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Acid Mucopolysaccharide Patterns in Aging Human Cartilage*

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The relative proportions and chemical structures of acid mucopolysaccharides (AMP) of cartilage change during embryonic development and early growth stages of vertebrates (1). Changes from normal in AMP patterns have also been noted in many pathological conditions in humans, where frequency and severity of pathology generally increase with age (2, 3). However, it has been evident to many investigators that a better knowledge of nonpathological cartilage of humans is an essential preliminary to assessing the significance of pathological findings.

Loewi (4) assayed hexosamine and sulfate in AMP extracted from costal cartilage and concluded that the tissue concentration and degree of polymerization of chondroitin sulfate (CS) vary inversely with age. Kuhn and Leppelmann (5) assayed articular cartilage hydrolysates and reported that with increasing age total hexosamine, as well as galactosamine, declines, whereas glucosamine increases slightly. Stidworthy, Masters, and Shetlar (6) found that galactosamine and uronic acid levels in costal cartilage fall with increasing age, whereas glucosamine levels rise during maturation but remain constant thereafter. They suggested that since galactose is also present in the hydrolysates, the glucosamine is associated with skeletal keratosulfate (SKS). Kaplan and Meyer (7) extracted AMP from costal cartilage and calculated the amounts of CS and SKS from analyses for hexosamine, uronic acid, hexose, and sulfate on partially separated fractions of AMP. They concluded that the total amount of chondroitin sulfate in cartilage decreases linearly with age, whereas SKS rises to a plateau value at 20 to 30 years and remains constant thereafter. It was also stated that newborn cartilage contains only chondroitin sulfate A (CS-A), whereas adult cartilage contains predominantly chondroitin sulfate C (CS-C).

However, there are several reasons why analyses for hexosamines, hexoses, and sulfate on whole tissue, or on partially purified fractions of AMP, are inadequate for calculation of the tissue content of individual AMP components. First, human cartilage has been shown by Anderson (8) to contain considerable quantities of amino sugars and hexoses that appear to be constituents of macromolecules other than AMP. Second, the SKS of cartilage, clearly different (9, 10) from corneal keratosulfate (CKS), contains, in addition to 1 mole galactose, up to 0.1 mole galactosamine per mole glucosamine. Third, cartilage SKS contains an excess of 0.3 to 0.8 mole sulfate above the expected 1.0 mole sulfate per mole glucosamine. Finally, differentiation between CS-A and CS-C as well as determination of molecular constants of AMP components requires well-purified and chemically characterized fractions.

In view of the above considerations, our investigation of the AMP composition of human cartilage was based upon isolation of purified AMP components.

Methods

Isolation of crude AMP. Human cartilage was obtained at postmortem examination 4 to 12 hours after

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death.[1] In each case, the larynx with 4 to 6 attached tracheal rings and approximately 2.0-cm parasternal lengths of the sixth, seventh, and eighth costal cartilages were placed in clean, dry jars and frozen. One to 4 weeks later, the cartilages were removed to room temperature and allowed to thaw. Costal cartilages were cut into discs, and the perichondrium and attached soft tissues were dissected away. After soft tissues were scraped from the thyroid, cricoid, and tracheal cartilages, these were cut into small strips approximately 0.2 cm thick. Grossly detectable calcific foci were discarded. Fetal costal cartilages were removed from nonmacerated fetuses delivered spontaneously and prepared in a similar manner. Death of children and adults was due to a variety of causes, in most instances associated with chronic diseases such as malignant neoplasms and degenerative cardiovascular diseases. Several individuals died suddenly due to cerebral hemorrhage or accidental trauma. None of the cartilages used were involved by any grossly evident disease process.

The tissue was digested with crystalline papain[2] in 0.1 M PO_4 buffer, pH 6.5, containing 0.01 M cysteine and 0.01 M EDTA at 60° C for 18 hours. For each gram of tissue, 5 mg of enzyme in 30 ml buffer was used. The resulting solution was dialyzed for 2 days in running tap water. Saturated NaOH was added to a final concentration of 0.5 mole per L and the mixture kept at 2° C for 18 hours. Glacial acetic acid was used to neutralize the solution to near pH 7 and ⅓ vol of 40% trichloroacetic acid solution added. After 2 to 3 hours at 2°, the solution was clarified by filtration with suction through Celite, neutralized with NaOH, and dialyzed against running tap water. The crude AMP was precipitated with a 4% solution of cetylpyridinium chloride in the presence of 0.04 M NaCl, the precipitate dissolved in 12 ml of a mixture of 2 vol of 2.5 M NaCl and 1 vol methanol, and the AMP precipitated as sodium salts by the addition of 70 ml of 85% (vol/vol) ethanol. After standing at 2° overnight, the precipitate was washed with ethanol and ether and dried in vacuum over P_2O_5.

Fractionation of crude AMP. About 300 mg of crude AMP was absorbed from 40 ml water solution upon a 40-cm column containing 250 ml of Dowex 1 × 2 resin, 200 to 400 mesh, chloride form.[3] The column was eluted successively with about 600-ml vol each of water and NaCl solutions of 1.0, 1.3, 1.5, 1.6, 1.7, and 5.0 moles per L for 24-hour periods. After dialysis to remove salts and concentration to small volumes, cetylpyridinium chloride was used to precipitate the AMP, which was finally recovered as the sodium salt by the above described procedure.

Digestion of AMP by hyaluronidase. Crude AMP preparations and fractions from resin were dissolved at a concentration of 0.30 ± 0.02 mg hexosamine per ml and digested with testicular hyaluronidase. The residual turbidity was measured in a procedure that was essentially as previously described (11) except that the enzyme concentration was 100 turbidity reducing units per tube and the period of incubation was 2 hours. In control experiments with mixtures of resin fractions characterized as highly purified CS and SKS, the per cent reduction of turbidity was directly proportional to the per cent of CS in the mixture to a precision of about 5%. The per cent reduction of turbidity in crude AMP preparations thus gave a measure of the relative proportions of CS and SKS. Calculation of these proportions from analytical values of uronic acid and hexosamine agreed with the enzymatic results within 10%.

Determination of CS-A and CS-C. The determination of CS-C, and of CS-A by difference, was performed as previously described (12) on fractions (usually the 1.5 M NaCl eluate) from resin columns shown by chemical analyses and hyaluronidase digestion to contain essentially only these two AMP. The fraction chosen represented frequently only two-thirds of the total CS in the crude AMP. However, it was considered representative in composition, since CS-A and CS-C are not appreciably separated by the resin column.

Molecular parameters. The procedures for viscometry and for osmometry (13) have been previously described. The concentration of solutions was calculated from the analytical data for each preparation based upon a repeating unit for CS of equimolar quantities of glucuronate, acetylgalactosamine, and ester sulfate. For SKS the repeating unit used was a hypothetical one containing equimolar galactose and acetylglucosamine with ester sulfate based upon analyses (9). The repeating disaccharide weight for SKS thus varies from 450 for sulfate/hexosamine = 1 to 536 for sulfate/hexosamine = 2. Thus, both intrinsic viscosity, $[\eta]$, and the number average molecular weight, M_n, reflect only a moiety of the SKS molecule and neglect both galactosamine and peptide.

Neutral sugars. Chromatography of neutral sugars was performed after hydrolysis of the sample in 0.4 N HCl at 100° C for 4 hours. The solution was neutralized with resin in carbonate form, passed over Dowex 50 × 12 resin (H^+ form) to remove amino sugars, and dried. Whatman 1 paper was used in descending chromatography for 72 hours with a solvent containing tertiary amyl alcohol, 99% isopropanol, and water in a volume ratio of 4:1:1.5.

Analyses. Procedures for the determination of sulfate, galactose, nitrogen, uronic acid, and hexosamine have been previously described (14). Analysis for amino acids and for galactosamine and glucosamine were performed on the Technicon autoanalyzer with samples hydrolyzed for 24 hours at 100° in 6 N HCl. Since hydrolysis destroys about 25% of the amino sugars, the ratios of amino acid to hexosamine reported are uniformly too high by this percentage. Hexose was determined by a phenol-H_2SO_4 method (15), sialic acid by the resorcinol method after purification by ion exchange resin (16).

[1] Most of the specimens were provided by the Department of Pathology of the University of Chicago. Three samples were obtained from Dr. G. Milles of Augustana Hospital. Fetuses were provided by Dr. V. E. Freese of the Chicago Lying-in Hospital. Cartilage from a patient with Marfan's syndrome was provided by Dr. G. Berenson.

[2] Nutritional Biochemical Corp., Cleveland, Ohio.

[3] AG 1 × 2, Calbiochem, Los Angeles, Calif.

TABLE I

Analyses of recoveries in resin column fractionation of costal acid mucopolysaccharides (AMP) of two specimens

	Fraction (molarity NaCl)						
	1.0	1.3	1.5	1.6	1.7	5.0	Total
	Specimen 14						
Uronic acid, *mg*	0.8	4.1	23.3	3.0	1.4	(2.7)*	32.6
Hexosamine, *mg*	1.1	4.5	21.1	3.5	3.2	44.1	77.5
Hexose, *mg*	1.9	3.0	14.0	3.4	4.3	52.8	79.3
% reduction in turbidity by hyaluronidase	100	100	100	100	70	2	
	Specimen 5						
Uronic acid, *mg*	1.6	6.7	31.4	6.9	1.3	(2.7)*	47.9
Hexosamine, *mg*	1.8	6.2	27.6	6.1	4.0	24.0	63.6
Hexose, *mg*	1.6	4.7	12.7	3.0	3.5	22.4	47.9
% reduction in turbidity by hyaluronidase	100	100	100	100	95	0	

* Color equivalent in uronic acid, which is actually absent (9).

Results

Isolation of crude AMP. The crude AMP obtained contains virtually the entire AMP of high molecular weight in the tissues. Between 2 and 5% of the total hexosamine of cartilage from various age groups is lost by dialysis. Less than 5% of the uronic acid and 10 to 20% (variable with age) of the hexosamine and ester sulfate of the original cartilage digest remain in the supernatant after precipitation of AMP with cetylpyridinium chloride. The hexosamine of the supernatant is almost entirely glucosamine; the molar ratio of sulfate to hexosamine varies from 1.1 to 1.3. Galactose, mannose, glucose, and traces of other monosaccharides are detectable after hydrolysis of this supernatant. It appears that the supernatant contains largely short chains of SKS, of molecular weight about 2,000, with smaller amounts of glycoprotein fragments and glycogen.

Resin fractionation of AMP. Analytical data on effluents from resin fractionation of two representative crude AMP preparations are presented in Table I. The bulk of the uronic acid and of the CS of the preparations appears in the 1.5 M NaCl effluent, whereas the SKS is almost entirely in the 5.0 M NaCl effluent. The 1.7 M NaCl effluent consists mainly of SKS of low molecular weight and of low molar ratio sulfate to hexosamine.

When extensively purified, CS from cartilage still yields an equivalent value for hexose in the phenol-H_2SO_4 method (14) of about one-third the value of the uronic acid determined by the carbazole method. This equivalent color as hexose is due mainly to interference by uronic acid, but may also be due to the presence of neutral sugars such as galactose and xylose in the region of linkage of the CS chain to peptide (17). The excess above this proportion in the first four fractions, and particularly in the first two fractions, which had a molar ratio of nitrogen to hexosamine exceeding 2, suggests contamination of crude AMP with variable, small amounts of acidic glycoprotein fragments.

Although the carbazole method for uronic acid applied to the 5.0 M NaCl fractions gives a solution absorbing light at 530 mμ, it has been shown that this color is not due to uronic acid (9). This fraction is thus free of CS. Calculation of the proportions of CS and SKS from the total values for uronic acid and for hexosamine of Table I yields values in good agreement with the proportions in crude AMP determined by the hyaluronidase digestion method.

Composition of purified CS and SKS. The analytical data on some preparations of CS and

TABLE II

*Chemical composition of typical preparations of costal CS and SKS (molar ratio to hexosamine)**

Specimen no.	N	Uronic	S	Hexose (as glucose)	Galactose	Methylpentose
Fetal-4 – CS	1.14	1.12	0.90	0.52	0.04	<0.02
14–CS	1.21	0.99	0.99	0.58	0.06	0.03
10-CS	1.22	1.01	1.05	0.52	0.04	0.03
14–SKS	1.84	<0.01	1.47	1.10	0.94	<0.02
10–SKS	1.49	<0.01	1.51	0.94	0.90	<0.02

* CS = chondroitin sulfate; SKS = skeletal keratosulfate.

TABLE III

Hexosamine, principal amino acids, and molecular weight of some preparations of costal CS and SKS (molar ratio to galactosamine for CS and to glucosamine for SKS)

AMP	Glucosamine	Galactosamine	Aspartic acid	Threonine	Serine	Glutamic acid	Proline	Glycine	M_n*
CS (14-costal)	0.03	1.00	0.015	0.009	0.031	0.013	0.021	0.017	9,000
CS (fetal-4)	0.02	1.00	0.008	0.004	0.027	0.008	0.000	0.009	
CS (Marfan-1)	0.03	1.00	0.008	0.005	0.025	0.007	0.014	0.010	
SKS (Marfan-1)	1.00	0.097	0.014	0.044	0.029	0.038	0.067	0.021	
SKS (5-cricoid)	1.00	0.104	0.020	0.075	0.049	0.072	0.109	0.032	
SKS† (9G)	1.00	0.060	0.022	0.061	0.042	0.059	0.083	0.031	10,000
SKS† (8F)	1.00	0.090	0.036	0.094	0.063	0.131	0.236	0.054	9,000

* M_n = number average molecular weight.
† Major fractions of pooled preparations repurified on resin columns.

SKS in Table II show deviations from "theoretical" composition. The excess nitrogen is especially high in SKS, but is only slightly reduced by further treatment with papain or by pronase. Values for sulfate/hexosamine are close to unity for all preparations of CS from costal cartilage, except for fetal CS. Cartilage SKS preparations differ greatly from corneal (CKS) preparations in possessing very high contents of ester sulfate (9).

Rechromatography of SKS on resin yielded fractions differing in chemical composition solely with respect to sulfate/hexosamine ratios, which ranged from 1.2 to 1.7. Heterogeneity of molecular size was moderate, since the intrinsic viscosities were all in the range of 0.2 to 0.3. Values for galactose in CS apparently reflect a high proportion of this sugar in short chain CS molecules corresponding to M_n near 10,000, for which the molar ratio of galactose to galactosamine may be about 0.1 (17). Thus, elevated values for hexose in CS preparations of Table III could be mainly due to hexoses in the linkage region to polypeptide.

Glucosamine values in CS preparations of Table III suggest minimal contamination with either glycoprotein or SKS. The prominence of serine among the residual amino acids is a characteristic shared generally with CS of other vertebrates (1). The galactosamine in cartilage SKS is also a common feature of cartilage SKS of other vertebrates, distinguishing these preparations from corneal CKS, which is free of galactosamine (9). The principal residual amino acids feature proline, threonine, glutamic acid, serine, glycine, and aspartic acid in order of decreasing content. This pattern differs greatly from that of corneal CKS, which contains mainly aspartic acid and little of the other amino acids (9). Apparent values of sialic acid in SKS did not exceed 0.02 mole per mole glucosamine but were not considered quantitatively reliable when determined at such low levels. Optical rotations, $[\alpha]_D$, of SKS preparations were very low, in the range of + 1° to − 1°.

TABLE IV

AMP of fetal costal cartilage

Specimen no.	Fetal age*	% CS†	% CS-A of CS	S/H	$[\eta]$‡
	years				
Fetal-1	0.22	100	40	0.83	
Fetal-2	0.25	100	40	0.82	
Fetal-3	0.30	100	40	0.84	0.67
Fetal-4	0.50	100	45	0.90	0.43

* Estimated from crown-to-rump length (18).
† CS with molar ratio sulfate/hexosamine (S/H) less than 1.
‡ $[\eta]$ = intrinsic viscosity.

Fetal cartilage. Since quantities of cartilage from fetuses were very small and SKS appeared absent, resin fractionation of crude AMP was omitted. After precipitation of crude AMP with cetylpyridinium chloride, the supernatant contained 5 to 10% of the uronic acid and 5 to 10% of the hexosamine of the original digest. Analyses indicated that the supernatants contained mainly low molecular weight CS. Unlike postnatal cartilage, fetal cartilage contains CS deficient in ester sulfate (Table IV).

Postnatal cartilage. Data on postnatal cartilage are given in Table V. The CS had a molar sulfate/hexosamine ratio of unity in all instances. With the exception of CS of No. 18, a fairly narrow range of average molecular weight is indicated by the range of $[\eta]$ from 0.31 to 0.61. For SKS, the sulfate/hexosamine ratio is in the range of 1.29 to 1.62 with no apparent correlation of this

TABLE V

*AMP composition of postnatal cartilage**

No.	Age	Sex	Costal					Cricoid		Thyroid		Tracheal	
			% CS	% CS-A in CS	S/H of SKS	$[\eta]$ of CS	$[\eta]$ of SKS	% CS	% CS-A in CS	% CS	% CS-A in CS	% CS	% CS-A in CS
	years												
1	0.2	F	100	50		0.45		100	50	100	60		
2	1.8	F	100	50		0.61							
2A	5	M	95	40									
2B	7	F	92	39									
3	9	F						95	55	90	50	90	50
3A	12	F	84	29									
4	20	M	81	40	1.37	0.41		82	40	74	40	85	40
5	23	M	75	40	1.45	0.39	0.26	70	45			78	
6	33	M	47	10				53		53		64	
7	35	M	56	20				55		54		64	
8	35	M	49	20				56		54		61	
9	35	F	51	30				56		56		59	
10	38	F	44	10	1.47	0.42	0.24	66	40	55	40	63	
11	39	F	53	20	1.40	0.35	0.25	69	30	63	40		
12	42	M	45	20	1.45	0.31	0.26	50		57		50	
13	45	F	41	10	1.35	0.43	0.24	53	20	50			
14	45	F	39	0	1.51	0.35	0.61	43	20	52	10	62	50
15	46	F	45	30	1.42		0.27	76		52		70	
16	61	F	49	30	1.29			65		57	40	60	
17	62	F	44	10	1.40		0.63	60				65	
18	72	M	44	40	1.62	1.20	0.62	40	20	46	10	53	
19	73	M	44	10	1.38	0.34	0.27	50	30	50	40	63	
20	85	M	39	10	1.51	0.40	0.23	55		56		58	
Marfan-1	14	M	83	40	1.50	0.47	0.23						

* Notes: Per cent CS-C in CS = 100 − per cent CS-A in CS. For 3-cricoid CS, $[\eta] = 0.47$; for 4-cricoid SKS, $[\eta] = 0.24$; for 5-cricoid SKS, S/H = 1.60, $[\eta] = 0.24$; for 5-cricoid CS, $[\eta] = 0.34$; for 18-cricoid CS, $[\eta] = 0.36$; for 18-thyroid CS, $[\eta] = 0.36$.

ratio with age. There appear to be two classes of SKS with respect to range of $[\eta]$, viz., 0.23 to 0.27 and 0.61 to 0.63. Although the residual peptide contribution to intrinsic viscosity is probably very small for CS, it might be important for SKS. However, the peptide contents of SKS preparations are not correlated with intrinsic viscosities.

In the first three decades, the per cent of CS in costal AMP drops rapidly with a concomitant rise in SKS. The per cent CS-A in CS also declines with increasing age. The general trends are revealed more clearly when the data are plotted as in Figure 1: CS-C undergoes only a small drop from birth to old age, whereas CS-A has declined rapidly.

The data of Table V indicate that, in almost every instance, the changes with age have occurred more rapidly in costal cartilage than in cricoid, thyroid, or tracheal cartilages. Again, the exception is the cartilage of No. 18, which is also unusual in $[\eta]$ of CS.

The single case of Marfan's syndrome does not appear unusual in AMP composition.

Discussion

Limitations of procedures. It is evident that separations from glycoprotein fragments are frequently incomplete and are a cause of small errors

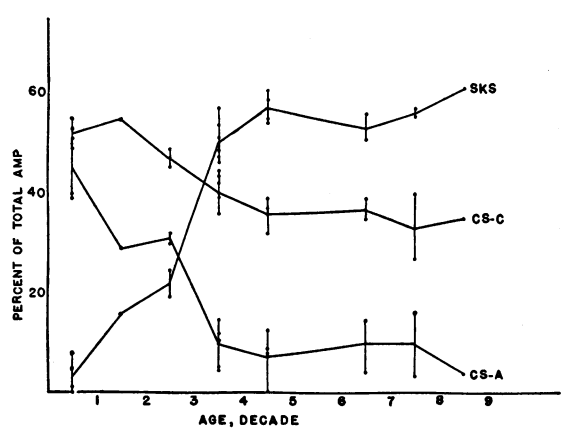

FIG. 1. COMPOSITION OF ACID MUCOPOLYSACCHARIDES (AMP) OF COSTAL CARTILAGE. Values for each decade are averaged from Table V to illustrate general trend for skeletal keratosulfate (SKS), chondroitin sulfate-C (CS-C), and chondroitin sulfate-A (CS-A).

in estimation of AMP components. Very low molecular weight SKS, possibly as much as 15% of the total AMP, has been omitted. Also, recoveries of AMP from resin columns are not complete. It is thus possible that the fractions isolated are not entirely representative with respect to molecular parameters. The inclusion in our calculations of the low molecular weight SKS of supernatants, which increases in amount with age, would yield only minor increases in the tabulated values of the proportion of SKS in AMP. Conclusions to be drawn from the results obtained are necessarily limited by these considerations.

Direct comparisons with data of other workers are difficult due to differences in methodology referred to earlier. Stidworthy and his associates (6) reported that the glucosamine proportion of the total hexosamine of human costal cartilage increases nearly linearly from birth, approaching 80% at 80 years. Kaplan and Meyer (7) reported that the SKS proportion of the total AMP (consisting of SKS and CS) of human costal cartilage increases linearly with age to approach 50% at 80 years. These reports differ from our findings (Figure 1) that the proportion of SKS reaches a plateau in the fourth decade. The conflict may be due in part to the contribution of hexosamine from increasing concentrations of glycoproteins in cartilage after the fourth decade to the analyses by the earlier workers. It is of interest, however, that Kuhn and Leppelmann (5) found that the major changes in the ratios of glucosamine to galactosamine for articular cartilage occur before the fourth decade.

Fetal cartilage. The AMP composition of cartilage of the human fetus is comparable to that of embryos of other higher vertebrates, which also contain only a mixture of CS-A and CS-C with low ratios of sulfate to hexosamine. However, differences are present in the proportion of CS-A in the mixture. In the ox at one-third term, the sulfate/hexosamine ratio is 0.85 with 30% CS-A. In the rabbit at one-half term, the sulfate/hexosamine ratio is 0.81 and CS-A is absent (1). At term, the CS-A content of the AMP rises to 60% in the ox but only to 20% in the rabbit. However, the CS-A in AMP of rabbit cartilage rises within 2 months to 60% also. Thus, although time schedules differ, the general developmental patterns are similar in different mammals with respect to increase in sulfate/hexosamine ratio and in per cent CS-A. Similar AMP patterns during development have been observed in the frog and in the chicken (1).

Postnatal cartilage. Data on AMP patterns in aging cartilage of vertebrates other than man are inadequate for complete taxonomic comparisons. However, SKS is characteristically present in cartilage of various species. In nasal septa of the ox and the camel at 15 to 18 years of age, SKS represents about 20% of the total AMP. This value is comparable to that for human cartilage at the same age. In specimens of some species of shark (of undetermined age) as in man, SKS may rise to 40% of the total AMP of cartilage. The remainder of the AMP is, however, mainly CS-C with a sulfate/hexosamine ratio of 1.20 to 1.30. A possibly unique variant is mature costal cartilage of the male rat at 9 months, which contains CS-A as the sole acid mucopolysaccharide (1). The rat is unusual among mammals in that the male may show continuous skeletal growth throughout life. It is evident that species differences may be great.

Although age-related patterns may be generally similar for all human hyaline cartilages, the data of Table V indicate that rates of changes may vary both with site and with the individual. Site differences are evident by comparisons of tissues in the same individuals, primarily beyond age 35. Thus for No. 10 to 20 of Table V, values of per cent CS and per cent CS-A in CS, in each individual case except one, are higher for cricoid, thyroid, and tracheal cartilage than for costal cartilage. The single unexplained exception, No. 18, is unusual also in possessing the highest values for S/H of SKS and for $[\eta]$ of CS. These differences may be due in part to local differences in the physical and nutritional environment of cartilage cells, associated with different anatomic locations or different zones within the same cartilage, or both. Stockwell and Scott (19) proposed a similar hypothesis based upon a histochemical study of staining changes in aging human costal cartilage. Differences were noted in distribution of the SKS between the immediate "territorial" region of cells and the "interterritorial" matrix. The importance of mechanical forces is indicated by the finding of Convery and Akeson (20) that the nonpressure area of human articular cartilage

contains more collagen but much less ester sulfate than the adjacent pressure area. Local hypoxia is associated with a shift in metabolism resulting in increased AMP production (21), or it may favor chondrogenesis of myocardium (22).

Age-dependent changes in AMP, similar to those occurring in hyaline cartilage, occur in human intervertebral discs, which consist of a type of fibrocartilage (annulus fibrosus) surrounding a gel rich in AMP (nucleus pulposus). Buddecke and Sziegoleit (23) reported that between the first and the eighth decades, the SKS of discs rises from about 10% to 50% of the total AMP, whereas the ratio of CS-A to CS-C changes from 2:1 to 1:2. However, Hallén (24) found that the glucosamine/galactosamine ratio increases primarily during the first 3 decades in the annulus fibrosus, but increases linearly with age throughout life in the nucleus pulposus. Evidence was presented by Van den Hooff (25) that as a result of degeneration of the nucleus with increasing age, abnormal pressures on the annulus result in disorientation of collagen fibers and in enhanced pericellular staining for AMP. The AMP is probably largely SKS, which appears also in the pericellular regions of aged costal cartilage (19).

In the single instance of Marfan's syndrome at age 14 (Table V), the AMP composition of cartilage does not appear abnormal. However, for three cases of this disorder between the ages of 20 and 44, Meyer (26) reported an abnormally high proportion of SKS in the total AMP.

Comparison of cartilage with endochondral bone indicates retention in aging of tissue specificity of AMP composition. Thus, in a 58-year-old male, the AMP composition of the articular cartilage of the femur, as of the costal cartilage, was 40% SKS and 60% CS-C, whereas the compact bone contained CS-A as virtually the sole AMP (27). These differences in AMP composition may also be related to local environmental factors. This suggestion gains support from observations that the appearance of either cartilage or bone in a culture of cells arising from bone was determined by oxygen tension and mechanical forces (28).

General biological significance. The rise in SKS occurs concomitantly (Figure 1) with a rapid decline in CS-A. It may be that the control of biosynthesis of these two components is related, possibly via the formation of uridinediphosphoglucuronic acid (19). However, proposed mechanisms must also account for the relatively small age-dependent change in CS-C. Since CS-A and CS-C differ only in position of substitution of the sulfate group, metabolic control may be exerted also at the level of specific sulfotransferases, which transfer sulfate to a common precursor polysaccharide chain. The selective stimulation of specific sulfotransferases was suggested as an explanation for the preferential synthesis of CS-A in cartilage of metamorphosing frog larva (29). Alternatively, changes in pool concentrations of matrix components may be determined by degradative processes or may involve different cell types.

The age-dependent changes in relative proportion of CS-A and CS-C in human adult cartilage appear as the reverse of the changes in these polysaccharides that occur during embryonic development and early growth of higher vertebrates generally (1). The type and timetable of change in AMP composition (Figure 1) suggest that these aging effects in cartilage may be related to alterations of physiological conditions associated with cessation of development and growth. Thus, various progressive changes occur in costal cartilage after growth ceases: the cells swell and viability declines, calcium salts deposit, the AMP content declines, a yellow pigment accumulates, rate of oxygen consumption per cell decreases, fibrous components show altered structures, and so on (30–33). It is interesting to note in this regard that chondrosarcomata of aged humans contain AMP consisting solely of CS-C (34), a characteristic associated with fetal cartilage. Also, growth hormone alters the AMP metabolism of mature rabbit nucleus pulposus resulting in a composition characteristic of a younger age (35).

It seems likely that at each stage the nature and organization of the matrix components have a profound effect on cellular activity and thus exert an influence on subsequent changes in the matrix. The structural role of matrix components of cartilage has been discussed recently in largely speculative papers (2, 36, 37). However, precise characterizations of physiological function are still lacking. As a consequence, interpretation of changes in composition with age is limited.

It should be further noted, particularly with references to studies of proteolytically digested tissues such as the present one, that the polysaccha-

ride components of matrix are present as AMP-protein macromolecules (38). A recent study of these substances has shown that their ease of extraction from human costal cartilage by mild procedures decreases with advancing age and that present methods of isolation and purification are inadequate (39). Detailed evaluation of factors, such as changes in average molecular weight of CS and SKS and the variability of sulfate to hexosamine ratios in SKS, awaits further elucidation of the chemical structures and physiological roles of AMP-protein macromolecules.

Summary

1. Human cartilage, digested with papain, yielded a mixture of acid mucopolysaccharides (AMP). The AMP were fractionated on anion exchange resin to yield a mixture of chondroitin sulfates A and C (CS-A and CS-C) and a highly sulfated skeletal keratosulfate (SKS) as a separate component. The relative amounts of CS-A and CS-C were determined.

2. Embryonic and early growth patterns of AMP are roughly similar for man and higher vertebrates, although rates of change are variable: SKS is absent, the degree of sulfation of CS-C or CS-A or both rises to equimolar sulfate and galactosamine, and the ratio CS-A/CS-C increases from less than 0.7 to more than 1.0.

3. From the first to the fourth decade, the proportion of SKS in the total AMP rises to a plateau near 55%, CS-C declines slightly, and the ratio CS-A/CS-C falls to 0.25 or less. Little change, if any, occurs thereafter. The changes in proportion of CS-A and CS-C appear as the reverse of AMP patterns of embryonic development and early growth of tetrapods, generally. It is possible that aging effects in cartilage are related responses to alterations of physiological conditions associated with cessation of development and growth.

4. Although age-related patterns are generally similar for all human cartilages, rates of change appear lower in cricoid, thyroid, and tracheal cartilages than in costal cartilage, and vary with the individual. These differences may be related in part to the local differences in the physical and nutritional environment of cartilage cells.

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