Chondroitin 4- and 6-Sulfaturia: A New Type of Inborn Error of Metabolism?

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HAYASHI, S., KIMURA, A., HOSHINO, R., TAKAHASHI, K. and Tsunumi, K.
Chondroitin 4- and 6-Sulfaturia: A New Type of Inborn Error of Metabolism?
Tohoku J. exp. Med., 1979, 127 (4), 327-338 — A 14-year-old boy was found to
excrete excessive amounts of acidic glycosaminoglycans which were predominantly
chondroitin 4-sulfate and chondroitin 6-sulfate. Clinical features included
dwarfism, mental retardation, coarse facies, deformities of the spine, hip joints
and thorax, and granulations in leucocytes. The clinical and biochemical features
found in this boy were compared with the known types of mucopolysaccharidosis
and it has been concluded that this case is a new type of mucopolysacchariduria.
—— chondroitin 4-/6-sulfaturia; mucopolysaccharidosis; urinary AGAG

In 1957 Dorfman and Lorincz first reported an excess and abnormal excretion
of acidic glycosaminoglycans (AGAG) in a six-year-old girl with Hurler syndrome.
Since then, the quantitative and qualitative studies on urinary AGAG in patients
who were diagnosed as then so-called “Hurler syndrome” have been made by many
investigators, and in 1966 McKusick proposed a classification of mucopolysaccharidoses
on the basis of the analytical data of the urinary AGAG and on clinical
or genetic criteria in patients with Hurler syndrome. Therefore the analysis of
urinary AGAG is one of the most important procedures to make a differential
diagnosis of mucopolysaccharidoses.

Acidic glycosaminoglycans excreted in excess are known to be dermatan sulfate
(DS) and heparan sulfate (HS) in mucopolysaccharidosis Type I (Hurler) (Type IH)
and Type II (Hunter), HS in Type III (Sanfilippo), keratan sulfate (KS) in Type IV
(Morquio), and DS in Type V (Scheie) (Type IS) and Type VI (Maroteaux-Lamy).

During the screening for patients with mucopolysaccharidoses by examination
of urinary AGAG we found an abnormal excretion of chondroitin 4- and 6-sulfates
in one patient with an appearance similar to so-called “gargoyl features”. However,
over excess excretion of DS, HS or KS was found. Although we did not
examine the enzymatic disturbance in skin fibroblasts from the patient, taking

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Meeting of the Japanese Society of Connective Tissue Researchers held in Kanazawa on
into consideration the urinary excretion pattern of AGAG together with the clinical findings, the present patient is considered to be a new type of metabolic error of AGAG, and we want to designate this abnormality as "chondroitin 4- and 6-sulfaturia".

CASE RECORD

The patient Y.S., a 14-year-old boy, was a pupil of a special school for weak children. His I.Q. was 66. The family history revealed that his grandfather and grandmother on the father's side were first cousins. No other similarly affected persons have been known in this family. He began to walk without support at the age of 2 years. At the age of 4 years he underwent an operation for right inguinal herniation; at the age of 5 years he was noted to have a limping gait and hospitalized for a month under the presumptive diagnosis of bilateral perthes disease. At the age of 8 years he entered the Hospital School for Handicapped Children in Koriyama City and received treatment of traction and no-weight bearing for 3 years. At that time he was suspected to have Morquio syndrome. However, the examination of urinary AGAG revealed no abnormal excretion of keratan sulfate. Since then he has been boarding in the School for Mentally Retarded Children, where he was noticed to be an excess excretor of chondroitin 4- and 6-sulfates.

Physical findings. The results of physical examination were as follows: a short boy 125.2 cm tall and weighing 37.0 kg; short neck, pigeon breast, large lips and saddle nose, coarse facies, hypertrichosis, dorsolumbar scoliosis. No rales, no bronchial breathing nor hepatosplenomegaly was observed. The heart sound was normal. No joint abnormality was observed except at hip joints where limitation of movement and flexor contracture were found. The skin was not coarse. The examination of the eyes revealed normal cornea and fundi.

X-ray examination of the bones, the scapulae, hands, feet and long tubular bones showed no special findings except the femur. The heads of the bilateral femurs were flat, constituting coxa valga at the hip joints, and the rim of acetabulum was irregular. The spinal roentgenogram showed slight lumbar kyphosis and a slight dorsal dislocation of the 4th lumbar vertebra. The lower thoracic and upper lumbar vertebral bodies have an anterior hook-like projection, so-called "beaking of the vertebra".

Laboratory examination. No glucosuria nor albuminuria was found. The urinary level of free amino acids was normal except a slight increase of hydroxyproline. Serum calcium, inorganic phosphate and alkaline phosphatase level were normal. Serum total protein value was 7.3 g/100 ml and serum electrophoresis showed an albumin of 66.2%, $\alpha_1$-globulin 3.2%, $\alpha_2$-globulin 7.0%, $\beta$-globulin 7.6% and $\gamma$-globulin 15.4%. Serum immunoelectrophoresis disclosed an increase in the Ig-M (306 mg/100 ml). The levels of other immunoglobulins were about normal: Ig-A, 280 mg/100 ml; Ig-G, 1610 mg/100 ml; transferrin, 170 mg/100 ml; $\alpha_2$-macroglobulin, 310 mg/100 ml; $\beta_1$-E, 31 mg/100 ml; $\beta_1$-A, 92 mg/100 ml.

The most characteristic feature of the examination of blood cells was the occurrence of Reily bodies in polymorphonuclear leucocytes, monocytes and lymphocytes. These white blood cells contained basophilic granules stained metachromatically with toluidine blue.

Serum protein-bound iodine was 5.4 $\mu$g/100 ml. Triosorb test was 23.8%. Liver function tests were normal. The results of the renal function tests were as follows: the creatinine clearance tests, 115 ml/min; PSP test, 30% (30 min); and the renal concentration test (by Fishberg method) over 1.022.

Histological studies. Bone marrow aspirates and skin, lymph node and liver biopsies from the patient were submitted to light and electron microscopy. Light microscopically,
variable numbers of foam cells were found in the bone marrow, subcutaneous tissue, hepatic sinusoids and in the lymphatic sinuses of the lymph nodes. In the liver, the parenchymal cells appeared swollen and vacuolated. In the cytoplasm of the foam cells, numerous, mostly round, membrane-bound inclusions containing a flocculent, finely reticulogranular or amorphous material of low electron density were electron microscopically observed, which closely resembled those usually seen in the well-established types of genetic mucopolysaccharidoses. Similar intracytoplasmic inclusions were present in the hepatic cells.

A preliminary report was presented previously (Hoshino et al. 1974); more extensive histological studies have been presented elsewhere (Takahashi et al. 1978).

**Experimental**

**Determination of urinary AGAG.** Fifteen samples of 24-hr-urine were obtained from the patient during 2.5 years. The urine was pooled with Thimerosal as preservative until use.

For the determination of AGAG the urine sample was diluted to obtain a specific gravity of 1.020 or less, 0.5 ml of 5% cetylpyridinium chloride (CPC) was added to 10.0 ml of the urine, pH of the mixture being adjusted to 6. After standing overnight at 4°C the precipitate (CP-AGAG complex) was separated, washed with NaCl-saturated ethanol and dissolved in 5 ml of 0.01 N NaOH. The uronic acid content of this solution was determined by the carbazole method of Bitter and Muir, glucuronic acid being used as standard. The daily excretion of AGAG was expressed as glucuronic acid value.

AGAG samples, used for the identification of urinary AGAG, were obtained from 24-hr urine by using the CPC-precipitation method. CP-AGAG complex was dissolved in 1.25 M MgCl₂ and AGAG was recovered by addition of 4 volumes of ethanol at pH 5, and the precipitate was washed with 80% ethanol, absolute ethanol and ether, successively, and dried. This AGAG mixture was dissolved in a physiological saline and subjected to proteolytic digestion for 24 hr after pH of the solution was adjusted to 8 with calcium hydroxide. After proteolytic digestion the solution was treated with 0.5 N NaOH for 4 hr in order to cleave the alkali-labile linkage between AGAG and peptide chain (alkaline treatment), followed by treatment with 10% trichloroacetic acid for 3 hr at 4°C (TCA treatment). After removing the resultant precipitate a crude AGAG fraction was obtained by addition of ethanol.

**Ethanol fractionation of the urinary AGAG.** 530 mg of the crude AGAG obtained by the above-mentioned procedures was fractionated by fractional precipitation with different ethanol concentrations in the presence of calcium ion: one per cent solution of the sample was made in 5% calcium acetate-0.5 N acetic acid buffer and after removing the insoluble material AGAG was precipitated with 3 volumes of ethanol. The recovered AGAG was redissolved in the calcium acetate-acetate buffer and ethanol was added to give a final ethanol concentration of 25%. The precipitate (25% EtOH Fr.) was separated and dried with ethanol and ether. Ethanol was further added to the supernate obtained above to give a final concentration of 80%. The resultant precipitate (80% EtOH Fr.) was separated and dried.

**Fractionation of AGAG by Dowex 1 chromatography.** Two AGAG fractions obtained by ethanol fractionation in the presence of calcium ion were fractionated by chromatography on a Dowex 1 (Cl⁻) column by stepwise elution with increasing concentration of sodium chloride.

**Electrophoresis of AGAG obtained by Dowex 1 chromatography.** For the identification of individual AGAG electrophoresis on cellulose acetate paper was performed in barium acetate solution according to a modified method of Wessler (Kimura et al. 1974), in calcium acetate solution (Seno et al. 1970) and in 0.1 M HCl solution (Wessler 1971). AGAG was located by staining with 1% toluidine blue followed by washing with 2% acetic acid.
On electrophoresis in 0.075 M barium acetate solution three bands, the fast-, the intermediate- and the slow-moving bands, are obtained, each band corresponding to chondroitin 4- or 6-sulfates (Ch4-/6-S), DS and HS, respectively. AGAG content in each band was determined by colorimetric measurement according to Kimura et al. (1974).

**Differentiation of AGAG with enzymes.** In order to specify the AGAG species the following enzymes were used: chondroitinase AC II, chondroitinase ABC and Streptomyces hyaluronidase. The enzymes were purchased from Seikagaku Kogyo Co., Ltd., Tokyo. AGAG which was not digested with specific enzymes was determined by electrophoresis. The quantitative determination of chondroitin 4- or 6-sulfate was carried out by digestion with chondroitinase AC II and ABC according to the method of Saito et al. (1968). The unsaturated disaccharides in the digest were separated by descending paper chromatography and estimated by spectrophotometry.

**Gel filtration.** For the examination of molecular-weight distribution of the AGAG, gel filtration on Sephadex G-200 was performed.

**Chemical analysis.** Uronic acid was determined by the carbazole method of Dische (1947) as well as by that of Bitter and Muir (1962) and the orcinol method (Brown 1949). Total hexosamine was assayed by the Elson and Morgan method (Blix 1948). Glucosamine and galactosamine contents were determined on an automatic amino acid analyzer (JLC-5AH, JEOL, Ltd., Tokyo) after hydrolysis with 2 N HCl at 100°C for 14 hr.

**RESULTS**

As shown in Table 1, the urinary excretion of AGAG in this patient was in the range of 30.2–73.1 mg/day, averaging 47.9 mg/day (as glucuronic acid). Electrophoresis of each crude fraction in 0.075 M barium acetate solution showed that the main component of the crude AGAG was Ch4-/6-S, which amounted to about 80% of total urinary AGAG.

For the further characterization of the urinary AGAG, eight crude AGAG fractions obtained from 2nd and 3rd experiments were combined and used for the ethanol fractionation described above. The 80% EtOH Fr., containing 92.6%

<table>
<thead>
<tr>
<th>Table 1. Excretion of urinary AGAG (as glucuronic acid mg/day*)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1st Experiment 60.6</td>
</tr>
<tr>
<td>2nd Experiment† 41.7</td>
</tr>
<tr>
<td>33.8</td>
</tr>
<tr>
<td>73.1</td>
</tr>
<tr>
<td>45.6</td>
</tr>
<tr>
<td>36.6</td>
</tr>
<tr>
<td>3rd Experiment† 69.8</td>
</tr>
<tr>
<td>49.5</td>
</tr>
<tr>
<td>55.7</td>
</tr>
<tr>
<td>4th Experiment† 54.4</td>
</tr>
<tr>
<td>49.5</td>
</tr>
<tr>
<td>44.4</td>
</tr>
<tr>
<td>5th Experiment† 30.2</td>
</tr>
<tr>
<td>35.5</td>
</tr>
<tr>
<td>44.4</td>
</tr>
</tbody>
</table>

* Determined by carbazole method of Bitter and Muir.
† 2, 3, 5, and 30 months after 1st experiment, respectively.
of the total glucuronic acid, was fractionated on a Dowex 1 column. Electrophoretic patterns of the subfractions of the 80% EtOH Fr. obtained by the Dowex 1 column are shown in Fig. 1. In the barium acetate solution the AGAG of the 0.5 M fr. and the faster-moving components of the 1.0 M fr. and 1.25 M fr. migrated between authentic DS and Ch4-/6-S with gradual increase of mobility in accordance with the concentration of the eluant. The AGAG of the 1.5 M fr. and 2.0 M fr. showed the same mobility as that of authentic Ch4-/6-S. These bands disappeared after the digestion with chondroitinase AC II. On the other hand, the slow-moving components of the 1.0 M fr. and 1.25 M fr. agreed with HS in electrophoretic mobility and in resistance to the action of chondroitinase ABC.

Table 2 shows the properties of the urinary AGAG fractionated by Dowex 1 column chromatography. These results show that the fast-moving component of the subfractions of the 80% EtOH Fr. on the electrophoresis was determined as Ch4-/6-S and the slow-moving component as HS.

For the quantitative determination of Ch4-S or Ch6-S, each of the subfractions of the 80% EtOH Fr. was digested with chondroitinase AC II and ABC, and the resulting unsaturated disaccharides were determined after separation by paper chromatography. As shown in Table 3, the main unsaturated disaccharides of the digestion products were 4Di-4S and 4Di-6S. In addition to these two unsaturated disaccharides, 4Di-0S was obtained from the digestion products of the 1.0 M fr.
and αDi-diS from the 1.5 M fr. and 2.0 M fr. The same results were obtained from the digestion studies with chondroitinase AC II and ABC. The chemical analysis of the subfractions of the 80% EtOH Fr., as seen in Table 3, shows heterogeneity in sulfate content of these fractions. This heterogeneity in sulfate content was also shown by electrophoresis in 0.1 M HCl solution, in which the mobility of the AGAG was proportional to their sulfate content (Fig. 2).

The 25% EtOH Fr. accounted for 7.4% of total AGAG. About 40% of the 25% EtOH Fr. was eluted with 1.5 M sodium chloride from a Dowex 1 column. The electrophoresis of the subfractions of the 25% EtOH Fr. showed the main component of the 1.0 M fr. to be HS, and the other three fractions consisted of DS and HS (Fig. 3).

### Table 2. Distribution of urinary AGAG on Dowex 1 chromatography

<table>
<thead>
<tr>
<th>Fraction (M NaCl)</th>
<th>Recovery* (%)</th>
<th>Distribution on electrophoresis†</th>
<th>GlcN : GalN C/O ratio‡</th>
</tr>
</thead>
<tbody>
<tr>
<td>25% EtOH Fr.</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>0.5 M</td>
<td>0</td>
<td></td>
<td></td>
</tr>
<tr>
<td>1.0 M</td>
<td>1.4</td>
<td>HS</td>
<td>100 : 0</td>
</tr>
<tr>
<td>1.25 M</td>
<td>1.8</td>
<td>HS &amp; DS (51:49)</td>
<td>38 : 62</td>
</tr>
<tr>
<td>1.5 M</td>
<td>3.1</td>
<td>HS &amp; DS (8:92)</td>
<td>5 : 95</td>
</tr>
<tr>
<td>2.0 M</td>
<td>1.1</td>
<td>HS &amp; DS (4:96)</td>
<td>2 : 98</td>
</tr>
<tr>
<td>3.0 M</td>
<td>0</td>
<td></td>
<td></td>
</tr>
<tr>
<td>80% EtOH Fr.</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>0.5 M</td>
<td>0.1</td>
<td></td>
<td></td>
</tr>
<tr>
<td>1.0 M-A</td>
<td>17.4</td>
<td>HS &amp; Ch4-/6-S (58:42)</td>
<td>41 : 59</td>
</tr>
<tr>
<td>-B</td>
<td>12.4</td>
<td>HS &amp; Ch4-/6-S (21:79)</td>
<td>10 : 90</td>
</tr>
<tr>
<td>1.25 M-A</td>
<td>13.7</td>
<td>HS &amp; Ch4-/6-S (6:94)</td>
<td>3 : 97</td>
</tr>
<tr>
<td>-B</td>
<td>11.0</td>
<td>Ch4-/6-S</td>
<td>0 : 100</td>
</tr>
<tr>
<td>1.5 M-A</td>
<td>15.3</td>
<td>Ch4-/6-S</td>
<td>0 : 100</td>
</tr>
<tr>
<td>-B</td>
<td>10.5</td>
<td>Ch4-/6-S</td>
<td>0 : 100</td>
</tr>
<tr>
<td>1.0 M-A</td>
<td>10.1</td>
<td>Ch4-/6-S</td>
<td>0 : 100</td>
</tr>
<tr>
<td>-B</td>
<td>2.1</td>
<td>Ch4-/6-S</td>
<td>0 : 100</td>
</tr>
<tr>
<td>3.0 M</td>
<td>0</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

* Based on the amount of uronic acid eluted from the column.
† Determined by colorimetry after electrophoresis in 0.075 M barium acetate solution.
‡ Carbazole-to-orcinol ratio.

and αDi-diS from the 1.5 M fr. and 2.0 M fr. The same results were obtained from the digestion studies with chondroitinase AC II and ABC. The chemical analysis of the subfractions of the 80% EtOH Fr., as seen in Table 3, shows heterogeneity in sulfate content of these fractions. This heterogeneity in sulfate content was also shown by electrophoresis in 0.1 M HCl solution, in which the mobility of the AGAG was proportional to their sulfate content (Fig. 2).

The 25% EtOH Fr. accounted for 7.4% of total AGAG. About 40% of the 25% EtOH Fr. was eluted with 1.5 M sodium chloride from a Dowex 1 column. The electrophoresis of the subfractions of the 25% EtOH Fr. showed the main component of the 1.0 M fr. to be HS, and the other three fractions consisted of DS and HS (Fig. 3).

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Fig. 2. Electrophoretic pattern in 0.1 M hydrochloric acid of the subfractions of the 80% EtOH Fr. St. Ch4-S (molar ratio of hexosamine to sulfate of 1:1). 1, 2, 3, 4, 5, 6, 7 and 8 correspond to 1.0 M-A, 1.0 M-B, 1.25 M-A, 1.25 M-B, 1.5 M-A, 1.5 M-B, 2.0 M-A and 2.0 M-B, respectively.
TABLE 3. Chemical analysis of the subfractions obtained by Dowex 1 column chromatography of the 80 % EtOH Fr

<table>
<thead>
<tr>
<th>Fraction (M NaCl)</th>
<th>Uronic acid*</th>
<th>Hexosamine</th>
<th>Sulfate</th>
<th>Disaccharides†</th>
</tr>
</thead>
<tbody>
<tr>
<td>1.0 M-A</td>
<td>40.8%</td>
<td>37.2%</td>
<td>12.4% (0.75)</td>
<td>OS : 4S : 6S : diS</td>
</tr>
<tr>
<td>-B</td>
<td>40.2</td>
<td>38.2</td>
<td>17.2 (1.01)</td>
<td>30 : 35 : 35</td>
</tr>
<tr>
<td>1.25 M-A</td>
<td>38.8</td>
<td>39.8</td>
<td>19.2 (1.08)</td>
<td>50 : 50</td>
</tr>
<tr>
<td>-B</td>
<td>32.3</td>
<td>36.0</td>
<td>19.0 (1.14)</td>
<td>62 : 38</td>
</tr>
<tr>
<td>1.5 M-A</td>
<td>30.0</td>
<td>30.6</td>
<td>14.3 (1.13)</td>
<td>49 : 51</td>
</tr>
<tr>
<td>-B</td>
<td>32.5</td>
<td>30.7</td>
<td>17.3 (1.27)</td>
<td>37 : 40 : 23</td>
</tr>
<tr>
<td>2.0 M-A</td>
<td>30.7</td>
<td>33.4</td>
<td>19.7 (1.32)</td>
<td>39 : 27 : 34</td>
</tr>
<tr>
<td>-B</td>
<td>30.7</td>
<td>35.1</td>
<td>25.1 (1.54)</td>
<td>26 : 28 : 46</td>
</tr>
</tbody>
</table>

* Determined by the carbazole method of Bitter and Muir.
† Unsaturated disaccharides formed by chondroitinase AC II digestion.
OS: 4Di-OS; 4S: 4Di-4S; 6S: 4Di-6S; diS: 4Di-diS.
‡ Molar ratios based on hexosamine=1.

Fig. 3. Electrophoresis on cellulose acetate of the 25% EtOH Fr. after separation on Dowex 1 column, in the supporting medium of 0.075 M barium acetate. St. 1 Ch4-S, Ch6-S and DS; St. 2 HS. 1, 2, 3 and 4 correspond to 1.0 M, 1.25 M, 1.5 M and 2.0 M, respectively.

Table 4 summarizes the quantitative distribution of the individual AGAG in this patient's urine. Ch4-S and Ch6-S, 40.3% and 38.8% of the total AGAG respectively, are the major urinary AGAG in this patient. HS and DS constitute 16.1% and 4.8% of the total amount of urinary AGAG respectively.

Fig. 4 shows the molecular-weight distribution patterns of the urinary AGAG from normal subjects and the patient. The molecular sizes of the crude AGAG obtained from this patient were smaller than those from normal subjects. Elution patterns of the AGAG fractionated by Dowex 1 are shown in Fig. 5 and indicate that the molecular-weight distribution patterns of the 1.5 M fr. and 2.0 M fr. were

Table 4. Composition of urinary AGAG

<table>
<thead>
<tr>
<th>AGAG</th>
<th>% of total AGAG</th>
</tr>
</thead>
<tbody>
<tr>
<td>Chondroitin 4-sulfate</td>
<td>40.3</td>
</tr>
<tr>
<td>Chondroitin 6-sulfate</td>
<td>38.8</td>
</tr>
<tr>
<td>Heparan sulfate</td>
<td>16.1</td>
</tr>
<tr>
<td>Dermatan sulfate</td>
<td>4.8</td>
</tr>
</tbody>
</table>
Fig. 4. Gel filtration patterns of the crude AGAG from normal subjects (●) and the patient (○). The AGAG was applied to a 0.9×60 cm column of Sephadex G-200 and eluted with 0.2 M sodium chloride. The eluate was analyzed for glucuronic acid. The column was calibrated with Blue Dextran and ammonium chloride in order to determine the void volume and the total volume, respectively.

Fig. 5. Gel filtration patterns of the subfractions of the 80% EtOH Fr. The chromatography was conducted in the same way as that of Fig. 4.

○—○, 1.0 M-A; ●—●, 1.25 M-A; ○—○, 1.5 M-A; ●—●, 2.0 M-A.

similar to those of the crude AGAG of normal subjects. However, the molecular sizes of the 1.0 M fr. and 1.25 M fr. were smaller than those of the crude AGAG from normal urine.
Patients with abnormal acidic glycosaminoglycans metabolism have varying degrees of mental and physical retardation in conjunction with excessive urinary excretion of DS, HS and keratan sulfate. Hers (1965) suggested that the electron microscopic findings and natural history of the mucopolysaccharidoses were consistent with their being "lysosomal diseases". Neufeld and her colleagues showed that cells from several of the mucopolysaccharidoses have a defect in the degradation of mucopolysaccharides and that a diffusible factor produced by normal cells or by cells from a different mucopolysaccharidosis corrects this metabolic defect in vitro (Fratantoni et al. 1968). These and other advances showed that these mucopolysaccharidoses were caused by defect of lysosomal enzymes (Dorfman and Matalon 1976).

The mean AGAG excretion in this patient was 47.9 mg/day and in normal children 6.2 mg/day in our laboratory. The 24-hr urinary excretion of AGAG in normal human, although children excretes larger quantity of AGAG than adults, is usually less than 10 mg/day. Therefore, the urinary AGAG excretion level of this patient is about 5–10 times that of normal and comparable to the level of mucopolysaccharidoses. The composition of AGAG in this patient’s urine, however, was different from that of the known mucopolysaccharidoses and was essentially in agreement with those reported by Varadii et al. (1967) and Orii (1968) on normal urine. The sulfate content and molecular-weight distribution of Ch4-S and Ch6-S in this patient were heterogeneous in comparison with those of normal subjects. It is well known that the distribution of sulfate in Ch4-/6-S molecules of urinary AGAG was heterogeneous. Vardaii et al. (1967) described that 25% of the repeating disaccharide units of chondroitin sulfate in normal urine were non-sulfated, and Murata et al. (1973) indicated by the enzymatic analysis an increases of ADi-OS with advance of age. Several authors reporting on urinary AGAG from mucopolysaccharidosis noted a shift in the molecular-weight distribution towards smaller molecules (Constantopoulos 1968; DiFerrante et al. 1971). As shown in Fig. 3, the elution diagrams of the urinary AGAG from this patient on Sephadex G-200 were broad elution patterns and showed a shift to smaller molecules.

Recently increased excretion of Ch4-S and/or Ch6-S has been noted in the pathological conditions by several authors. However, few reports on molecular characteristics of the excreted compounds are found. It may be difficult to find a biochemical abnormality from the excretion mode or chemical properties of these urinary AGAG, because Ch4-S and Ch6-S constitute the major AGAG in normal urine and are heterogeneous in molecular sizes and in sulfate content as mentioned above. The term "chondroitin 4-sulfate mucopolysaccharidosis" was introduced by Thompson et al. in 1968. This case was a 48-year-old female who showed dwarfism, mental retardation, pectus carinatum, congenital dislocation of the hip, clouding of the cornea, hepatosplenomegaly, ausculatory signs of aortic and mitral valve diseases, deafness and abnormal vertebrae. Two unrelated patients with "chondroitin 4-sulfate mucopolysaccharidosis" were reported by Philippart and
Sugarman (1969) and Schuster and Spranger (1971). Schimke et al. (1971) described a case of chondroitin 6-sulfaturia. In 1971 Onizawa et al. reported a case closely related to our case. Their case's clinical aspects resemble Morquio syndrome but the major urinary AGAG was Ch4-S and Ch6-S which had a hybrid structure of uronic acid composition. In these patients with "chondroitin 4-/6-sulfaturia", however, the defect of a specific enzyme responsible for the abnormal metabolism of Ch4-/6-S has not been reported.

In addition, Sly et al. (1973), and Danes and Degnan (1974) reported the patients with β-glucuronidase deficiency mucopolysaccharidosis with a mild Ch4-/6-sulfaturia. Although it is well known that β-glucuronidase hydrolyzes the nonreducing terminal glucuronic acid residues from the oligosaccharides produced by hyaluronidase, there is little available evidence regarding the role of β-glucuronidase in the degradation of the fragments with large molecular weight produced by hyaluronidase.

Chondroitin 4-/6-sulfate was degraded by hyaluronidase together with the concerted actions of β-glucuronidase, β-N-acetylgalactosaminidase and sulfatase. Hayashi (1977, 1978) reported the contribution of β-glucuronidase to the degradation of Ch4-S, chondroitin and HA on the basis of characterization of the degradation products by the lysosomal enzymes. Decasaccharide from Ch4-S or chondroitin was the largest-size substrate for β-glucuronidase in the degradation of Ch4-S or chondroitin by the lysosomal enzymes and hexasaccharide from HA in the degradation of HA. When a specific inhibitor of β-glucuronidase was added to the incubation mixture, the depolymerization of chondroitin and HA was significantly inhibited (Hayashi et al., 1979). The depolymerization of chondroitin was significantly inhibited by the addition of the inhibitor in comparison with that of HA. These results suggest that β-glucuronidase plays an important role in the depolymerization of Ch4-/6-S by hyaluronidase. On the basis of these findings, it is suggested that not only reduced activity of hyaluronidase but also insufficiency of β-glucuronidase action leads to an increased excretion of Ch4-/6-S of molecular sizes similar to Ch4-/6-S from normal urine.

References

A New Type of Mucopolysacchariduria


