Alkaline borohydride-reduced keratan sulfate chains were isolated from human articular cartilage aggrecan from individuals of various ages (0–85 years old). The chains were structurally characterized using $^1$H NMR spectroscopy, gel permeation chromatography, and oligosaccharide profiling (after digestion with the enzymes keratanase and keratanase II). The results show that from birth to early adolescence (0–9 years) the levels of $\alpha$(1–3)-fucosylation, $\alpha$(2–3)-sialylation, and galactose sulfation increase. Also, the weight-average molecular weight of the chains increases. During maturation (9–18 years) the levels of fucosylation and galactose sulfation continue to increase and $\alpha$(2–6)-sialylation of the chains occurs. In adult life (18–85 years) there is little change in the weight-average molecular weight of the chains, and the levels of fucosylation, sialylation, and sulfation remain fairly constant.

Cartilage consists of a sparse array of cells (chondrocytes) distributed within an extracellular matrix of macromolecules that they secrete. Polyamionic proteoglycans are trapped within a network of collagen fibers, and these serve to attract and organize water molecules to form a viscoelastic gel that provides tissue resilience and compressibility. The major proteoglycan within cartilage is the large aggregating proteoglycan, aggrecan. This consists of a central protein core (250–300 kDa) that is substituted with the glycosaminoglycans, keratan sulfate, and chondroitin sulfate. Up to 50 KS1 chains can be present, most of which are clustered together in a KS-rich domain close to the N terminus of the protein core (1, 2).

The concentration of keratan sulfate in human cartilage increases markedly during maturation, but it continues to increase throughout life (3–6). This increase is mostly due to the decrease in aggrecan size that occurs during aging (7–9), which results in a proteoglycan containing proportionally more keratan sulfate. This may be partly due to the KS chains themselves increasing in size (10, 11), although some studies have reported finding no change in chain size with age (12). Other research has suggested that the level of sulfation of KS increases during normal aging, although these investigations were not extensive and did not provide any details of the structural changes occurring in the glycosaminoglycan (13–15).

It is now apparent that catabolic products of aggrecan can be detected in serum in the process of cartilage breakdown, and these may be important for the early diagnosis of degenerative joint disease. Several groups have demonstrated that osteoarthritic patients have significantly higher levels of KS in both serum (16–19) and synovial fluid of the affected joint (18, 20, 21). However, other studies have recorded no change in the circulating and synovial fluid concentrations of KS in osteoarthritic patients (22, 23). The analysis of KS in body fluids has almost invariably been measured using enzyme-linked immunosorbent assay with monoclonal antibodies recognizing specific sulfation epitopes. These are likely to be structural features of only a small proportion of the glycosaminoglycan chains, and this, together with the natural heterogeneous expression of osteoarthritis, is probably the main reason for the apparently conflicting published data.

The structure of keratan sulfate is based upon a poly-N-acetyllactosamine backbone of Gal$\beta$(1–4)GlcNAc$\beta$(1–3) which is almost always sulfated on C(6) of N-acetylgalactosamine but to a variable extent on C(6) of galactose (24). KS derived from articular cartilage contains sialic acid (N-acetylsialameric acid) and fucose as minor structural components. The sialic acid residues can be either $\alpha$(2–3)- or $\alpha$(2–6)-linked to galactose and occupy nonreducing terminal positions. Both types are present at the end of the poly-N-acetyllactosamine repeat sequence (25), and $\alpha$(2–3)-NeuAc is found in the linkage region (to protein). The fucose residues are linked $\alpha$(1–3) to N-acetylgalactosamine and occur within the main poly-N-acetyllactosamine repeat (26, 27). Thus, there are numerous structural aspects of the molecule that could give rise to unique “markers” of normal aging and also specifically reflect abnormal metabolic responses in diseased tissue. In the present study, keratan sulfates have been isolated from aggrecan derived from human articular cartilage of different ages, and their detailed structure has been investigated using $^1$H NMR spectroscopy and degradative studies.

**EXPERIMENTAL PROCEDURES**

**Materials**—Guandine hydrochloride (practical grade), benzamidine hydrochloride, N-ethylmaleimide, and sodium borohydride were obtained from Sigma. Caesium chloride, di-sodium EDTA, and 6-amino-hexanoic acid were purchased from BDH Chemicals (Poole, UK). Lithium perchlorate (A.C.S. grade), piperezine, anhydrous hydradine, and hydrazine sulfate were from Aldrich. All other chemicals were of analytical grade.

Diphenyl carbamyl chloride-treated trypsin (bovine pancreas, EC 3.4.21.4) was purchased from Sigma. Chondroitin ABC lyase (Proteus vulgaris, EC 4.2.2.4) and keratanase II (Bacillus sp.) were obtained from ICM Biomedicals Ltd. (High Wycombe, Buckinghamshire, UK). Sepharose CL-6B and SephadeX G-50 (medium grade) were obtained...
from Pharmacia Fine Chemicals (Uppsala, Sweden). Bio-Gel P2 (fine grade) was from Bio-Rad. The Mono-Q HR 5/5 (5 × 0.5 mm) column was obtained from Pharmacia. The Bio-Gel TSK 30 XL (30 × 0.78 cm) and Aminex HPX-87H columns were from Bio-Rad. Visking dialysis tubing (3) patella; see Table I) using 4M guanidine hydrochloride including pro-

Preparation of Human Articular Cartilage Keratan Sulfates—Kera-
tan sulfate chains were prepared essentially by the method described by Dickenson et al. (28). This involved the extraction of the proteoglycans from comminuted cartilage (from the femoral head, femoral condyle, or patella; see Table I) using 4M guanidine hydrochloride including pro-

tease inhibitors followed by associative CaCl2 density gradient centrifu-
gation. Typically over 95% of the extractable KS present was in the high buoyant density fraction. The proteoglycan aggregate fraction was di-
gested with chondroitin ABC lyase followed by diethyl carbamyl chlo-
ride-treated trypsin and then subjected to gel permeation chromatog-

raphy on a column of Sepharose CL-6B (114 × 2.6 cm) eluted with 0.5 M sodium acetate/10 mM EDTA. Fractions were pooled to give the peptide-glycan fragments 6B1 (i.e. the KS-rich region, typical yield >85% of total KS present). KS chains were subsequently isolated by alkaline borohydride reduction (29) followed by chondroitin ABC lyase digestion and gel permeation chromatography on a column of Sephadox G-50 (82 × 1.5 cm) eluted with 0.15 M NaCl (typical yield, >90% total KS present). After dialysis against water, the KS chains were recovered by lyophilization and then purified further from any remaining O-

linked oligosaccharides (30) by ion exchange chromatography on a Pharmacia Mono-Q HR 5/5 column (5 × 0.5 cm) eluted with a linear gradient of 0–0.5 M LiClO4/10 mM piperazine (typical yield, >95% total KS present). Whenever possible column fractions were pooled identi-
cally for each sample to ensure comparability.

Keratan Sulfate Hydrodynamic Size—Estimations of keratan sulfate hydrodynamic size were made using a Bio-Gel TSK 30 XL column (30 × 0.78 cm) as described earlier (31). Weight-average molecular weights (Mw) of keratan sulfates were calculated using the following formula:

\[ \text{log} M_w = 4.588 - (2.128 \times K_v), \]

where \( V_v \) and \( V_f \) are 11.4 and 20.0 min, respectively.

Carbohydrate Analysis—Fucose and galactose contents and ratios were determined by carbohydrate analysis as described by Tai et al. (26) using methods similar to those described by Lohmander (32) with post-column derivatization using 2-cyanacetic acid (33).

Keratanase Digestion—Keratan sulfate chains (200 μg) were dis-

olved in 200 μl of 0.2 M sodium acetate, pH 7.4, and 0.07 units of enzyme were added (equivalent to 1 unit/2.8 mg KS). Digestion was performed at 37 °C for 24 h. These conditions had previously been determined to give a limit digest. The digest was reduced by the addi-
tion of 2 μl of NaBH4/0.1 μl of NaOH at 45 °C for 24 h. The reaction was terminated by the careful addition of 0.5 M CH3COOH, and the oligosaccharides were desalted on a column of Bio-Gel P2 and lyophi-
lized. The reduced oligosaccharides were subjected to gel filtration on a TSK 30 XL column as described previously.

Keratanase II Digestion—Keratan sulfate chains (100 μg) were dis-

olved in sodium acetate buffer (100 μl), and 200 microunits enzyme were added. Digestion was performed at 37 °C for 30 h. These condi-
tions had previously been determined to give a limit digest. The pH of the mixture was raised to approximately 7.5 by adding 2 μl of NH4OH, and 3.8 mg of NaBH4 were added (equivalent to 1 μl). Reduction was performed at room temperature for 3 h. The reaction was terminated by the careful addition of 0.5 M CH3COOH, and the oligosaccharides were desalted on a column of Bio-Gel P2 and lyophilized. Approximately 10 μg of the reduced oligosaccharides were chromatographed on a Dionex AS4A-SC column using the procedure of Brown et al. (34).

NMR Spectroscopy—Samples were prepared for high field NMR spectroscopy as described previously (38). Spectra were determined at either 23 or 55 °C, the chemical shift of the residual HOH signal varying with temperature. All chemical shifts are quoted relative to internal sodium 3-(trimethylsilyl)-[2H4]propionate at 0.0 ppm. Spectra were reprocessed for presentation using the NMRi software package, NMR1, version 1-3-4, supplied by New Methods Research Inc. (Syra-
cuse, NY). Axes for spectra are in ppm.

RESULTS

KS Chain Hydrodynamic Size

The hydrodynamic sizes of the human articular cartilage keratan sulfate chains were determined on a TSK 30 XL column previously calibrated using keratan sulfate oligosaccha-
rides (Table I). The results shown in Fig. 1 demonstrate that

### Table I

| Age       | Cartilage source | \( M_w \) of KS chains* |
|-----------|------------------|------------------------|
| Newborn fetal (i) | femoral condyle | 4000                  |
| Newborn fetal (ii) | femoral condyle | 4700                  |
| 5 (i)      | femoral condyle  | 4850                  |
| 5 (ii)     | femoral condyle | 5580                  |
| 9 (i)      | femoral condyle | 6700                  |
| 9 (ii)     | femoral condyle | 8250                  |
| 11        | tibial plateau and patella | 6400            |
| 14        | femoral condyle | 7100                  |
| 18        | femoral condyle | 7550                  |
| 19 (i)     | femoral condyle | 6700                  |
| 19 (ii)    | femoral condyle | 8050                  |
| 23        | femoral condyle | 6500                  |
| 24        | femoral condyle | 6300                  |
| 31        | femoral condyle | 6600                  |
| 38 (i)     | femoral condyle | 6000                  |
| 38 (ii)    | femoral condyle | 6450                  |
| 55        | femoral condyle | 6650                  |
| 85        | femoral head | 5700                  |

* Determined by gel permeation chromatography on a calibrated TSK 30 XL column.

FIG. 1. Weight-average molecular weights of human articular cartilage keratan sulfates as a function of age. The TSK 30 XL column (30 × 7.8 cm) was eluted with 0.2 M NaCl at a flow rate of 0.5 ml/min at 30 °C. \( V_v \) and \( V_f \) are 11.4 and 20.0 min, respectively.

The KS chains isolated from the two full term fetal (\( M_w = 4000 \) and 4700) and 5-year-old (\( M_w = 4850 \) and 5850) cartilage samples are significantly smaller in size than those from the older specimens over the age of 9 years old (\( M_w \) average = 6750 ± 700). A significant increase in average chain size occurs during adolescence (0–9 years), and although KS preparations from individual adult specimens display a high degree of vari-
ability (\( M_w \) range, 5700–8250), the data suggest that over 9 years of age there is little change in KS molecular weight.

NMR Spectroscopic Studies

Sulfation Levels—Several signals in the 1H NMR spectra of keratan sulfates yield information on the level and position (Gal or GlcNAc) of sulfation. The GlcNAc H(1) anomeric reso-
nance occurs at –4.75 ppm and are known to be sensitive to their immediate sulfation environment (36). When the galac-
tose residue on the reducing side of the GlcNAc is sulfated the H(1) resonance occurs at –4.76 ppm; however, when the galac-
tose is unsulfated the signal occurs at 4.76 ppm; however, when the galac-
tose residue on the reducing side of the GlcNAc is sulfated the H(1) resonance occurs at –4.76 ppm; however, when the galac-
tose is unsulfated the signal occurs at 4.76 ppm. These shift differences usually give rise to a signal resembling a triplet consisting of two overlapping doublets. In addition, as galac-
Fucose occurrence as doublet resonances at 2.64 ppm; as N-acetylglucosamine anomeric resonances. N-acetylglucosamine residues have been detected.

All of the human KS samples have been analyzed by \(^{1}H\) NMR spectroscopy. Representative partial high field spectra of keratan sulfates isolated from full term fetal and 11-, 31-, and 38-year-old human articular cartilage are shown in Fig. 2. It can be seen that the pattern of signals at \(-3.97\) ppm varies between samples reflecting differences in galactose sulfation levels. The fetal samples possess the lowest galactose sulfation level (\(-20–30\%\)), whereas the 31- and 38-year-old samples are much more highly sulfated (\(-70–80\%\)). The 11-year-old sample is intermediate at 50–60\%. In general, the samples up to 11 years of age exhibit galactose sulfation levels of 20–50\%, whereas those of samples within the 18–85-year-old range are between 60 and 80\%. This suggests that galactose sulfation levels increase during adolescence and early maturation and then remain fairly constant. However, it is also possible that KS chains with increased galactose sulfation levels have a longer half-life than their less sulfated counterparts and that this apparent increase in sulfation of the KS population as a whole reflects the faster turnover of fetal-type chains.

Fucosylation—The H(1) and H(6) protons of α(1–3)-linked fucose occur as doublet resonances at \(-5.12\) and \(-1.17\) ppm, respectively (37). These signals occur within regions of the \(^{1}H\) NMR spectrum free of other KS signals and so tend to be clearly visible if present. Examination of the \(^{1}H\) NMR spectra of the human keratan sulfates reveals the presence of α(1–3)-linked fucose in all of the samples; however, the degree of substitution varies considerably. It is evident that the fetal KS samples contain the lowest amount of this component (0.1% w/w, determined by carbohydrate analysis) with a H(1) doublet resonance only just visible in the \(^{1}H\) spectrum at \(-5.12\) ppm (Fig. 2a). Similarly, all the KS samples up to 11 years old (Fig. 2b) also contain markedly lower levels of fucose (0.1–0.4% w/w) than the other human samples studied from the older ages (18–85 years old), which generally contain fucose at the \(-1.5\%\) level (Fig. 2c). The most notable exception is that isolated from a 38-year-old (sample (i)), which contains a much higher level at \(-2.7\%\) (Fig. 2d). Clearly, the fucose content of human articular cartilage keratan sulfates increases during adolescence. During subsequent aging, KS chains from most adult individuals have similar fucose contents; however, there are exceptions, and some samples show a markedly different composition, the cause of which is unknown.

Sialylation—The H(3ax) and H(3eq) protons of α(2–3)-linked sialic acid give rise to characteristic resonances at \(-1.80\) and \(-2.77\) ppm, respectively, whereas those slightly further upfield at \(-1.70\) and 2.70 ppm derive from sialic acid that is α(2–6)-linked. Although quantitative interpretations of these signals are difficult due to the complexity of the overlapping resonances, careful analysis of high field spectra can identify the presence (or absence) of sialic acid in a discrete environment. Table II gives details of the sialic acid H(3ax) and H(3eq) proton chemical shifts in the several sialic acid-containing environments identified for bovine articular cartilage keratan sulfates occurring as either capping sequence or linkage region oligosaccharides (38).

Examination of the \(^{1}H\) NMR spectra of the human keratan sulfate samples (Fig. 3) reveals the presence of sialic acid in several environments. It is apparent that the fetal KS samples contain no detectable α(2–6)-linked sialic acid and that the level of α(2–3)-linked sialic acid is much lower than in the other human samples studied (Fig. 3a). This finding is surprising because these samples might have been expected to contain proportionally more sialic acid than those from the older ages because the hydrodynamic size of the chains appears to be much smaller (M<sub>w</sub> = 4000 and 4700). Analysis of the α(2–3)-linked sialic acid signals at \(-1.8\) ppm indicates the presence of at least two environments. The major component at 1.80 ppm corresponds to sialic acid in the capping sequence NeuAcα(2–3)Galβ1–4GlcNAc(6S)β1–1, whereas the minor component (at 1.794 ppm) represents the α(2–3)-linked sialic acid in the linkage region. The highly sulfated cap, NeuAcα(2–3)Galβ1–4GlcNAc(6S)β1–1, is not detectable in the \(^{1}H\) NMR spectrum of fetal KS, presumably due to the low level of sulfation within these samples. The 5-year-old samples also contain no detectable α(2–6)-linked sialic acid; however, the α(2–3)-linked sialic acid content is broadly equivalent with that found in most of the older samples (Fig. 3b). The 9- and 11-year-old samples also contain little if any α(2–6)-linked sialic acid, although levels of α(2–3)-linked sialic acid are normal. The remainder of the human samples all demonstrate the presence of five sialic acid environments summarized in Table II, with the sequence NeuAcα(2–3)Galβ1–4GlcNAc(6S)β1–1 as the dominant structure (Fig. 3, c and d), although the relative proportions vary considerably, particularly the α(2–6)-linked sialic acid content.

**Fig. 2.** Partial high field \(^{1}H\) NMR spectra of human articular cartilage keratan sulfates showing fucose and N-acetylglucosamine anomeric resonances. a, fetal (full term, sample (ii)); b, 11-year-old sample; c, 31-year-old sample; d, 38-year-old (sample (i)). Spectra a, b, and c were measured at 600 MHz, and d was measured at 500 MHz.
Degradative Studies

Keratanase Digestion—Four human keratan sulfate samples (namely 19(i)-, 24-, 38(i)-, and 85-year-old) containing similar galactose sulfation levels as judged by NMR spectroscopy were digested with keratanase (an endo-β-galactosidase) to assess the size of the fully sulfated block structures. Examination of the gel permeation chromatograms shown in Fig. 4 indicates considerable variation in the pattern of enzyme digestion. It can be seen that the sample from the 19-year-old is relatively resistant to degradation by the enzyme, resulting in a relatively high proportion of large oligosaccharides. This has two potential implications for the detailed microstructure of the chains. Either the sample possesses a large proportion of fully sulfated blocks, possibly separated by keratanase-sensitive oligosaccharide sequences possessing low galactose sulfation, or it contains a high proportion of fucose residues that are known to inhibit the action of the enzyme (39). Providing that the fucose is evenly distributed within the repeat sequence, the latter hypothesis can be discounted because this sample contained a fucose content (1.4%) similar to that in the 24- and 85-year-old samples (1.3 and 1.5%, respectively), which were fragmented to a much greater extent (Fig. 4, b and d). By contrast, the sample from the 38-year-old (Fig. 4c) produced a larger quantity of smaller oligosaccharides, suggesting that galactose sulfation is more evenly distributed within the keratan sulfate chains.

Keratanase II Digestion—The detailed structures of all the human samples were analyzed using the keratanase II/Dionex anion exchange chromatography oligosaccharide profiling technique described by Brown et al. (34). It can be seen from the comparison of partial chromatograms shown in Fig. 5 that these analyses confirm those obtained by NMR spectroscopy regarding the age-related increases in the proportions of α(2-
and a (2–6)-linked sialic acid and a (1–3)-fucose. Interestingly, oligosaccharide profiling of one of the fetal samples (Fig. 6) reveals that almost all of the fucose and sialic acid is present in the capping structure NeuAc α(2–3)Galβ1–4GlcNAcα(6S)β1–3Galβ1–4GlcNAcα(6S)-ol (i.e. a sulfated VIM-2 epitope; (40)). This is surprising because the fucose content is low in this sample, and this structure is only present at very low levels in all the other KS samples studied from the older cartilages. The significance of this finding is not yet understood; however, it is possible that such antigens may be important in the early stages of tissue development (41).

**DISCUSSION**

This is the first detailed study documenting the changes that occur in the structure of aggrecan-derived keratan sulfate as a function of age. It is clear that the fetal KS samples are significantly different from those isolated from older cartilages. The analytical data show that (i) the sulfation level on galactose is low (<20–30%); (ii) the fucose content is very low (0.1% w/w); (iii) α(2–3)-sialylation is low; and (iv) α(2–6)-sialylation is absent. In addition, the 1H NMR spectrum of one of the fetal KS samples displays a doublet resonance at ~4.98 ppm (Fig. 5a) that is not present in any other human sample studied. Although the origin of this signal is unknown, it clearly derives from the anomeric proton of an α-linked sugar (the coupling constant is small, ~3 Hz). This resonance has been seen before on the 1H NMR spectra of keratan sulfates isolated in this
laboratory from very young bovine articular cartilage (36) but has not yet been identified. It is possible that this fetal-specific feature represents an alternative to sialic acid as a chain cap (quite possibly considering the relative deficiency of sialic acid caps in this sample), and the Dionex fingerprint of this sample did reveal the presence of several minor unknown peaks (data not shown).

The KS samples from all of the younger cartilages studied (5–11 years old) also display significant differences from those derived from older material. The α(2-6)-linked sialic acid is either not present or present at very low levels, and the fucose content is very low (0.1–0.4% w/w). The sulfation level on galactose (~30–50%) is generally intermediate between that in the fetal samples and that in the older samples.

From the results presented here, the aging process has several implications for the detailed structure of human articular cartilage keratan sulfates (Scheme 1) and can be divided into three stages: birth to early adolescence (0–9 years), maturation (9–18 years), and adulthood (18–85 years) (Table III).

The functions of sialic acid and fucose in keratan sulfates are still not clear. It is possible that sialic acid residues, present as chain caps, have significance for chain degradation. Sialic acid masks the galactose residue to which it is attached, preventing the removal of the glycoprotein from the circulation via Gal-specific receptors on the cell surface of hepatocytes (42). Removal of the sialic acids by sialidases causes the rapid removal of the glycoprotein from the circulation. It is possible that the sialic acid chain caps in KS, present in several discrete environments, dictate the lifetime of these molecules. At present, nothing is known about the rates of removal of α(2-3)- versus α(2-6)-linked sialic acid glycoproteins and glycosaminoglycans in serum. The presence of fucose may also have potential significance for the degradation of the molecules. Studies using the KS degrading enzymes keratanase and keratanase II have shown that fucose residues in the vicinity of susceptible glycosidic bonds inhibit cleavage (39). If glycosidases responsible for the breakdown of KS in vivo also possess this substrate specificity, fucosylated chains may be less susceptible to degradation.

The interest in keratan sulfate study has been partially stimulated by the possible assay of these molecules in the early diagnosis of arthritic diseases and in the monitoring of cartilage catabolism. It is known that the degradation of proteoglycans occurs early in joint damage (43) and that fragments are released into the synovial fluid and subsequently the serum (44, 45). The quantitation of keratan sulfates in both serum and synovial fluid has therefore been proposed as a measure of cartilage breakdown. Currently, most measurements involve an enzyme-linked immunosorbent assay approach using the anti-KS antibodies, 5D4 and MZ15, which recognize fully sulfated blocks of hexaascarhide size and larger (46). The results obtained from keratanase analysis of the KS chains show that even in samples where the overall galactose sulfation levels are similar, considerable variation in the distribution of sulfate groups can occur within the poly-N-acetyllactosamine repeat sequence. This has considerable significance for their study and quantitation by these monoclonal antibodies. It is clear that for the samples studied here, the KS chains from the 19-year-old would bind to these antibodies much more strongly than those from the 38-year-old. It is clear that new quantitative techniques for the assay of KS concentration need to be developed if keratan sulfate is to be used as an accurate measure of cartilage breakdown. The identification of oligosaccharide sequences unique (or present in high abundance) to KSs in high load-bearing areas may be important to this goal. The lesions leading to osteoarthritis are known to appear first in the most highly loaded areas of the cartilage surface (47), and it is possible that “load-related epitopes” could be present in high concentrations in synovial fluid (and possibly blood) of patients with arthritic disease. At Lancaster, studies are now in progress to identify such load-related epitopes and determine whether they are present in keratan sulfates and whether their detection and quantitation can be used to monitor cartilage loading and hence breakdown of the extracellular matrix. The discovery that both α(1–3)-linked fucose and α(2-6)-linked sialic acid are present only in articular (i.e. load-bearing) cartilage keratan sulfates (48) and that their proportions change significantly during adolescence at a time when extensive cartilage remodelling is occurring suggests that putative load-related epitopes may contain one or more of these structures.

Acknowledgments—Tai Guihua, Bob Lauder, and Haydn Morris are thanked for helpful discussions.

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\(^2\) K. M. Whitham, unpublished results.

Human Keratan Sulfates

| Table III | Developmental changes that occur in human keratan sulfates |
|-----------|----------------------------------------------------------|
| Stage     | Characteristics                                           |
| Birth to early adolescence (0–9 years) | i. The levels of fucosylation and galactose sulfation increase. |
|           | ii. The molecular weight of the KS chains increases significantly. |
|           | iii. α(2-3)-linked sialic acid content increases. |
| Maturation (9–18 years) | i. The levels of fucosylation and galactose sulfation continue to increase. |
|           | ii. α(2-6)-sialylation occurs. |
| Adulthood (18–85 years) | i. The molecular weight of the KS chains remains fairly constant. |
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