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Urinary Hyaluronidase Activity in Mucopolysaccharidosis

By

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Hyaluronidase activity has been investigated in the urine of 15 patients with mucopolysaccharidosis. A decreased activity was found in Sanfilippo's, Hunter's, Hurler's, Dyggve's and Morquio's syndromes, while in Öckerman's syndrome the values were normal. No relationship could be established between the quality of urinary mucopolysaccharide fractions and hyaluronidase activity. The amount of hyaluronidase was inversely related to the mucopolysaccharide level in urine.

Mucopolysaccharidosis or gargoylism is a condition associated with the abnormal accumulation in the tissues and increased urinary excretion of mucopolysaccharides. The biochemical background of the disease is unclear; an increased rate of mucopolysaccharide production or the impaired metabolic breakdown of normally present mucopolysaccharide fractions are alternative possibilities. Since the low molecular weight of excreted mucopolysaccharides must be the result of a depolymerisation process, increased mucopolysaccharide production has been considered more probable [8]. This fact, however, cannot rule out the possibility of an impaired function or a complete absence of some of the enzymes involved in mucopolysaccharide metabolism.

According to BADOUAL [3], mucopolysaccharidosis is associated with the disturbed function of lysosomal enzymes. Whereas some enzymes having an important role in mucopolysaccharide metabolism, such as beta-glucuronidase, beta-N-acetylaminodesoxyglucosidase, beta-galactosidase or glucoseaminidase, display an increased activity, others such as sulphatase, cysteinedesulphurase, etc., involved in the breakdown of mucopolysaccharide, show a diminished activity or complete absence [2, 3, 4, 11].

Since in mucopolysaccharidosis the mucopolysaccharide excreted in urine consists not only of hyaluronidase resistent, but also of hyaluronidase sensitive fractions, it may be assumed that the metabolism of this latter fraction is also subject to pathologic changes, even in cases when it is not excreted with urine.

In the lack of pertaining data in the literature, it seemed interesting to study the behaviour of hyaluroni-

dase activity in the urine of patients with mucopolysaccharidosis.

Materials and Methods

The urinary hyaluronidase level has been determined in a total of 30 subjects, 15 of whom were suffering from mucopolysaccharidosis, while 15 healthy infants and children served as control. No restrictions in daily activity, diet or fluid intake were introduced. Renal disease or circulatory failure has been excluded in all patients. Specific weight of urine ranged between 1010 and 1020; it contained no pathologic components (leukocytes, glucose etc.). Renal function tests performed only in some cases revealed normal values. Urine collected after spontaneous discharge in older children, or by means of an indwelling catheter in smaller ones, was stored in the refrigerator.

Mucopolysaccharide content of the urine was determined in all cases; the fractions were isolated according to DiFerrante and Rich [6], while quantitative estimation was done by our own method [8]. The levels were expressed in terms of uronic acid (or iduronic acid in the case of chondroitin B) content of the fractions. Hyaluronidase activity was estimated by two different techniques. The first one, described by Abramson and Friedman [1] and modified by McClean [9], depends on the observation that native non-depolymerized hyaluronic acid in acetic acid solution precipitates with protein, but depolymerized hyaluronic acid does not precipitate. Hyaluronidase activity (mucin clot prevention units) was expressed as the reciprocal of the highest dilution completely preventing precipitation.

The second method used was that of DiFerrante [5], involving the estimation of the amount of hyaluronic acid broken down by 10 ml of urine in 30 minutes. Although the values yielded by the two methods were sometimes divergent, their complement value was useful. Both assays required hyaluronic acid obtained from human umbilical cord, as substrate (Reanal, Budapest). Mean specific weight of the preparation used was 622,000.

Results

Hyaluronidase activity of the control urines ranged between 250 and 400 mucin clot prevention units, or 55—79 μg/10 ml of urine by the DiFerrante method (see Table I).

In the patients with mucopolysaccharidosis, hyaluronidase activity was considerably reduced. While the Abramson—Friedman method revealed hyaluronidase activity in all cases, a complete lack of activity was found in three patients by the DiFerrante technique. An inverse relationship could be established between urinary mucopolysaccharide content and hyaluronidase activity, and the latter proved to be related to the composition of urinary mucopolysaccharide, too. In Sanfilippo's syndrome, elevated levels of heparitin sulphate were associated with a reduced urinary hyaluronidase activity. In Morquio's syndrome hyaluronidase activity was decreased, while in Öckerman's syndrome it was approximately normal. Vitamin A tolerance tests performed in these patients resulted in an increased mucopolysaccharide excretion but left hyaluronidase activity unaffected (Table II).

Discussion

Biochemical and enzymological changes in the mucopolysaccharidoses

TABLE I

Hyaluronidase activity in the urine of healthy control children

	Sex	Age year	Daily urine volume (ml)	Total MPS, mg	HA, mg	CSA, mg	CSB, mg	HS, mg	KS, mg	Hyaluronidase activity		Note
										MCPE	μg/10 ml	
1	M	3	510	19.6	—	19.1	—	0.5	—	256	57	—
2	M	2	380	16.2	—	16.2	—	—	—	300	62	—
3	M	3	470	21.0	—	21.0	—	—	—	250	59	—
4	M	1	210	17.5	—	17.5	—	—	—	300	60	—
5	M	8/12	160	15.8	—	15.8	—	—	—	300	60	—
6	F	6	630	15.0	—	15.0	—	—	—	350	59	—
			610	14.2	—	14.2	—	—	—	300	62	—
7	F	2	390	10.4	—	10.4	—	—	—	350	65	—
			350	12.3	—	12.3	—	—	—	350	60	After vitamin A
8	M	5	520	13.0	—	13.0	—	—	—	400	67	—
			590	13.5	—	13.5	—	—	—	350	72	—
9	M	4	370	13.0	—	13.0	—	—	—	256	55	—
10	M	6	700	12.8	—	12.8	—	—	—	256	67	—
11	F	4	410	12.9	—	12.9	—	—	—	250	62	—
			380	12.4	—	12.4	—	—	—	256	59	—
12	F	9	820	15.0	—	15.0	—	—	—	300	57	—
13	F	2	300	12.0	—	12.0	—	—	—	350	55	—
14	F	5	610	10.5	—	10.5	—	—	—	350	79	—
15	F	11	900	11.5	—	11.5	—	—	—	300	69	—
			870	10.0	—	10.0	—	—	—	300	62	After vitamin A

M = male
F = female

have yet to be clarified. Deficiency or reduced activity of several enzymes involved in mucopolysaccharide metabolism were repeatedly reported, but the role and function of hyaluronidase have not been investigated. The present data suggest that in mucopolysaccharidosis the basic pathochemical disorder is a congenital defect of the enzymes, giving rise not only to the hyperactivity of synthesizing enzymes, but to the dysfunction of those involved in breakdown. Theoretically, the decrease of hyaluronidase activity could even be due to the formation of iso-enzymes the activity of which remains below that of normal hyaluronidase.

The reduced urinary hyaluronidase activity in mucopolysaccharidosis

TABLE II

Hyaluronidase activity in the urine

	Sex	Age years	Daily urine volume ml	Mucopolysaccharide			
				Total MPS, mg	HA, mg	CSA, mg	CSB, mg
16	male	6	690	24.2	—	22.3	1.3
			620	20.5	—	19.5	0.5
			700	18.0	—	11.5	2.5
17	female	8	1140	116.3	—	7.7	1.4
			960	92.0	—	6.0	8.0
18	female	9	850	58.3	—	11.0	0.3
19	female	$^{4}/_{12}$	90	30.1	—	26.2	2.1
		$^{9}/_{12}$	130	28.4	—	24.0	3.4
			110	39.2	—	23.5	13.6
		$^{11}/_{12}$	190	19.5	—	12.5	4.5
			180	22.0	—	12.1	7.0
			220	29.0	—	12.5	12.5
20	female	1	200	34.0	—	13.5	7.5
		$1\ ^{1}/_{2}$	260	24.0	—	8.0	5.0
			280	39.0	—	4.5	15.0
21	female	4	410	32.0	—	7.8	—
22	male	13	1070	190.0	—	20.0	141.0
			980	230.0	—	40.0	161.0
			1150	209.0	—	32.0	137.0
23	female	10	940	45.5	—	14.5	12.0
24	male	3	430	26.0	—	5.5	11.5
		4	440	18.5	—	7.0	6.0
			460	32.0	—	5.0	13.0
25	male	7	680	41.0	29.0	12.0	—
			660	74.0	60.0	14.0	—
26	female	5	590	19.2	—	7.2	—
27	male	9	910	72.0	—	7.0	23.0
			840	74.5	—	7.5	26.5
			890	96.0	—	5.0	29.0
28	female	5	550	25.0	—	9.0	—
29	female	6	640	40.5	—	12.0	0.5
			620	45.0	—	13.5	—
30	female	$^{8}/_{12}$	120	29.3	2.1	13.2	8.0
		$^{9}/_{12}$	105	24.0	—	11.5	6.5
			170	21.0	1.5	6.5	4.5
		1	190	31.0	2.0	10.5	6.5

Abbreviations:

MPS: mucopolysaccharide
HA: hyaluronic acid
CSA: chondroitin sulphate A+C
CSB: chondroitin sulphate B
HS: heparitin sulphate
KS: keratosulphate

of mucopolysaccharidosis patients

excreted by urine		Unknown	Diagnosis	Hyaluronidase activity		Note
HS, mg	KS, mg			MCPE	μg/10 ml	
0.6	—	—		16	11	
0.5	—	—	Hunter	64	16	Prednisolone
4.0	—	—		100	19	
107.2	—	—	Sanfilippo	2	0	Vitamin A
78.0	—	—		4	0	
47.0	—	—	Sanfilippo	2	0	—
1.7	—	—		8	12	
1.0	—	—		4	5	
2.1	—	—	Hunter	16	12	
2.5	—	—		16	7	Prednisolone
2.9	—	—		8	9	Prednisolone
4.0	—	—		16	10	Prednisolone
13.0	—	—		100	19	
11.0	—	—	Hunter	64	21	
19.5	—	—		64	19	Vitamin A
24.2	—	—	Sanfilippo	8	0	—
29.0	—	—		64	12	Azathioptine*
29.0	—	—	Hurler	16	4	Prednisolone
40.0	—	—		16	6	
—	19.0*	—	Morquio	150	37	—
14.5	—	—	Hunter +	64	13	—
5.5	—	—	Glycogenosis III.	64	12	
14.0	—	—		50	12	—
—	—	—	Dyggve	64	18	—
—	—	—		128	27	Vitamin A
12.0	—	—	Sanfilippo	64	17	—
42.0	—	—		100	14	
41.0	—	—	Hunter	100	15	—
62.0	—	—		32	10	Vitamin A
—	16.0*	—	Morquio	128	29	—
2.0	—	26.0**	Öckerman	200	32	—
3.5	—	28.0		250	32	Vitamin A
6.0	—	—		32	9	
6.0	—	—	Hunter?	50	10	—
8.5	—	—		50	6	
12.0	—	—		64	9	

* hexosamine content of isolated keratosulphate fraction, mg.
** mannose content of isolated fraction, mg.

suggests a deficient production of hyaluronidase and, also, that the accumulation of mucopolysaccharides in the tissues is due, in addition to their increased formation, also to their insufficient metabolic breakdown.

References

1. Abramson, C., Friedman, H.: Direct localisation and visualisation of hyaluronate lyase activity by agar-gel electrophoresis. Proc. Soc. exp. Biol. (N. Y.) **125,** 256 (1967).
2. Austin, J.: cit.: Badoual [3].
3. Badoual, J.: Les mucopolysaccharidoses. Presse méd. 74 : 2835 (1966).
4. Bona, C., Stanescu, V., Streja, D., Ionescu, V.: Histochemical and histoenzymatical study of tibial growing cartilage in Hurler's syndrome. Acta histochem. (Jena). **23**:231 (1966).
5. DiFerrante, N.: Quantitative colorimetric assay of acid mucopolysaccharides. J. biol. Chem. **209**:579 (1954).
6. Diferrante, N., Rich, C.: The determination of acid mucopolysaccharides in urine. J. Lab. clin. Med. 48:91 (1956).
7. Greiling, H., Stuhlsatz, H. W., Eberhard, T.: Zur Heterogenität der Hyaluronatlyase. Hoppe Seylers Z. physiol. Chem. **340,** 243 (1965).
8. Józsa, L.: A mucopolysaccharidosisok laboratóriumi és histochemiai diagnosztikája. Kórházi Évkönyv, Kecskemét 1967.
9. McClean, D.: Biochem. J. **37,** 169 (1943). cit.: Abramson [1].
10. Orlikowska, W.: Hyaluronidase egészségesek és betegek vizeletében. Orv. Hetil. **103,** 2196 (1962).
11. Öckerman, P.: A generalised storage disorder resembling Hurler's syndrome. Lancet **2,** 239 (1967).
12. Szabó, L., Polgár, J., Vass, Z., Józsa, L.: A Hurler's syndrome variant. Lancet **2,** 1314 (1967).

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