Screening Study on Excretion Pattern of Urinary Glycosaminoglycans from Orthopedic Patients

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Orthopedic Patients.* Tohoku J. exp. Med., 1982, **137** (2), 179–190 — To obtain
a clue for the metabolic disorder of glycosaminoglycans (GAG) in orthopedic
diseases, a screening study on excretion pattern of urinary GAG from orthopedic
patients was performed by the procedures of Nagatsuka et al. (1980). All the
urines examined gave three regular GAG-bands. Besides the regular bands,
some samples gave irregular bands. Of 123 cases examined in the present study,
the numbers of cases with an abnormal excretion pattern of urinary GAG in the
regular bands (A) and with the irregular band(s) (B) were as follows: 23(A) and
7(B) in 38 cases of rheumatoid arthritis (RA); 8(A) and 5(B) in 13 cases of
malignant bone tumor; 11(A) and 3(B) in 12 cases of benign bone tumor; 16(A)
and 3(B) in 25 cases of bone metabolic and connective tissue diseases; 6(A) and
4(B) in 9 cases of osteoarthritis; 6(A) and 3(B) in 8 cases of spine diseases; and
14(A) and 8(B) in 18 cases of other orthopedic diseases. The excretion patterns
of urinary GAG suggested that the metabolic rates of chondroitin sulfates tended
to elevate in most cases of (A). Also, certain metabolic disorder of dermatan
sulfate and heparan sulfate was suggested in several cases of (A). In addition,
the abnormal metabolism of acidic glycoprotein(s) was suggested in many cases
of (B). Excretion of a large amount of dermatan sulfate was elucidated in one
case (Case 20 of bone metabolic and connective tissue diseases) by digestion with
chondroitinases after the screening study. Therefore, the metabolic disorder of
dermatan sulfate was indicated in this patient. The low activity of α -L-iduronidase
in her urine supported this indication. This patient was finally diagnosed as
mucopolysaccharidosis I-S (Scheie syndrome). — glycosaminoglycan; urinary
glycosaminoglycans; orthopedic patients

To obtain a clue for the metabolic disorder of glycosaminoglycans (GAG)
in connective tissue diseases, a device of screening test for excretion pattern of
urinary GAG was achieved (Nagatsuka et al. 1980). The results of application of

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Abbreviations: GAG, glycosaminoglycans; HexN, hexosamine; UA, hexuronic acid;
 Δ Di-OS, 2-acetamido-2-deoxy-3-O-(4-deoxy- α -L-threo-hex-4-enopyranosyluronic acid)-D-galac-
tose; Δ Di-4S and Δ Di-6S, 4-sulfate and 6-sulfate, respectively, of Δ Di-OS; RA, rheumatoid
arthritis.

the screening test to normal human urines and to urines from 50 cases of orthopedic patients have been reported (Nagatsuka et al. 1980, 1981).

This paper reports the results of further screening study on excretion pattern of urinary GAG from 123 cases of orthopedic patients.

MATERIALS AND METHODS

Urines. Urines were collected separately from 123 cases of orthopedic patients without any dietary restriction as described previously (Nagatsuka et al. 1981). The cases examined are as follows: 38 cases of rheumatoid arthritis (RA); 13 cases of malignant bone tumor; 12 cases of benign bone tumor; 25 cases of bone metabolic and connective tissue diseases; 9 cases of osteoarthritis (arthritis deformans); 8 cases of spine diseases; and 18 cases of other orthopedic diseases.

Materials. Pronase P, Separax (cellulose acetate membrane), Toluidine blue, Alcian blue 8GS, and cetylpyridinium chloride were those described previously (Nagatsuka et al. 1980). Phenyl- α -L-iduronide was purchased from Zeriashinyaku Co., Tokyo. Other materials were commercial products.

Methods. Separation of crude GAG from urine, quantitation of GAG by Separax electrophoresis, quantitation of hexosamine, enzymatic digestion with chondroitinases and heparitinase were performed as described previously (Nagatsuka et al. 1980). Uronic acid was determined by the method of Bitter and Muir (1962). Protein was determined by the procedure of Lowry et al. (1951) using bovine serum albumin as a standard.

Assay for α -L-iduronidase. The activity of α -L-iduronidase was assayed by the procedure of Liem and Hooghwinkel (1975) as follows: An enzyme solution (250 μ l) prepared from urine was added to an incubation mixture of 100 μ l of 5 mM phenyl- α -L-iduronide solution and 25 μ l of 1.2 M sodium formate buffer (pH 3.5) containing 0.45 M sodium chloride and 0.3% sodium azide. After incubation for 18 hr at 25°C, 1.25 ml of Folin Ciocalteu phenol reagent was added to the incubation mixture. The mixture was centrifuged, and 1.2 ml of the supernatant was mixed with 0.3 ml of 24% sodium carbonate. After standing for 20 min at 37°C, the absorbance at 660 nm was read against the blank prepared by the same procedures without substrate. The enzyme activity was expressed as nmole phenol/mg protein/18 hr.

RESULTS AND DISCUSSION

All the urines so far examined gave three regular GAG-bands, which were designated as bands 1, 2 and 3, in the order from greatest to least of the electrophoretic mobility, as reported previously (Nagatsuka et al. 1980). However, band 1 often spread over band 2 zone as reported previously (Nagatsuka et al. 1980). In these cases, determination of hexosamine was made as bands 1+2. Besides the three regular bands, some samples gave irregular bands migrated between band 3 and origin, which were designated as bands 4, 5, 6, 7 and 8, in the order from greatest to least of the electrophoretic mobility, as reported previously (Nagatsuka et al. 1980). Excretion patterns of urinary GAG, in terms of hexosamine, in these bands were expressed as percent distribution in total. In addition, the amounts of GAG, expressed as mg/day of hexosamine and hexuronic acid, were also determined.

The components of bands 1, 2 and 3 were identified as partially desulfated chondroitin sulfates A and C, dermatan sulfate (major) plus low-sulfated chondroitin sulfates (minor) and heparan sulfate (major) plus low-sulfated chondroitin

sulfates (minor), respectively, as reported previously (Nagatsuka et al. 1980). Band 4 corresponded to that of hyaluronic acid. Bands 5, 6, 7 and 8 were suggested to contain acidic glycoproteins (Nagatsuka et al. 1980).

Excretion pattern of urinary GAG from the patients with RA

The cases examined are listed in Table 1. Diagnostic criteria for RA (Ropes

TABLE 1. *Excretion pattern of urinary GAG from the patients with rheumatoid arthritis (RA)*

Case	Name	Age	Sex	Stage	Class	Total GAG (HexN/UA)*	Distribution of GAG (as HexN) in bands†				Recovery‡
							1	2	3	Others	
1	S.B.	58	M	1	2	4.63/4.44	43	21	36		89
2	T.S.	63	M	1	2	4.30/4.19	45	20	35		85
3	K.T.	26	M	2	2	6.20/5.97		67§	33		75
4	C.S.	33	M	2	2	4.18/4.10	53	19	28		95
5	K.K.	44	M	2	2	1.89/1.75	54	15	31		100
6	T.K.	61	M	2	2	5.15/4.95		56	44		91
7	K.K.	63	M	2	2	3.13/2.96	69	6	25		78
8	H.S.	73	M	2	2	4.58/4.00	59	14	27		90
9	T.T.	69	F	2	2	7.51/4.57	49	10	22	19(5)//	97
10	K.K.	73	F	2	2	2.04/2.04	57	17	26		90
11	T.N.	62	M	3	2	3.76/3.35	55	15	30		96
12	S.N.	67	M	3	2	2.12/1.93	56	22	22		93
13	M.H.	73	M	3	2	3.15/2.98	53	22	25		88
14	E.A.	27	F	3	2	6.09/4.32	34	12	17	20(5) 17(6)	80
15	E.T.	29	F	3	2	5.37/3.09	41	15	14	20(7) 10(8)	76
16	R.Y.	38	F	3	2	3.44/3.23	57	15	28		95
17	E.H.	39	F	3	2	3.24/2.55	39	15	26	20(7)	86
18	K.H.	42	F	3	2	1.05/0.97		68	32		98
19	H.A.	60	F	3	2	2.93/2.73	62	10	28		88
20	M.T.	67	F	3	2	1.96/1.63	46	23	31		84
21	T.E.	70	F	3	2	2.60/2.58	52	20	28		98
22	T.K.	43	F	3	3	2.73/2.16	32	24	27	17(6)	93
23	F.K.	55	F	3	3	3.48/2.61	56	12	19	13(5)	82
24	R.A.	57	F	3	3	1.86/1.44	56	23	21		90
25	R.G.	66	F	3	3	3.16/2.81	39	22	39		79
26	T.S.	29	M	4	2	2.97/2.89	56	16	28		97
27	Y.O.	50	M	4	2	4.10/3.49	56	14	30		84
28	K.K.	27	F	4	3	1.58/1.41		61	39		81
29	S.N.	42	F	4	3	5.35/4.50	45	24	31		91
30	S.C.	60	F	4	3	2.48/2.22		64	36		86
31	F.K.	65	F	4	3	1.99/1.53	57	14	29		94
32	K.S.	69	F	4	3	4.06/3.80	68	10	22		72
33	K.Y.	41	F	4	4	3.37/2.96		68	32		102
34	M.C.	49	F	4	4	1.06/0.96		69	31		97
35	M.T.	50	F	4	4	1.96/1.41		54	46		89
36	M.O.	53	F	4	4	3.57/3.27		57	43		91
37	Y.S.	59	F	4	4	3.72/3.20	46	18	36		89
38	T.N.	53	F	(Malignant RA)		3.10/2.05	38	21	21	20(8)	75

* Expressed as mg/day of hexosamine and hexuronic acid. † Expressed as percent distribution in total. ‡ Percent recovery from the crude GAG applied on Separax. § Bands 1+2. // Name of the other bands.

TABLE 2. *Excretion of urinary GAG, in terms of hexosamine and hexuronic acid, from the patients with RA with stage*

Stage	Number of Cases	HexN (mg/day)	UA (mg/day)
1	2	4.47*	4.32*
2	8	4.34 $\pm$ 1.96†	3.79 $\pm$ 1.45†
3	15	3.13 $\pm$ 1.29	2.63 $\pm$ 1.01
4	11	2.95 $\pm$ 1.29	2.59 $\pm$ 1.15

* Mean values. † Standard deviation.

et al. 1958) of all these cases were definite or classical. Stage and class of these cases defined by the classification of Steinbrocker et al. (1949) are described in Table 1. As shown in Table 1, 7 cases gave irregular bands. Of 38 cases, 23 cases showed abnormal excretion patterns of urinary GAG as compared with the normal range of percent distribution of bands 1, 2 and 3 (Nagatsuka et al. 1980). In these cases, the proportion of band 1 tended to increase, whereas the opposite was the case in bands 2 and 3. This finding suggested that the metabolic rates of chondroitin sulfates tended to elevate in these cases.

The amounts of urinary GAG from the patients with RA in the earlier stages (stages 1 and 2) were larger than those in the later stages (stages 3 and 4) (Table 2), suggesting that GAG in RA were more actively metabolized in the former than in the latter. However, the proportion of bands 1, 2 and 3 of stages 1+2 ($n=8$) was 55.0 ± 8.37 , 15.5 ± 4.93 and 29.5 ± 4.10 , respectively, while that of stages 3+4 ($n=20$) was 53.9 ± 7.39 , 18.4 ± 4.95 and 27.6 ± 4.57 , respectively. This observation suggests that the proportion of the relative metabolic rates of chondroitin sulfates, dermatan sulfate and heparan sulfate in RA does not change significantly throughout the stages.

Although several investigators (Di Ferrante 1957; Loewi 1959; Wessler 1967; Ishikawa 1970) reported an increase of urinary GAG in RA, no significant increase of urinary GAG was found in the present cases of RA as compared with normal cases (Ohkawa et al. 1972).

Excretion pattern of urinary GAG from the patients with malignant bone tumor

The cases examined are listed in Table 3. Five cases gave irregular bands, and 8 cases showed abnormal excretion patterns of GAG as compared with the normal range of percent distribution of the regular three bands (Nagatsuka et al. 1980).

Case 4 of osteosarcoma excreted a large amount of urinary GAG and gave an irregular band. The data suggested elevation of the metabolic rates of GAG and acidic glycoprotein in this patient, although certain disorder in renal function could not be excluded. Alteration of the proportions of the regular bands of Case 7 of chondrosarcoma was the most prominent among 13 cases. Judging from the data, conspicuous elevation of the metabolic rates of chondroitin sulfates and a significant depression of that of dermatan sulfate were conceivable in this patient. In addition, the presence of band 5 suggested an abnormal metabolism of acidic

TABLE 3. *Excretion pattern of urinary GAG from the patients with malignant bone tumor*

Case	Name	Age	Sex	Diagnosis (site)	Total GAG (HexN/UA)*	Distribution of GAG (as HexN) in bands†				Recovery‡
						1	2	3	Others	
1	K.T.	13	M	Osteosarcoma (Femur)	3.52/2.96	75§		25		90
2	K.S.	16	M	Osteosarcoma (Femur)	2.89/2.90	58	16	26		88
3	Y.S.	22	F	Osteosarcoma¶ (Femur)	3.24/2.00	42	17	24	17(6)//	80
4	S.K.	56	F	Osteosarcoma (Femur)	21.19/19.28	63	26	11(5)		91
5	M.O.	13	F	Osteosarcoma (Spine)	4.33/4.23	64	14	22		89
6	S.C.	22	F	Osteosarcoma¶ (Sacrum)	2.97/2.69	59	13	28		78
7	Y.S.	5	F	Chondrosarcoma (Femur)	6.02/5.44	66	5	11	17(5)	101
8	J.S.	17	F	Chondrosarcoma (Femur)	2.75/1.40	34	10	23	$\begin{cases} 13(5) \\ 7(6) \\ 13(8) \end{cases}$	90
9	S.N.	56	F	Chondrosarcoma (Pubic bone)	7.80/7.40	35	22	43		102
10	N.K.	12	F	Ewing's sarcoma (Femur)	2.54/1.50	50	11	15	$\begin{cases} 10(4) \\ 14(5) \end{cases}$	89
11	Y.H.	18	M	Undifferentiated sarcoma (Fibula)	4.66/4.33	63	19	18		86
12	M.H.	18	M	Malignant lymphoma (Clavicle)	1.89/1.48	59	10	31		99
13	N.A.	77	F	Metastatic bone tumor (Tibia)	5.36/4.56	60	8	32		96

*, †, ‡, §, // See Table 1. ¶Lung metastasis was observed.

glycoprotein in this patient. On the other hand, the proportion of band 3 of Case 9 of chondrosarcoma was relatively higher than those of others. Therefore, an elevation of the metabolic rate of heparan sulfate might be conceivable in this patient. This patient had wide invasion of the tumor into the surrounding tissues.

Excretion pattern of urinary GAG from the patients with benign bone tumor

The cases examined are listed in Table 4. Three cases gave irregular bands. Of 12 cases, 11 cases showed abnormal excretion patterns of urinary GAG different from the normal range of percent distribution of the regular three bands (Nagatsuka et al. 1980). In these cases, the proportion of band 1 increased and that of band 2 decreased, suggesting elevation of the metabolic rates of chondroitin sulfates and a depression of that of dermatan sulfate.

Excretion pattern of urinary GAG from the patients with bone metabolic and connective tissue diseases

The cases examined are listed in Table 5. Three cases gave irregular bands and 16 cases showed abnormal excretion patterns of urinary GAG as compared with

TABLE 4. *Excretion pattern of urinary GAG from the patients with benign bone tumor*

Case	Name	Age	Sex	Diagnosis (Site)	Total GAG (HexN/UA)*	Distribution of GAG (as HexN) in bands†				Recovery‡
						1	2	3	Others	
1	S.T.	33	M	Giant cell tumor (Femur)	3.50/3.30	47	15	38		93
2	K.H.	22	F	Giant cell tumor (Femur)	4.38/4.14	43	8	33	16(5)//	99
3	K.O.	23	F	Giant cell tumor (Femur)	1.80/1.50	62	11	27		101
4	M.S.	36	M	Giant cell tumor (Femur)	3.03/2.90	49	12	39		95
5	S.Y.	18	M	Osteoid osteoma (Tibia)	5.63/5.27	55	9	26	10(6)	91
6	T.O.	49	M	Osteoid osteoma (Tibia)	4.23/3.10	50	10	28	3(4) 9(7)	88
7	S.A.	16	M	Bone cyst (Femur)	5.30/4.10	52	23	25		85
8	K.C.	38	M	Bone cyst (Femur)	3.80/2.65	53	18	29		90
9	Y.M.	9	M	Fibrous dysplasia (Femur, Tibia)	1.76/1.73	58	17	25		85
10	C.S.	7	F	Fibrous dysplasia (Tibia)	2.83/2.81	88§		12		98
11	T.U.	20	F	Metaphyseal cortical defect (Tibia)	5.77/5.25	58	16	26		97
12	A.K.	13	F	Multiple cartilaginous exostosis	8.33/8.07	70	14	16		89

*, †, ‡, §, // See Table 1

the normal range of percent distribution of the regular three bands (Nagatsuka et al. 1980). Of these cases, following cases are worthy of being noted.

Paget's disease and related one. Cases 10, 11 and 12 were Paget's disease and related one. A large amount of GAG was excreted into urine of Case 10. The proportion of band 1 of Cases 10 and 12 exceeded 80% and those of bands 2 and 3 decreased remarkably. However, the proportion of band 2 to band 3 in these cases were similar to those in the normal cases (Nagatsuka et al. 1980). These observations suggested marked elevation of the metabolic rates of chondroitin sulfates in these cases. Similar excretion pattern of urinary GAG was found in Case 5 of juvenile osteoporosis.

Werner's syndrome. We reported previously an abnormal excretion of hyaluronic acid into urine of the patients with Werner's syndrome (Tokunaga et al. 1975). Of two cases of Werner's syndrome in the present study, two irregular bands were observed in Case 13, in which band 4 corresponded to that of hyaluronic acid. However, no irregular band was found in Case 14, although the proportion of band 1 increased and that of band 2 decreased. These observations suggest various abnormalities may exist in the metabolism of GAG in the patients with Werner's syndrome.

TABLE 5. *Excretion pattern of urinary GAG from the patients with bone metabolic and connective tissue diseases*

Case	Name	Age	Sex	Diagnosis	Total GAG (HexN/UA)*	Distribution of GAG (as HexN) in bands†				Recovery‡
						1	2	3	Others	
1	T.T.	54	F	Osteoporosis	4.26/3.86	48	19	34		91
2	Y.M.	60	F	Osteoporosis	2.81/2.48	47	15	38		89
3	T.S.	62	F	Osteoporosis	5.80/N.D.¶	47	23	30		95
4	M.T.	12	M	Juvenile osteoporosis	5.25/5.08	89§		11		76
5	K.A.	12	F	Juvenile osteoporosis	8.16/N.D.	87	4	9		82
6	M.Y.	33	M	Hypogonadal osteoporosis	4.90/4.67	42	7	25	26 (5)//	89
7	K.S.	43	F	Cushing osteoporosis	3.40/N.D.	56	22	22		99
8	T.S.	12	M	Juvenile rheumatoid arthritis	6.55/6.06	87		13		93
9	M.C.	14	F	Juvenile rheumatoid arthritis	5.81/5.30	46	11	43		99
10	K.O.	14	M	Juvenile Paget's disease	17.43/15.89	81	7	12		80
11	K.K.	33	M	Paget's disease with osteosarcoma	3.76/3.76	53	19	28		98
12	A.Y.	21	F	Hyperphosphatasia	4.20/3.64	83	7	10		88
13	C.M.	44	M	Werner's syndrome	3.97**/3.20**	41	13	20	{16 (4) 11 (6)}	76
14	C.S.	32	F	Werner's syndrome	4.27/3.93	57	13	30		92
15	T.S.	8	M	Stickler's syndrome	2.47/2.04	63		37		89
16	E.I.	14	M	Osteopathia striata	7.50/N.D.	61	20	19		101
17	H.W.	27	M	Achondroplasia	4.90/4.40	80		20		98
18	T.T.	31	M	Melorheostosis	2.35/1.87	67		33		78
19	S.K.	54	M	Osteomalacia	3.32/2.73	38	26	36		89
20	S.M.	8	F	Mucopolysac- charidosis I-S	16.97/15.07	18	61	21		96
21	Y.T.	14	F	A syndrome of progressive muscle spasm	1.71/1.48	44	26	30		91
22	T.A.	21	F	Osteogenesis imperfecta	1.25/N.D.	50	6	16	18 (6)	105
23	M.T.	28	F	Multiple epiphyseal dysplasia	2.20/1.82	49	20	31		80
24	N.S.	50	M	Unknown	3.78**/3.19**	44	21	35		97
25	Y.O.	50	F	Unknown	2.05/1.76	41	18	41		91

*, †, ‡, §, // See Table 1. ¶Not determined.

** Expressed as mg/liter.

Mucopolysaccharidosis I-S (Scheie syndrome). Since clinical observations on Case 20 suggested a mucopolysaccharidosis, she was studied comprehensively. A large amount of urinary GAG was found. Since the proportion of band 2 was 61%, the metabolic disorder of dermatan sulfate was conceivable in this patient. Electrophoretograms of urinary GAG of Case 20 before and after digestion with chondroitinases ABC and AC-II are shown in Fig. 1. Although bands 1 and 2 disappeared after digestion with chondroitinase ABC, band 2 remained after digestion with chondroitinase AC-II. The digestion products were examined by paper chromatography according to the procedures of Saito et al. (1968) and Suzuki et al.

Fig. 1. Electrophoretograms of urinary GAG of Case 20 (S.M.) in Table 5 before and after digestion with chondroitinase ABC and chondroitinase AC-II. Electrophoresis was carried out on Separax in 0.2 M aqueous calcium acetate at 0.5 mA/cm for 4.5 hr. GAG were stained with Toluidine blue. ABC, Digest of chondroitinase ABC; AC, digest of chondroitinase AC-II; R, references; CC, chondroitin sulfate C; CA, chondroitin sulfate A; DS, dermatan sulfate; HS, heparan sulfate; 1, band 1; 2, band 2; 3, band 3.

(1968), using *n*-butanol-0.5 M ammonia (5:3, v/v) solvent for 48 hr at 25°C. The substances were located by staining with alkaline-silver reagent. The results indicated the presence of Δ Di-4S as the major unsaturated disaccharide together with Δ Di-6S and Δ Di-0S as very scanty ones in the digest with chondroitinase ABC, while very small amounts of Δ Di-6S and Δ Di-0S were only detected in the digest with chondroitinase AC-II (data not shown). These observations indicated that this patient excreted a large amount of dermatan sulfate into urine. Consequently, the deficiency of an enzyme involved in the degradation of dermatan sulfate was conceivable in this patient. Therefore, the activity of α -L-iduronidase in urine of this patient was assayed.

An enzyme solution was prepared by the method of Isemura et al. (1978) as follows: A portion (200 ml) of fresh urine collected from this patient was made to 70% saturation with ammonium sulfate at 4°C. The precipitate was collected after standing overnight at 4°C, and dissolved in 0.9% sodium chloride. Some insoluble matter was removed by centrifugation, and the protein was reprecipitated with ammonium sulfate as above. The precipitate was dissolved in 0.9% sodium chloride, and dialyzed against 0.1 M sodium acetate buffer (pH 4.5) containing 0.9% sodium chloride. The non-dialyzable fraction was used as an enzyme solution. As a control, an enzyme solution was prepared from urine of healthy male aged 34.

The activity of α -L-iduronidase was determined as described in MATERIALS and METHODS. The enzyme activities thus obtained of Case 20 and the control were 8 and 250 nmoles phenol/mg protein/18 hr, respectively. The low activity of α -L-iduronidase in the urine of Case 20 supported the above conception

of the metabolic disorder of dermatan sulfate in this patient. Moreover, relatively high level of the proportion of band 3 was also supported by the low activity of this enzyme.

Judging from the above findings together with clinical observations, the patient of Case 20 was finally diagnosed as mucopolysaccharidosis type I-S (Scheie syndrome).

Osteoarthritis (arthritis deformans). Nine cases were examined (Table 6) and 4 cases gave irregular bands. Of these cases, 3 cases gave bands 8, suggesting abnormal metabolism of acidic glycoprotein(s) in these patients. Six cases showed abnormal excretion patterns of urinary GAG in the regular bands.

Spine diseases. Eight cases were examined and 3 cases gave irregular bands (Table 7). Six cases showed abnormal excretion patterns of urinary GAG in the regular bands. Case 6 of spondylolysis excreted a large amount of urinary GAG. Since the proportions of bands 2 and 3 were 6 and 61%, respectively, a

TABLE 6. *Excretion pattern of urinary GAG from the patients with osteoarthritis (arthritis deformans)*

Case	Name	Age	Sex	Site	Total GAG (HexN/UA)*	Distribution of GAG (as HexN) in bands†				Recovery‡
						1	2	3	Others	
1	T.T.	35	M	Hip	5.04/4.40	50	22	28		95
2	S.Y.	46	M	Hip	3.55/N.D.¶	49	36	15		97
3	M.S.	30	F	Hip	4.15/3.21	36	6	23	35(8)//	100
4	S.U.	46	F	Hip	4.56/3.54	34	19	22	15(5)	91
5	M.K.	57	F	Hip	5.10/3.79	25	9	16	{29(5) 20(8)	81
6	M.O.	57	F	Hip	6.62/4.40	41	14	18	{15(4) 13(8)	75
7	K.T.	62	F	Hip	4.34/3.58	62	13	25		77
8	H.K.	52	F	Hip, Knee	4.35/3.87	56	17	27		86
9	F.O.	74	F	Knee	3.98/3.23	52	18	30		88

*, †, ‡, // See Table 1. ¶ See Table 5.

TABLE 7. *Excretion pattern of urinary GAG from the patients with spine diseases*

Case	Name	Age	Sex	Diagnosis	Total GAG (HexN/UA)*	Distribution of GAG (as HexN) in bands†				Recovery‡
						1	2	3	Others	
1	S.S.	21	M	Disc hernia	6.21/5.53	61	8	20	11(5)//	91
2	T.H.	34	M	Disc hernia	5.19/4.50	48	23	29		86
3	S.S.	46	M	Disc hernia	6.50/5.81	43	9	33	15(5)	88
4	N.E.	36	M	Spinal canal stenosis	5.14/4.91	57	8	35		93
5	T.H.	53	F	Spinal canal stenosis	4.82/2.71	43	17	40		89
6	H.N.	41	M	Spondylolysis	13.27/12.53	33	6	61		91
7	S.A.	55	M	Ossification of posterior longitudinal ligament	5.88/3.45	33	6	22	{9(4) 23(7) 7(8)	83
8	M.S.	60	F	Pseudospondylolisthesis	2.13/2.02	45	18	37		90

*, †, ‡, // See Table 1.

marked depression of the metabolic rate of dermatan sulfate and a conspicuous elevation of that of heparan sulfate were conceivable in this patient.

Other orthopedic diseases. The cases examined are listed in Table 8. Eight cases gave irregular bands and 14 cases showed abnormal excretion patterns of urinary GAG in the regular bands. Of these cases, the proportion of band 3 of Cases 9 and 15 was significantly higher than the normal range. Therefore, marked elevation of the metabolic rates of heparan sulfate was conceivable in these patients.

The present screening study on 123 cases of the orthopedic patients provides with a certain clue for the metabolism of GAG in the various types of orthopedic diseases. Of 123 cases, 84 cases showed abnormal excretion patterns of urinary GAG

TABLE 8. *Excretion pattern of urinary GAG from the patients with other orthopedic diseases*

Case	Name	Age	Sex	Diagnosis	Total GAG (HexN/UA)*	Distribution of GAG (as HexN) in bands†				Recovery‡
						1	2	3	Others	
1	K.I.	30	M	Aseptic necrosis of the femoral head	5.58/3.37	53	9	24	14(6)//	97
2	M.T.	44	M	Aseptic necrosis of the femoral head	6.06/5.02	65	8	27		72
3	K.H.	70	M	Aseptic necrosis of the femoral head	7.00/5.99	51	22	27		103
4	S.K.	7	F	Congenital dislocation of the hip	5.98/4.27	52	20	22	6(5)	80
5	K.S.	13	F	Congenital dislocation of the hip	6.78/4.84	27	21	30	22(6)	87
6	C.K.	28	F	Traumatic dislocation of the hip	4.87/2.99	39	18	24	19(5)	90
7	S.S.	16	F	Recurrent dislocation of the patella	4.90/3.84	36	8	21	$\begin{cases} 17(4) \\ 7(7) \\ 11(8) \end{cases}$	81
8	K.C.	15	M	Fracture of the ischium	3.54/3.42	57	20	23		89
9	T.K.	19	M	Fracture of the femur	7.75/7.00	36	11	53		93
10	H.S.	12	M	Osteochondrosis of the elbow	6.09/5.62	61	10	29		84
11	H.T.	14	M	Progressive muscular dystrophy	9.48/8.37	54	9	14	$\begin{cases} 9(4) \\ 6(7) \\ 8(8) \end{cases}$	90
12	S.T.	18	M	Gout (glycogen storage disease)	3.65/3.12	61	10	20	9(7)	100
13	K.T.	22	M	Neurotic arthropathy (syringomyelia)	3.41/2.91	45	27	28		97
14	S.O.	23	M	Spinal cord tumor	3.12/2.70	54	12	23	11(7)	78
15	S.M.	31	M	Pyogenic arthritis of the hip	3.55/3.02	27	18	55		89
16	K.T.	72	M	Metastatic tumor of the neck (tonsil tumor)	3.61/3.32	61	20	19		95
17	T.S.	36	F	Chondromalacia patellae	1.91/1.27	57	19	24		84
18	T.Y.	62	F	Osteomyelitis of the femur	2.85/2.67	59§		41		81

*, †, ‡, §, // See Table 1.

in the three regular bands and 33 cases gave irregular bands. The proportion of band 1 increased in 70 cases and those of bands 2 and 3 decreased in 54 and 42 cases, respectively. On the other hand, the proportion of band 1 decreased in 5 cases and those of bands 2 and 3 increased in 4 and 5 cases, respectively. Of these cases, alteration of the proportion of the regular bands was most prominent in the cases of bone metabolic and connective tissue diseases. These observations suggested therefore that the metabolic rates of chondroitin sulfates tended to elevate in orthopedic diseases. In addition, certain metabolic disorder of dermatan sulfate and heparan sulfate was suggested in several cases. The presence of irregular band(s) suggested abnormal metabolism of acidic glycoprotein(s) except for the cases at puberty (Nagatsuka et al. 1980).

Case 20 of bone metabolic and connective tissue diseases was only one studied comprehensively, since it was conceivable to be a mucopolysaccharidosis from the excretion pattern of urinary GAG and clinical observations. Enzymatic digestion of its urinary GAG with chondroitinases and assay of the activity of α -L-iduronidase in urine of this patient indicated that this case was mucopolysaccharidosis I-S (Scheie syndrome). Elucidation of the metabolic disorder of GAG in the patients with abnormal metabolic patterns of urinary GAG is a project of further study.

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