The Early Laboratory Diagnosis of Mucopolysaccharidoses

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ABSTRACT

The early and accurate diagnosis of the mucopolysaccharidoses remains a problem for the clinical laboratory. Reported here is the systematic comparison of the ability of three common glycosaminoglycanuria screening procedure (the Berry spot test, the AMES MPS® spot test, and the gross acid albumin turbidity test) to detect the mucopolysaccharidoses. These tests were run on random urine samples obtained from 34 well characterized cases of mucopolysaccharidosis representing nine different mucopolysaccharidosis types. A few random urine samples from normals as well as from selected individuals with other genetic diseases were also tested in the same manner. The Berry spot test with the 10 µl application of urine detected all 34 of the confirmed cases of mucopolysaccharidosis. One of 12 normals and six of eight of the other genetic disorders tested positively at this level. The AMES MPS® spot test failed to detect several Type IV and Type VI mucopolysaccharidoses, and the gross acid albumin turbidity test missed several Type III, Type IV, and Type VI cases.

The failure of the gross acid albumin turbidity test to detect reliably the most frequently occurring Type III mucopolysaccharidoses, as well as the rarer Type IV and Type VI disorders, renders questionable the use of this procedure for glycosaminoglycanuria screening. The AMES MPS® spot test appears to be capable in detecting Types I, II, and III disorders. Also, this test affords less false positives than the Berry spot test. Unfortunately, it cannot be relied upon to detect the glycosaminoglycanuria found in Type IV and VI disorders.

The Berry spot test clearly has the highest sensitivity; however, because of its low specificity, it is concluded that it is imperative that it be used in conjunction with additional urinary glycosaminoglycan analyses in order to establish definitively the presence of glycosaminoglycanuria.

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Introduction

The group of lysosomal storage disorders known as the mucopolysaccharidoses, contrary to popular opinion, are not easily diagnosed either clinically or in the laboratory. Three levels of laboratory assessment exist: (1) urinary screening procedures for evidence of increased glycosaminoglycanuria; (2) definitive quantitative and qualitative measures of glycosaminoglycan excreted, and (3) assays for a specific lysosomal hydrolase enzyme activity deficiency, at least ten of which have already been identified in the spectrum of known mucopolysaccharidoses types.

In this paper are briefly reviewed the biochemical methods used for the diagnosis of these disorders. Since the original reports of screening methodologies, several additional mucopolysaccharidoses types have been identified. Therefore, a systematic application of these screening methods to a spectrum of mucopolysaccharidoses as currently defined was undertaken, particularly to determine whether or not some types could be detected by these methods.

This group of genetically determined disorders has in common the abnormal excretion, both qualitatively and quantitatively, of urinary glycosaminoglycans (GAGs; mucopolysaccharides). Current classifications include over 15 clinical types, each of which is usually associated with one of at least ten specific and different lysosomal hydrolase enzymatic activity deficiencies (table I). These enzymatic activities can be assayed in serum, leukocytes, or in cultivated cells (fibroblasts or amniotic fetal cells). Establishing a definitive diagnosis requires demonstration of glycosaminoglycanuria, including the determination of increased excretion of dermatan sulfate, heparan sulfate, or keratan sulfate, and the deficiency of a specific associated enzymatic activity. Only a few specialized laboratories in this country perform these enzymatic assays, and no one laboratory assays all ten enzymes. The costs for performing these enzymatic assays are high, particularly for those requiring cell culture.

The incidence and prevalence of mucopolysaccharidoses is greater than has previously been reported. When all the different types of mucopolysaccharidoses are taken in aggregate, their incidence may approximate that of PKU, or about 1 in 16,000. The clinical manifestations range from profound mental retardation and dwarfed stature to above average intellect, near normal stature, and only minimal skeletal deformities. The diagnostic problems are complex since nearly all affected individuals appear phenotypically "normal" at birth and in early infancy. It is not uncommon for significant physical manifestations to become diagnostically evident only after five or six years of age. However, in the Type III (Sanfilippo) forms, profound functional retardation may be detected considerably earlier.

Although there are no currently known cures for this group of disorders, the need for accurate early diagnosis is particularly important for three reasons. (1) Early detection permits genetic counseling of parents so that birth of subsequent affected children may be prevented. (2) Some of the clinical types can be provided specific therapies, e.g., cardiac valve surgery, orthopedic correction of hand deformities, or ventricular shunts, which can greatly increase the quality of life, particularly for individuals with normal intelligence. (3) Inappropriate "labeling" in those individuals with an outlook for normal intelligence may be prevented.

The probability of effective cure for treatment of the mucopolysaccharidoses looms ever closer. Enzyme replacement via bone marrow transplantation has been reported to inhibit development of physical and mental deterioration in one case of mucopolysaccharidosis IH. Genetic
### TABLE I
Diagnosis of Mucopolysaccharidoses

<table>
<thead>
<tr>
<th>Type of Mucopolysaccharidosis</th>
<th>Urine GAG</th>
<th>Enzymatic Activity Deficiency</th>
</tr>
</thead>
<tbody>
<tr>
<td>MPS I H</td>
<td>Dermatan sulfate</td>
<td>α-L-iduronidase</td>
</tr>
<tr>
<td>(Hurler syndrome)</td>
<td>Heparan sulfate</td>
<td>α-L-iduronidase</td>
</tr>
<tr>
<td>MPS I S</td>
<td>Dermatan sulfate</td>
<td>α-L-iduronidase</td>
</tr>
<tr>
<td>(Scheie syndrome)</td>
<td>Heparan sulfate</td>
<td>α-L-iduronidase</td>
</tr>
<tr>
<td>MPS I H/S</td>
<td>Dermatan sulfate</td>
<td>α-L-iduronidase</td>
</tr>
<tr>
<td>(Hurler-Scheie compound)</td>
<td>Heparan sulfate</td>
<td>α-L-iduronidase</td>
</tr>
<tr>
<td>MPS II, severe</td>
<td>Dermatan sulfate</td>
<td>L-iduronate-2-sulfate sulfatase</td>
</tr>
<tr>
<td>(Hunter syndrome, severe)</td>
<td>Heparan sulfate</td>
<td>L-iduronate-2-sulfate sulfatase</td>
</tr>
<tr>
<td>MPS II, mild</td>
<td>Dermatan sulfate</td>
<td>L-iduronate-2-sulfate sulfatase</td>
</tr>
<tr>
<td>(Hunter syndrome, mild)</td>
<td>Heparan sulfate</td>
<td>L-iduronate-2-sulfate sulfatase</td>
</tr>
<tr>
<td>MPS III A</td>
<td>Heparan sulfate</td>
<td>Heparan N-sulfatase</td>
</tr>
<tr>
<td>(Sanfilippo syndrome A)</td>
<td>Heparan sulfate</td>
<td>α-N-acetyl-D-glucosaminidase</td>
</tr>
<tr>
<td>MPS III B</td>
<td>Heparan sulfate</td>
<td>Acetyl-CoA:α-glucosaminidase</td>
</tr>
<tr>
<td>(Sanfilippo syndrome B)</td>
<td>N-acetyl transferase</td>
<td>N-acetylglucosamine-6-sulfate sulfatase</td>
</tr>
<tr>
<td>MPS III C</td>
<td>Heparan sulfate</td>
<td>N-acetylglucosamine-6-sulfatase</td>
</tr>
<tr>
<td>(Sanfilippo syndrome C)</td>
<td>Keratan sulfate</td>
<td>N-acetylglucosamine-6-sulfatase</td>
</tr>
<tr>
<td>MPS III D</td>
<td>Keratan sulfate</td>
<td>β-Galactosidase</td>
</tr>
<tr>
<td>(Sanfilippo syndrome D)</td>
<td>Keratan sulfate</td>
<td>β-Galactosidase</td>
</tr>
<tr>
<td>MPS IV A</td>
<td>Keratan sulfate</td>
<td>N-acetylglucosamine-6-sulfatase</td>
</tr>
<tr>
<td>(Morquio syndrome)</td>
<td>Keratan sulfate</td>
<td>β-Galactosidase</td>
</tr>
<tr>
<td>MPS IV B</td>
<td>Keratan sulfate</td>
<td>N-acetylglucosamine-6-sulfatase</td>
</tr>
<tr>
<td>(Morquio syndrome)</td>
<td>Keratan sulfate</td>
<td>β-Galactosidase</td>
</tr>
<tr>
<td>MPS VI, severe</td>
<td>Dermatan sulfate</td>
<td>Arylsulfatase B (N-acetylgalactosamine 4-sulfatase)</td>
</tr>
<tr>
<td>(Maroteaux-Lamy syndrome, classic severe form)</td>
<td>Arylsulfatase B (N-acetylgalactosamine 4-sulfatase)</td>
<td></td>
</tr>
<tr>
<td>MPS VI, intermediate</td>
<td>Dermatan sulfate</td>
<td>Arylsulfatase B (N-acetylgalactosamine 4-sulfatase)</td>
</tr>
<tr>
<td>(Maroteaux-Lamy syndrome, intermediate form)</td>
<td>Arylsulfatase B (N-acetylgalactosamine 4-sulfatase)</td>
<td></td>
</tr>
<tr>
<td>MPS VI, mild</td>
<td>Dermatan sulfate</td>
<td>Arylsulfatase B (N-acetylgalactosamine 4-sulfatase)</td>
</tr>
<tr>
<td>(Maroteaux-Lamy syndrome, mild form)</td>
<td>Arylsulfatase B (N-acetylgalactosamine 4-sulfatase)</td>
<td></td>
</tr>
<tr>
<td>MPS VII</td>
<td>Dermatan sulfate</td>
<td>β-Glucuronidase</td>
</tr>
<tr>
<td>(β-Glucuronidase deficiency)</td>
<td>Heparan sulfate</td>
<td>β-Glucuronidase</td>
</tr>
<tr>
<td>MPS VIII</td>
<td>Keratan sulfate</td>
<td>N-acetylglucosamine-6-sulfatase sulfatase</td>
</tr>
<tr>
<td>(Matalon syndrome)</td>
<td>Keratan sulfate</td>
<td>N-acetylglucosamine-6-sulfatase sulfatase</td>
</tr>
</tbody>
</table>

Engineering techniques offer the hope of restoring the biosynthesis of normal enzyme in affected individuals. Should the mucopolysaccharidoses become treatable metabolic diseases, the laboratory must be prepared to make available diagnostic procedures that can be applied to large populations, perhaps even to include all newborns.

**Prenatal Diagnosis**

The biochemical manifestations of the mucopolysaccharidoses have been shown to be present in utero through examination of tissue obtained from affected fetuses, through the direct qualitative and quantitative measurement of abnormal levels of amniotic fluid GAG, and through the demonstration of deficient specific enzymatic activity in cultured amniotic fluid cells. Although the biochemical manifestations of the mucopolysaccharidoses are present in utero, the prenatal diagnosis of these disorders is not a simple problem. Some of the problems associated with prenatal diagnosis utilizing transabdominal amniocentesis have been reviewed in detail.\textsuperscript{16, 20}

The direct analysis of GAG present in the amniotic fluid cannot be relied upon until sometime after the 20th week of ges-
Prior to this time many investigators have found a significant incidence of false negative findings. Similarly, the use of uncultured amniotic fluid cells has not proven useful in the determination of specific enzyme activities.

Use of Cultured Amniotic Fluid Cells

Of the two major types of amniotic fluid cells (the epithelial-like type and the fibroblast-like type), generally only the fibroblast-like type cells can be maintained in culture until sufficient numbers of cells are available for enzyme analyses. Most investigators agree that the use of different techniques in the culture procedure can lead to large differences in measured enzyme activity levels.

Despite these limitations, the accurate prenatal diagnosis of the various mucopolysaccharidoses has been achieved or considered possible for all of the presently known types. In centers with diagnostic laboratory expertise, the use of cultured amniotic fibroblast-like cells for specific enzymatic activity has resulted in a high level of diagnostic accuracy. Amniocentesis can be performed in the 14th to 16th week of gestation. Usually a minimum of two to three weeks of uncontaminated culture growth is necessary to obtain a sufficient number of cells for analysis. Another serious technical limitation for these studies is the need for suitable substrates to assay some of the enzymes found to be deficient in the mucopolysaccharidoses. Frequently, these substrates are available only in a few highly specialized lysosomal storage disease laboratories.

The possibility of directly assessing a few unfixed amniotic fluid cells via biophysical cytochemistry techniques to establish prenatal diagnosis of mucopolysaccharidoses has been suggested but not definitively established.

Screening Tests for Glycosaminoglycanuria

Since the original demonstration of mucopolysacchariduria in the Hurler phenotype in 1957 by Dorfman and Lorincz, many different approaches have evolved to detect altered urinary GAG excretion. The most commonly used screening tests for glycosaminoglycans are essentially of two types.

Metachromatic Reactions

Metachromasia of various basic dyes, such as toluidine blue, Alcian blue, and azure A, has been used to detect excess GAGs in urine. The metachromasia results from the organization of the cationic dye molecules along a regular polyanionic matrix. In addition to GAG, other polyanions, such as nucleic acids, polyphosphates, bacterial polysaccharides or even excessive cellular material, can produce a metachromatic reaction. Another consideration is that a certain minimum polymer size is required for the production of metachromasia. Therefore, highly degraded GAG, e.g., some heparan sulfates excreted in certain mucopolysaccharidoses, may yield false-negative results.

The toluidine blue procedure has been reported to yield false positives according to Sabater who screened 15,000 newborns. Of the 103 tested positive by 20 days of age, only two were subsequently confirmed as positive at one year of age. At that time, these two were considered clinically normal.

False negative tests may also result from testing urine that is too dilute (i.e., specific gravity below 1.005). This problem is particularly pertinent for the study of newborns, who although capable of concentrating urine to a much higher level usually excrete urine with a specific gravity less than 1.005.
Turbidimetric Reactions

The second major type of screening procedure is based on turbidimetric reactions, in which excess GAGs interact to produce turbidity with either acidified albumin solutions with basic dyes such as 6,9-diamino-2-ethoxyacridine lactate monohydrate (Rivanol), or with quaternary ammonium compounds such as cetylpyridinium chloride or cetyltrimethylammonium bromide. These procedures may also yield false positive results; however, false negative reactions are less common.

Materials and Methods

Fifty-four random urine specimens, collected without added preservatives, were used in this study. Many specimens had been stored frozen for several years. After thawing, a 12 to 15 ml of aliquot was centrifuged at 1800 relative centrifugal force for 10 minutes. The clear supernatant was utilized for this study. All samples were randomly coded, and only urine samples with a specific gravity greater than 1.005 as determined with an American Optical TS refractometer, were analyzed.

Berry Spot Test

This was performed according to the procedure of Berry and Spinager. Five, 10 and 25 µl of centrifuged urine supernatant were placed on separate spots on a piece of Whatman No. 1 filter paper. A micropipet was used to add five µl at a time. Each application was allowed to dry thoroughly before the next was made. The paper was dipped in an aqueous solution of 0.04 percent toluidine blue buffered at pH 2.0 for about one minute, was drained, and then rinsed in 95 percent ethyl alcohol. For interpretation of a positive test, five µl of solution of chondroitin 4-sulfate containing 0.10 mg per ml give a positive purple spot against a light blue background.

Gross Acid Albumin Turbidity Test

This was performed according to the procedure of Carter et al. To 1.0 ml of clear urine supernatant, two drops of 5N HCl and two ml of acidified bovine serum albumin reagent were added. After mixing and standing at room temperature for exactly 10 minutes, the resulting turbidity was subjectively graded 0, 1+, 2+, 3+, or 4+. A series of standards for these visual gradings was prepared by diluting 0.01, 0.02, 0.03, 0.05, and 0.20 ml of 0.1 mg per ml chondroitin 4-sulfate to one ml. The lowest dilution did not produce visually detectable turbidity.

Ames MPS Spot Test

The MPS test-papers* consisted of specially treated paper impregnated with azure A dye. One drop of clear urine supernatant was added to the center of the test square. After waiting three minutes, the test square was covered with the recommended wash solvent (0.01 ml glacial acetic acid, 20 ml anhydrous methanol, and 200 ml deionized water). After gentle agitation in the wash solvent for 10 minutes, the test square was blotted to remove excess solvent, and the result was visually interpreted. A positive interpretation requires presence of a purple spot where the urine was applied. No purple color or the presence of a faint blue spot was considered negative.

All test results were objectively interpreted by at least two different observers. Repeatability and agreement in interpretation of all tests was uniformly excellent for all three screening methodologies.

Of the 54 specimens, 34 were from clinically and biochemically well charac-

* Obtained from the Ames Co., Division of Miles Laboratories, Inc., Elkhart, IN 46514.
terized cases of mucopolysaccharidoses with previously demonstrated glyco-
saminoglycanuria and confirmed specific enzymatic deficiency (exceptions were
four cases of Type II Hunter syndrome where specific enzyme assays ruled out
any Type I, and Type IIIA or Type IIIB mucopolysaccharidosis). Twelve random
specimens from adult university employees, were used as "presumed normal"
controls, and eight urine specimens from selected individuals with other genetic
disorders, e.g., osteogenesis imperfecta, fucosidosis, and undiagnosed develop-
mental retardation, were also tested. Urine GAG analysis showed that none contained excess macromolecular uronate.

Results

The results of applying the three screening tests are summarized in table II.

BERRY SPOT TEST

All 34 mucopolysaccharidosis cases had positive tests with the 10 μl and 25 μl
applications, and 33 of 34 were positive at the five μl spot. Of the normal adults, 0 of
12 was positive with the five μl level, one of 12 was positive at the 10 μl level, and
eight of 12 were positive at the 25 μl level. In the clinically non-mucopolysacchari-
dosis affected group of eight, three of the eight were positive at the five μl level; six
of eight were positive at the 10 μl level, and seven were positive at the 25 μl level.

GROSS ALBUMIN TURBIDITY TEST

Twenty-six of the 34 mucopolysaccharidosis cases had a positive test. Of the
eight negative mucopolysaccharidosis cases, five were Type III, two were Type IV,
and one was a mild form of Type VI.

None of the 12 normals had a positive test, and none of the eight clinically af-
fected non-mucopolysaccharidosis group tested positively.

<table>
<thead>
<tr>
<th>Clinical Type</th>
<th>Number of Cases Tested</th>
<th>Berry Spot Test</th>
<th>Ames MPS® Spot Test</th>
<th>Gross Albumin Turbidity</th>
</tr>
</thead>
<tbody>
<tr>
<td>Type I H</td>
<td>3/3</td>
<td>3/3</td>
<td>3/3</td>
<td>3/3</td>
</tr>
<tr>
<td>Type I S</td>
<td>1/1</td>
<td>1/1</td>
<td>1/1</td>
<td>1/1</td>
</tr>
<tr>
<td>Type I H/S</td>
<td>6/6</td>
<td>6/6</td>
<td>6/6</td>
<td>6/6</td>
</tr>
<tr>
<td>Type II</td>
<td>7/7</td>
<td>7/7</td>
<td>7/7</td>
<td>7/7</td>
</tr>
<tr>
<td>Type III A</td>
<td>4/4</td>
<td>4/4</td>
<td>4/4</td>
<td>4/4</td>
</tr>
<tr>
<td>Type III B</td>
<td>4/4</td>
<td>4/4</td>
<td>4/4</td>
<td>4/4</td>
</tr>
<tr>
<td>Type III C</td>
<td>4/4</td>
<td>4/4</td>
<td>4/4</td>
<td>4/4</td>
</tr>
<tr>
<td>Type IV</td>
<td>3/3</td>
<td>3/3</td>
<td>3/3</td>
<td>3/3</td>
</tr>
<tr>
<td>Type VI</td>
<td>5/5</td>
<td>5/5</td>
<td>5/5</td>
<td>5/5</td>
</tr>
<tr>
<td>Presumed normals</td>
<td>0/12</td>
<td>1/12</td>
<td>8/12</td>
<td>0/12</td>
</tr>
<tr>
<td>Other genetic disorders</td>
<td>8/8</td>
<td>6/8</td>
<td>7/8</td>
<td>1/8</td>
</tr>
</tbody>
</table>

Results are expressed as the number of cases with a positive test per number of cases of a particular type that were tested.

AMES MPS® SPOT TEST

Thirty-one of the 34 mucopolysaccharidosis cases gave a positive test. The two
Type IV and the one mild form of Type VI tested negatively. These three were
the same cases that also had a negative gross albumin turbidity test. None of the 12
normals had a positive test, and only one of eight of the clinically affected non-
mucopolysaccharidosis group tested positively.

In comparing the performance of the three screening tests in detecting the
definitively diagnosed mucopolysaccharidosis, the Berry spot test afforded only
one false negative at the five μl level and detected all affected cases at the 10 and 25
μl levels. The Ames test accurately detected all of the Types I, II, and III, but it
was unreliable in detecting Types IV and VI. The gross albumin turbidity test effec-
tively detected Types I and II, but it was not reliable in detecting the Types III, IV, or VI.
DISCUSSION

The Berry spot test showed the largest number of false positives in the 12 presumed normals, particularly at the 25 μl level. Though the urines from the eight individuals with other genetic disorders did not contain excess GAG, the rate of false positives was significantly higher with the Berry spot test than with the Ames and gross acid albumin turbidity tests. None of the three tests is specific for GAG, so that a certain number of false positive results, which many investigators have previously reported, are to be expected. By design, our study focused upon sensitivity (false negatives) and did not attempt to address the significant and complex epidemiological problem of determining specificity of these screening procedures.

The establishment of levels of abnormal or normal levels of GAG excretion in urine is a problem affecting both screening tests as well as quantitative assays. Essentially, if any substance selected to separate these populations is found in both normal and affected populations, any level is arbitrary. Establishing such levels is a classic textbook problem of epidemiology which has no absolute solution. It requires the adjustment of specificity and sensitivity of a particular test. This is usually done on the basis of considerations of the costs in separating true positives from false positives, as well as the costs accruing from any false negatives. For diseases like the mucopolysaccharidoses, the number of true positives may only represent a small fraction of the total population that tests positive.

One solution to the problem of overlapping normal and abnormal populations is to reduce the variance of one or both populations by some data-transformation. It has been assumed that differences in "urine concentration" are a major contributor to variability in urinary GAG concentrations. Normalizing the GAG concentration to creatinine concentration has been used by many. An implicit assumption in the use of creatinine ratios is that the substance of interest and creatinine are excreted and resolved by similar, or at least, linked mechanisms. On the other hand, it has been reported by us that expressing urinary GAG concentrations relative to creatinine has at best only a marginal effect on the variance of either mucopolysaccharidosis affected or normal populations. Moreover, although the use of GAG to creatinine ratios clearly forced some very dilute urine samples from mucopolysaccharidosis affected individuals into the abnormal range, so likewise were some very dilute urines from normals. The consequence of this was to reduce false negatives, yet it increased the number of false positives. Therefore, the present authors have elected to avoid problems of excessive urine dilution by arbitrarily limiting their studies to urine samples with a specific gravity greater than 1.005. Pennock et al similarly recommended excluding random urine samples with creatinine concentrations below 10 mg per dl for their glycosaminoglycan screening test. It has been found, as described following, that using ratios of GAG constituents to each other was quite superior to using concentrations or creatinine ratios alone when doing more detailed urine analyses on GAG.

There remains a large gap between the application of these urinary screening methods for GAG and the laboratory assessment of specific enzymatic activity deficiency evaluations. It remains a major problem for the laboratory to provide an early, accurate, definitive diagnosis of the mucopolysaccharidoses.

Since the enzymatic assays require very specialized substrates, it is difficult, even for the specialized lysosomal storage disease laboratory, to do a systematic comprehensive battery of enzymatic assays. Fortunately, it is not usually necessary to
perform all the assays for any given case, since some clues as to which enzymatic assays should be employed can be inferred from clinical manifestations.

**COMPLEMENTARY GAG ANALYSES**

A complementary approach through characterization of the excreted GAGs can inexpensively confirm glycosaminoglycanuria and further reduce the list of possible enzyme deficiencies. Because degradation of GAGs is a complex sequential process, lack of any given enzyme activity in the sequence should lead to excretion of a unique product(s). Given sufficiently sensitive chemical methods, the unique chemical or structural characteristics of these "limit" excretion products could be used in the effective detection of specific enzymatic deficiencies. A number of the mucopolysaccharidoses result in excretion of elevated amounts of heparan sulfate. It was reported earlier that this can be very successfully exploited. The molar ratio of sulfamino-hexose to uronic acid in the urinary macromolecular fraction, isolated by gel filtration, was found to have a unique and reproducible value for Type I, Type II, and Type III mucopolysaccharidosis. This ratio is totally independent of the concentration of the urine, and replicate measurements with the urine of the same individual over several years, or of different individuals with the same disorder, have been reproducible to within ± 10 percent.

The possibilities for further exploiting this principle appear promising, because the available evidence suggests further unique structural differences exist among excreted GAGs. Ramage and Cunningham demonstrated unique sulfation differences in the dermatan sulfates excreted in Type I and II diseases, while sulfation differences in the heparan sulfates excreted in Type I, II and IIIA diseases were demonstrated by us. Additional unpublished findings suggest that the heparan sulfates excreted in the different Type III disorders may have different structures. If it can be shown that different diseases result in excretion of chemically distinct substances, then the diagnostic problem can become much simpler, as in detection of sickle-cell disease where demonstration of the presence of a mutant hemoglobin, irrespective of concentration is sufficient. Further research into the structural characteristics of urinary glycosaminoglycans excreted in the mucopolysaccharidosis is needed.

**Conclusions**

There is clearly a pressing need for an even more accurate specific screening procedure capable of a much higher validity than is available with current procedures. Until such tests become available, from the observations reported here, it can be concluded that if a positive screening test is obtained with the Berry Spot test, and/or subsequently confirmed to be positive with the AMES MPS® Spot test, it is then necessary to undertake a more detailed laboratory isolation and characterization of the urinary GAGs excreted.

Gel filtration coupled with colorimetric assessment of sulfamino-hexose and uro-nate in the macromolecular fraction as well as electrophoresis and/or high performance liquid chromatography (HPLC) analyses of this same fraction can better assess the qualitative as well as quantitative nature of the urinary GAGs excreted. This information coupled with knowledge of clinical manifestations should permit the better selection of an enzymatic hydrolase deficiency assay to establish conclusively the specific type of mucopolysaccharidosis.

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References


