Increase in Lens Gangliosides due to Aging and Cataract Progression in Human Senile Cataract

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Gangliosides were isolated from human senile cataractous lenses by solvent extraction, DEAE-Sephadex column chromatography, and thin-layer chromatography. The content and composition of gangliosides were examined in individual lens tissues. Three predominant gangliosides, GM3, GM1, and GD1a, were tentatively identified in comparison with authentic brain gangliosides, and several unidentified gangliosides were also recognized. The increase in ganglioside content per mg of protein content in cataractous lenses was found to be influenced by two physiologic parameters: aging and cataract progression. The mature cataractous lenses showed a higher ganglioside level on a protein basis than the immature lenses compared with the same age group. On the basis of statistical analysis, an age-dependent increase in ganglioside concentration was recognized in both mature and immature lens groups. The relative increase in slow-moving polysialogangliosides on thin-layer chromatography seemed to be caused by the maturation of cataract. The sugar composition of one of the polysialogangliosides was found to be glucose, galactose, and sialic acid in the molar ratio of 2:1:4; this suggests the presence of a unique ganglioside species in human cataractous lens. Invest Ophthalmol Vis Sci 31:2171-2179, 1990

Ocular gangliosides in lens tissue were first reported by Feldman and Feldman1 and have been characterized in cow, pig, rat,2,3 and rabbit4 lenses. The human lens is reported to have a total ganglioside concentration 4-11 times higher than cow, pig, or rat lenses.5 Various clinical studies have also been done on gangliosides in human senile cataractous lenses.1,2,5-7 Windeler and Feldman3 and Sarkar and Cenedella2 reported the presence of only two gangliosides, GM3 and GM1, on the basis of thin-layer chromatographic (TLC) analysis. No significant differences were observed in the content or composition of gangliosides from normal and cataractous lenses.

In a more elaborate study using TLC analysis, Tao et al7 found that the human cataractous lens showed a complex ganglioside pattern containing 11 resorcinol-positive bands. The presence of several fucose- and N-acetylglucosamine-containing ganglioside species was shown by combined TLC and gas-liquid chromatography (GLC). In a subsequent study, Tao and Lee8 identified the neuraminic acid moiety of these gangliosides as N-acetylneuraminic acid. More recently, Swindell et al,6 also using TLC, tentatively identified GM3, GM1, and GD1a as major gangliosides and demonstrated the presence of several lesser-intensity resorcinol-positive bands in cataractous nuclei. No statistically significant differences in the three major gangliosides were observed between cataractous nuclei and normal or cataractous whole lenses. However, some minor but possibly significant differences were found in band intensities by densitometric scanning. At present, no conclusive evidence has been obtained to demonstrate whether a more complex ganglioside pattern is implicated in the human cataractogenic process.

Recently, we found an age-dependent increase in lenticular gangliosides in normal lenses from monkeys between the ages of 6-15 yr.9 The ganglioside content in individual clear lenses of rhesus monkeys increased with age, and polysialogangliosides appeared in aged lenses. The total gangliosidic sialic acid content of the monkey lenses was more than one half that of the human lens. The life span of the monkey is reported to be about 15 yr, with 1 yr in the life of the monkey equivalent to approximately 4.5 yr in a human life.10 Thus, monkey lenses provided more comprehensive information on changes in lenticular gangliosides due to aging over a long life span.

In the current study, quantitative and qualitative
changes in lens gangliosides of the human senile cataract were explored taking account of the age-dependent increase. Ganglioside content increased according to two physiologic parameters: aging, as previously described in the monkey lens, and cataractous progression leading to lens opacification. The appearance of slow-moving polysialogangliosides on TLC was occasionally accompanied by the maturation of senile cataracts. In addition, one of them was estimated to have a characteristic sugar composition by GLC analysis. Preliminary reports of these studies have been presented.

Materials and Methods

Human Cataractous Lenses

Human senile cataractous lenses from patients aged 37–84 yr were obtained from the operating room of Toho University Hospital after cataract surgery and kept frozen at −80°C until use. In some experiments, the nuclear and cortical regions were separated under a binocular. The cortical region contained capsular and epithelial cells.

Extraction and Quantitation of Gangliosides

Gangliosides were extracted from individual lenses using organic solvent extraction as described elsewhere. The ganglioside fraction was separated from total lipid extract by DEAE-Sephadex (Pharmacia-LKB, A-25, acetate form; Uppsala, Sweden) column chromatography, and partially purified by a reverse-phase SEP-PAK C18 cartridge (Waters Associates, Millford, MA) after mild alkaline hydrolysis. Total gangliosidic sialic acid content was estimated as the content of N-acetylneuraminic acid (NANA) using the thiobarbituric acid assay described by Warren. The values calibrated by Warren’s equation were corrected against the contents of ganglioside standards used. Protein content was determined using bovine serum albumin as a standard according to the method of Lowry et al.

TLC of Gangliosides

Samples corresponding to one-tenth each of the ganglioside fraction were developed using a precoated high-performance TLC (HP-TLC) silica gel 60 plate (5641; Merck, Darmstadt, FRG, Rahway, NJ) in a solvent system of chloroform/methanol/0.25% CaCl2 solution (55:45:10, v/v). The ganglioside spots were visualized by heating at 95°C after spraying with resorcinol HCl reagent. The intensity of spots was quantitated by densitometric scanning of the TLC plate at 580 nm with a Shimadzu CS-930 TLC scanner (Shimadzu, Kyoto, Japan). Two-dimensional TLC was done according to the method of Ohashi. The solvent systems were chloroform/methanol/0.02% CaCl2 (60:35:8, v/v) as the first dimension and 1-propanol/28% ammonia water/water (75:5:25, v/v) as the second. The ganglioside nomenclature used here is that of Svennerholm.

Ganglioside Mapping

To purify ganglioside species, crude ganglioside fractions underwent ganglioside mapping by high-performance liquid chromatography (HPLC) with an DEAE-Iatrobeads column (4.6 × 250 mm; Iatron, Tokyo, Japan). The gangliosides were applied to a column equilibrated with methanol and separated with a programmed gradient of ammonium acetate (40–50 mM) in methanol at the flow rate of 1 ml/min. One-milliliter fractions were collected, and an aliquot of each fraction was analyzed by TLC. The polysialoganglioside fraction was collected and purified by preparative TLC on a HP-TLC plate.

GLC of Gangliosides

The GLC was used to identify the products obtained after methanolysis of the recovered polysialoganglioside. An aliquot of the ganglioside fraction was treated with 1 M of anhydrous methanolic hydrochloric acid at 80°C for 16 hr. The glycosides of liberated hexosamines and methylneuraminate were converted to N-acetyl derivatives according to the method of Kozulic et al. Fatty acid methyl esters were recovered from the acid methanolyzate by hexane extraction and analyzed isothermally by a Shimadzu gas chromatograph GC-7A at 200 and 250°C on a fused silica capillary column of ULBON HR-101 (0.24 mm × 25 m; Chromato Packings Center, Tokyo, Japan). Methylglycosides were analyzed as their trimethylsilyl (TMS) derivatives on the same column. The column temperature was programmed to increase from 170–230°C at 2°C/min.

Results

Total Gangliosidic Sialic Acid Content in Human Senile Cataractous Lenses

Ganglioside fractions from 13 individual whole cataractous lenses obtained by intracapsular cataract extraction were prepared by the successive steps of solvent extraction, DEAE-Sephadex column chromatography, and SEP-PAK C18 cartridge purification. The cataractous lenses were obtained from patients ranging in age from 49–84 yr. An aliquot of each sample was analyzed for total NANA content...
Table 1. Relationship between lens ganglioside content and progression of human senile cataracts

<table>
<thead>
<tr>
<th>Age (years)</th>
<th>Stage</th>
<th>Sialic acid (nmoles)</th>
<th>Protein (mg)</th>
<th>Sialic acid/protein (pmoles/mg)</th>
</tr>
</thead>
<tbody>
<tr>
<td>49*</td>
<td>incipient</td>
<td>40.1</td>
<td>51.2</td>
<td>783</td>
</tr>
<tr>
<td>60</td>
<td>incipient</td>
<td>48.0</td>
<td>52.6</td>
<td>913</td>
</tr>
<tr>
<td>79</td>
<td>immature</td>
<td>43.5</td>
<td>56.5</td>
<td>770</td>
</tr>
<tr>
<td>72</td>
<td>immature</td>
<td>33.8</td>
<td>50.8</td>
<td>665</td>
</tr>
<tr>
<td>73</td>
<td>immature</td>
<td>40.9</td>
<td>51.5</td>
<td>795</td>
</tr>
<tr>
<td>74</td>
<td>immature</td>
<td>41.1</td>
<td>50.8</td>
<td>810</td>
</tr>
<tr>
<td>78</td>
<td>immature</td>
<td>54.3</td>
<td>53.2</td>
<td>1020</td>
</tr>
<tr>
<td>81</td>
<td>immature</td>
<td>69.6</td>
<td>54.0</td>
<td>1288</td>
</tr>
<tr>
<td>84</td>
<td>immature</td>
<td>62.3</td>
<td>55.6</td>
<td>1120</td>
</tr>
<tr>
<td>64</td>
<td>mature</td>
<td>46.8</td>
<td>48.2</td>
<td>970</td>
</tr>
<tr>
<td>72</td>
<td>mature</td>
<td>67.6</td>
<td>53.3</td>
<td>1268</td>
</tr>
<tr>
<td>73</td>
<td>mature</td>
<td>48.5</td>
<td>53.4</td>
<td>908</td>
</tr>
<tr>
<td>74</td>
<td>mature</td>
<td>68.5</td>
<td>50.0</td>
<td>1370</td>
</tr>
</tbody>
</table>

* Diabetic cataract.

The ganglioside content in individual lenses was determined in duplicate as the amount of NANA liberated from gangliosides in 50 mM H2SO4 at 80°C for 1 hour. Protein content was determined using the protein residue after solvent extraction. The data are arranged according to cataractous stage of the lens and by age within stages.

Average NANA content and NANA content per mg of lens protein showed wide distributions, ranging from 33.8-69.6 pmol and from 665–1370 pmol/mg, respectively (Table 1). The concentration of gangliosides in mature cataracts (1129 ± 112 pmol/mg) was not significantly different from that in immature cataracts (950 ± 95 pmol/mg) (P > 0.05). In this study, protein content was used to compensate for the loss of water content in the specimens kept at −80°C and determined in protein residue after solvent extraction.

TLC Analysis of Lens Gangliosides

An aliquot corresponding to one-tenth of the ganglioside fraction from each individual lens was applied to TLC analysis using an HP-TLC plate (Fig. 1). Several resorcinol-positive bands were revealed in all the fractions from cataractous whole lenses. Three predominant gangliosides, GM3, GM1, and GD1a, were tentatively identified in comparison with the chromatographic behavior of authentic brain gangliosides. Lesser amounts of minor components were recognized in different samples but were not identified. Slow-moving gangliosides, designated as lower than GD1a in the text, were observed, especially in mature cataracts. Two-dimensional TLC provided more comprehensive information on the characteristics of the ganglioside species (Fig. 2). The mixing experiment (Fig. 2B) indicated that the predominant species in the slow-moving gangliosides did not co-migrate with authentic brain GD1b and GT1b, suggesting a different structure for polysialogangliosides. Estimates of the relative amounts of each ganglioside species were made by the densitometric scanning method of Ando et al.16 Apparent differences in ganglioside composition between immature and mature cataracts were indicated only by the appearance of

Fig. 1. HP-TLC separation of gangliosides from lenses of 49- to 84-year-old cataractous patients. An aliquot corresponding to one-tenth of individual lens gangliosides was developed in a solvent system of chloroform/methanol/0.25% CaCl2 (55:45:10, v/v). The gangliosides were visualized by resorcinol-HCl spray. The arrow indicates the slow-moving ganglioside designated in the text. IN = incipient; IM = immature; M = mature cataract.
Fig. 2. Two-dimensional HP-TLC profiles of lens gangliosides. (A) Gangliosides from the mature cataractous lens of a 72-year-old patient were developed twice in solvent systems of chloroform/methanol/0.02% CaCl₂ (60:35:8, v/v) (first dimension) and once 1-propanol/28% ammonia water/water (75:5:25, v/v) (second dimension). (B) A mixture of lens gangliosides and authentic brain gangliosides, GM₁(a), GD₁a (b), GD₁b (c) and GT₁b (d), was developed.

slow-moving gangliosides, and no differences were found in the peak area ratio of mono- to distaloligandosides (data not shown).

Relationship Between Ganglioside Content and Lens Opacification

Human cataractous lenses were classified into three phases, incipient, immature, and mature, according to the location and degree of opacity, as determined by slit-lamp observation. The incipient, immature, and mature groups roughly corresponded to Groups I, I-II, and II-IV described by Pirie. When the data on lens gangliosides were arranged according to this classification, higher values of ganglioside content expressed per mg of protein were seen in mature cataractous lenses from younger patients than in immature lenses (Table 1). The close relationship between aging and lens opacification was studied by plotting the ganglioside values as a function of age (Fig. 3A). For statistical comparison, an additional 16 specimens were examined. In both groups, the correlation coefficients (r) between ganglioside content and aging were calculated to be 0.73 (P < 0.01) for mature lenses (n = 13) and 0.87 (P < 0.01) for immature lenses (n = 12). Diabetic cataractous lenses from 37-, 49-, and 55-year-old patients had ganglioside contents within the range of the correlation. A similar correlation (r = 0.88, P < 0.01) observed in normal monkey lenses has been expressed in terms of human age and inserted in Figure 3A (dotted line). There was a significant difference between mature and immature groups (P < 0.01) when the ganglioside concentration were adjusted to those of 60-year-old humans, according to the corresponding regression lines (Fig. 3B).

Distribution of Gangliosides in Cataractous Lenses

To examine the increase in gangliosides, whole cataractous lenses were carefully separated into nuclear and cortical regions. Mature lenses appeared to accumulate gangliosides in the cortical region rather than in the nuclear region (Table 2). In senile cataracts, the ratio of ganglioside content in the cortical to that in the nuclear region was greater than 1 with the single exception of an 82-year-old immature cataract lens. When the total ganglioside content of each region was expressed per mg of protein, the nuclear region showed lower values than the cortical region. In diabetic patients, enrichment of gangliosides was observed in the nuclear region when nuclear opacity was produced. A significant difference (P < 0.05) between senile and diabetic cataracts was only found in the ratio of ganglioside content in the cortical region to that in the nuclear region. The age-dependent increase of gangliosides in each region was not examined because of the small numbers of specimens.
Fig. 3. Relationship between ganglioside content and aging in whole cataractous lenses. (A) Ganglioside content expressed as NANA per mg protein was plotted against age. Regression lines were calculated for immature (open circles) and mature (closed circles) cataractous lenses. The regression line calculated for normal monkey lenses and adjusted for age difference has been inserted as a dotted line. Asterisks indicate diabetic cataractous lenses. x = incipient. (B) The values were calculated to be equivalent to those at age 60 years on the basis of the ratio to the regression lines (method 1) and the proportional change in content obtained from the regression lines (method 2). Dotted lines indicate the equivalent values for age 60. Immature (open circles) and mature (closed circles) cataractous lenses.

Qualitative changes in ganglioside composition were ascertained by HP-TLC, and band intensities corresponding to ganglioside species were compared between nuclear and cortical regions (Fig. 4). No regional differences were seen. Slow-moving gangliosides were recognized in both regions and in both mature and immature cataract lenses, when the ganglioside samples were applied at 3-4 nmol NANA equivalent.

Properties of Slow-Moving Gangliosides

Slow-moving gangliosides observed in mature cataracts in high proportions were separated by ganglioside mapping using HPLC with a DEAE-Iatrobeads column before compositional analysis by GLC. Figure 5 presents a map of lens gangliosides from cataractous lenses. More than 11 resorcinol-positive bands were recognized on HP-TLC separation, and slow-

Table 2. Distribution of gangliosides in cataractous lenses

<table>
<thead>
<tr>
<th>Age (years)</th>
<th>Stage</th>
<th>Opaque sites</th>
<th>Cortex (A)</th>
<th>Nucleus (B)</th>
<th>A/B</th>
</tr>
</thead>
<tbody>
<tr>
<td>54*</td>
<td>immature</td>
<td>+</td>
<td>28.6 (998)</td>
<td>54.9 (1,651)</td>
<td>0.52</td>
</tr>
<tr>
<td>56*</td>
<td>incipient</td>
<td>+</td>
<td>20.0 (579)</td>
<td>24.5 (348)</td>
<td>0.82</td>
</tr>
<tr>
<td>62</td>
<td>immature</td>
<td>+</td>
<td>26.7 (833)</td>
<td>10.3 (469)</td>
<td>2.59</td>
</tr>
<tr>
<td>64*</td>
<td>immature</td>
<td>+</td>
<td>8.4 (665)</td>
<td>58.3 (1,475)</td>
<td>0.14</td>
</tr>
<tr>
<td>66*</td>
<td>incipient</td>
<td>+</td>
<td>77.9 (2,273)</td>
<td>11.2 (179)</td>
<td>6.96</td>
</tr>
<tr>
<td>66</td>
<td>immature</td>
<td>+</td>
<td>101.8 (2,436)</td>
<td>13.8 (349)</td>
<td>7.38</td>
</tr>
<tr>
<td>66</td>
<td>mature</td>
<td>+</td>
<td>93.3 (3,638)</td>
<td>47.5 (1,339)</td>
<td>1.97</td>
</tr>
<tr>
<td>66</td>
<td>mature</td>
<td>+</td>
<td>72.1 (2,503)</td>
<td>52.0 (1,505)</td>
<td>1.39</td>
</tr>
<tr>
<td>70</td>
<td>mature</td>
<td>+</td>
<td>46.5 (1,495)</td>
<td>35.3 (1,193)</td>
<td>1.32</td>
</tr>
<tr>
<td>73</td>
<td>immature</td>
<td>+</td>
<td>44.5 (1,426)</td>
<td>19.2 (617)</td>
<td>2.32</td>
</tr>
<tr>
<td>75</td>
<td>immature</td>
<td>+</td>
<td>47.4 (1,342)</td>
<td>27.1 (938)</td>
<td>1.75</td>
</tr>
<tr>
<td>80*</td>
<td>immature</td>
<td>+</td>
<td>55.3 (1,945)</td>
<td>49.2 (1,411)</td>
<td>1.12</td>
</tr>
<tr>
<td>82</td>
<td>immature</td>
<td>+</td>
<td>22.5 (686)</td>
<td>23.9 (1,052)</td>
<td>0.94</td>
</tr>
</tbody>
</table>

Whole cataractous lenses were separated into cortical and nuclear regions. Ganglioside content was determined as described in Table 1. The values are expressed as total sialic acid content (nmoles), and as that per mg protein (pmoles/mg) in parentheses.
Fig. 4. Distribution of gangliosides in mature and immature cataractous lenses. An aliquot of each ganglioside fraction from 70-year-old mature and 73-year-old immature lenses were developed as described in Figure 1. Ganglioside patterns from cortical (1) and nuclear (2) regions were compared.

Fig. 5. Mapping of lens gangliosides from cataractous lenses. The lower panel indicates the gradient profile of ammonium acetate used for elution from the DEAE-latrobeads column. One milliliter fractions were collected, and an aliquot of each fraction was applied to TLC. The plate was developed as described in Figure 1. The arrowheads indicate GM3 and a slow-moving ganglioside, which were partially purified by preparative TLC, respectively.

Fig. 6. Gas-liquid chromatogram of O-TMS derivatives of methylglycosides from the slow-moving ganglioside of human cataracts. Conditions of separation are given in "Materials and Methods." Gal = galactose; Glc = glucose; SA = N-acetylneuraminic acid.

Discussion

The TLC separation in whole cataractous lenses found GM3, GM1, GD1a, and minor unidentified gangliosides (Fig. 1). Ganglioside mapping revealed a more complex pattern of gangliosides, consisting of at least 15 components (Fig. 7), than that described by Tao et al.7 In earlier studies by Windeler and Feldman3 and Sarkar and Cenedella,2 human cataractous lens showed a simple ganglioside pattern consisting of GM3 and GM1. In contrast, recent reports by Tao et
Table 3. Fatty acid compositions of gangliosides isolated from human cataractous lenses

<table>
<thead>
<tr>
<th>Fatty acid</th>
<th>GM3</th>
<th>Slow-moving ganglioside</th>
</tr>
</thead>
<tbody>
<tr>
<td>12:0</td>
<td>1.7 (3.6)</td>
<td>3.7 (3.8)</td>
</tr>
<tr>
<td>12:1</td>
<td>2.3 (4.8)</td>
<td>4.7 (4.8)</td>
</tr>
<tr>
<td>14:0</td>
<td>9.8 (20.2)</td>
<td>17.5 (18.0)</td>
</tr>
<tr>
<td>14:1</td>
<td>1.5 (3.1)</td>
<td>4.7 (4.8)</td>
</tr>
<tr>
<td>16:0</td>
<td>25.7 (53.0)</td>
<td>36.9 (37.9)</td>
</tr>
<tr>
<td>16:1</td>
<td>2.1 (4.4)</td>
<td>7.5 (7.7)</td>
</tr>
<tr>
<td>18:0</td>
<td>3.0 (6.1)</td>
<td>12.6 (13.0)</td>
</tr>
<tr>
<td>18:1</td>
<td>2.4 (4.9)</td>
<td>9.8 (10.0)</td>
</tr>
<tr>
<td>22:0</td>
<td>4.8</td>
<td>2.6</td>
</tr>
<tr>
<td>24:0</td>
<td>9.4</td>
<td></td>
</tr>
<tr>
<td>24:1</td>
<td>37.3</td>
<td></td>
</tr>
</tbody>
</table>

Fatty acids are denoted as chain length: number of double bonds. *Values in parentheses are percentages of the sum of values from 12:0 to 18:1.

Sarkar and Cenedella described a more complicated pattern of gangliosides, including polysialogangliosides. The discrepancy with earlier studies may be explained by differences in procedural techniques, for example, the application of small amounts of samples in the dialysis step and the Folch-Suzuki partition procedure. In the current study, the dialysis step was replaced by the SEP-PAK C18 cartridge method since there appeared to be less than a critical amount needed for the gangliosides to form micelles.

Sarkar and Cenedella reported a high ganglioside content in human lens (272.5 μg of NANA/g of wet weight), and similar values for total NANA content were estimated in cataractous nuclei (294 μg of NANA/g of wet weight), whole cataractous lenses (243 μg/g) and whole normal lenses (357 μg/g). We estimated that the ganglioside content in whole cataractous lenses ranged from 248–1486 pmol of NANA/mg of protein. Assuming that the wet weight of lens is 180 mg, ganglioside content as NANA would be 19.2–114.8 μg/g. The lower values are considered to be due to differences in ganglioside determination. The thiobarbituric acid method for total sialic acid estimation is dependent on the release of free sialic acids from gangliosides and provides lower values than those estimated by the resorcinol method as modified by Miettinen and Takki-Luukkainen, but it consumes less of the sample than the latter. In our previous study, much lower estimates of ganglioside content in human cataractous lenses were made, presumably because of the phosphate-buffered tetrahydrofuran method and the dialysis step. In addition, whole normal lenses from 31–37-yr-olds had no less than two thirds of the total ganglioside content of whole cataractous lenses from 55–89-yr-olds. At present, physiologic function of gangliosides in cataractous lenses and the relation of ganglioside content and pattern to cataract formation remain unclear.

This study indicated that two factors, one related to aging and the other to lens opacification, influence the increase in lens gangliosides on a protein basis. The protein content of cataractous lenses fluctuated slightly between 49–84 yr and appeared to increase with aging (Table 1). The age-dependent increase in ganglioside content was shown in individual mature and immature cataractous lenses (Fig. 3A). A high correlation was observed in the immature lens group (r = 0.87), and the mature groups were distributed over the regression line calculated from immature lenses. The greater accumulation of gangliosides in mature compared with immature lenses appears to lead to the maturation of senile cataract. When the age-dependent increase in ganglioside content was not considered, there was no significant difference in terms of cataract progression. However, correction for the age-dependent increase revealed a significant difference between mature and immature cataractous lenses (Fig. 3B). The TLC analysis (Fig. 1) showed that the increase in gangliosides with maturation of the cataract was occasionally accompanied with the appearance of polysialogangliosides. However, immature lenses also showed the presence of the gangliosides when ganglioside samples were quantitatively applied to TLC (Fig. 4), suggesting that they are more or less capable of synthesizing the gangliosides. Although the detailed mechanism has not been clarified, it can be hypothesized that gangliosides accumulate in the lens with aging and the progression of senile cataracts, causing the relative increase in polysialogangliosides in mature cataractous lenses.

In their study of the normal bovine lens, Sarkar and Cenedella reported that the epithelial cell fraction contained an approximately tenfold greater concentration of gangliosides than either the lens cortex or nucleus. Swanson and Albers work with bovine lenses indicated differences in ganglioside patterns between the cortical and nuclear regions of the lens based on TLC analysis. Our study with monkey lenses provided similar evidence: the total ganglioside sialic acid concentration of the cortical region was about four times greater than that of the nuclear region. A complex ganglioside pattern similar to that from whole lens was obtained in the ganglioside fraction from the cortical region. In contrast to lenses in several other animal species, no significant differences in resorcinol-positive bands on TLC were observed between human cataractous nuclei and whole lenses. In addition, ganglioside content expressed per g of wet weight was higher in cataractous nuclei than in whole cataractous lenses, suggesting a higher con-
centration of gangliosides in the nuclear region. Thus, human cataracts appear to possess ganglioside distributions that differ from those of the normal lenses of several animals, as shown in Figure 4. Table 2 indicates that ganglioside accumulation occurs in the cortical region of senile cataract, probably due to an age-dependent increase in plasma membranes. If the age-dependent increase in ganglioside content in each region were clarified, more comprehensive information on lens gangliosides could be obtained. The discrepancy between the sum of ganglioside content in both regions and the values from whole lenses remains unknown, but presumably it is attributable to contamination by nonsialic acids together with small amounts of sialic acids. Diabetic cataractous lenses were characterized by a high accumulation of gangliosides in the nuclear region, suggesting that a different mechanism from that in senile cataracts is operating in the accumulation of lens gangliosides. An attempt to locate lens gangliosides using the immunohistochemical approaches is currently underway.

For structural analysis of predominant ganglioside species, combined TLC-immunostaining using antiasialo-GM1 antiserum (there was no apparent cross-reactivity to gangliosides by enzyme-linked immunosorbsent assay; Seikagaku Kogyo, Tokyo, Japan) and acid hydrolysis to remove the sialic acid moiety from the ganglioside suggested that, except for GM3 and GM2, they consisted of the gangliotetraosyl backbone.11 However, slow-moving gangliosides, noted in this and a previous study,11 were regarded as a mixture of gangliosides with RF-values lower than that of GD1a. As shown in Figures 1 and 2, trace levels of GD1b and GT1b were detected. Judging from these facts, antiasialo-GM1 antiserum is considered to react immunologically exclusively with the backbones originating from contaminated GD1b and GT1b. In the current study, GLC analysis revealed that one of the slow-moving gangliosides was composed of glucose, galactose, and sialic acid in the molar ratio of 2:1:4 (Fig. 6). Surprisingly, the ganglioside was eluted in the disialoganglioside fraction by DEAE-Iatrobeads ganglioside mapping despite the high proportion of sialic acid in the molecule. In addition, other slow-moving gangliosides also had a similar chromatographic behavior on two-dimensional TLC and ganglioside mapping (Figs. 2, 5). Thus the cataractous human lens seems to contain certain ganglioside species, which have unreported sugar-chain structures as minor components. The long-chain base, complete structure, and precursor(s) of the slow-moving ganglioside examined in the current study are under investigation.

In their study of the fatty acid composition of cataractous lenticular gangliosides, Feldman et al5 found that palmitic (16:0) and nervonic (24:1) acids predominated in GM3 and GM1. Tao et al7 also reported that nervonic acid was the predominant fatty acid in GM3 and GM1. More recently, Swindell et al6 determined fatty acid percentages from total lenticular ganglioside extract and indicated that nervonic acid is increased in both cataractous nuclei and whole cataractous lenses compared with whole normal lenses. Our data on GM3 were compatible with those of earlier reports, but data on the slow-moving ganglioside relating to the maturation of cataracts were not. Our findings indicate that the slow-moving ganglioside which lacks long-chain fatty acids such as nervonic acid is synthesized by a different pathway. The fatty acid composition of other ganglioside species was not undertaken in this study, but it was expected to be the same as that of GM3 because GM1 and GD1a are synthesized from GM3 by the a-pathway of gangliosides.

Key words: human cataractous lens, gangliosides, senile cataract

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References