ACID MUCOPOLYSACCHARIDES IN CULTURED FIBROBLASTS OF CYSTIC FIBROSIS OF THE PANCREAS*

Reuben Matalon and Albert Dorfman

From the Departments of Pediatrics and Biochemistry,
the Joseph P. Kennedy, Jr. Mental Retardation Research Center
and LaRabida-University of Chicago Institute,
Pritzker School of Medicine,
University of Chicago, Chicago, Illinois

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Cystic fibrosis of the pancreas (fibrocystic disease, mucoviscidosis) is a heritable disease affecting the exocrine glands which was first described by Fanconi et al. (1936). Current knowledge regarding the clinical manifestations and the pathophysiology has been extensively reviewed by di Sant'Agnese and Talamo (1967). The disease is characterized by pancreatic insufficiency, recurrent pulmonary infection and elevated sweat electrolytes as well as by other complications secondary to abnormalities of exocrine function. Cystic fibrosis, the most common of the lethal genetic defects in Caucasians, is transmitted as a Mendelian autosomal recessive (Steinberg and Brown (1960) and Merritt et al. (1962)). Although numerous studies have been concerned with the chemistry of glycoproteins in this syndrome there has been no clearcut delineation of the underlying biochemical defect. On the basis of immunological evidence, Lowe et al. (1966) claimed the presence of an abnormal mucopolysaccharide in the feces of affected patients. Danes and Bearn (1968) reported the presence of metachromatic

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granules in fibroblasts derived from cystic fibrosis patients as well as from individuals heterozygous for this disease. This paper reports the quantitative and qualitative distribution of acid mucopolysaccharides in fibroblasts derived from patients with cystic fibrosis.

Materials and Methods

Fibroblast cultures were established from skin of four patients with cystic fibrosis. These were compared with cultures derived from patients with Hurler's and Marfan's syndrome and normal individuals. The cells were grown in 100 mm Petri dishes under conditions previously described (Matalon and Dorfman, 1966). Staining of fibroblasts with toluidine blue, isolation and characterization of acid mucopolysaccharides, electrophoresis, and analyses for uronic acid and hexosamine were performed as previously described (Matalon and Dorfman, 1966, 1968). Amino sugars were determined with the Technicon amino acid analyzer.

Results and Discussion

Fibroblasts, derived from skin biopsies of four children who displayed clinical characteristics of cystic fibrosis contained large amounts of material which stained metachromatically with toluidine blue. The inclusions were somewhat larger and less uniformly distributed than those observed in Hurler's disease and Marfan's disease.

The total amount of polysaccharide isolated from the fibroblasts of three of the patients was approximately 8-10 times that present in normal fibroblasts. In one case (L.S., Table 2) the increase was only two fold.

Table 1 summarizes the properties of mucopolysaccharide fractions obtained from two patients. Electrophoresis showed the three fractions to correspond in mobility to known standards. These data indicate the presence of chondroitin 4/6 - SO_4, hyaluronic acid and dermatan sulfate.

TABLE 1

Identification of AMPS Isolated from Cystic Fibrosis Cells

	Hyaluronic Acid	Dermatan Sulfate	Chondroitin Sulfate 4/6
Eluction from Dowex-1 Cl^-	0.5 M NaCl	1.75 M NaCl	1.5 M NaCl
Hexosamine	Glucosamine	Galactosamine	Galactosamine
Uronic Acid:Hexosamine	1.18	0.68	1.12
Carbazole:Orcinol		0.48	
Testicular Hyaluronidase	+	-	+
$[\alpha]_D^{24^\circ}$	-68°		

TABLE 2

Acid Mucopolysaccharides Isolated from Normal, Cystic Fibrosis, Hurler and Marfan Fibroblasts

Cell Type	Total AMPS	AMPS Fractions		
		Hyaluronic Acid	Dermatan Sulfate	Chondroitin Sulfate 4/6
	mg*	% of total	% of total	% of total
Normal	0.6	67	16	16
Cystic Fibrosis (L. S.)	1.2	68	19	13
Cystic Fibrosis (D.L.)	5.5	71	18	11
Hurler	5.5	22	73	5
Marfan	5.2	92	2	6

* Results are based on isolation of AMPS from 10 tissue culture plates (100 mm), with 12×10^6 to 14×10^6 cells per plate. Quantity of AMPS is based on 33% hexosamine content.

Table 2 indicates that the relative amounts of hyaluronic acid, dermatan sulfate and chondroitin sulfate in cystic fibrosis closely parallel the distribution of mucopolysaccharides in normal cells. In contrast the increased mucopolysaccharide content in Hurler cells is largely due to increased quantity of dermatan sulfate and in Marfan cells is due to increased quantity of hyaluronic acid.

The finding of increased acid mucopolysaccharides in fibroblasts from fibrocystic disease was somewhat surprising in view of the lack of previous evidence of abnormalities of mucopolysaccharide metabolism. The fact that intracellular acid mucopolysaccharide concentration is increased in a number of conditions is becoming increasingly apparent. Striking however is the finding that the chemical nature of the accumulated material differs in cells all of which show metachromasia.

The impressive increase in mucopolysaccharides represents the first clearcut chemical difference between cystic fibrosis cells and normal cells and establishes the fact that whatever the pathogenesis, the genetic defect is expressed in fibroblasts as well as exocrine cells. Neither the nature of the biochemical defect which leads to accumulation of acid mucopolysaccharides nor its relation to the abnormality of exocrine function is clear. In view of the genetics of this disease, however, it might be expected that both are related to a single biochemical abnormality.

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References

Danes, B. S. and Bearn, A. G., Lancet, 1, 1061 (1968).
Fanconi, G., Uehlinger, E. and Knauer, C., Wien, Med. Wchnsehr., 86, 753 (1936).
Lowe, C. V., Adler, W., Broberger, O., Walsh, J. and Meter, E., Science, 153, 1124 (1966).
Matalon, R. and Dorfman, A., Proc. Natl. Acad. Sci., U. S., 56, 1310 (1966).
Matalon, R. and Dorfman, A., Biochem. Biophys. Res. Commun., 32, 150 (1968).
Merritt, A. D., Hanna, B. L., Todd, C. W., Jr., and Myers, T. L., J. Lab. Clin. Med., 60, 998 (1962).
di Sant'Agnese, P. A. and Talamo, R. C., New Eng. J. Med., 277, 1287 (1967).
Steinberg, A. C. and Brown, D. C., Am. J. Hum. Genet., 12, 416 (1960).