Laboratory Diagnosis of the Neuromuscular Glycogen Storage Diseases

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ABSTRACT

Of the 12 known genetic disorders of glycogen metabolism, five consistently involve the neuromuscular system. Pompe’s disease is a generalized, fatal, lysosomal storage disease caused by absence of acid maltase. Structurally abnormal glycogen accumulates in Forbes-Cori and Andersen’s diseases, resulting from deficient debranching and branching enzymes, respectively. Exercise intolerance, muscle cramps, and myoglobinuria characterize McArdle’s syndrome or myophosphorylase deficiency. In Tauri’s disease, absence of phosphofructokinase leads to glycogen accumulation indirectly owing to a metabolic block in glycolysis. Diagnosis of the symptomatic patient, antenatal diagnosis, and detection of heterozygous genetic carriers are accomplished using a variety of laboratory methods. Tissue enzyme assays, chemical analysis of glycogen, and studies of carbohydrate metabolism are available. Recent advances in biophysics, such as nuclear magnetic resonance, have opened up a new approach for the study of metabolic diseases.

Introduction

Genetic disorders of glycogen storage have been known since the 1920’s with the original publications by Wagner and Paras,61 Van Creveld,58 and Von Gierke.50 Refinements in clinical and laboratory methods over the years have more precisely defined these conditions so that at present at least 12 different abnormalities of glycogen metabolism are known.55 As the enzymatic defects responsible for separate clinical presentations were discovered, the Cori began a system of numbering to describe them in shorthand. This Cori numeral system is used interchangeably with eponyms when referring to disorders of glycogen metabolism. Collectively, the study of these diseases offers an unique opportunity to view the various manifestations of several different enzymatic deficiencies along a single metabolic pathway. Depending on the enzymatic block, symptoms may be caused
by hypoglycemia, separately or in conjunction with glycogen accumulation in tissues. Organ involvement may be generalized or selective. The neuromuscular system is involved in five types (Cori types 2, 3, 4, 5, and 7).

These glycogenoses present a threefold challenge to the clinical scientist: precise diagnosis of the symptomatic patient, antenatal diagnosis, and detection of the heterozygous carrier state. In this task, he is aided by improved assay methods for enzymes in liver and muscle biopsy material and, to a larger extent, to the use of blood cells and cultured skin fibroblasts.

A complete discussion of every glycogen storage disease is beyond the scope of this communication. Recent informative reviews have been written by Howell and by Huijing. The glycogenoses which affect the neuromuscular system form a concise group of interests to the neurologist and the laboratory diagnostician. This publication will deal exclusively with these neuromuscular glycogenosis.

Pompe's Disease (Cori Type 2)

Pompe's disease, or generalized glycogenosis, is a fatal genetic disorder characterized by muscle weakness, psychomotor retardation, shallow breathing, and congestive heart failure. Enlargement of the liver, tongue, and heart is present, and death supervenes in the first year of life, typically from intractible myocardial failure. The disease is transmitted as an autosomal recessive; infantile, childhood, and adult forms have been described. Excessive accumulation of glycogen in skeletal and heart muscle and in the nervous system is responsible for the clinical presentation. Visceral organs are also involved. The disease results from a deficiency of acid maltase, an enzyme responsible for the catabolism of glycogen. Ultrastructural studies of involved muscle demonstrate massive replacement of the sarcoplasm by glycogen which is found free and within membrane bound vacuoles. These vacuoles are lysosomes engorged with glycogen which cannot be broken down because of the absence of lysosomal acid maltase. The accumulation of the free glycogen is difficult to explain, since there is normal activity of cytoplasmic myophosphorylase, an enzyme capable of breaking 1,4 linkages in glycogen and which participates in the physiologic glycogenolytic pathway.

No effective treatment is available for Pompe's disease. Unsuccessful attempts have been made at replacement with enzymes prepared from Aspergillus niger and human placenta and with "lysosome labilizing" agents. Accurate diagnosis is required for genetic and pre-natal counseling. Laboratory methods available for diagnosis include histochemistry, glycogen analysis, and enzyme determination. Muscle biopsies of affected infants demonstrate massive accumulation of periodic acid shift (PAS) positive, diastase positive material, but this finding is non-specific and may be seen in other glycogenoses. Biochemical analysis reveals an excess of glycogen which is structurally normal. The absence of acid maltase activity must be demonstrated for precise diagnosis. This can be accomplished by enzymatic assay of skeletal muscle, cultured skin fibroblasts, leukocytes, and urine. Rapid prenatal diagnosis of Pompe's disease is possible using cultivated amniotic fluid cells. Amniotic fluid itself is not useful since it contains an isoenzyme of 1,4-glucosidase which originates from fetal urine. Relatives of patients with Pompe's disease have acid maltase activities which are intermediate between patients and normal controls. This finding now permits the detection of the heterozygous carrier state.

Forbes-Cori Disease

Forbes-Cori disease, or type 3 glycogenosis, is characterized by infantile hep-
atomegaly, delayed physical development with normal intellect, a preference for starchy foods, fasting hypoglycemia, seizures, and an increased susceptibility to infections. For reasons which are not clear, symptoms usually disappear by puberty, and normal survival into adult life is typical. Mild hypotonia and weakness are reported in children, but muscle dysfunction is over-shadowed by liver disease. Only rarely is myopathy the predominant clinical feature in children. On the other hand, the adult form of the disease may show progressive muscular weakness and congestive heart failure. Therapy is limited and symptomatic.

The incidence of this disease is difficult to determine. Approximately 65 cases have been reported. However, in some series it is the most common glycogen storage disease, and Pearson suggests that many cases may have been misdiagnosed as Von Gierke's Disease. Family studies indicate an autosomal recessive mode of inheritance.

The condition is caused by a deficiency of amylo-1-6-glucosidase or glycogen debranching enzyme. This defect leads to the formation of an abnormal glycogen with short outer chains, having a structure similar to that of a phosphorylase limit dextrin. For this reason the disease is known as "limit dextrinosis." The clinical picture results from the accumulation of this abnormal glycogen in liver and muscle and from a disturbance of carbohydrate metabolism secondary to impaired glycogenolysis.

A number of laboratory tests characterize Forbes-Cori disease: (1) Patients typically have fasting hypoglycemia, and blood lactate rises after the oral administration of glucose. This is due to increased glycolysis and increased glycogen synthesis. (2) Blood glucose fails to rise following epinephrine or glucagon administration in the fasting state but increases after ingestion of galactose. This indicates a block of liver glycogenesis. (3) Ischemic exercise causes no increase in venous lactate, demonstrating that glycogen breakdown is also impaired in muscle.

Routine histochemical stains reveal accumulations of glycogen in biopsies of skeletal muscle and liver. Periportal hepatic fibrosis and inflammation are uncommon in children but have been reported. Glycogen granules seen by electron microscopy have a normal ultrastructure in spite of their altered biochemical composition. In the atypical myopathic variant in children and in the adult forms of Forbes-Cori Disease, degenerative changes in muscle are found. Electron microscopic studies demonstrate disruption of the myofibrillar pattern by glycogen and disintegration of the Z disc.

Biochemical studies demonstrate excessive concentrations of glycogen in tissues, especially erythrocytes. The abnormal structure of this glycogen is revealed by a number of techniques including beta amylase degradation, infrared spectrum, nuclear magnetic resonance, and iodine absorption analysis. In the presence of iodine, glycogen isolated from patients with Forbes-Cori disease has an abnormal spectrum with a peak absorbance between 350 and 400 mm instead of at 450 mm, as with normal glycogen.

Assays for debranched enzyme activity can be performed on biopsy material and, more conveniently, on leukocytes or erythrocytes. Two techniques are available. One is based on a reversal of the physiologic reaction and measures the incorporation of 14C labeled glucose into glycogen. It is highly specific but not very sensitive. Another method, dynamic spectrophotometric assay, is based on the phosphorolytic degradation of limit dextrin; it is sensitive and reflects the physiologic role of the enzyme.

Debrancher enzyme activity is totally absent in patients with Forbes-Cori disease, but family members may have reduced activity providing for the identification of heterozygotes.
Andersen’s Disease

Brancher enzyme deficiency, Andersen’s disease or type 4 glycogenosis, is rare. About 19 cases have been reported. Affected infants appear normal at birth but soon develop physical and mental retardation, hepatosplenomegaly, hypotonia, muscle atrophy, heart failure, and cirrhosis. In contrast to Forbes-Cori disease, tests for carbohydrate metabolism are normal. Death from cardiac or liver failure usually occurs in the first year of life.1,4

Deficiency of branching enzyme results in the synthesis of an abnormal glycogen molecule with decreased branch points and increased chain length.16 This glycogen has a structure superficially similar to starch, giving the disease the name “amylopectinosis.”31 The organ involvement is generalized, but liver, skeletal, and cardiac muscle bear the brunt of the damage. In histochemical preparations, the atypical glycogen is resistant to salivary amylase, is digested by pectinase, and stains purple with iodine.42 Ultrastructurally, it is also abnormal. Instead of the usual glycogen rosettes, granulo fibrillar material predominates in the tissue deposits when viewed by the electron microscope.4,39 Biochemical analysis of polysaccharides isolated from these tissues shows increased phosphorylase degradation and an iodine-complex absorption spectrum similar to amylopectin.

Brancher enzyme deficiency can be demonstrated in liver, leukocytes, and cultured skin fibroblasts.7,28 Kindred studies suggest an autosomal recessive inheritance. Reduced activity of brancher enzyme in relatives of affected children and in amniotic fluid from pregnancies at risk provides a valuable tool in genetic counseling and antenatal diagnosis.28

Attempts have been made to remove the amylopectin-like glycogen from affected tissues in an effort to forestall the development of cirrhosis. Injecting the livers of patients with preparations of fungal glucosidase has been successful in reducing the concentration of hepatic glycogen, but has not produced any clinical improvement.18,31

McArdle’s Syndrome

Glycogenesis type 5 was first described in 1951 by McArdle.38 Symptomatology depends upon the age of the patient. Children and adolescents may complain only of easy fatigability. In adult life, severe cramps and myoglobinuria develop during strenuous muscular activity. After the age of 40, progressive weakness and wasting of skeletal muscle ensues.33

The syndrome results from a deficiency of myophosphorylase which catalyses the breakdown of the alpha 1-4 glucosidic linkages in glycogen to form glucose-1-phosphate. In muscle at rest or under light work loads, no symptoms occur, since energy is then derived from the oxidation of fatty acids rather than carbohydrate. Under conditions of heavy exercise in normal muscle, glucose consumption and glycogenolysis become vitally important in energy metabolism. In patients with myophosphorylase deficiency, the exercising muscle cannot produce sufficient adenosine triphosphate (ATP), which falls below critical levels, leading to muscle cramps and myofiber necrosis.31 Acute renal failure may result from severe myoglobinuria.23 A “second wind” phenomenon may occur in patients who continue to exercise after the appearance of fatigue and cramps. This is related to elevated levels of free fatty acids and increased blood flow which improve the energy supply for muscle activity.47

Multiple isoenzymes of phosphorylase exist in different tissues under the control of several structural genes. Cardiac muscle contains both skeletal and smooth muscle isoenzymes, and patients with McArdle’s syndrome have not developed congestive failure or other myocardial complications.10 There is an apparent paradox in that regenerating fibers in biopsied muscle and muscle cell cultures from
patients with this syndrome do demonstrate phosphorylase activity. DiMauro and associates have demonstrated that this activity is not due to normal adult phosphorylase, but rather to a fetal isoenzyme which is under separate genetic control, and which usually disappears during the process of skeletal muscle maturation. This isoenzyme is then reexpressed under the special conditions of regeneration and tissue culture. The presence of this or other isoenzymes of myophosphorylase may explain why children with McArdle's syndrome typically have minor symptoms which become more severe as they mature into adulthood and the fetal enzyme disappears.

Additional information indicates that McArdle's syndrome is not a single genetic entity. The condition most often appears to be transmitted as an autosomal recessive trait, but the prevalence of males suggests a limited mode of inheritance. An autosomal dominant pattern has been observed in one family. In further evidence of genetic heterogeneity, some patients have no enzyme protein detectable by immunologic methods, whereas others show the presence of the protein but in an inactive form.

Diagnosis is suggested by the history of painful cramps and myoglobinuria following strenuous exercise. Electrocardiographic changes may occur even though cardiac symptoms are absent. The ischemic exercise test is performed by inflating a blood pressure cuff on the patient's arm and asking the patient to squeeze a rubber ball while the cuff pressure is maintained. Blood is then withdrawn from the ante cubital vein at five minute intervals. Patients with McArdle's syndrome show no venous lactate production owing to failure of glycogenolysis and glycolysis.

Phosphorylase deficiency in muscle biopsy specimens can be demonstrated by a simple histochemical method. Light microscopic examination of these biopsies reveals a variable degree of degenerating and necrotic fibers as well as subsarcolemmal blebs of PAS positive glycogen granules. An excess of structurally normal glycogen, disorganization of the I band, and distortion of the myofibrils is found on electron microscopy.

Analysis of leukocytes, liver, and other tissues is of no diagnostic help since they contain normal concentrations of glycogen and isoenzymes of phosphorylase. Biochemical determination of myophosphorylase activity can be performed on muscle biopsy specimens. A recent advance in the diagnosis of McArdle's syndrome is the application of nuclear magnetic resonance (NMR). This technique uses large super conducting magnets to "polarize" atomic nuclei and observe their behavior with low energy radiowaves. This method permits the non-invasive measurements of intracellular pH and of phosphorous containing metabolites of ATP. In one study using NMR, a patient with McArdle's syndrome showed no fall in intramuscular pH and an excessive reduction in phosphocreatin during exercise. These results are characteristic of myophosphorylase deficiency, given the inability to catabolize glycogen and generate lactic acid.

Tauri's Disease

Tauri's disease or type 7 glycogenosis is due to a defect in muscle phosphofructokinase (PFK). The disease is rare since only six cases in four families have been reported. Pedigree studies and diminished PFK activities in family members suggest an autosomal recessive mode of transmission. The symptomatology is similar to that of McArdle's syndrome with exercise intolerance, contractures and myoglobinuria. A "second wind" phenomenon and failure of lactate elevation on ischemic exercise testing are characteristic. Pathologic changes in skeletal muscle biopsies also resemble McArdle's
syndrome: scattered fiber necrosis with subsarcolemmal and intramyofibrillar glycogen deposition.\(^{57}\)

The two conditions are distinguished by the presence in Tauri's disease of a mild chronic hemolytic tendency, including reticulocytosis, erythroid hyperplasia of marrow, shortened red cell life span, and elevated indirect bilirubin.\(^{56}\) These hematologic features are due to a partial defect in erythrocyte PFK. Phosphofructokinase is important in the glycolytic pathway. However, it does not catalyze a reaction in which glycogen itself is the substrate as opposed to phosphorylase, branching or debranching enzyme, and alpha glucosidase. The PFK deficiency causes an accumulation of hexose intermediates, especially glucose-6-phosphate.\(^{14}\) This activates glycogen synthesis and inhibits glycogen breakdown as an allosteric effect.

Experimental results indicate that erythrocyte PFK is a hybrid enzyme containing muscle type (M) and red blood cell type (R) specific subunits, while muscle is composed of M sub units only.\(^{36}\) Patients with Tauri's disease have almost total absence of PFK in muscle and a partial defect (the M subunit) of PFK in erythrocytes. Since the red blood cell defect is incomplete, mild hemolysis occurs but without anemia or splenomegaly.\(^{56}\)

Diagnosis is suggested by the clinical presentation and muscle biopsy, but it depends upon the demonstration of reduced PFK activities in red blood cells and muscle. Several assays including a spectrophotometric analysis are available.\(^{25,26}\)

**Summary**

The glycogen storage diseases represent a growing group of metabolic disorders which frequently involve the neuromuscular system. Since clinical manifestations are overlapping, precise diagnosis, detection of carrier states, and antenatal diagnosis for genetic counsel-

References

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