THE ACCUMULATION OF HYALURONIC ACID IN CULTURED FIBROBLASTS OF THE MARFAN SYNDROME*

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The Marfan syndrome is a disease of the connective tissue, manifested by skeletal, cardiovascular and ocular lesions (McKusick, 1966). The disease is transmitted as a Mendelian autosomal dominant (Rados, 1942; Lutman and Neel, 1949), but the biochemical basis of the defect has not been established. Early pathological changes, referred to as cystic medial necrosis, are observed in the great vessels and are characterized by degeneration of elastic fibers with cystic areas filled with metachromatically staining material (Erdheim, 1930).

Previous studies in this laboratory have established that fibroblasts from patients with the Hurler-Hunter syndromes can be studied by tissue culture methods. Such cells accumulate acid mucopolysaccharides (AMPS), and may contain ten times as much AMPS as do normal controls (Matalon and Dorfman, 1966). The primary increase is in dermatan sulfate although some increase in hyaluronic acid (HA) and chondroitin sulfate A is also observed. Such cells exhibit metachromasia when stained with toluidine blue (Danes and Bearn, 1966). In a genetic variant of Hurler's syndrome, cultured fibroblasts contained increased amounts of lipids, with a striking increase in the ganglioside fraction,

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in addition to augmented amounts of AMPS (Matalon et al., 1968).

The present study was undertaken to determine the metabolic defect in the Marfan syndrome utilizing tissue culture techniques. The results indicate that Marfan fibroblasts contain markedly increased amounts of AMPS with a predominance of HA. Marfan fibroblasts can also be recognized morphologically by an abundance of metachromatic granules in their cytoplasm.

Materials and Methods

Fibroblast cultures were established from the skin of three patients with the Marfan syndrome, patients with Hurler's syndrome and normal controls. The cells were grown in 100 mm plastic Petri dishes as well as in the Bellco roller apparatus in bottles with a growth surface of 840 cm^2. The culture conditions and the medium used were those described previously (Matalon and Dorfman, 1966). Isolation and characterization of AMPS from the fibroblasts were carried out as previously described (Matalon and Dorfman, 1966). Hyaluronic acid was digested with streptococcal hyaluronidase under the conditions described by Mathews et al. (1951). Electrophoresis of AMPS, analyses for uronic acid and hexosamine were performed as previously described (Matalon and Dorfman, 1966). The method of Lagunoff and Warren (1962) was used for the detection of heparitin sulfate. Amino sugars were determined with the Technicon amino acid analyzer. The intrinsic viscosity, $[\eta]$, of the HA was kindly determined by Dr. M. B. Mathews. Fibroblasts were stained by the technique of Haust and Landing (1961), with slight modification. The fixation time was 5 min and the staining ranged from 30 to 60 sec depending on the cell density on the slide.

Results and Discussion

Toluidine blue staining of the Marfan fibroblasts showed a striking

difference from normal cells. The Marfan cells contained abundant metachromatically staining granules, which differed from those observed in Hurler fibroblasts with regard to their staining properties and their distribution within the cell. The metachromatic granules in the Marfan cells were more purplish and filled the entire cytoplasm.

Isolation of AMPS yielded eight to ten times as much polysaccharides from Marfan and Hurler cells as from the same number of normal cells. Electrophoretic separation of the Marfan AMPS revealed that the major component moved as does standard HA, while the major component of Hurler cells migrated as does dermatan sulfate. This observation was confirmed by fractionation and quantitation of the various polysaccharides isolated (Table I). Although the total amounts of AMPS are comparable in the Hurler and Marfan cells, there is a difference in quantitative composition. In the case of Hurler cells, the increased AMPS content is largely accounted for by dermatan sulfate, while in the Marfan cells the increase is due to HA. The dermatan sulfate concentration of Marfan cells is similar to that of normal cells. No heparitin

TABLE I

Acid Mucopolysaccharides Isolated from Normal, Hurler and Marfan Fibroblasts

Cell Type	Total AMPS	AMPS Fractions					
		Hyaluronic Acid		Dermatan Sulfate		Chondroitin Sulfate	
	mg*	mg*	% of total	mg*	% of total	mg*	% of total
Normal	0.6	0.4	67	0.1	16	0.1	16
Marfan	5.2	4.8	92	0.1	2	0.3	6
Hurler	5.5	1.2	22	4.0	73	0.3	5

* 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.

sulfate was detected in any of the polysaccharide fractions. The separated HA fraction showed a uronic acid to hexosamine ratio of 1.16:1. Ion exchange chromatography of an HA hydrolysate indicated that glucosamine was the sole amino sugar, confirming the absence of chondroitin sulfates and dermatan sulfate. The optical rotation was $[\alpha]_D^{22} = -70^\circ$; the intrinsic viscosity was $[\eta] = 3.5$, indicating a molecular weight based on this determination of $(M_v) =$ 130.000 (Mathews and Dorfman, 1953). The HA isolated from the Marfan cells was completely digested by streptococcal hyaluronidase as indicated by the acid albumin assay as well as by electrophoresis on cellulose polyacetate, thus confirming the identity of HA.

No previous reports have implicated the accumulation of hyaluronic acid in the Marfan syndrome. Bacchus (1958) reported low levels of serum mucoprotein in the Marfan patients. However, Leeming and McKusick (1962) and Lehman (1960) could demonstrate no abnormality in serum mucoprotein on a larger number of patients. Prockop and Sjoerdsma (1961) found increased excretion of peptide bound hydroxyproline in the urine of Marfan patients. However, the importance of this finding is not clear since increased growth is accompanied by elevated urinary hydroxyproline (Jasin et al., 1962; Jones et al., 1964; Meilman et al., 1963; and Smiley and Ziff, 1964). Berenson and Dalferes (1965) report a two to four fold increase in urinary excretion of AMPS in Marfan patients with a quantitative pattern similar to normal controls.

In summary, Marfan cells differ both biochemically and morphologically from Hurler cells or cells of a Hurler variant studied in tissue culture (Matalon and Dorfman, 1966; Matalon et al., 1968). There is a striking accumulation of AMPS in Marfan fibroblasts, with HA representing over 90% of the total AMPS. This increase may afford an explanation to the pathology of

the disease and may serve as a model for the study of the pathogenesis of the Marfan syndrome as well as for its diagnosis and genetics.

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