CYSTIC FIBROSIS: DISTRIBUTION OF MUCOPOLYSACCHARIDES IN FIBROBLAST CULTURES

B. Shannon Danes and Alexander G. Bearn

The Division of Human Genetics, Department of Medicine,
Cornell University Medical College, New York

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SUMMARY

The mucopolysaccharide content of skin fibroblast cultures from patients and heterozygous carriers of cystic fibrosis was increased as compared to that from normal, non-carrier individuals. The distribution of mucopolysaccharides in these cultures (with the intracellular uronic acid similar to control cells and that in the extracellular matrix and medium increased) was markedly different from that seen in cultures derived from normals and patients with Hurler's syndrome.

Although the recognized clinical phenotype for cystic fibrosis of the pancreas is presumed to be secondary to a disorder of the exocrine system, the presence of the abnormal genotype can be revealed in at least two types of cultured cells.

Cytoplasmic metachromasia after staining with toluidine blue O was observed in fibroblasts and white blood cells from cystic fibrosis patients and their heterozygous parents (1,2). From these studies, there appeared to be three morphological classes (2). In the first two classes, Class IA and IB, the metachromasia was confined to vesicles and varied only quantitatively. In Class II the metachromasia was dispersed

diffusely throughout the cytoplasm and was indistinguishable from that found in patients with the mucopolysaccharidoses and certain other inherited diseases.

In fibroblast cultures from patients with cystic fibrosis, Matalon and Dorfman (3) found an increase in the intracellular mucopolysaccharide content with the relative amounts of hyaluronic acid, dermatan sulfate and chondroitin sulfate similar to that found in normal cultured fibroblasts. As the increase above normal ranged from 2 to 10 times, they remarked on this wide variability in intracellular content. Danes and Bearn (2) reported that an increase in the intracellular mucopolysaccharides occurred only in cultures of Class II. On the basis of these two studies, it seemed appropriate to determine the distribution of mucopolysaccharides in the total culture (cells, extracellular matrix and medium) from families with cystic fibrosis, patients with Hurler's syndrome and normal presumed non-carrier individuals.

Materials and Methods

Skin fibroblast cultures were established from 23 patients (4 Class IA, 7 Class IB and 12 Class II), 24 heterozygous parents (5 Class IA, 5 Class IB, 12 Class II), 3patients with Hurler's syndrome and 12 normal, presumed non-carriers. The establishment of cell lines from skin biopsies by standard culture methods and the metachromatic staining procedure have been described previously in detail (2). Cells (initial innoculum 4×10^6 cells) were grown in round bottles in a roller apparatus for chemical studies. After one month, when the cells formed a dense multilayered culture, the cells were harvested in 2 ways: the first method used was to analyze only the washed cells as previously described (2). The second method, the medium was decanted and the cell layer was dispersed with trypsin (0.25%) for 10 minutes and the sheet of cells

shaken into a suspension. The cell suspension was centrifuged and the three fractions (cells, trypsin, and medium) were analyzed for mucopolysaccharides as described previously in detail (4). Total polysaccharide was estimated as uronic acid by the carbazole method (5), and cellular protein by the method of Lowry (6).

Results and Discussion

Using washed cells from affected individuals (Table I) essentially the same uronic acid content was found as those derived from normals. Only the cultures derived from heterozygous carriers Class II showed a marked increase in the intracellular uronic acid content.

Using trypsin dispersed cells (Table II) in which the total culture content was analyzed, the total uronic acid content of cultures derived from affected patients Class IB was twice, and of Class II seven times greater than control cultures. The uronic acid content of the cells in these cultures was essentially normal; the uronic acid content of the extracellular matrix (trypsin) and medium was significantly increased. The cultures derived from the heterozygous carriers in Class II showed a significant increase in uronic acid in all three fractions.

The distribution of uronic acid in the cultures from patients with Hurler's syndrome was markedly different. Intracellular uronic acid was increased whereas the amount in the extracellular matrix (trypsin-wash) and medium was similar to control cells.

On the basis of these studies it appears that the cellular metachromasia observed in fibroblast cultures derived from patients and heterozygous carriers for cystic fibrosis reflects an increased synthesis of mucopolysaccharides with release into the extracellular space (matrix and medium). The original observations (2,3) reporting an increase in cellular mucopolysaccharide content probably reflected membrane and extra-

TABLE I

CELLULAR URONIC ACID OF SKIN FIBROBLAST CULTURES
(Cells isolated by Method I)

Subjects		no. of individuals studied	total no. of determinations	µg uronic acid/mg cellular protein
Normal individuals		8	18	4.51 ± 1.8
Cystic Fibrosis				
Class IA	Propositus	4	8	5.41 ± 1.9
	Heterozygote	5	10	5.43 ± 1.3
Class IB	Propositus	4	8	4.62 ± 1.6
	Heterozygote	6	12	5.86 ± 2.0
Class II	Propositus	6	12	7.10 ± 2.3
	Heterozygote	9	14	11.23 ± 2.5

TABLE II

DISTRIBUTION OF URONIC ACID IN SKIN FIBROBLAST CULTURES
(Culture contents isolated by Method II)

Subjects	No. of individuals studied	μg uronic acid/mg cellular protein			
		cells	trypsin	medium	total
Normal individuals	4	5.82 ± 1.55	0.64 ± 0.22	35.2 ± 8.90	41.4 ± 10.70
Hurler's syndrome	3	11.21 ± 1.80	0.72 ± 0.33	33.4 ± 12.40	45.3 ± 14.53
Cystic Fibrosis					
Class IB - affected	3	4.48 ± 0.92	1.70 ± 0.37	74.8 ± 5.20	79.9 ± 6.49
Class II - affected	6	7.65 ± 2.90	2.90 ± 1.65	324.1 ± 45.50	339.3 ± 61.08
Class II - heterozygotes	3	16.2 ± 1.96	5.77 ± 1.46	279.0 ± 62.5	281.7 ± 65.92

cellular matrix uronic acid. Storage of mucopolysaccharides within fibroblasts in cystic fibrosis does not occur and in this respect differs from the mucopolysaccharidoses.

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References

1. Danes, B.S. and Bearn, A.G., Lancet, i, 1061, (1968).

2. Danes, B.S. and Bearn, A.G., J. Exp. Med., 129, 775, (1969).

3. Matalon, R. and Dorfman, A., Biochem. & Biophys. Res. Com., 33, 954, (1968).

4. Danes, B.S. and Bearn, A.G., J. Exp. Med., 124, 1181 (1966).

5. Dische, Z., J. Biol. Chem., 167, 189, (1947).

6. Lowry, O.H., Rosebrough, N.J., Farr, A.L. and Randall, R.J., J. Biol. Chem., 193, 265, (1951).