myopathy due to absence of muscle phosphorylase, Amer. J. Med. **30**: 502, 1961.
12. Hartmann, A. F., Sr.: Personal communication.
13. Broberger, O., and Zetterstrom, R.: Hypoglycemia with an inability to increase the epinephrine secretion in insulin-induced hypoglycemia. A further report, J. Pediat. **59**: 215, 1961.
14. Lewis, G. M., Spencer-Peet, J., and Stewart, K. M.: Infantile hypoglycemia due to inherited deficiency of glycogen synthetase in liver, Arch. Dis. Childhood **38**: 40, 1963.
15. Swanson, M. A.: Glucose 6 phosphatase from the liver, in Methods in Enzymology, edited by Colowick. S. P., and Kaplan, N. O., New York, Academic Press, Inc., 1957, vol. III, p. 541.
16. Hauk, R., Illingworth, B., Brown, D. H., and Cori, C. F.: Enzymes of glycogen synthesis in glycogen deposition disease, Biochim. Biophys. Acta **33**: 554, 1959.
17. Hug, G.: Degradation of glycogen in tissues from generalized glycogen storage disease, Biochim. Biophys. Acta **47**: 271, 1961.