Table 2. URINARY EXCRETION OF ACID MUCOPOLYSACCHARIDES IN PATIENTS WITH DIFFERENT MUCOPOLYSACCHARIDOSES

The values are expressed in percentages of total hexuronic acid eluted from the columns and determined by the carbazole reaction.

Carbazole	1 per cent HMS	2 per cent CSA	3 per cent CSB	4 KS
	GARGOYLISM			
E. M., ♂, 10 yr	52·5	17·5	30·0	
D. K., ♂, 6 yr	55·3	22·2	22·5	
R. F., ♀, 8 yr	54·0	22·0	24·0	
L. B., ♀, 9 6/12 yr	52·5	23·0	24·5	
Mean values	53·6	21·2	25·2	
	POLYDYSTROPHIC OLIGOPHRENIA			
S. C., ♀, 9 yr	94·3	5·7	–	

individuals and patients with mucopolysaccharidoses. According to our present experience the urinary AMP pattern is of valuable assistance in the diagnosis of some hereditary bone dysplasias. In addition it affords certain clues as to their basic biochemical defect.

Fractionation of urinary mucopolysaccharides was achieved by the following procedure: dialysis of the urine; precipitation with cetyltrimethylammonium bromide; washing of the precipitate with 95 per cent ethanol; column chromatography on 'Dowex 1 *X* 2'; and colorimetric determination of hexuronic acid and/or neutral sugar content of each fraction.

Eight normal persons of various ages revealed the following mean excretion of acid mucopolysaccharides: heparitin sulphate, 27·5 per cent; chondroitin sulphate *A*, 58·5 per cent; chondroitin sulphate *B*, 14 per cent; keratosulphate, trace. The values are expressed in percentages of total carbazole and anthrone material eluted from the columns. In four patients with gargoylism the mean excretion was as follows: heparitin sulphate, 53·6 per cent; chondroitin sulphate *A*, 21·2 per cent; chondroitin sulphate *B*, 25·2 per cent. One patient with polydystrophic oligophrenia excreted heparitin sulphate almost exclusively (94·3 per cent).

I think that the urinary mucopolysaccharide pattern is pathognomonic of certain hereditary bone diseases and may serve as an aid in their diagnosis.

This investigation was supported by a grant from the Deutsche Forschungsgemeinschaft, Bad Godesberg, which is gratefully acknowledged.

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[1] Maroteaux, P., and Lamy, M., *J. Pediat.*, **67**, 312 (1965).
[2] Schiller, S., Slover, G. A., and Dorfman, A., *J. Biol. Chem.*, **236**, 983 (1961).
[3] Dische, Z., *J. Biol. Chem.*, **167**, 189 (1947).
[4] Roe, J. H., *J. Biol. Chem.*, **212**, 335 (1955).
[5] Teller, W., and Ziemann, A., *Klin. Wschr.*, **44**, 1142, (1966).
[6] Balazs, E. A., and Jeanloz, R. W., *The Aminosugars*, IIa (Academic Press, New York–London, 1965).

Disk Electrophoresis of Acid Mucopolysaccharides

WITHIN the past four years the application of disk electrophoresis for the separation of serum proteins[1] and lipoproteins (S. Lakshmanan, personal communication, 1966) has become more prevalent. The present report deals with the application of disk electrophoresis to the separation of acid mucopolysaccharides, in particular chondroitin sulphate *B* (CSB) and heparitin monosulphate (HMS).

Purified CSB and HMS which were homogeneous to paper chromatography and moving boundary electrophoresis were applied to gel columns separately and as a mixture. Acid mucopolysaccharides obtained from the urine of Hurler's syndrome patients were also investigated. Paper chromatography, moving boundary electrophoresis and uronic acid determination revealed the polysaccharide

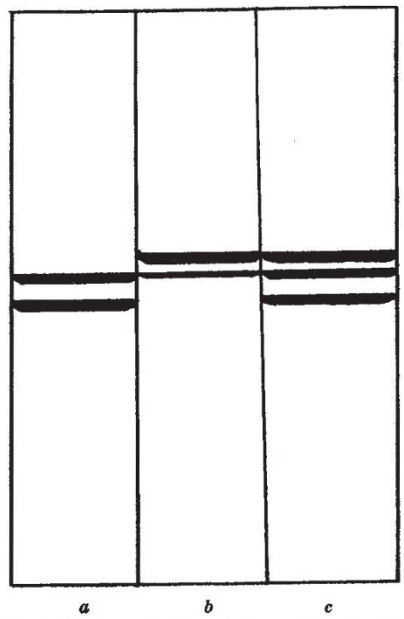

Fig. 1. Disk electrophoresis of acid mucopolysaccharides. *a*, CSB; *b*, HMS; *c*, Hurler's AMPS, 60 per cent CSB, 40 per cent HMS.

from one of these patients to consist of 60 per cent CSB and 40 per cent HMS.

A 'Canalco' disk electrophoresis apparatus was used. The polyacrylamide gel was prepared in the conventional fashion. The buffer used consisted of: *tris*-3·0 g, glycine – 14·4 g, diluted to 1 l. The electrophoresis was run at a current of 5 m.amp/column, and no interference resulted from the use of tracking dye. The disks were identified by staining with a solution of 1 per cent toluidine blue in 3·5 per cent acetic acid for 1 h, and subsequent destaining.

The electrophoresis of homogeneous CSB resolves two bands as shown in Fig. 1*a*. A slow moving major component (CSB–2) and a fast moving component (CSB–1) are separated. Similar results are obtained with HMS. In this case the major component is fast (HMS–1) and the minor component (HMS–2) is slow. Calculation of the R_F indicates that HMS–2 and CSB–1 have the same rate of migration in the buffer system employed. Electrophoresis of a mixture of CSB and HMS results in the resolution of three bands. The same result is obtained from the electrophoresis of mucopolysaccharide from a Hurler's patient (Fig. 1*c*).

These data, as well as others conducted in this laboratory, indicate that this is not an artefact caused by the procedure. Cifonelli and Dorfman have demonstrated that small amounts of iduronic acid may be found in HMS[2]. If the additional bands demonstrated by the method are not impurities it is possible that the component common to CSB and HMS contains iduronic acid. One is tempted to speculate further that the region of the glycopeptide linkage of mucopolysaccharides may be found in this common component.

The samples of CSB and HMS were given by Dr. A. Stone. I wish to thank Dr. S. Lakshmanan for his encouragement and great interest.

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Received September 27; revised December 2, 1966.

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[1] Reisfeld, R. A., Lewis, U. J., and Williams, D. E., *Nature*, **195**, 281 (1962).
[2] Cifonelli, J. A., and Dorfman, A., *Biochem. Biophys. Res. Commun.*, **7**, 41 (1962).