Distribution of Acidic Glycosaminoglycans, Lipids and Water in Normal Human Cerebral Arteries at Various Ages

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SUMMARY Functional alterations in arterial acidic glycosaminoglycans (AGAG) may be related to the pathogenesis of some forms of cerebrovascular disease. We measured the AGAG, lipid and water content of human cerebral artery of 275 normal males at various ages. These measures were separately carried out in the main trunk and distal branches. The AGAG components were analyzed by an enzymatic assay method employing specific enzymes which digest AGAG to assess topographic change and aging variations. The total AGAG content was higher in the main cerebral artery than in the distal branches. The main AGAG component of the normal main cerebral artery was heparan sulfates (HS), constituting half the total AGAG, followed by moderate amounts of dermatan sulfate (DS), chondroitin-6-sulfate (C-6S) and chondroitin-4-sulfate (C-4S). Hyaluronic acid (HA) was a minor component and it was more prominent in young arteries. Heparin could be occasionally detected. With advancing age, the relative amounts of HS, HA, chondroitin and C-4S both in the main trunk and distal branches decreased but those of DS and C-6S increased. The total lipid, cholesterol ester and triglyceride content was greater in the main trunk than in the distal branches; the total lipid content increased with age. A possible function of the cerebral arterial AGAG is discussed with respect to change in lipid and water content according to topographic sites and aging.

A DECLINE OF STROKE MORTALITY has been recently reported. Evidence presently available suggests that both incidence and mortality of stroke increase with age and are higher in Japan than in the United States or Europe. Whether this difference is attributable to genetic or environmental factors is unknown. Alterations in the vascular components of the cerebral arteries may be one of the causes for stroke.

Previous studies have indicated a relationship between elevated serum lipid levels and ischemic heart disease. Such relation has not been demonstrated with stroke. We have noted a positive relationship between serum lipids, total cholesterol and triglyceride, and advancing age. The lipid content in human cerebral arteries has not been studied extensively. The alterations in acidic glycosaminoglycans (AGAG) in human cerebral arteries as a result of aging have not been reported in detail.

Chemical analysis of the AGAG of cerebral arteries and lipids in relation to age may elucidate the pathogenesis of some types of stroke, since some AGAG form complexes with lipoproteins and arterial AGAG components are affected with age. Alterations in the cerebral arterial AGAG as well as water and lipid content have not been studied extensively.

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Materials and Methods

The cerebral arterial trees were prepared from 275 male subjects ranging from 12 to 88 year old. The samples were obtained at autopsy from previously healthy subjects, within 9 h following death due to an accident or some unknown cause, at the Tokyo Metropolitan Medical Examiner’s Office. After being separated from the surrounding tissues, the samples were kept at -20°C until use. For analysis of the chemical content, the cerebral arteries were divided into the main cerebral trunk and the distal branches of both hemispheres. The main cerebral trunk consisted of a basilar artery along with the circle of Willis, with 0.5 cm of the attached anterior, posterior and middle cerebral arteries and was carefully freed from the adventitia. The distal branches were separated from their proximal points where their outer diameter was approximately 2.5 mm and at the distal points where the outer diameter was approximately 1 mm. Normal specimens showed a whitish smooth surface and were quite free of fibrous plaques and fat deposits. Any visibly affected parts were not included in the present

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Components were used for electrophoretic characterization. The latter eluate was desalted and the AGAG applied on a Dowex 1-X2 column (Cl\textsuperscript{−} form, 200-400 mesh, 0.7 X 20 cm) and eluted with 0.25 M and 3.0 M sodium acetate solution. The AGAG components were precipitated by adding 5% cetylpyridinium chloride (CPC) and kept overnight. The resulting supernatant was decanted into a dialyzing tube (Visking, 0.7 X 20 cm) and dialyzed against running tap and distilled water for 12 h each. The macromolecular AGAG components were precipitated by adding 4 vols of ethanol. After drying, the yield of uronic acid in units of AGAG was determined by the method of Allain et al\textsuperscript{[16]} and Bucolo and David\textsuperscript{[17]} for each.

**Preparation and Extraction of Cerebral Arterial AGAG**

Preparation of cerebral arterial AGAG was performed essentially by the same method reported previously.\textsuperscript{[18, 20, 26, 31]} The basic specimens for determining AGAG were 5-15 defatted pooled samples from individuals matched for both age and topographic site. The homogenized delipidized sample (1-2 g defatted dry weight) was boiled for 5 min in distilled water. Two M sodium hydroxide was then added to a final concentration of 0.4 M and the mixture was kept at 4°C overnight. Each specimen was then digested with pronase, protease, or pepsin (1,000,000 tyrosine units/g, Kakenyakukako Co., Tokyo) at the rate of 50 mg/g defatted dry weight in 0.067 M phosphate buffer, pH 7.8, at 37°C for 12 h. The digestion was repeated four times. Cold trichloroacetic acid was added to a final concentration of 10%, and the mixture left at 4°C overnight and centrifuged. The supernatant was decanted into a dialyzing tube (Visking Co.) and dialyzed against running tap and distilled water for 12 h each. The macromolecular AGAG components were precipitated by adding 5% cetylpyridinium chloride (CPC) and kept overnight. The resulting AGAG-CPC precipitate was then dissolved in 10% acetic acid and centrifuged. This lipid extraction procedure was repeated twice. The total lipid weight thus extracted was determined by the method of Allain et al.\textsuperscript{[16]} and Bucolo and David\textsuperscript{[17]} for each.

**Preparation of Enzymes**

Chondroitinase-AC II (EC 4.2.2.5) was prepared from *Arthrobacter aurescens*,\textsuperscript{[19]} and chondroitinase-ABC (EC 4.2.2.4) from *Proteus vulgaris*.\textsuperscript{[30]} Chondroitinase-AC II cleaves hexosaminidic linkages to produce \(\triangleleft\) glucuronic acid and converts chondroitin, C-4S and C-6S chains to the corresponding unsaturated disaccharide units. This enzyme also digests HA to form unsaturated non-sulfated disaccharide units.\textsuperscript{[32-33]} Chondroitinase-ABC cleaved hexosaminidic linkages to form not only \(\triangleleft\) glucuronic acid but also \(\triangleleft\) iduronic acid, converting both CS isomers and DS chains to the corresponding unsaturated disaccharide units.\textsuperscript{[32, 33]} Streptomyces hyaluronidase (EC 4.2.2.1) which was extracted from *Streptomyces hyalolyticus nov.* digests only HA.\textsuperscript{[40]} This enzyme was originally prepared by Amano Pharmaceutical Co., Nagoya. HA, approximately 400 \(\mu\)g as uronic acid, was degraded with *Streptomyces hyaluronidase* (30 turbidity reducing units) in 0.1 M acetate buffer, pH 5.0 at 37°C for 120 min.\textsuperscript{[40]} Heparitinase was prepared from *Flavobacterium heparinum* by the method of Silva et al.\textsuperscript{[41]} HS, in the amount of 300 \(\mu\)g as uronic acid, was digested with 1 unit of heparitinase in 0.1 M acetate buffer, pH 7.0, for 20 h at 37°C.\textsuperscript{[38]} By high performance liquid chromatography,\textsuperscript{[42]} the degradation products of HS could be qualitatively measurable from the ultraviolet activity absorbance value by comparing them with similarly treated standard HS. The enzymatic activity was examined using standard C-4S, C-6S, HA and DS as substrates when the enzymes were of practical use.

**Standard Acidic Glycosaminoglycans**

The reference AGAG, that is, HA, HS, DS and heparin were kindly provided by Dr. M. B. Mathews and J. A. Cifonelli, University of Chicago, Illinois. The C-4S and C-6S standards and the unsaturated disaccharide standards (\(\Delta\)D-S) were prepared at the Tokyo Institute of Seikagaku Kogyo Co., Tokyo. The following abbreviations are used: Unsaturated non-sulfated disaccharide (\(\Delta\)D - 0S) = 2-acetamido-2-deoxy-3-O-(4-deoxy-\(\alpha\)-L-threo) \(\Delta\)D\textsubscript{6S}-hex-4-enepyranosyluronic acid)-D-glucose, unsaturated 4-sulfated disaccharide (1D\textsubscript{4S}- 4S) = 2-acetamido-2-deoxy-3-O-(4-deoxy-\(\alpha\)-L-threo) \(\Delta\)D\textsubscript{4S}-hex-4-enepyranosyluronic acid)-D-sulfoglucosamine, unsaturated 6-sulfated disaccharide (1D\textsubscript{6S}- 6S) = 2-acetamido-2-deoxy-3-O-(4-deoxy-\(\alpha\)-L-threo) \(\Delta\)D\textsubscript{6S}-hex-4-enepyranosyluronic acid)-D-6-sulfoglucosamine, and unsaturated di-sulfated disaccharide (1D\textsubscript{6S}-diS) = 2-acetamido-2-deoxy-3-O-(4-deoxy-\(\alpha\)-L-threo) \(\Delta\)D\textsubscript{6S}-hex-4-enepyranosyluronic acid)-2-sulfate)-D-6-sulfoglucosamine.

**Enzymatic Assay Method for AGAG Components**

The enzymatic procedure using chondroitinases was carried out in the manner described previously.\textsuperscript{[18, 20, 26, 31]} The AGAG, approximately 350 \(\mu\)g in amount as uronic acid, were exhaustively digested with chondroitinase-AC II (1 unit) in 100 \(\mu\)l of 0.2 M acetic acid buffer, pH 6.0, at 37°C for 120 min.\textsuperscript{[32, 33]} Digestion with chondroitinase-ABC was similarly performed with ABC-lyase (0.75 units) in 100 \(\mu\)l of Tris-
HCl buffer, pH 7.8, at 37°C for 120 min.\(^{32,33}\) After boiling for 3 min, the solution was cooled for separation by paper chromatography. The susceptibility of HA to Streptomyces hyaluronidase was also examined. HA, as approximately 100 \(\mu g\) of uronic acid, was digested with Streptomyces hyaluronidase (15 turbidity reducing units) in 50 \(\mu l\) of 0.1 M acetate buffer, pH 5.0, at 37°C for 120 min.\(^{40}\) HS, as 300 \(\mu g\) of uronic acid, was digested with heparitinase (1 unit) in 70 \(\mu l\) of 0.1 M acetate buffer, pH 7.0, at 42°C for 18 h.\(^{35}\) Identification of hexosamine moieties was carried out by thin-layer chromatography, using Eastman Kodak 511 sheets by the method of Moczar et al.\(^{42}\)

The unsaturated disaccharides derived from the CS isomers by digestion with chondroitinases were applied as a band 1 cm in width to Whatman No. 1 filter paper. After desalting by paper chromatography, descending paper chromatography was carried out in 1-butyric acid – 0.5 M ammonia (5:3, \(v/v\)) for 60 h at room temperature.\(^{32}\) Localization of the separated unsaturated disaccharides was estimated under a MineraLight at 232 nm. The visualized spots and the area of origin were cut out and eluted with distilled water. Aliquots of the disaccharides and AGAG undigestedorigin were cut out and eluted with distilled water. Electrophoretic Characterization

The AGAG components were characterized for electrophoretic separation before and after enzymatic digestion. Electrophoresis was carried out on cellulose acetate membranes as follows: In 0.1 M formic acid – pyridine buffer, pH 3.1, at 0.5 mA/cm for 60 min,\(^{43}\) in 0.1 M calcium acetate at 0.5 mA/cm for 180 min,\(^{44}\) and in 0.1 M barium acetate, pH 7.4, at 5 V/cm for 180 min.\(^{45}\) Two-dimensional electrophoresis was also carried out in the formic acid – pyridine buffer as first run and in the calcium or barium acetate as second run.\(^{31}\)

Correlation coefficients for alterations of each substance or item with age were statistically tested. \(P\) values less than 0.05% were accepted as significant. Differences of the values between the main trunk and its terminal branches were also compared for each age period by paired \(t\) tests.

**Results**

The water content of the normal human cerebral arteries from the 2nd to 9th decades ranged from 72.2% to 74.3% of the total weight of the main trunk and 74.6% to 77.8% of the distal branches (table 1). The water content was greatest at the 5th decade. The distal branches contained more water than the corresponding main cerebral arteries at every decade, being significant between the 3rd and the 7th decade (\(p < 0.05\)).

Total lipid, cholesterol esters and triglyceride content increased more in the aged cerebral arteries compared with the younger ones. The main cerebral arteries always contained greater total lipid, cholesterol ester and triglyceride than the corresponding distal branches. A significant difference in the cholesterol ester content was obtained at the 6th and 7th decades (\(p < 0.05\)).

A comparison of the lipid content at various ages showed total lipid, cholesterol ester and triglyceride content to be significantly greater in proportion to age both in the main cerebral artery and distal branches (\(p < 0.01\)). Also the ratio of cholesterol ester to triglyceride was significantly higher both in the main trunk and in the distal branches with age (\(p < 0.05\)).

The AGAG content was greater in the main cerebral artery.

### Table 1: Contents of Water and Lipid Components in Human Cerebral Arteries of Normal Males at Various Ages

<table>
<thead>
<tr>
<th>Age in years</th>
<th>Number of samples</th>
<th>Water content(%)</th>
<th>Total lipid(%)</th>
<th>Cholesterol ester(%)</th>
<th>Triglyceride(%)</th>
<th>C/T ratio</th>
<th>Water content(%)</th>
<th>Total lipid(%)</th>
<th>Cholesterol ester(%)</th>
<th>Triglyceride(%)</th>
<th>C/T ratio</th>
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<tr>
<td>10–19</td>
<td>5</td>
<td>72.17 ± 1.04(%)</td>
<td>5.5</td>
<td>6.3 ± 1.9(%)</td>
<td>7.4 ± 2.8(%)</td>
<td>0.85</td>
<td>74.60 ± 1.25(%)</td>
<td>4.1</td>
<td>5.1 ± 2.2(%)</td>
<td>6.2 ± 2.7(%)</td>
<td>0.83</td>
</tr>
<tr>
<td>20–29</td>
<td>22</td>
<td>72.66 ± 0.79(%)</td>
<td>6.3</td>
<td>6.9 ± 1.7(%)</td>
<td>7.6 ± 2.1(%)</td>
<td>0.91</td>
<td>76.08 ± 0.95(%)</td>
<td>5.3</td>
<td>5.3 ± 1.8(%)</td>
<td>6.1 ± 1.8(%)</td>
<td>0.87</td>
</tr>
<tr>
<td>30–39</td>
<td>35</td>
<td>73.50 ± 0.42(%)</td>
<td>8.6</td>
<td>8.6 ± 1.0(%)</td>
<td>7.8 ± 1.0(%)</td>
<td>1.10</td>
<td>76.91 ± 0.36(%)</td>
<td>7.7</td>
<td>6.8 ± 1.3(%)</td>
<td>6.3 ± 1.2(%)</td>
<td>0.92</td>
</tr>
<tr>
<td>40–44</td>
<td>59</td>
<td>74.33 ± 0.48(%)</td>
<td>8.8</td>
<td>10.6 ± 1.8(%)</td>
<td>7.9 ± 1.1(%)</td>
<td>1.34</td>
<td>77.35 ± 0.32(%)</td>
<td>7.9</td>
<td>7.2 ± 2.1(%)</td>
<td>6.5 ± 1.5(%)</td>
<td>1.11</td>
</tr>
<tr>
<td>45–49</td>
<td>33</td>
<td>73.26 ± 0.59(%)</td>
<td>8.9</td>
<td>11.5 ± 1.9(%)</td>
<td>7.9 ± 1.5(%)</td>
<td>1.47</td>
<td>77.82 ± 0.49(%)</td>
<td>8.2</td>
<td>7.4 ± 1.9(%)</td>
<td>6.6 ± 1.8(%)</td>
<td>1.12</td>
</tr>
<tr>
<td>50–59</td>
<td>44</td>
<td>72.60 ± 1.06(%)</td>
<td>9.6</td>
<td>12.3 ± 1.2(%)</td>
<td>8.1 ± 1.8(%)</td>
<td>1.52</td>
<td>76.79 ± 0.54(%)</td>
<td>9.1</td>
<td>8.0 ± 1.8(%)</td>
<td>6.8 ± 1.2(%)</td>
<td>1.18</td>
</tr>
<tr>
<td>60–69</td>
<td>64</td>
<td>72.46 ± 1.52(%)</td>
<td>10.2</td>
<td>13.8 ± 1.7(%)</td>
<td>8.3 ± 1.9(%)</td>
<td>1.66</td>
<td>76.59 ± 0.66(%)</td>
<td>9.3</td>
<td>8.3 ± 0.9(%)</td>
<td>6.9 ± 1.2(%)</td>
<td>1.20</td>
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<tr>
<td>70–79</td>
<td>24</td>
<td>72.48 ± 1.85(%)</td>
<td>10.4</td>
<td>13.3 ± 2.2(%)</td>
<td>8.8 ± 1.6(%)</td>
<td>1.51</td>
<td>76.53 ± 2.28(%)</td>
<td>9.3</td>
<td>8.5 ± 1.7(%)</td>
<td>6.9 ± 1.5(%)</td>
<td>1.23</td>
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<tr>
<td>80–89</td>
<td>9</td>
<td>72.17 ± 1.79(%)</td>
<td>10.5</td>
<td>12.9 ± 2.9(%)</td>
<td>8.7 ± 2.2(%)</td>
<td>1.48</td>
<td>76.54 ± 1.83(%)</td>
<td>9.4</td>
<td>8.5 ± 2.0(%)</td>
<td>6.8 ± 2.8(%)</td>
<td>1.25</td>
</tr>
</tbody>
</table>

\(r\) to age and \(p\) value: 0.254 * 0.934 * 0.924 * 0.976 * 0.857 * 0.402 * 0.899 * 0.944 * 0.909 * 0.940

* Comparisons were made between the content of main cerebral artery and distal branches for each age period.
* Significant difference: \(p < 0.05\), \(p < 0.01\).
* All values are means and standard error.
* Expressed as % of total wet tissue weight.
* Expressed as mg/g dry tissue weight.
trunks than that of the distal branches at all decades (table 2). At younger ages, the main trunk contained approximately 3.2 mg/g defatted dry tissue weight, whereas the distal branches had 2.8 mg/g defatted dry weight at the same ages. The AGAG content of both the main cerebral artery and the distal branches increased significantly with advancing age (p < 0.05).

Electrophoretic patterns of the AGAG components showed major AGAG components to be HS, DS and CS isomers both in the main cerebral artery and distal branches. In the pyridine-formic acid buffer, the first and second spots moved in the similar manner of standard CS isomers and DS (fig. 1). The former did not stain following digestion with both CS-lyases and the latter with chondroitinase-ABC. The third spot, which remained undigested with chondroitinase-ABC, migrated slower than standard HS, indicating that the arterial HS contains less sulfate content than standard HS as already reported.19 It is interesting to note that HA spot was found only in the AGAG components of young age. In the calcium acetate buffer, the spot migrating in the manner of standard DS seemed to stain more densely with advancing age.

The two-dimensional electrophoretic observation for AGAG components in normal young cerebral arteries showed a spot migrating in the manner of standard HA, indicating the presence of a small amount of HA (fig. 2). The HA spot stained less densely with advancing age and finally could not be detected. In contrast, the spot corresponding to standard DS and CS isomers stained more densely with age. After digestion with chondroitinase-ABC, only a single spot corresponding to standard HS appeared. The AGAG extracted from the spot on the paper chromatography was susceptible to degradation by heparitinase and the hydrolysate migrated as glucosamine on thin layer chromatography,42 indicating the AGAG component to be HS. Since chondroitinase-AC II digests C-4S, C-6S and HA, the two spots corresponding to standard DS and HS remained their stain.

The paper chromatographic pattern of the ADi-S derived from the AGAG components of the main cerebral artery following digestion with chondroitinase-ABC and AC-lyase showed five ADi-S: two ADi-0S derived from HA and chondroitin, ADi-4S from C-4S and/or DS, ADi-6S from C-6S and ADi-diS from oversulfated DS (fig. 3). Since the spot corresponding to the ADi-4S was stained more densely after digestion with chondroitinase-ABC than with chondroitinase-AC II, the relative amount of DS was thus considered to be relatively greater than that of C-4S. These paper chromatographic observations showed that the human cerebral artery contains the following AGAG components, digested by the above enzymes: mainly C-6S and DS followed by an intermediate amount of C-4S, and small amounts of HA and oversulfated DS. In addition, HS which was not digested was found to be the most predominant AGAG components.

The results obtained from the enzymatic analysis of the AGAG components of cerebral arteries at various decades and topographic sites are summarized in table 2. The major AGAG of the normal main cerebral arteries was HS, which constituted nearly half the total AGAG and DS, a less prominent AGAG component, comprising \( \frac{3}{10} - \frac{1}{4} \) of the total AGAG. C-4S and C-6S accounted, respectively, for 6–12% and 10–19% of the total AGAG. HA, chondroitin and oversulfated DS were present only as minor components.
arterial AGAG was digested with Streptomyces hyaluronidase substantiates the fact that HA is present only in a small but noticeable amount. The AGAG components undigested by chondroitinase-ABC, most probably HS, constituted 45–65% of the total AGAG. After the AGAG component was digested with heparitinase, the degradation products could be detected by high performance liquid chromatography.34, 35, 41 This observation indicates the AGAG component to be HS. As mentioned above, two-dimensional electrophoresis31 showed three spots corresponding to standard HS, DS and CS isomers. The ratio of the staining intensity of DS to HS was greater in the increase with age. The spot migrating in the manner of standard heparin could be detected occasionally on two-dimensional electrophoresis of the cerebral arterial AGAG, especially prepared from the main trunk in the normal state, as already found in human coronary artery.30

Discussion

Some types of stroke may be induced by alteration in the AGAG components of the cerebral arteries and lipids. Information available on AGAG, lipid and water content in human cerebral arteries is limited.25, 26, 46, 47 The physiological function of arterial AGAG related to the pathogenesis of cerebrovascular disease has been the focus of considerable interest. A topographic comparison of AGAG constituent in cerebral arteries corresponding to various age should facilitate the elucidation of the pathogenesis of hemorrhage and thrombo-embolic type strokes. In this study, an attempt was made first to determine the AGAG components of the main cerebral arteries and their distal branches at various ages.

Our data clearly indicate that AGAG components vary according to age and topographic site. Water content is highest at ages 40 to 49. That of the cerebral arteries was somewhat higher than in the coronary arteries46, 47 and aorta48 but lower than in the heart valves.49 Our determination of water content in the cerebral arteries agreed with data from other authors.46, 47 Water content in the distal branches was higher than in the main cerebral arteries. The exact reason for this is not clear, but an appreciable amount of fluids may be retained in the distal branches surrounding the pia mater of both hemispheres. Such AGAG components, particularly HA, may possess the capacity to bind with water in situ.24

We are able to confirm that total lipid, cholesterol ester and triglyceride content in human cerebral arteries is greater in aged than in young arteries. The cholesterol ester content of the main cerebral arteries as determined in this study was essentially the same as that found previously.25, 47, 51 The content in both the main cerebral artery and its distal branches increases with age. The triglyceride content was close to that of Scott et al17 and Bottcher et al41 but somewhat higher than most of Nakamura et al.25 Total lipid, cholesterol ester and triglyceride content was significantly less in the distal branches than in the main trunk; both cholesterol ester and triglyceride content in human cerebral
arteries is lower than that in the aorta and coronary arteries. These findings indicate that the main trunk of cerebral artery is progressively affected with atherosclerotic lesions not apparent with advancing age.

Certain histochemical studies have shown AGAG components of human cerebral arteries to differ from those of the aorta and coronary arteries and the cerebral artery to contain only a small amount of HA. In our previous study, in the AGAG components of cerebral arteries, the relative amounts of HS were always found higher and HA lower than in other arteries.

Human cerebral arterial AGAG components reportedly differ from those of other arteries. Normally the AGAG content of cerebral arteries is somewhat less than that of the aorta and coronary arteries. This may be explained by the fact that blood pressure is lower in the cerebral arteries than in larger arteries. We found that AGAG components of cerebral arteries are similar to that in the aortic outer layer and venous tissue, but the HS content in the cerebral arteries is much higher than in other large vessels and that of HA is considerably lower.

Although a final conclusion may be premature, a comparison of the AGAG components of the main cerebral artery with those of their distal branches showed some topographic changes in AGAG components. Total AGAG content in the distal branches was significantly lower than in the main cerebral artery. This may also be caused by blood pressure that is lower in the distal branches than in the trunk. The distribution of cerebral arterial AGAG components was such that the relative amount of HS in the distal branches was obviously higher than that in the main cerebral arteries. In the main cerebral artery, the relative amounts of C-4S, chondroitin and HA were somewhat higher in younger arteries but the reverse was the case for C-6S and DS. HA was present in very small amounts in the cerebral arteries but rather abundant in younger main trunks.

The present data show that either the total AGAG content or individual AGAG distribution in human cerebral arteries varies according to the arterial site and according to age. Constitutional changes in AGAG components may reflect proliferative reactions or aging. Such change in AGAG components influenced by aging or topographic site may serve a physiological function in anti-coagulation and anti-thrombogenesis. These components, after having passed in younger main trunks.

### Table 2

<table>
<thead>
<tr>
<th>Decades—*</th>
<th>Glycosaminoglycans</th>
<th>Second</th>
<th>Third</th>
<th>Fourth</th>
<th>Fifth</th>
<th>Sixth</th>
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<tr>
<td>Main cerebral arteries</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
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</tr>
<tr>
<td>Hyaluronic acid</td>
<td>100 (3.2)</td>
<td>78 (2.5)</td>
<td>114 (3.4)</td>
<td>115 (3.3)</td>
<td>75 (2.1)</td>
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<tr>
<td>Chondroitin</td>
<td>72 (2.3)</td>
<td>60 (1.9)</td>
<td>50 (1.5)</td>
<td>42 (1.2)</td>
<td>43 (1.2)</td>
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<tr>
<td>Chondroitin 4-sulfate</td>
<td>376 (12.1)</td>
<td>354 (11.2)</td>
<td>343 (10.2)</td>
<td>365 (10.5)</td>
<td>330 (9.3)</td>
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</tr>
<tr>
<td>Dermatan sulfate</td>
<td>515 (16.5)</td>
<td>628 (19.9)</td>
<td>686 (20.4)</td>
<td>705 (20.3)</td>
<td>746 (21.0)</td>
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</tr>
<tr>
<td>Chondroitin 6-sulfate</td>
<td>306 (9.8)</td>
<td>332 (10.5)</td>
<td>363 (10.8)</td>
<td>385 (11.1)</td>
<td>565 (15.9)</td>
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</tr>
<tr>
<td>Oversulfated dermatan sulfates</td>
<td>31 (1.0)</td>
<td>16 (0.5)</td>
<td>27 (0.8)</td>
<td>42 (1.2)</td>
<td>14 (0.4)</td>
<td></td>
</tr>
<tr>
<td>Heparan sulfate</td>
<td>1719 (55.1)</td>
<td>1690 (53.5)</td>
<td>1778 (52.9)</td>
<td>1819 (52.4)</td>
<td>1779 (50.1)</td>
<td></td>
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<tr>
<td>Total AGAG</td>
<td>3119(100.0)</td>
<td>3158(100.0)</td>
<td>3361(100.0)</td>
<td>3473(100.0)</td>
<td>3552(100.0)</td>
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<td>Distal branches</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Hyaluronic acid</td>
<td>—</td>
<td>93 (3.3)</td>
<td>67 (2.4)</td>
<td>101 (3.5)</td>
<td>126 (4.3)</td>
<td></td>
</tr>
<tr>
<td>Chondroitin</td>
<td>—</td>
<td>96 (3.4)</td>
<td>90 (3.2)</td>
<td>89 (3.1)</td>
<td>144 (4.9)</td>
<td></td>
</tr>
<tr>
<td>Chondroitin 4-sulfate</td>
<td>—</td>
<td>307 (10.9)</td>
<td>230 (8.2)</td>
<td>239 (8.3)</td>
<td>232 (7.9)</td>
<td></td>
</tr>
<tr>
<td>Dermatan sulfate</td>
<td>—</td>
<td>296 (10.5)</td>
<td>331 (11.8)</td>
<td>351 (12.2)</td>
<td>347 (11.8)</td>
<td></td>
</tr>
<tr>
<td>Chondroitin 6-sulfate</td>
<td>—</td>
<td>166 (5.9)</td>
<td>244 (8.7)</td>
<td>268 (9.3)</td>
<td>250 (8.5)</td>
<td></td>
</tr>
<tr>
<td>Oversulfated dermatan sulfates</td>
<td>—</td>
<td>14 (0.5)</td>
<td>25 (0.9)</td>
<td>40 (1.4)</td>
<td>47 (1.6)</td>
<td></td>
</tr>
<tr>
<td>Heparan sulfate</td>
<td>—</td>
<td>1847 (65.5)</td>
<td>1820 (64.8)</td>
<td>1789 (62.2)</td>
<td>1792 (61.0)</td>
<td></td>
</tr>
<tr>
<td>Total AGAG</td>
<td>—</td>
<td>2819(100.0)</td>
<td>2807(100.0)</td>
<td>2877(100.0)</td>
<td>2938(100.0)</td>
<td></td>
</tr>
</tbody>
</table>

All numbers are mean values measured in duplicates by the carbazole reaction and expressed as µg/g defatted dry tissue weight. Numbers in parentheses are expressed in %.

Hyaluronic acid, chondroitin and chondroitin 6-sulfate were calculated from the mean values of the unsaturated non-sulfated and 6-sulfated disaccharides derived from hyaluronic acid, chondroitin and chondroitin 6-sulfate by digestion with chondroitinase-ABC and -AC. The yield of dermatan sulfate was estimated by subtraction of the value for the unsaturated 4-sulfated disaccharide from digestion with chondroitinase-AC from that with chondroitinase-ABC. Oversulfated dermatan sulfate was estimated from the value for the unsaturated disulfated disaccharide after digesting with chondroitinase-ABC. Heparan sulfates were calculated from the value of glycosaminoglycans undigested with chondroitinase-ABC.

*p < 0.05; †p < 0.01.
GLYCOSAMINOGLYCANS IN HUMAN CEREBRAL ARTERY/Murata 693

Table 2 (Continued)

<table>
<thead>
<tr>
<th>Seventh</th>
<th>Eighth</th>
<th>Ninth</th>
<th>$r$ of actual content with age</th>
</tr>
</thead>
<tbody>
<tr>
<td>31 (0.8)</td>
<td>19 (0.5)</td>
<td>11 (0.3)</td>
<td>-0.832*</td>
</tr>
<tr>
<td>42 (1.1)</td>
<td>38 (1.0)</td>
<td>46 (1.2)</td>
<td>-0.803*</td>
</tr>
<tr>
<td>266 (6.9)</td>
<td>268 (7.1)</td>
<td>248 (6.5)</td>
<td>-0.922*</td>
</tr>
<tr>
<td>850 (22.0)</td>
<td>962 (25.5)</td>
<td>944 (24.7)</td>
<td>0.978†</td>
</tr>
<tr>
<td>626 (16.2)</td>
<td>645 (17.1)</td>
<td>715 (18.7)</td>
<td>0.969†</td>
</tr>
<tr>
<td>119 (3.1)</td>
<td>15 (0.4)</td>
<td>115 (3.0)</td>
<td>0.551</td>
</tr>
<tr>
<td>1927 (49.9)</td>
<td>1830 (48.5)</td>
<td>1742 (45.6)</td>
<td>0.498</td>
</tr>
<tr>
<td>3861(100.0)</td>
<td>3777(100.1)</td>
<td>3821(100.0)</td>
<td>0.961†</td>
</tr>
</tbody>
</table>

| 83 (2.6) | 115 (3.4) | 54 (1.6) | -0.118 |
| 86 (2.7) | 98 (2.9) | 115 (3.4) | 0.262 |
| 214 (6.7) | 230 (6.8) | 283 (8.3) | -0.222 |
| 485 (15.2) | 528 (15.6) | 528 (15.5) | 0.943* |
| 379 (11.9) | 413 (12.2) | 436 (12.8) | 0.958† |
| 96 (3.0) | 34 (1.0) | 78 (2.3) | 0.700 |
| 1846 (57.9) | 1966 (58.1) | 1910 (56.1) | 0.643 |
| 3189(100.0) | 3384(100.0) | 3404(100.0) | 0.950* |

Acknowledgment

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K Murata

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