

*Brief Definitive Reports*

THE HURLER SYNDROME: A STUDY OF CULTURED LYMPHOID  
CELL LINES\*

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The Hurler syndrome is a genetic disorder in mucopolysaccharide metabolism which results in storage of mucopolysaccharides in various tissues of the body (1). The lymphocytes in the peripheral blood of patients with the Hurler syndrome show metachromatic inclusions which are considered to be diagnostic (2). It has not been established whether these inclusions reflect the uptake of mucopolysaccharides from the plasma or synthesis by the lymphocytes.

With the development of techniques for the establishment and maintenance of permanent lymphoid cell lines (3), it has been possible to study the mucopolysaccharide metabolism of such suspension cell lines derived from cells of the peripheral blood of both patients with the Hurler syndrome and normal individuals in an attempt to answer such questions.

*Materials and Methods*

Suspension cultures from three patients with the Hurler syndrome and four normal individuals were established as previously described in detail (4). These lymphoid suspension lines were grown routinely in culture medium RPMI 1640 containing 20% heat-inactivated fetal calf serum, 2 mM glutamine/ml, 100  $\mu$ g of streptomycin, and 100 units of penicillin/ml in culture flasks (Falcon Plastics, Division of B-D Laboratories, Inc., Los Angeles, Calif.). Before the mucopolysaccharide studies, suspensions (cell number  $10^8$ /bottle) were grown in roller bottles on a roller apparatus (Bellco Glass, Inc., Vineland, N. J.) in the same medium. The medium volume was doubled every 72 hr so that at the end of 1 culture wk, there was sufficient cellular material for chemical analyses.

Fibroblast monolayer cultures were established from skin biopsy specimens from the same Hurler and normal individuals by standard culture methods (5) in order to compare the mucopolysaccharides present in cultured cells from two different tissue sources. The culture medium used for the fibroblasts was Eagle's medium containing 20% by volume of heat-inactivated fetal calf serum.

Staining methods for metachromasia and alcianophilia used have been previously described

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(6). For determination of mucopolysaccharides, each lymphoid suspension culture was centrifuged and the cell pellet and medium were analyzed separately. After trypsinization into suspension, the monolayer fibroblast cultures were analyzed by the same methods. Each was digested with pronase, dialyzed, and precipitated with cetylpyridinium chloride (CPC) as previously described in detail (7). Total polysaccharide was estimated in all samples as uronic acid by the carbazole method (8). For qualitative studies the CPC precipitate was redissolved and reprecipitated with  $\text{CuSO}_4$  (9). The precipitate (containing dermatan sulfate and hyaluronic acid) and the supernatant (containing heparan sulfate and chondroitin sulfates) were further purified (9). Portions of both fractions were subjected to testicular hyaluronidase digestion and the undigested material was reprecipitated with ethanol-sodium acetate. Hyaluronic acid and chondroitin sulfates were determined as the material susceptible to testicular hyaluronidase. Heparan sulfate was detected directly by the nitrous acid reaction for *N*-sulfated hexosamines (10). Dermatan sulfate was calculated as the difference between the total and the three other mucopolysaccharides measured. The electrophoretic pattern of the purified CPC-precipitated mucopolysaccharides was observed on cellulose acetate strips in a Beckman microzone cell (model R-101; Beckman Instruments, Inc., Fullerton, Calif.) using two buffer systems to identify individual mucopolysaccharides (11). In the calcium lactate buffer, hyaluronic acid and heparan sulfate could be separated; whereas, dermatan sulfate and chondroitin sulfates were electrophoretically indistinguishable. In the barium acetate buffer, heparan sulfate and chondroitin sulfates migrated separately and hyaluronic acid moved together with dermatan sulfate.

## RESULTS

The cultured lymphoid cells as well as the cultured skin fibroblasts from the normal individuals studied showed orthochromasia (ametachromasia) and no alcianophilia at 0.3 M  $\text{MgCl}_2$ . The majority of the lymphocytes in the peripheral blood from the three patients with the Hurler syndrome showed metachromatic inclusions before culture. The cultured lymphoid cells from the Hurler patients showed both metachromasia and alcianophilia at 0.3 M  $\text{MgCl}_2$ . Cytoplasmic

TABLE I  
*Mucopolysaccharide Content (Measured as Uronic Acid) of Cultured Lymphoid and Skin Fibroblast Lines Derived from Three Patients with the Hurler Syndrome and Four Normal Individuals*

Subjects	Lymphoid lines		Fibroblast lines	
	Cells	Medium	Cells	Medium
<i>μg uronic acid/mg cell protein</i>				
Normals				
No. 1	0.7	25.2	1.5	60.5
No. 2	0.7	26.1	1.9	80.4
No. 3	0.7	39.0	1.4	90.3
No. 4	0.6	23.3	1.7	80.6
Mean	0.7	28.4	1.6	78.0
Hurler syndrome				
No. 5	2.1	41.7	19.0	128.7
No. 6	3.2	49.1	11.6	106.4
No. 7	4.0	50.2	18.2	116.0
Mean	3.1	47.0	16.3	117.0

TABLE II  
*Intracellular Mucopolysaccharides in Cultured Lymphoid and Skin Fibroblast Lines Derived from a Patient with the Hurler Syndrome (No. 5) and a Normal Individual (No. 1).*

Subjects	Cell line	% of total cellular mucopolysaccharides			
		HA	CS	DS	HMS
Normal (No. 1)	Lymphoid	0	90	5	5
	Skin fibroblast	68	12	16	4
Hurler syndrome (No. 5)	Lymphoid	0	60	35	5
	Skin fibroblast	28	6	64	2

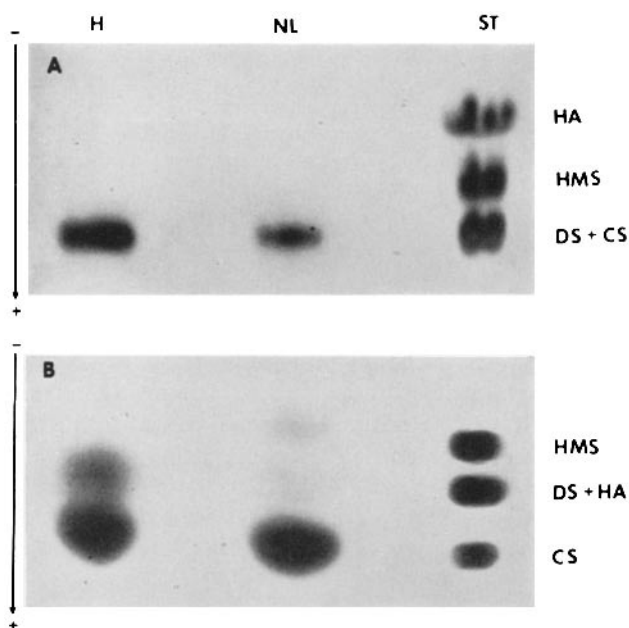


FIG. 1. Electrophoretic patterns of CPC-precipitated cellular mucopolysaccharides from cultured lymphoid lines from a patient with the Hurler syndrome (No. 5) (*H*) and a normal individual (No. 1) (*NL*) (run on cellulose acetate strip in calcium lactate buffer [*A*] and barium acetate buffer [*B*]). *ST*, standards (provided by Dr. M. B. Mathews, Dept. Pediatrics, University of Chicago): *HA*, hyaluronic acid; *HMS*, heparan sulfate; *DS*, dermatan sulfate; *CS* chondroitin sulfates.

staining was diffuse; discrete metachromatic inclusions, as seen in the peripheral blood, were not seen in the cytoplasm of cultured lymphoid lines.

The cultured lymphoid lines established from the Hurler patients had increased intracellular mucopolysaccharides compared with that of the matched normal cultures (Table I). The Hurler suspension cultures averaged approxi-

mately four times more uronic acid ( $3.1 \mu\text{g}$  uronic acid/mg cell protein) than those from normals ( $0.7 \mu\text{g}$  uronic acid/mg cell protein). The uronic acid content of the culture media of the Hurler lymphoid lines averaged  $47.0 \mu\text{g}$  uronic acid/mg cell protein which was slightly higher than the uronic acid content of the media of normal lymphoid suspension cultures ( $28.4 \mu\text{g}$  uronic acid/mg cell protein). Compared with the skin fibroblast cultures, the lymphoid suspension cells from both normal and Hurler patients contained less uronic acid (Table I).

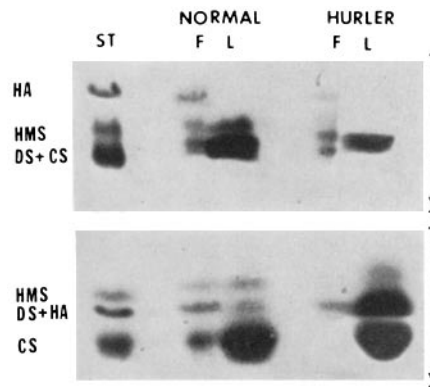


FIG. 2. Electrophoretic patterns of CPC-precipitated cellular mucopolysaccharides from cultured lymphoid (*L*) and skin fibroblast (*F*) lines from a patient with the Hurler syndrome (No. 6) and a normal individual (No. 2) (run on cellulose acetate strips in calcium lactate buffer [top] and barium acetate buffer [bottom]).

By chemical analyses (Table II) and electrophoresis (Figs. 1 and 2), the major mucopolysaccharide in the lymphoid suspension cell from normal individuals was chondroitin sulfates (90%) with smaller amounts of heparan sulfate (5%) and dermatan sulfate (5%). There appeared to be no hyaluronic acid present. The major mucopolysaccharide in the cultured normal fibroblast was hyaluronic acid (68%) with smaller amounts of dermatan sulfate (16%), chondroitin sulfates (12%), and heparan sulfate (4%).

The major mucopolysaccharides in the Hurler lymphoid lines were chondroitin sulfates (60%) and dermatan sulfate (35%) with a small amount of heparan sulfate (5%). No hyaluronic acid could be detected. The mucopolysaccharides in the cultured Hurler fibroblasts were composed primarily of dermatan sulfate (64%) with smaller amounts of hyaluronic acid (28%), chondroitin sulfates (6%), and heparan sulfate (2%).

#### DISCUSSION

Since the demonstration that the cultured skin fibroblast from patients with the Hurler syndrome showed increased intracellular mucopolysaccharides (7,

12), the cultured fibroblast has been used as an *in vitro* model. With the establishment of permanent lymphoid lines from three patients with the Hurler syndrome, an alternate cell system is now available for study of mucopolysaccharide metabolism.

As in the cultured skin fibroblast, the Hurler lymphoid cell in suspension culture could be distinguished from normals by (a) its staining characteristics (metachromasia and alcianophilia at 0.3 M  $MgCl_2$ ) and (b) qualitative and quantitative distribution of cellular mucopolysaccharides (Tables I and II). The major mucopolysaccharide in the three Hurler lymphoid suspension lines was also chondroitin sulfates but dermatan sulfate was markedly increased (Table II).

Metachromatic inclusions (2) are the hematological "hallmark" for the Hurler syndrome. Although histochemical staining has demonstrated that these inclusions are composed of mucopolysaccharides, their origin has not been clear. Bowman et al. (13) noted persistence of metachromatic inclusions for 3 culture wk in cultures of peripheral white blood cells from patients with the Hurler syndrome. Short-term cultures of white cells derived from the peripheral blood of individuals homozygous and heterozygous for the Hurler syndrome have been shown (14) to reveal distinct intracellular metachromatic staining. However, there was no chemical verification that this staining reflected *de novo* mucopolysaccharide synthesis. The permanent lymphoid suspension cultures established from both normals and Hurler patients have been shown to synthesize mucopolysaccharides (Tables I and II). Furthermore, the Hurler lymphoid lines showed increased intracellular mucopolysaccharide content with a marked increase in dermatan sulfate.

The availability of such biochemically marked permanent cell lines for the Hurler syndrome is timely. The recent finding (A. Dorfman and E. F. Neufeld, unpublished data) that the "corrective Hurler factor" is probably  $\alpha$ -L-iduronidase adds credence to the suggestion of Matalon et al. (15) that this enzyme is involved in the basic defect in the Hurler syndrome. Clinical evidence is accumulating that mobilization of stored mucopolysaccharides may be enhanced in the Hurler patient by normal plasma infusions (16). The advantage of having suspension cultures which will yield large quantities of material for assay will be helpful in not only the isolation of enzymatically active material for clinical trials but also for the further elucidation of this group of inherited mucopolysaccharide disorders.

#### SUMMARY

Lymphoid suspension lines have been established from three patients with the Hurler syndrome and four normals. The Hurler lines can be distinguished from normals by (a) staining characteristics, (b) increase in total cellular mucopolysaccharide content, and (c) increase in dermatan sulfate. Hyaluronic acid is absent in cultured lymphoid cells from normal persons and patients with

the Hurler syndrome. The availability of biochemically marked suspension cultures should prove useful for enzymatic studies as well as for further elucidation of this clinical syndrome.

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