Syndrome of the Defective Lysosome—the Genetic Mucopolysaccharidoses

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ABSTRACT

The genetic mucopolysaccharidoses represent the group of disorders recognized in the early 1900's. In half a century, it was recognized that these were disorders of polyanionic macromolecules known as glycosaminoglycans. Within the past five years, these disorders have been identified as prototypes of lysosomal diseases, are a genetically heterogeneous group and this heterogeneity is recognizable in vitro systems. Finally, these disorders represent prototypes for the development of methods of enzyme replacement therapy.

Introduction

The original prototypes of what are now recognized to be the genetic mucopolysaccharidoses were the Hunter and Hurler Syndromes. The first reported description of the sex-linked, recessive disorder, the Hunter Syndrome, was in 1917 by Charles Hunter, and the progressive autosomal recessive variant by Gertrude Hurler in 1919. Until 1952, references to this group of disorders identified them as either inborn errors of lipid metabolism, chondrodystrophies as exemplified by the present day listing in Index Medicus of lipochondrodystrophy or as a primary bone disorder in grouping dysostosis multiplex. It was at this time that the cogent observation was made by the Swedish pathologist Brante that vacuolar inclusions that characterize the pathology of affected tissues and organs represented not anomalous lipid, which did not stain with lipophilic agents, but rather that the vacuoles were a consequence of the dissolution of cellular inclusion material due to the use of aqueous fixatives for the pathologic preparation of specimens. It was from this point on that it was correctly recognized that the disorders identified as Hunter-Hurler Syndrome were indeed genetic disorders of metabolism of the complex carbohydrates, acid mucopolysaccharides, or glycosaminoglycans.

Subsequent literature deals with clinical delineation of the genetic heterogeneity of this group of disorders with the ultimate recognition of at least six different genetic entities comprising the Hunter-Hurler Syndrome. Application of fibroblast tissue culture techniques to the study of this group of disorders provides (1) the first example in clinical medicine of the development of a refined laboratory model for establishment of genetic pedigrees within a group of genetic disorders; (2) a model system for the study of enzymatic defect and the molecular
pathophysiology; (3) development of a method which could be applied for antenatal diagnosis; (4) development of a test tube model system for evaluation of potential therapeutic agents; and (5) a model system which gave experimental evidence for the possible efficacy of enzyme replacement therapy in the field of inborn errors of metabolism. Within a period of less than one decade, the words of William Harvey\textsuperscript{18} from 1657 have reached fruition. Harvey concluded, regarding the value of the investigation of rarer forms of disease, that "... it has been found in almost all things that what they contain of useful or applicable nature is hardly perceived unless we are deprived of them or they become deranged in some way."

Numerous reviews have discussed either the clinical or biochemical features characterizing this group of disorders.\textsuperscript{13,24} This report, therefore, will abbreviate a discussion of the clinical features of this syndrome and will concentrate largely on defining the newer genetic classification that has been applied to this group of disorders as well as discussing newer concepts which are an outgrowth of laboratory investigations in this general area.

Clinical Features

The onset of symptoms is recognized between the ages of six months and two years. The development of facial coarseness, frontal prominence, hirsutism, reversal of the normal lumbar lordosis (apparent as the infant begins to sit) or hepatomegaly may herald the diagnosis. The presence of these clinical features in association with a history of normal mental development throughout the first year of life, then cessation of mental development and subsequent regression, almost certainly defines the diagnosis of the genetic mucopolysaccharidoses. The presence of significant central nervous system involvement makes the diagnosis of Types I, II or III mucopolysaccharidoses likely (table I).

The major differential diagnostic considerations are the genetic mucolipidoses.\textsuperscript{40} These disorders are characterized by an overlapping phenotype with the mucopolysaccharidoses and patients demonstrating visceral storage of glycosaminoglycans and complex lipids. In recent years, the molecular defects in these disorders have been elucidated to be the result of defective catabolism of complex lipids (glycolipids).\textsuperscript{17}

The present classification of the genetic mucopolysaccharidoses is shown in table I. The enzymatic defect, the mode of inheritance and the major clinical features are indicated. The major findings which allow a genotypic diagnosis relate largely to (1) the presence or absence of corneal clouding, and (2) the degree of visceral and skeletal involvement in contrast to the central nervous system involvement.\textsuperscript{6}

Radiologic Findings

Variable expression of the gene defect is apparent on radiologic examination. The most frequently affected regions demonstrable on roentgenologic study are the ribs, the vertebral bodies, calvarium and the diaphyseal regions of the extremities and hands. Characteristic findings in the Hunter-Hurler Syndrome (Types I and II) are a J-shaped sella turcica, anterior wedging and beaking of the vertebral bodies of the lumbar and lower thoracic region as well as widening (spoon-shaped) ends of the anterior borders of the ribs. In the extremities and hands, unusually broad (short-appearing) bones with increased trabeculation are seen.\textsuperscript{10} These findings are present to a variable degree in Type II of the genetic mucopolysaccharidoses.\textsuperscript{10}

In Morquio's Syndrome (Type IV), anterior deformity of the vertebral bodies, hypoplasia of the odontoid process and changes in the femora and acetabula of the lower extremities are also seen.\textsuperscript{25} Additionally, in Type VI, deformities are found of the epiphysis of upper and lower extremities
TABLE I
Classification of Genetic Mucopolysaccharidoses

<table>
<thead>
<tr>
<th>Type</th>
<th>Designation</th>
<th>Genetics (Recessive)</th>
<th>Enzymatic Defect</th>
<th>Major Clinical Features</th>
</tr>
</thead>
<tbody>
<tr>
<td>I</td>
<td>Hurler's Disease (H)</td>
<td>Autosomal</td>
<td>α-Iduronidase</td>
<td>Accumulation of dermatan sulfate &amp; heparan sulfate, corneal clouding CNS, visceral &amp; skeletal involvement Death with 1-2nd decade</td>
</tr>
<tr>
<td></td>
<td>Scheie's Disease (S)</td>
<td>Autosomal</td>
<td>α-Iduronidase</td>
<td>Accumulation of dermatan sulfate &amp; heparan sulfate Skeletal involvement, particularly joints, corneal clouding, cardiac involvement, normal mentation Normal life span</td>
</tr>
<tr>
<td>II</td>
<td>Hunter's Syndrome³⁹-⁴⁰</td>
<td>Severe</td>
<td>Sex-linked</td>
<td>Idurono-sulfatase</td>
</tr>
<tr>
<td></td>
<td>Mild</td>
<td>Sex-linked</td>
<td>Idurono-sulfatase</td>
<td>Milder involvement but similar to Type I (H) with exception of corneal clouding Severe type death &lt; 20 years, mild type - survival 3-5th decade</td>
</tr>
<tr>
<td>III</td>
<td>Sanfilippo Syndrome</td>
<td>Type A¹¹</td>
<td>Autosomal</td>
<td>Heparan sulfate sulfatase (sulfamidase)</td>
</tr>
<tr>
<td></td>
<td>Type B²²</td>
<td>Autosomal</td>
<td>N-acetyl-α-D-glucosaminidase</td>
<td></td>
</tr>
<tr>
<td>IV</td>
<td>Morquio Syndrome²³</td>
<td>Autosomal</td>
<td>Chondroitin sulfate</td>
<td>Keratan sulfate accumulation Severe skeletal deformity, corneal clouding, cardiac involvement</td>
</tr>
<tr>
<td></td>
<td></td>
<td>N-acetylhexosamine sulfatase</td>
<td></td>
<td></td>
</tr>
<tr>
<td>V</td>
<td>Maroteaux-Lamy Syndrome</td>
<td>Classic form</td>
<td>Autosomal</td>
<td>Arylsulfatase B</td>
</tr>
<tr>
<td></td>
<td>Mild form</td>
<td>Autosomal</td>
<td>Arylsulfatase B</td>
<td>Accumulation of dermatan sulfate Skeletal deformity, corneal involvement, normal mentation</td>
</tr>
<tr>
<td>VI</td>
<td></td>
<td>Autosomal</td>
<td>β-Glucuronidase</td>
<td>Accumulation of dermatan sulfate Hepato-splenomegaly, skeletal involvement, mental retardation</td>
</tr>
</tbody>
</table>

and changes in the radius and ulna not usually seen in Types I and II.²⁵

Laboratory Diagnosis
The major tool for the delineation of the genetic mucopolysaccharidoses resides in the demonstration of excessive excretion of urinary glycosaminoglycans. Irrespective of the methodology used, the normal urinary excretion of acid mucopolysaccharides is under 20 mg per 24 hours.¹¹,²⁴ In Types I through VI of the genetic mucopolysaccharidoses, the glycosaminoglycan excretion markedly exceeds this value. Additionally (table 1) a qualitative shift is found in the acid mucopolysaccharides excreted as contrasted to normal.³⁵,³⁷ In Types I and II, a marked increase in the excretion of dermatan sulfate and heparan sulfate occurs, that is, mixed acid mucopolysaccharide excretion. In Type III, a preponderance of heparan sulfate is found in the urine.²⁴,³⁴ In Type IV, keratan sulfate is excreted in excess. This is a glycosaminoglycan which does not contain uronic acid.

In Types V and VI, predominant accu-
mulation and excretion of dermatan sulfate are found.\textsuperscript{13,24} Thus, on the basis of the quantitative and qualitative analysis of urinary mucopolysaccharide excretion and the clinical course, it is possible to arrive at a tentative genotypic diagnosis. The presumptive diagnosis of increased mucopolysacchariduria may be defined by laboratory screening tests: the acid albumin turbidity test,\textsuperscript{14} a quaternary ammonium salt precipitation test,\textsuperscript{33} and the toluidin blue metachromasia test of urine.\textsuperscript{4} In this writer's view, the most accurate screening may be obtained with the acid albumin turbidity test. The subsequent analysis of the mucopolysaccharide excretion requires precipitation with quaternary ammonium salts and separation into individual fractions by the use of chromatography. Finally, the identification of the composition of this material is assayed by (1) uronic acid, (2) hexosamine, (3) degree of sulfation, and (4) N-sulfate content and electrophoretic separation of the individual mucopolysaccharides.\textsuperscript{34}

**Biology of Protein-polysaccharides**

The polyanionic macromolecules which accumulate in the genetic mucopolysaccharidoses have historically been referred to as acid mucopolysaccharides. This term was originally introduced by Meyer to describe polysaccharides of animal origin which contained hexosamines.\textsuperscript{29} The elucidation of the chemistry of these complex carbohydrates resulted in the application of several eponyms for their generic name: glycosaminoglycans, protein-polysaccharide complexes. These anionic polymers are produced by cells of mesenchymal origin and represent the major components of the intercellular matrix or ground substance.

One of their main functions, the regulation of water and electrolyte transport across membranes, may be traced phylogenetically to their occurrence and distribution in unicellular organisms. In mammals, specifically man, these complex substances are ubiquitous. They are important molecular components of the cardiovascular, optic, musculoskeletal and reticuloendothelial systems.\textsuperscript{21} In the normal state, they are covalently bound to protein as a protein-polysaccharide complex.\textsuperscript{28}

Additional physiologic functions for this molecular species are the maintenance of intracellular integrity and their function as intercellular glues. They regulate the structural organization of fibrillar elements in tissue as a consequence of their chemical and physical interaction with collagen and the capacity of these substances to trap water and exclude large molecules.\textsuperscript{3} Their presence in synovial fluids, predominantly hyaluronic acid, exemplifies their lubrication function with respect to joint mobility.

Finally, in terms of skeletal distribution and occurrence, they appear to be regulators of endochondral ossification. It can be demonstrated that concomitant with mineralization and calcium deposition, a decrease may be found in the acid mucopolysaccharide content of enchondral tissue. It is interesting to speculate that the normal inhibitors of mineralization and calcification may potentially be degraded in an aberrant fashion in disorders associated with metastatic or inappropriate calcification, — for example, fibrodysplasia ossificans progressiva.

With the exception of keratan sulfate, the basic structure of glycosaminoglycans consists of alternating hexosamine and uronic acid groups. With respect to the chondroitin 4- and 6-sulfate and hyaluronic acid, the major uronic acid moiety is glucuronic acid. In heparan sulfate and heparin, the predominant uronic acid is glucuronic acid but small portions of iduronic acid also occur. Dermatan sulfate, the mucopolysaccharide which accumulates in mucopolysaccharidoses Type I, Type II, Type V and VI, the uronic acid is iduronic acid. Hyaluronic acid, chondroitin 4- and 6-sulfate and dermatan sulfate all contain the hexosamine N-acetylg glucosamine.

The three sulfated mucopolysaccharides
contain the sulfate attached to the hexosamine derivative. Heparan sulfate, the glycosaminoglycan which accumulates predominantly in Type III, contains the sulfate group attached to the N-acetyl group of the hexosamine. The presence of heparan sulfate may be chemically identified by the detection of N-sulfate groups.\textsuperscript{23} Keratan sulfate, the material in excess in Type IV mucopolysaccharidoses, contains no uronic acid and consists of the repeating dimer of galactose and N-acetylglucosamine-6-sulfate.

In the normal state the polysaccharide polymer is covalently linked to protein. Roden and co-workers\textsuperscript{42} identified the linkage region of polysaccharide protein complex to consist of an O-glycosidic bond linking the beta hydroxyl of serine to xylose which is linked to galactose-galactose-uronic acid. This linkage region appears to be identical for the chondroitin and dermatan sulfates and for heparan sulfate. The protein linkage region for the corneal and skeletal keratan sulfate appears to differ and contains an N-glycoside bond between the amide group of asparagine and N-acetylglucosamine.\textsuperscript{36}

Recent investigations on the physical and chemical nature of the protein polysaccharide complex suggest that multiple polysaccharide chains extend laterally from the core protein. Heterogeneity with respect to the number of polysaccharide chains, the nature of the polysaccharide chains and a variation in the core protein appear to relate to their cellular distribution and their in situ functions.

These inborn errors of mucopolysaccharide chemistry served as a stimulus for the characterization of the metabolic pathway of these substances. Tissue culture techniques utilizing mesenchymal cells such as the fibroblast and studies employing the mast cell\textsuperscript{41} have aided in the elucidation of the glycosaminoglycan pathway. Uridine nucleotides serve as the glycoside donors for biosynthesis. The hexosamine is derived from fructose-6-phosphate through a series of conversions forming N-acetylglucosamine-1-phosphate. Subsequently, in the presence of the nucleotide triphosphate, UDP-N-acetylgalactosamine is formed. The uronic acid is derived from UDP-glucose which is converted to UDP-glucuronic acid and then an epimerase yields UDP-iduronic acid.

The nucleotide sugars are the immediate precursors for the synthesis of polysaccharide. The epimerization of glucuronic acid to iduronic acid occurs at the polymer level. Sulfation of these macromolecules occurs via the organic sulfate donor 3'-phosphoadenosine-5'-phosphosulfate. The sulfation process appears to occur at the macromolecular level. The addition of the polysaccharide chains occurs on a preformed protein core and active protein synthesis is required. The formation of the protein-polysaccharide complex occurs in the rough endoplasmic reticulum; extension and sulfation of the polysaccharide chain occurs in the golgi apparatus. Recent evidence suggests that the sequential order involved for protein-polysaccharide synthesis occurs on membrane bound enzyme complexes which confer thermodynamic stability to the process.

Studies on the biosynthesis of glycosaminoglycans in the genetic mucopolysaccharidoses have unequivocally indicated that no defect resides in this portion of the metabolic cycle. Additionally, some years ago a potential defect in the glycoprotein linkage region was postulated to account for aberrant mucopolysaccharide metabolism, — these postulations are not tenable.

The identification and elucidation of the catabolic pathway for the degradation of mucopolysaccharides has been a consequence of investigations on the genetic mucopolysaccharidoses. The observation of Van Hoof and Hers in 1964\textsuperscript{43} was the first suggestion that these inborn errors might be a consequence of a defect in degradation. These authors observed the accumulation of metachromatic material around and within unit bound membrane structures identified
by ultrastructural techniques to be lysosomes. They postulated that these disorders might be caused by a defect in lysosomal acid hydrolases.

In 1966, Danes and Bearn reported on the presence of abnormal cytoplasmic metachromatic material in fibroblast cultures obtained by skin biopsy in patients with the Hunter-Hurler Syndrome. Several features emerged from these studies. First, skin biopsy material in tissue cultures exhibited the genetic defect characteristic of the disease. Second, the histochemical and biochemical techniques applied to these model systems indicated that genetic patterns could be identified. Application of tissue culture techniques in suspected carriers permitted identification of the heterozygote state and the differentiation between the autosomal recessive and sex-linked recessive inheritance patterns.

The major discovery relating the genetic mucopolysaccharidoses resulted from the study of Neufeld and collaborators. These investigators studied 35 S-sulfate kinetics in culture to demonstrate faulty degradation of acid mucopolysaccharides. They subsequently employed cocultivation techniques using fibroblasts from normals and patients with the genetic mucopolysaccharidoses. Such a maneuver resulted in correction of abnormal sulfate kinetics in the mutant fibroblast culture. Co-cultivation of cells from patients with different forms of the genetic mucopolysaccharidoses resulted in mutual correction of the defect whereas if cells had the same genotype one had no complementation.

Expansion of this approach indicated that fibroblasts secrete factors into the extracellular medium which are lysosomal enzymes and these may be pinocytosed and subsequently are intracellularly active. Neufeld and Fratatoni demonstrated that other biologic fluids (blood, urine) normally contained these factors. These observations indicated that (1) lysosomal degradative enzymes are found in biologic fluids in vitro and in situ which are capable of degrading acid mucopolysaccharides; (2) these substances may be taken up by normal and mutant cells and are biologically active in the recipient cell; (3) mucopolysaccharide degradative factors are absent in the different types of genetic mucopolysaccharidoses; and (4) the technique of co-cultivation permits the establishment of genotypic identity or dissimilarity.

It is now possible to identify these corrective factors to show that they are lysosomal enzymes and that they may be assayed for molecular diagnosis for the genetic mucopolysaccharidoses.

The clarification of the pathophysiology of the genetic mucopolysaccharidoses is a milestone in the development of in vitro models for the study of inborn errors of metabolism and their application to develop potential therapeutic regimens. Based upon the existence of corrective factors (lysosomal degradative enzymes) in culture media from normal cells and cross-correction in co-cultivation experiments, Di Ferrante and coworkers attempted plasma infusion therapy in patients with the Hunter-Hurler Syndrome. These efforts were an extension of laboratory studies and their application to the clinical situation. Other avenues of therapy of genetic disease based upon such premises are shown in table II.

Di Ferrante et al speculated that the Hurler corrective factors present in normal plasma when infused intravenously would result in replacement of the deficient factor and, thus, correction of aberrant mucopolysaccharide metabolism. End points for success were clinical improvement and increased excretion of low molecular weight

| TABLE II |
| Therapeutic Approaches of Lysosomal Disorders |
| Substitution therapy with biologic fluids containing missing enzyme-plasma infusion therapy |
| Therapy with purified enzyme |
| Organ transplantation as a form of therapy |
| Encapsulation of purified missing factors |
| Enzyme activation: (1) co-factors |
| (2) conformational change |
mucopolysaccharide components. These initial attempts showed some evidence of correction of the metabolic defect.

However, subsequent investigators, such as our group,22 and Nadler,5 suggested that antecedent to the application of such therapeutic attempts, several features must be defined. These include (1) precise documentation of the quantity of missing factor activity present in the infused material; (2) extreme precision of the side effects and knowledge of the duration of its effect; and (3) careful clinical and chemical monitoring. These attempts were reasonable and feasible. In practice, however, they have little value for the chemotherapy of these inborn errors of metabolism. The first aim of therapy for genetic deficiency diseases would be the development of enzyme replacement therapy.

Several fashions in which such attempts may be undertaken are indicated in table II. At present, however, none of these have proved to be of practical use. The only entity in which enzyme replacement therapy has been of significant therapeutic benefit is the use of organ transplantation therapy (renal) in Fabrey’s disease.32 There has been significant improvement in the clinical and chemical course in the afflicted patient. At present, with regard to the genetic mucopolysaccharidoses, the defective degradative lysosomal enzymes have not yet been purified and characterized and there is no immediate prospect for their chemotherapeutic application. The major investigative developments are the characterization of these enzymes and the development of appropriate artificial and natural substrates for their assay.2

The defect in N-acetyl-α-D-glucosaminidase (table I), Type B Sanfilippo Syndrome, is routinely used for clinical delineation of this syndrome. However, the assays for heparan sulfate sulfatase, the sulfamidase defect and the α-iduronidase assay are not yet available in most laboratories. The validity of the arylsulfatase B defect in Type V mucopolysaccharidosis remains to be verified.16 The β-glucuronidase36 defect characterizing Type VI mucopolysaccharidosis is a laboratory procedure that is easily performed. Directions are focused in many laboratories for the development of precise assays and the use of complementation techniques of co-cultivation for genotypic diagnosis. The future directions involve the development and purification of the missing factor and the biology of these factors in culture systems, i.e., (1) whether or not it is taken up by the host cell, (2) whether or not it is biologically active in situ, and (3) what the half-life of the enzyme is in the host cytoplasm.

References

362


