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*OCCURRENCE OF URINARY ACID MUCOPOLYSACCHARIDES IN THE HURLER SYNDROME**

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The presence of abnormal amounts of a chemically unidentified substance in tissues of patients with the Hurler syndrome (lipochondrodystrophy gargoylism dysostosis multiplex) has been recognized.[1] Brante[2] recovered a substance with characteristics of an acid mucopolysaccharide from tissues obtained at autopsy (liver, meninges) of patients with this disorder. He suggested that this material, which was metachromatic and contained 3.9 per cent sulfur, 27 per cent hexosamine, and 26 per cent glucuronic acid, was similar to or identical with chondroitinsulfuric acid. Dawson[3] and Uzman[4] have also suggested that the "storage material" in affected tissues is a complex polysaccharide. Since acid mucopolysaccharides are not known to be synthesized in the parenchymal cells affected in this disease (e.g., liver, spleen, nervous system) it seemed possible that widespread deposition in tissues results from an increase in circulating mucopolysaccharides. Accordingly, an attempt was made to determine whether urine of afflicted individuals contains unusual amounts or types of mucopolysaccharides. This communication describes the isolation and identification of two acid mucopolysaccharides from the urine or a six-year-old Negro girl who has the typical grotesque skeletal and facial deformities, hepatosplenomegaly, and corneal clouding characteristic of this disorder. Preliminary studies on a second, male, patient with the Hurler syndrome indicated the presence of similar materials.

Experimental.—After dialysis against cold running tap water, 48-hour urine samples containing thymol were concentrated *in vacuo* at 50° C. to $^1/_{10}$ volume. Sodium hydroxide was added to make a 2 per cent solution, and, after 24 hours at room temperature, dialysis against cold running tap water was repeated. Ten milligrams of one-time crystallized trypsin was added and the mixture dialyzed against 0.1 *M*, pH 8.0, phosphate buffer at 38° C., with external stirring for 4–5 days. Protein was precipitated by the addition of $^1/_2$ volume of 40 per cent trichloroacetic acid and was removed by centrifugation after standing at 4° C. for 1 hour. After dialysis against distilled water, the solution was concentrated to approximately $^1/_{10}$ volume, and the "crude mucopolysaccharide" fraction was precipitated by the addition of 4 volumes of 95 per cent ethanol in the presence of 2 per cent sodium acetate. The precipitate which formed after 48 hours at 4° C.

was collected by centrifugation, washed with absolute ethanol and ether, and dried *in vacuo* in a desiccator containing calcium chloride and paraffin.

These "crude mucopolysaccharide" fractions were purified by slab electrophoresis as described by Schiller *et al.*[5] One hundred and fifty to 200 mg. of "crude mucopolysaccharide" per 48-hour sample was isolated from the urine of this patient, as compared with 20–50 mg. per 48-hour sample obtained from the urine of normal individuals of comparable age and sex. The "crude mucopolysaccharide" obtained from normals, when subjected to slab electrophoresis, yielded insufficient material for further identification. Recently, Di Ferrante and Rich[6] isolated from 23 liters of pooled normal human urine 140 mg. of an acid mucopolysaccharide which was indistinguishable from chondroitinsulfuric acid–A (CSA-A).

Slab electrophoresis of the "crude mucopolysaccharide" fraction derived from the urine of the patient with the Hurler syndrome indicated two peaks. Most of the material was present in the rapidly migrating peak, which had a mobility comparable to that observed for CSA-A and chondroitinsulfuric acid–B (CSA-B, β-heparin).[5] Similarity of behavior to CSA-A and CSA-B was also noted by paper electrophoresis, with respect both to mobility and to metachromatic staining with toluidine blue.

The analyses (Table 1) of the more rapidly migrating peak are consistent with an

TABLE 1

COMPOSITION OF MUCOPOLYSACCHARIDES ISOLATED FROM URINE*

	Hexosamine†	Nitrogen‡	Uronic Acid§	Ester Sulfur‖	N-Acetyl#
"Crude fraction," normal	1.00	3.42	0.71	...	...
"Crude fraction," Hurler syndrome	1.00	2.30	0.76	...	...
"Purified fraction," rapidly migrating peak, Hurler syndrome	1.00	1.32	0.55	1.22	1.37
"Purified fraction," slowly migrating peak, Hurler syndrome	1.00	1.46	1.40	1.17	0.87

* All analyses on each fraction were performed on the same solution, with the exception of the N-acetyl determination on the rapidly migrating peak. Analyses are expressed as molar ratios with respect to hexosamine.

† Hexosamine was determined by the method of Boas (*J. Biol. Chem.*, **204**, 553, 1953), omitting the Dowex treatment.

‡ Nitrogen was determined by a micro-Kjeldahl procedure.

§ Uronic acid was determined by the method of Dische (*J. Biol. Chem.*, **167**, 189, 1947)

‖ Ester sulfur was determined by the method of L. Anderson (*Acta Chem. Scand.*, **7**, 689, 1953).

N-acetyl determination by chromic oxidation according to the procedure of E. Wiesenberger (*Mikrochim. Acta*, **33**, 51, 1948).

acid mucopolysaccharide containing one sulfate group per disaccharide repeating unit. The values for hexosamine by the method used[7] are generally about 10 per cent low. Some contamination by protein is present, as indicated by the slightly elevated nitrogen value and by the demonstration of free amino acids in hydrolyzates subjected to paper chromatography. The chromatograms were developed with tertiary amyl alcohol–formic acid–water (8:2:3), and the amino acids were demonstrated by the ninhydrin color reaction.

The low uronic acid value obtained by the carbazole method[8] is characteristic of CSA-B.[5] As has been found for CSA-B,[5,9] this material is resistant to the action of testicular hyaluronidase, as demonstrated by the method of Mathews, Roseman, and Dorfman.[10] The optical rotation was $[\alpha]_D^{25} = -68°$, calculated on the basis of hexosamine content of the solution. Galactosamine was demonstrated by the method of Stoffyn and Jeanloz.[11]

CSA-B has been isolated previously from skin,[5, 12] gastric mucosa,[13] and beef lung.[14] It differs from CSA-A in that it contains L-iduronic acid[15, 16] instead of D-glucuronic acid. The presence of L-iduronic acid in this material has been demonstrated by paper chromatography following mild acid hydrolysis.[15] The absence of sialic acid was indicated by a negative direct Ehrlich reaction.[17] Preliminary studies[18] indicate that this material has antithrombic activity similar to that of CSA-B[19] obtained from beef lung.

The more slowly migrating peak had an electrophoretic mobility somewhat less than CSA-A and CSA-B but greater than that of hyaluronic acid. Analyses of this material (Table 1) were consistent with an acid mucopolysaccharide containing one sulfate group per disaccharide unit. The high uronic acid value obtained by the carbazole method is of interest in view of the known high values which are obtained with heparin.[8] This material was also resistant to the action of testicular hyaluronidase. Glucosamine was demonstrated by the method of Stoffyn and Jeanloz.[11] The optical rotation was $[\alpha]_D^{20} = +39°$, based on hexosamine content. These data suggest that this substance is similar to that isolated by Jorpes and Gardell[20] from ox liver, which they named "heparin monosulfuric acid." Meyer[21] has recently indicated the existence of a similar substance in aorta and amyloid.

Discussion.—The data presented indicate that a marked quantitative deviation in the excretion of acid mucopolysaccharides occurs in the Hurler syndrome. Although the two mucopolysaccharides identified occur in normal connective tissues, they have not been previously isolated from urine. Available evidence indicates that normal urine contains only small amounts of CSA-A, in contrast to the finding in the Hurler syndrome of relatively large amounts of CSA-B (β-heparin) and small amounts of a second mucopolysaccharide, tentatively identified as heparin monosulfuric acid.

The site of origin of these polysaccharides is unknown, but it is unlikely that they are formed in all of the parenchymal cells in which abnormal accumulation occurs. More reasonable is the assumption that the fundamental defect resides in the connective tissues. The nature of this defect cannot be ascertained in the absence of more adequate knowledge of the metabolism of these mucopolysaccharides. In any case, it is probable that the distortion of tissues in this heritable disorder is related to an abnormality of mucopolysaccharide metabolism. The Hurler syndrome thus may be considered the first known example of a human disorder involving such a metabolic anomaly.

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ASYMMETRIC DISTRIBUTION OF C^{14} IN SUGARS FORMED DURING PHOTOSYNTHESIS

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The concept of the conversion of $C^{14}O_2$ to labeled sugars during photosynthesis as reported by Calvin and coworkers[1, 2] is the following: (1) carbon dioxide fixation involving the carboxylation of a symmetrically labeled two-carbon piece derived from ribulose diphosphate to yield phosphoglyceric acid predominantly labeled in the carboxyl carbon (CH_2*OPO_3H_2-C*HOH-C**OOH), (2) a cyclic transketolase-transaldolase sequence involving fructose-6-phosphate, sedoheptulose-7-phosphate, and pentulose-5-phosphate to produce the symmetrically labeled CO_2 acceptor, and (3) conversion of the phosphoglyceric acid via the Embden-Meyerhof sequence to yield a hexose sugar predominantly labeled in carbon atoms 3, 4 (C-3, C-4) with carbon atoms 1, 6 equal to 2, 5 (C*-C*-C**-C**-C*-C*).[3–6] In earlier papers the hexose degradation data were obtained by the *Lactobacillus casei*[7] procedure, which yields pairs of carbon atoms. In the present report, we have investigated the distribution of tracer in sugars produced during photosynthesis in $C^{14}O_2$ using the *Leuconostoc mesenteroides*[8, 9] degradation method, which permits a determination of the C^{14} content of the individual carbon atoms. The results of these experiments and their implications for the pathway of carbon dioxide to carbohydrate during photosynthesis are reported in this communication.

Methods and Materials.—The *Chlorella* was grown as described by Kandler,[10] except that the light source was fluorescent lamps with an intensity of approximately 1,000 foot-candles. The organisms were harvested by centrifugation, washed twice with water, and suspended in distilled water or potassium phosphate buffer. The tobacco, sunflower, and Canna leaves, including petioles, were removed from mature greenhouse stock plants.

The 10- and 60-second experiments with algae were carried out in a "lollipop"