Simple Laboratory Determination of Excess Oligosacchariduria
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I describe a simple set of procedures for the screening of patients' urine to detect oligosaccharide-storage diseases. Urines from patients with mucolipidosis I, mannosidosis, fucosidosis, asparaglycosaminuria, and type VI glycoprotein-storage disease can be distinguished by thin-layer chromatography. Patients with $\alpha$-galactosidase deficiency can be detected by use of a combination of ion-exchange and thin-layer chromatography. Excess sialyloligosaccharide excretion is detected by using gel filtration and a quantitative assay for neuraminic acid. The advantages of the system are detection of virtually all known disorders in which oligosaccharides are over-excreted, production of characteristic patterns, and small sample requirement.

Additional Keyphrases: oligosaccharide-storage diseases • chromatography, thin-layer • chromatography, ion-exchange • neuraminic acid • urine • disorders involving oligosaccharide excretion • screening • heritable disorders • lysosomal enzyme defects • pediatric chemistry

Disorders involving hyper-oligosacchariduria have in the last few years become of increasing interest. Patients with these diseases exhibit many of the features of the mucopolysaccharide storage disorders: skeletal changes, psychomotor retardation, peculiar facial appearance, and organomegaly. An inherited lysosomal enzyme defect leads to an accumulation of complex carbohydrates and to a specific urinary excretion pattern characteristic of incomplete glycoprotein catabolism (1). These disorders include the mucolipidoses (2), mannosidosis (3), fucosidosis (4), and asparaglycosaminuria (5).

Obviously, laboratory screening for these disorders in selected populations is beneficial. To date, methods based on thin-layer chromatography (TLC) have been reported (6–8); however, each of these fails to detect at least one of the disorders. Because a system whereby all of these disorders could be distinguished would be highly advantageous, I have developed a simple procedure involving TLC and gel filtration whereby most of the oligosaccharide-excretion disorders can be resolved. This procedure is suited to routine use in pediatric clinical chemistry.

Materials and Methods

Complete 24-h urine collections from all patients and controls were supplemented with a few drops of toluene as a preservative and kept frozen at $-20^\circ$C until required. Full clinical data were obtained from the physician referring the patient.

Portions of urine (about 10 mL) were centrifuged (Laborfuge II; Christ-Heraeus GmbH, Osterode am Harz, F.R.G.) at 3000 rpm at room temperature for 10 min and then decolorized by filtration through filter paper with a charcoal insert (no. 508; Schleicher and Schull, Dassel, F.R.G.). I determined urinary creatinine by conventional means, and performed mixed ion-exchange chromatography according to Friedman et al. (9). TLC was done exactly as described by Sewell (7) in conventional glass tanks and with precoated silica gel plates (no. 5715; Merck Ltd., Darmstadt, F.R.G.). Filtered urine (5 mL) was applied to a 1.6 X 70 cm column (Pharmacia Ltd., Freiburg, F.R.G.) packed with Biogel P6, 50–100 mesh (Bio-Rad Labs., Munich, F.R.G.), and eluted with distilled water at room temperature. The void volume was 41 mL, the flow rate 10 mL/min; 5-mL fractions were collected. I determined neuraminic acid in each fraction by the method of Warren (10) after hydrolyzing at 80 $^\circ$C for 1 h in 50 mmol/L sulfuric acid.

Standard sugars were obtained from Sigma GmbH, Munich, F.R.G.

Results

Figure 1 shows typical examples of oligosaccharide patterns found in patients with storage disorders. Each disease gives a characteristic pattern, enabling its rapid identification. Normal urine shows very few bands below that of the raffinose standard.

The patterns depicted here are typical of over 500 such samples tested. Patients with mucolipidosis I show at least two heavily stained bands migrating more slowly than raffinose. Patients with mannosidosis show as many as six bands specific for this disease that stain purple/brown with orcinol. Urines from patients with fucosidosis and asparaglycosaminuria also give type-specific patterns. Urine from a patient with mucolipidosis III shows quite heavily staining bands in this system, but an examination of neuraminic acid-containing oligosaccharides provides a more specific result in this disease (Sewell, unpublished results). Patient A.P. represents a case of type VI glycoprotein-storage disease (Hers disease). The pattern is fairly typical in that at least two of the major bands have been observed in at least six other cases (Sewell, unpublished results). Patient C.B. shows one of the bands seen in type VI glycoprotein storage disease, which may well arise from glycoprotein degradation. The Rf values (with respect to raffinose) of the major oligosaccharide bands are given in Table I.

Figure 2 depicts the excretion patterns of three patients with $\alpha$-galactosidase deficiency. After the sample has been subjected to ion-exchange chromatography, three different patterns emerge, depending upon the severity of clinical symptoms. Patient A.T. represents the most severe state, showing the usual three oligosaccharide bands near the origin. Patients S.S. and W.K. represent other clinical forms of the disease, with differing degrees of skeletal involvement. W.K. shows almost a normal pattern and patient S.S. a possible intermediate picture.

Figure 3 depicts the gel-filtration patterns of urine from a patient with mucolipidosis II and from a normal control. Two peaks of neuraminic acid-containing material are observed in both samples; one coincides with the excluded volume, the other is retarded by the gel. The patient with mucolipidosis II shows a neuraminic acid content in material represented by the first peak that is at least 40-fold that of the control. Because the exclusion limit of the gel is 6000 daltons, I conclude that the neuraminic acid content of the first peak is from oligosaccharides of high molecular mass and demonstrates the excessive excretion of such materials in mucolipidosis II.
Discussion

Palo and Savolainen (11) first reported the use of oligosaccharide excretion to detect a storage disorder. They used a one-dimensional TLC procedure to screen for aspartylglycosaminuria. This method was later used to detect the abnormal oligosacchariduria in mannosidosis, fucosidosis, and GM₁ gangliosidosis (6). A further method for determining both simple sugars and oligosaccharides involved a developing time of less than 5 h (8). The method I reported previously (7) did not detect mucolipidosis II, and failed to give an abnormal pattern for a variant of \(\beta\)-galactosidase deficiency. Because no one existing method would discriminate between most of the oligosaccharide-excreting disorders, I decided to integrate several methods into a system such that most of the diseases could be detected.

In addition to the already-described excretion patterns in fucosidosis, mannosidosis, aspartylglycosaminuria, and muco-

| Table 1. Location of Most-Prominent Oligosaccharide Bands in Storage Disorders |
|-----------------------------|-----------------|
| Disorder                   | Relative \(R_f\) of bands * |
| Mucolipidosis I            | 0.41; 0.29      |
| Manносidosis                | 0.93; 0.71; 0.66; 0.57; 0.49; 0.41 |
| Fucosidosis                | 0.49; 0.36; 0.065 |
| Aspartylglycosaminuria     | 0.45; 0.29; 0.23 |
| GM₁ gangliosidosis         | 0.58; 0.26; 0.16; 0.10 |
| Glycogenosis type VI       | 0.78; 0.43      |

* Relative to \(R_f\) of raffinose.
Hypersialyloligosacchariduria is a prominent feature of the mucolipidoses (14, 15) and the cherry-red spot/myoclonus syndrome (16, 17). The latter disease and mucolipidosis I can be detected by TLC (17), whereas more time-consuming methods involving paper chromatography are necessary to characterize the oligosaccharides excreted in mucolipidosis II. The use of gel filtration was first described in conjunction with high-voltage electrophoresis (18). The simple separation involving a small quantity of urine and the assay for neuraminic acid are useful because they provide a quantitative result and therefore a method of distinguishing the diseases. Other lysosomal storage disorders, such as mannosidosis, fucosidosis, and aspartylglycosaminuria do not exhibit above-normal sialyloligosaccharide excretion (Sewell, unpublished results).

In conclusion, the system herein described has been most beneficial in diagnosing patients suspected of having an oligosaccharide-storage disorder. Although this routine TLC method is not very rapid, speed is sacrificed to obtain a better separation and thus a more unequivocal result. The gel filtration procedure, used when excess sialyloligosacchariduria is suspected on clinical grounds, requires about 24 h. The patterns observed in this system are not taken as independent diagnostic criteria, but in conjunction with the clinical features and enzyme determinations provide a useful discriminating procedure in patients in which a storage disorder must be excluded.

I am indebted to the Stiftung Volkswagenwerk for financial support.

References