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Characterization of Dermatan Sulfate and Heparan Sulfate in the Urine of a Patient with the Hunter Syndrome

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KIMURA, A., HAYASHI, S. and TSURUMI, K. *Characterization of Dermatan Sulfate and Heparan Sulfate in the Urine of a Patient with the Hunter Syndrome.* Tohoku J. exp. Med., 1980, **131** (3), 227-239 —— Glycosaminoglycan isolated from the urine of a patient with the Hunter syndrome was composed of heparan sulfate (59.9%), dermatan sulfate (30.6%) and chondroitin sulfate (9.5%), and was heterogeneous in molecular weight (1,500–10,000) and in sulfate content (0.35–2.05 moles/mole of hexosamine). About 60% of dermatan sulfate and 10% of heparan sulfate had molecular weight of 7,000 to 10,000, while about 10% of the former and 60% of the latter had those of 1,500 to 3,500. Sulfate contents of dermatan sulfate and heparan sulfate were inversely related to their molecular weights. Higher total- and N-sulfate contents were measured in smaller molecular-weight heparan sulfate, and higher acetyl content was in larger molecular-weight heparan sulfate. On the basis of the chemical properties of dermatan sulfate and heparan sulfate isolated in this experiment, their catabolic processes in the Hunter syndrome were discussed. ——— mucopolysaccharidosis; Hunter syndrome; urinary glycosaminoglycans; heparan sulfate; dermatan sulfate

In 1952 Brante first demonstrated that the accumulation of glycosaminoglycans (GAG) in tissues of patients with "Hurler syndrome" (the old term, "gargoylism") is one characteristic feature of this disease. In 1957 Dorfman and Lorincz discovered that patients with this syndrome excreted abnormal GAG in excess. Since then a number of studies were done on the nature of urinary GAG in patients with this syndrome as well as in healthy persons. With the progress of the study, a new finding on the pattern of the urinary GAG was given by Terry and Linker (1964): they reported that there existed four distinct types of the excretion pattern of the urinary GAG among the patients who were diagnosed as "Hurler syndrome". In 1965 McKusick et al. proposed a classification of mucopolysaccharidosis (MPS) on the basis of clinical features, genetics and the pattern of excessive urinary GAG. The cause of the metabolic error in all of the MPS has recently been known to be due to the deficiency of the specific lysosomal enzyme which is required to break down GAG. A new classification of MPS, including the enzymatic aspect, has been proposed in 1972 by McKusick.

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The separation and the identification of urinary GAG are important for diagnosing the type of MPS and also for the study of catabolism of GAG.

The present study has aimed to characterize the chemical composition of the total urinary GAG excreted by a patient with the Hunter syndrome (MPS type II).

A Brief Note on Clinical Features of the Present Patient

The patient was a four-year-old Japanese boy. Hypertelorism was noticed at birth and a deformed thorax and vertebra at one year of age. Clinical examination showed a coarse face with short neck, funnel chest, kyphosis at a dorso-lumbar region, limitation of joint movement, clawhands and hepatomegaly but not splenomegaly. The ears located at the lower position in comparison with normal persons. Nasal obstruction, speech disturbance and deafness were present. No corneal clouding was observed. He was slow in action and rather unconcerned at the surrounding events. His IQ was 56.

x-Ray examination revealed the existence of the deformity of the vertebral bodies. The Reilly granulations were not demonstrated in the bone marrow cells.

The urinary analysis showed that the cetylpyridinium chloride (CPC)-precipitable GAG level was 72 mg/day (as uronic acid value). The major urinary GAG consisted of dermatan sulfate (DS) and heparan sulfate (HS) in a ratio of about 4:3.

Experimental

Analytical procedure. Total uronic acid was determined by the carbazole reaction of Dische (1947) and by its modification of Bitter and Muir (1962) as well as by the orcinol reaction (Khym and Doherty 1952). The uronic acid at the reducing end of GAG molecule was assayed by the method of Milner and Avigad (1967). Glucosamine, galactosamine and amino acids were analyzed by the use of an automatic amino acid analyzer (JLC–5AH, JEOL, Tokyo), and total hexosamine was by the Blix modification (1948) of the Elson-Morgan method. For the analysis of amino acids, the material was hydrolyzed with 6 N hydrochloric acid at 110°C for 22 hr in a sealed tube, and for the one of hexosamines with 2 N hydrochloric acid at 100°C for 14 hr. Total sulfate was determined by the barium chloride-gelatin method of Dodgson and Price (1962), and N-sulfate (or more precisely, non-acetylated hexosamine) by the procedure of Margolis and Atherton (1972) using a heparin standard of known glucosamine content. N-acetyl group was analyzed by the gas-liquid chromatographic method of Nagai and Watanabe (1969). Specific optical rotation was measured in a 1-cm cell with DIP-180 automatic polarimeter (Japan Spectroscopic Co., Ltd., Tokyo). For identification and determination of chondroitin sulfate (CS) and DS, they were digested with chondroitinase-AC II and -ABC, and resulting 4,5-unsaturated glucuronyl residue was measured by the TBA method of Koseki et al. (1978).

Isolation and fractionation of the urinary GAG. The urine was pooled from the patient and stored frozen at –15°C until used. The urine specimen was concentrated to about one-tenth of the original volume in vacuo at 40°C. After centrifugation, the clear supernatant was desalted by passing through a column of Sephadex G-10. The excluded portion was concentrated to 2% of the original volume of the urine. Calcium acetate and acetic acid were added to the concentrate to give 2.5% and 0.5 N, respectively, and then four volumes of ethanol were added to it. After standing overnight in a refrigerator, the precipitate was collected by centrifugation and was redissolved in a solution of 5% calcium acetate–0.5 N acetic acid. Two fractions were obtained by addition of ethanol to the solution up to 25% and 80% (Meyer et al. 1953). As the 25% ethanol-precipitate did not contain uronic acid, only the 80% ethanol-precipitate was used for the further experiment.

The 80% ethanol-precipitate was applied to a Dowex 1–X2 (Cl^-, 200–400 mesh) column after passing through a column of Dowex 50W–X2 (H^+, 200–400 mesh). Uronic acid-containing substances were eluted with 0.3, 0.5, 0.8, 1.0, 1.25, 1.5 and 2.0 M sodium chloride.

All the fractions, except the fraction eluted with 0.3 M sodium chloride, were separately concentrated in vacuo, and chromatographed on a Sephadex G–50 (fine) column. The fractions obtained were desalted by filtration through Sephadex G–10 gel and precipitated with 10 volumes of sodium acetate-containing ethanol.

Electrophoresis. Electrophoresis on a cellulose acetate sheet (Separax, Jookoo Sangyo Co., Ltd., Tokyo) was carried out under the following two conditions: in 0.075 M barium acetate at a constant current of 1 mA/cm for 1 hr (Kimura et al. 1974), and in 0.1 M veronal–0.15 M ammonium hydroxide at 0.5 mA/cm for 25 min (Kimura and Tsurumi 1969). After the electrophoresis, the sheet was stained with 0.1 % toluidine blue and washed with 1% acetic acid. The components separated by the electrophoresis in the barium acetate were quantified by measuring the color-intensity spectrophotometrically (Kimura et al. 1974).

Determination of molecular weight. The average molecular weight of fractionated GAG was estimated by gel filtration according to the method of Wasteson (1971) using Sephadex G–50 and G–100. For calibration of the gel column, oligosaccharides derived from CS were used. The oligosaccharides were prepared by partial digestion of chondroitin 4-sulfate (super special grade, Seikagaku Kogyo Co., Ltd., Tokyo) with testicular hyaluronidase followed by fractionation on a Sephadex G–50 column. Their molecular weights were calculated from the ratio of 4,5-unsaturated glucuronyl N-acetylgalactosamine 4-sulfate to glucuronyl N-acetylgalactosamine 4-sulfate after digestion with chondroitinase AC II. These disaccharides were separated from each other on a column of Dowex 1–X2 (Cl^-, 200–400 mesh); the saturated disaccharide was eluted with 0.5 M sodium chloride and the unsaturated one was with 0.8 M sodium chloride. They were monitored by the carbazole reaction and UV-absorption at 232 nm. The details of the procedure will be described in the following paper (Kimura et al. 1980).

Results and Discussion

Isolation and fractionation of urinary GAG. Our preliminary experiment showed that GAG with molecular weight under about 3,000 was not completely precipitated with CPC or cetyltrimethylammonium bromide in urine, and the recovery rate of such low molecular weight GAG varied depending on the experimental conditions. The loss of such low molecular GAG occurred also during dialysis through cellophane membrane. In the present investigation, the urine was desalted by gel filtration through Sephadex G–10 to avoid such a loss of GAG with low molecular weight.

For isolation of GAG, the fractional ethanol precipitation method was applied to the desalted urine, and 1.5 g of brownish powder was obtained from 5.2 liters of urine as the 25% ethanol-precipitable fraction, i.e., "chondroitin sulfate B" (DS) fraction of Meyer et al. (1953). However, no uronic acid was detected in the fraction. This unexpected result may be due to the low molecular weight of the urinary DS and to the contamination of impurities, because after removal of non-GAG components, DS in higher molecular-weight fractions was precipitated (51% of DS in the 1.5 M–1 and 85% in the 2.0 M–1), but DS in lower molecular-weight fractions (1.5 M–2, –3, and 2.0 M–3) was not precipitated under the same condition.

The GAG contained in the 80% ethanol-precipitate (9.8 g from 5.2 liters of the urine) was fractionated on a column of Dowex 1. The distribution of GAG among the 0.5, 0.8, 1.0, 1.25, 1.5, and 2.0 M fractions was 8.7, 9.4, 12.2, 22.4, 19.4, and

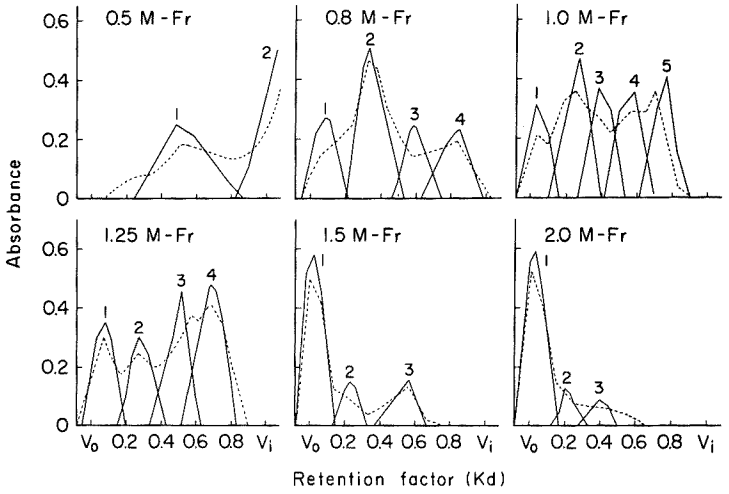

Fig. 1. Gel filtration patterns of the GAG preparations fractionated by Dowex 1 column chromatography.
A column (2.6×100 cm) of Sephadex G-50 (fine) was used for the chromatography, and an aliquot of 10 ml-fractions was analzyed for uronic acid. ....., before fractionation; ——, after fractionation into subfractions (1–5).

TABLE 1. *Analysis and identification*

Fraction	Yield* (mg)	UA†	Sulfate†			Acetyl†
			Total	N-S	O-S‡	
0.5M-1	52	0.56	0.08			
2	200	1.44	0.73			
0.8M-1	38	1.06	0.42	0.17	0.25	0.95
2	145	1.24	0.35	0.23	0.12	0.96
3	40	1.05	0.72	0.30	0.42	0.51
4	126	1.32	1.88	0.55	1.33	0.31
1.0M-1	52	1.69	0.65	0.26	0.39	0.70
2	87	1.41	0.72	0.30	0.42	0.87
3	42	1.43	0.95	0.48	0.47	0.67
4	73	1.45	1.37	0.64	0.73	0.53
5	101	1.27	1.77	0.69	1.08	0.27
1.25M-1	43	1.34	0.88	0.14	0.74	0.95
2	36	1.26	1.06	0.30	0.76	0.69
3	70	1.34	1.37	0.55	0.82	0.58
4	123	1.32	2.05	0.47	1.58	0.40
1.5M-1	123	1.08	0.81	0.02	0.79	1.02
2	27	0.94	1.21	0.05	1.16	1.02
3	90	1.19	1.83	0.55	1.28	0.59
2.0M-1	237	0.90	1.08	0	1.08	1.05
2	59	1.01	1.32	0	1.32	1.15
3	35	0.99	1.47	0.06	1.41	0.82

* Obtained from 5.2 liters of the urine specimen

† Molar ratio with hexosamine as 1.00

‡ Calculated as the difference between total sulfate and N-sulfate contents

§ Ratio of uronic acid values determined by the carbazole method of Dische (C) and by the orcinol method (O)

27.9% of the total, respectively. Although the 0.3 M fraction gave a positive uronic acid-reaction, it was not investigated further, since it was composed only of low molecular substances (M.W.<1,000) and showed no metachromatic reaction with toluidine blue.

Fig. 1 demonstrates the gel filtration pattern of the fractions separated by the Dowex 1 column chromatography. In the 0.5 M fraction, most of the substances appeared in the inner volume (V_i), while most of the substances in the 1.5 M and 2.0 M fractions appeared in the void volume (V_0). In the 0.5 M fraction, the substances eluted before V_i were very small in amount, therefore they were combined in one fraction. Other fractions were divided into three to five subfractions depending on their elution patterns.

Identification of the fractionated GAG. Table 1 summarizes the analytical data and the results of identification of the fractionated GAG. For identification and determination of the individual GAG, the electrophoretic method was used as well as chemical analyses. The electrophoretic patterns are shown in Fig. 2.

The 0.5 M-1 fraction was a mixture of substances different in molecular weight and in chemical component. The molecular weight was distributed from 1,000 to 5,000 with 3,000 as a prominence. The chemical analyses showed that the

of fractionated GAG

GlcN: GalN	C/O_S	$[\alpha]_D$ (degrees)	M.W. ($\times 10^3$)	Identification//		
				HS	DS	CS
70.8: 29.2	0.57	− 9	(3.0)			
83.0: 17.0	1.09		(<1.0)			
92.0: 8.0	1.28	+27	8.0	92	0	8
91.3: 8.7	1.11	+50	5.0	91	0	9
100	1.15	+37	2.8	100	0	0
100	0.96	+13	1.8	100	0	0
90.8: 9.2	1.20	+90	8.9	91	0	9
90.2: 9.8	1.62	+75	5.3	90	0	10
96.4: 3.6	1.53	+70	4.0	96		?
95.3: 4.7	1.35	+58	2.7	95		?
99.2: 0.8	0.77	+42	1.8	99		?
30.1: 69.9	1.27	+11	8.9	30	0	70
64.7: 35.3	1.35	+29	5.2	65	0	35
74.0: 26.0	2.22	+47	3.4	74	13	13
100	1.18	+23	2.4	100	0	0
100	0.53	−31	8.9	0	47	53
6.0: 94.0	0.42	−34	5.3	6	94	0
43.8: 56.2	1.06	+ 2	2.9	44	56	0
100	0.30	−61	10.0	0	100	0
100	0.21	−49	5.3	0	100	0
7.3: 92.7	0.41	−32	3.8	7	93	0

// HS, DS, and CS were identified and determined by chemical, enzymatic and electrophoretic analyses, and their amounts were expressed as percentage of the total GAG in each fraction. ? Not identified

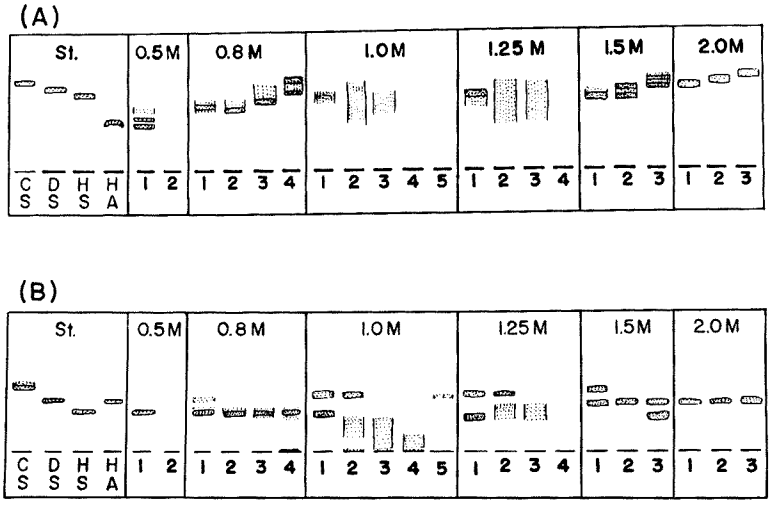

Fig. 2. Electrophoresis of the fractionated GAG.
(A), in 0.1 M veronal–0.15 M NH_4OH at a constant current of 0.5 mA/cm for 25 min; (B), in 0.075 M barium acetate at a constant current of 1 mA/cm for 1 hr; 0.5 M —2.0 M, concentration of the eluting solution on the Dowex 1 chromatography; 1–5, subfraction number (see Fig. 1); St., GAG standard; CS, chondroitin 4-sulfate; DS, dermatan sulfate; HS, heparan sulfate; HA, hyaluronic acid.

preparation contained hexose and sialic acid as well as peptide other than GAG components, and that the purity as GAG was less than 50%. By the electrophoresis in the barium acetate, only one band corresponding to the authentic HS was detected after staining with toluidine blue. This substance seemed to be under-sulfated HS, because its electrophoretic mobility in the veronal-ammonium hydroxide was lower than that of authentic HS, and because the sulfate content was very low compared to the uronic acid content. In addition to the HS, about 8% of chondroitinase AC-digestable GAG was determined. No DS was detected in this fraction.

Although 0.5 M–2 fraction exhibited no metachromatic band on electrophoresis, the chemical and enzymatic analyses indicated that oligosaccharide derived from HS was a predominant component in this fraction.

All the subfractions in 0.8 M and 1.0 M fractions were composed almost completely of HS with various molecular weights. The subfractions in 1.0 M fraction were noticed to have higher sulfate content, lower acetyl content and higher positive optical rotation in comparison with the corresponding subfractions in 0.8 M fraction.

The 1.25 M fraction contained HS and CS; the subfractions with larger molecular weight was rich in CS and ones with smaller molecular weight was rich in HS. The CS in this fraction migrated with the same mobility as the authentic CS on electrophoresis in the barium acetate, but CS in the 0.8 M and 1.0 M fractions showed slower mobility. Ohkawa et al. (1972) reported that low-sulfated CS

moved more slowly than authentic CS on electrophoresis in barium acetate and in pyridine-formate buffer.

The 1.5 M fraction contained DS as a major component, and CS and HS as minor components. The 2.0 M fraction comprised almost only DS.

According to the above findings, the components of urinary GAG excreted by our patient with the Hunter syndrome were finally estimated as follows; HS was 59.9%, DS 30.6% and CS 9.5% of the total GAG. The different result was obtained when the distribution ratio was determined only on the CPC-precipitable GAG in the same urine specimen according to the electrophoretic method of Kimura et al. (1974). In that case the ratio of HS decreased to 35.1%, whereas those of DS and CS increased to 48.0% and 16.9%, respectively. The result may be explained by the findings that 61.8% of the HS and 10.8% of DS had molecular weight less than 3,500 as described below, and that the GAG of such low molecular weight was difficult to precipitate as the CPC-complex under our experimental conditions.

Distribution of molecular weight of HS and DS. The molecular weights of the GAG obtained in the present experiment were distributed over the range of 1,500 to 10,000. A large difference in the distribution pattern was observed between DS- and HS-fractions, when the GAG-fractions were divided into three groups according to their molecular weights. The bulk of DS was included in the group with the largest molecular weight (7,000–10,000) in contrast with HS, most of which was included in the group with the smallest molecular weight (1,500–3,500) (Fig. 3). Kindler et al. (1977) isolated HS with molecular weight 2,000 to 6,000 from organs with MPS III A, and explained the possibility of the accumulation of HS-oligosaccharides with such molecular weight from the property of the HS-specific endoglucuronidase, which was partially purified from human placenta (Klein and von Figura 1976), and was found to act in vitro only on HS-chains with

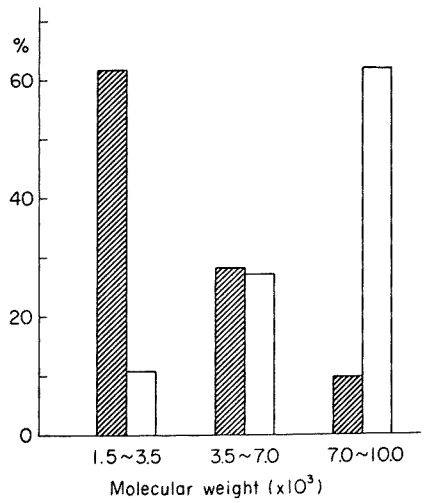

Fig. 3. Molecular-weight distribution of DS and HS.
Open column, DS; striped column, HS.

molecular weights above about 3,000 (Klein and von Figura, unpublished results, cited in Ref. 17).

Chemical characteristics of HS and DS. Of the variants of HS obtained in this experiment, the smaller molecular-weight HS had smaller amounts of acetyl and larger amounts of N- and O-sulfates. One of the smallest molecular HS which was purified from the 1.5 M–3 fraction was similar to heparin in chemical composition and had 2.6 moles of total sulfate and one mole of N-sulfate per one mole glucosamine. Knecht et al. (1967) also isolated low molecular-weight GAG similar to heparin in chemical composition from liver and urine of patients with "Hurler syndrome."

The sulfate content of DS fractions was also related inversely to the molecular weight. The relation of sulfate content to molecular weight of the preparations obtained in this experiment is demonstrated in Fig. 4. The findings suggest an uneven distribution of sulfate groups in the original polysaccharide chains of HS and DS, as shown in pig skin DS by Fransson et al. (1974).

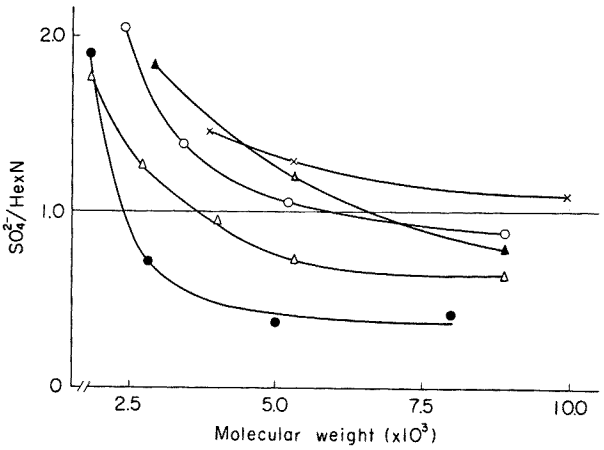

Fig. 4. Relation of sulfate content to molecular weight in various GAG fractions. ●—●, 0.8 M; △—△, 1.0 M; ○—○, 1.25 M; ▲—▲, 1.5 M; ×—×, 2.0 M fractions.

Amino acid composition. To avoid degradation of the peptide linked to GAG, the use of proteolytic enzyme or alkali was omitted from the process of isolation and purification. The contents of the total amino acid and of prominent amino acids in each fraction are listed in Table 2.

In most fractions containing amino acid less than 3% in weight, serine was obviously a predominant amino acid followed by glycine, glutamic acid and aspartic acid. The comparable amount of threonine was detected in some fractions. These amino acids may compose the peptide chain in the linkage region for a carbohydrate chain.

The total amount of amino acid in these fractions was too small to attach to all

TABLE 2. *Amino acid analysis of GAG preparations*

Fraction	Total amino acid*	Major amino acids†						
		Asp	Thr	Ser	Glu	Pro	Gly	Ala
0.5M-1	17.2	14.3	14.8	10.3	14.1	10.4	9.4	7.0
2	27.1	19.3	14.8	6.1	31.9	2.5	12.5	2.4
0.8M-1	6.9	13.2	14.2	16.0	12.0	9.0	8.2	5.1
2	12.0	11.9	13.8	11.2	15.2	8.1	12.0	6.4
3	8.5	23.4	7.5	12.5	17.3	5.0	13.2	3.8
4	9.9	20.4	4.1	7.8	27.4	3.3	17.2	2.4
1.0M-1	2.7	11.3	17.2	14.4	13.1	8.4	14.5	6.5
2	1.8	11.5	12.2	26.2	11.9	7.4	12.0	5.1
3	1.9	16.1	9.6	15.0	13.2	6.2	13.0	5.3
4	2.3	14.7	6.2	12.1	14.7	3.9	17.7	3.7
5	2.4	19.5	5.8	8.8	20.7	—	23.4	3.1
1.25M-1	2.0	7.8	12.1	22.0	10.7	12.9	13.1	6.3
2	2.0	9.6	13.8	26.3	10.9	10.0	12.3	5.3
3	1.3	11.0	9.6	17.6	14.7	7.4	16.9	7.4
4	1.2	18.5	7.8	11.3	18.2	—	23.7	3.0
1.5M-1	0.8	8.3	11.0	33.3	12.5	—	15.8	6.6
2	0.8	10.5	12.8	36.4	11.2	—	16.0	6.7
3	0.6	17.7	21.0	19.7	5.6	—	23.6	4.9
2.0M‡-1	0.4	5.4	7.2	34.8	8.1	—	16.3	6.3
2	0.6	12.5	7.5	15.9	13.4	—	25.2	7.8
3	0.6	6.5	7.2	18.8	7.6	—	13.4	4.7

* The amounts of total amino acid were expressed as per cent of dry weight.

† The values were expressed as moles of each amino acid per 100 moles of total amino acid.

‡ In addition to the amino acids listed here Lys was contained in the fractions 2.0 M-1 (27.1), 2.0 M-2 (17.8) and 2.0 M-3 (19.9), and His was in the fraction 2.0 M-3 (13.8) as major amino acids.

of the GAG molecules. Therefore, considerable GAG molecules in these fractions seemed to be free from peptide. The amino acid contents in 0.5 M and 0.8 M fractions were higher than those in the fractions discussed above. A portion of the amino acids, however, may not be related to the GAG, since serine was not predominant in these fractions except 0.8 M-1 fraction.

Speculation on the production of the HS- and the DS-oligosaccharides in the Hunter syndrome. Two endoglycosidases, i.e., endoglucosaminidase and endohexuronidase, have recently been suggested to participate in the degradation of HS in cultured human skin fibroblasts (Klein et al. 1976). One of them, endohexuronidase, was partially purified from human placenta and characterized as lysosomal endoglucuronidase acting specifically on heparan sulfate to degrade it into oligosaccharides (Klein and von Figura 1976).

The analysis of reducing terminal sugar of our HS preparations revealed that 15 to 35% of the HS had a uronic acid residue at their reducing terminal (Table 3). The result agreed with the report by Klein et al. (1976). They found uronic acid at the reducing terminal of 20 to 30% of HS which was isolated from cultured skin fibroblasts of patients with MPS type I S and type III B.

TABLE 3. *Determination of polysaccharide having a reducing hexuronic acid residue**

Subfraction	Fraction				
	0.8M	1.0M	1.25M	1.5M	2.0M
1	17	15	15	17	13
2	15	19	16	13	13
3	27	18	20	16	14
4	35	26	24		
		26			

* The data were expressed as moles per 100 moles of the polysaccharide.

Although the amount was smaller than that in the HS fractions, reducing terminal hexuronic acid was also found in the DS fractions (Table 3). The findings suggest participation of endohexuronidase(s) in the degradation process of DS.

As a preliminary experiment to estimate the contribution of endoglycosidases on the degradation of GAG in tissues, one of the DS fractions with the largest molecular weight (2.0 M–1) was subjected to exhaustive digestion with testicular hyaluronidase, which has been known to have almost the same properties as those of lysosomal hyaluronidase (Aronson and Davidson 1967). Only 6.5% of the total uronic acid in the fraction were shifted to a fraction of lower molecular weight by the digestion. On the other hand 26.2% of uronic acid of pig skin DS were shifted to a fraction with molecular weight less than 5,000 after the incubation under the same condition. The result indicates that the urinary DS excreted by patients with the Hunter syndrome may be attacked thoroughly by lysosomal hyaluronidase before excretion in urine.

After HS and DS have been attacked by endoglycosidases, the resulting oligosaccharides may be degraded by stepwise removal of monosaccharide units and sulfate groups by exoglycosidases and sulfatases as suggested by Dorfman et al. (1972). Since in the Hunter syndrome iduronosulfate (IU–S) sulfatase is deficient (Bach et al. 1973; Sjöberg et al. 1973), the catabolic pathways of DS and HS, which have been known to have IU–S residues in their molecules (Malmström and Fransson 1971; Cifonelli and King 1975), must be prevented at the step to be catalyzed by this enzyme. A possible pathway of DS catabolism is demonstrated in Fig. 5, which indicates that the accumulation of DS-oligosaccharides possessing an IU-S residue at the non-reducing terminal may occur in this disease. The assumption is consistent with the findings of Coppa et al. (1973) and of Sjöberg et al. (1973). The former isolated disulfated disaccharide containing an IU–S residue from urine of a patient with the Hunter syndrome, and the latter found the residue at non-reducing terminal of DS molecule isolated from cultured Hunter fibroblasts. We will report in the following paper our findings that the residue is a main non-reducing terminal sugar of DS molecules obtained in this experiment (Kimura et al. 1980).

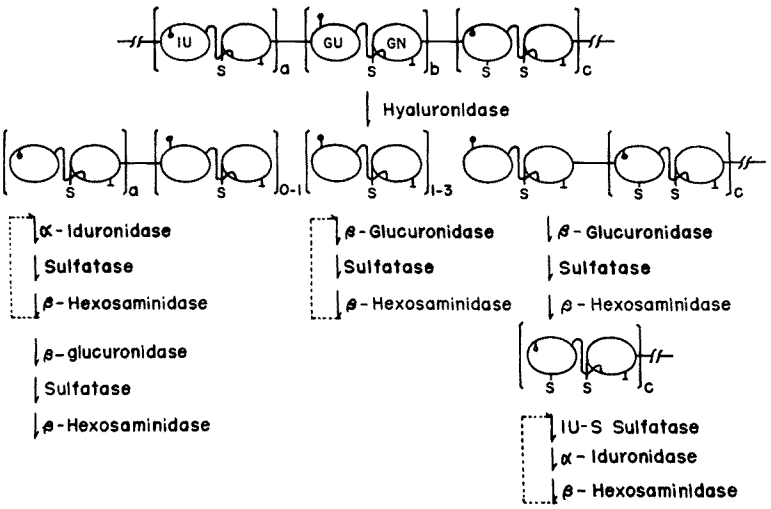

Fig. 5. A possible process of degradation of DS.
The copolymeric structure proposed by Fransson et al. (1974) has been chosen arbitrarily to illustrate the catabolic process performed by concerted action of various glycosidases and sulfatases. IU, iduronic acid; GU, glucuronic acid; GN, N-acetylgalactosamine; S, sulfate.

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