may be important in connexion with the genetic disease cystic fibrosis, in which clinical symptoms of defects in ion transport and mucous secretion seem to arise as a result of a single gene mutation (Marsden, 1969). Evidence has been presented to show that acid mucopolysaccharides are also implicated in this condition (Langgard et al., 1968). Our findings indicate that the presence of an ion-transport abnormality in this disease, possibly mediated by a circulating humoral factor (Spock et al., 1967; Mangos & McSherry, 1967), could influence the metabolism of both epithelial and connective-tissue cells.

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Investigation of Urinary Glycosaminoglycans in the Mucopolysaccharidoses

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A programme of clinical and biochemical investigation of suspected mucopolysaccharidosis patients is in progress. Specimens of 24h urine from 16 patients representing five of the mucopolysaccharidosis types were collected without preservative and kept at -20°C until analysed. Urinary glycosaminoglycans were examined by two simple screening tests, the Alcian Blue spot test and a turbidity test with bovine albumin (Steiness, 1961), a quantitative version of the Alcian Blue test (Whiteman, 1972), assay of total cetylpyridinium chloride-precipitable uronic acid (DiFerrante, 1967) and a newly devised electrophoretic separation technique.

Six types of mucopolysaccharidosis have been described in detail, and some rare cases suggest the existence of more (Spranger, 1972). Among the six accepted types, two have been shown to have subclasses that differ either in their clinical course or in their laboratory presentations. A third type, Morquio syndrome, has a wide spectrum of presenting symptoms and may well represent several aetiologically distinct connective-tissue disorders.

One or more of the three glycosaminoglycans dermatan sulphate, heparan sulphate and keratan sulphate is excreted in excess in the urine of mucopolysaccharidosis patients. More recently cases have been described who excrete an excess of chondroitin 4-sulphate (Philippart & Sugarman, 1969), and a chondroitin sulphate has been found together with keratan sulphate in the urine of some Morquio-syndrome patients (Onisawa et al., 1971; Applegarth, 1973).

These glycosaminoglycans are all polysulphated and form an intensely staining complex with the cationic dye Alcian Blue. With urine spotted on to filter paper a visual assessment of the intensity can be made, whereas the quantitative version of this test, in which the complex is precipitated and redissolved, allows a more precise measurement of the amount of glycosaminoglycan excreted.

A buffered solution of bovine albumin forms a similar complex with glycosaminoglycans and gives a measurable turbidity with urine when these compounds are present...
The test as originally described did not include a blank, but we have found it necessary to use a blank of dialysed urine to avoid false positives. Quaternary ammonium salts, in particular cetylpyridinium chloride, have been used as the basis of another turbidity test (Manley & Hawksworth, 1966), but in our hands this has given an unacceptable proportion of false positives, and after extensive investigation it has been abandoned.

The Alcian Blue spot test and the bovine albumin turbidity test have, with two exceptions, been consistently positive in patients believed to have Hurler, Hunter, Sanfilippo or Scheie syndromes. In suspected Morquio-syndrome patients the results are equivocal. However, one group of Morquio-syndrome patients, the Morquio-Ullrich type, consistently gives positive results. The two exceptions referred to are two patients, thought to be cases of Sanfilippo syndrome and Scheie syndrome, who consistently gave negative bovine albumin turbidity tests in the presence of positive spot tests and increased total uronic acid excretions.

The assay of cetylpyridinium chloride-precipitable uronic acid (DiFerrante, 1967) has been applied to urine from children in hospital for conditions unrelated to connective-tissue disorders. We have confirmed that the uronic acid parameter, related to urinary creatinine, falls with age up to the mid-teens. Unequivocally raised values were obtained for all the cases of Hurler, Hunter, Sanfilippo and Scheie syndromes. The Morquio-syndrome patients gave normal results, as expected, since keratan sulphate does not contain any uronic acid. The Morquio-Ullrich-syndrome patients all gave abnormally high results, supporting the evidence for the excessive excretion of a chondroitin sulphate in this condition.

The quantitative Alcian Blue test (Whiteman, 1972), when related to creatinine excretion, shows a declining upper limit of the normal range with age. This determination has an advantage over that of cetylpyridinium chloride-precipitable uronic acid as it gives comparable chromogenicity with all the glycosaminoglycans involved in the mucopolysaccharidoses, including keratan sulphate.

Clearly abnormal results were given by all the patients with Hurler, Hunter, Sanfilippo, Scheie and Morquio-Ullrich syndromes. Others, thought to have the Morquio syndrome, gave normal values. This supports the findings obtained by other groups, who have found no excessive mucopolysacchariduria in Morquio-syndrome patients.

When the results of these two quantitative assays were correlated the results for the Morquio-Ullrich-syndrome patients fell significantly clear of the regression line, indicating the excretion of both keratan sulphate and a chondroitin sulphate in this disorder.

Urine concentrates were examined by a cellulose acetate electrophoretic technique. Urine (50ml) was centrifuged at 1150g for 10min and 40ml of the supernatant was concentrated to approx. 400μl in Visking tubing (6mm) suspended in Carbowax in distilled water (300g/l). Electrophoresis of the concentrates was carried out in 0.1M-barium acetate (Wessler, 1968). Cellulose acetate sheets (Oxoid) were soaked in 0.1M-barium acetate for 30min, pressed briefly between filter paper and then equilibrated in the electrophoresis tank for 30min. The concentrate (5μl) was applied in a 1cm line and run alongside a standard preparation containing chondroitin 4-sulphate, chondroitin 6-sulphate, dermatan sulphate, heparan sulphate, keratan sulphate and hyaluronic acid (Dr. M. B. Matthews, Chicago). A constant voltage of 5V/cm was applied for 4.5h. The strips were stained in 1% Alcian Blue 8GX (Imperial Chemical Industries Ltd.) in 2% (v/v) acetic acid for 30min and washed in 2% acetic acid.

Five patterns of bands staining with Alcian Blue were found (Fig. 1). Where there was a confident clinical diagnosis of a specific mucopolysaccharidosis type (11 of 16 cases) there was a complete correlation between the disorder type and one of the electrophoretic patterns.

The glycosaminoglycans of the standard preparations come from animal tissue sources and cannot be assumed to have the same degree of polymerization or sulphation as the material in human urine. Therefore the separation of this standard
Fig. 1. Summary of the results of chemical investigation of the urinary glycosaminoglycans in 16 mucopolysaccharidosis patients

Types A–E represent the five electrophoretic patterns found. Type E was given by the 20 normal urines examined. Results of the other determinations are represented as: +, abnormal; −, normal. AB, Alcian Blue spot test; Alb. T, bovine albumin turbidity; UA/C, cetylpyridinium chloride-precipitable uronic acid/creatinine ratio; GAG/C, glycosaminoglycan/creatinine ratio determined by the quantitative Alcian Blue technique. Where a confident clinical diagnosis was made it is indicated by: HH, Hurler or Hunter syndrome; Sf, Sanfilippo syndrome; MU, Morquio–Ullrich syndrome; Sh, Scheie syndrome; M, Morquio syndrome.

<table>
<thead>
<tr>
<th>Type A</th>
<th>Type B</th>
<th>Type C</th>
<th>Type D</th>
<th>Type E</th>
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<td>UA/C</td>
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<tr>
<td>GAG/C</td>
<td>GAG/C</td>
<td>GAG/C</td>
<td>GAG/C</td>
<td>GAG/C</td>
</tr>
<tr>
<td>Three bands</td>
<td>One broad band</td>
<td>Fast leading band</td>
<td>As A, but faint leading band</td>
<td>Two bands</td>
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</table>

mixture by the technique serves only as a control of the quality of the electrophoretic runs and as a guide to the mobility of the bands resolved from the patient's urine.

Further analysis of the bands eluted from the cellulose acetate will determine whether each band represents a homogeneous glycosaminoglycan and, if so, which type. The technique provides a valuable qualitative differential test for at least four of the mucopolysaccharidoses.