

## **Proteinpolysaccharides from human articular and costal cartilage.**

L Rosenberg, ... , B Johnson, M Schubert

*J Clin Invest.* 1965;[44\(10\)](#):1647-1656. <https://doi.org/10.1172/JCI105271>.

Research Article

**Find the latest version:**

<https://jci.me/105271/pdf>



## Proteinpolysaccharides from Human Articular and Costal Cartilage \*

LAWRENCE ROSENBERG,† BLANCHE JOHNSON, AND MAXWELL SCHUBERT ‡

(From the Department of Medicine, and the Study Group for Rheumatic Diseases, New York University School of Medicine, and the Department of Surgery, Division of Orthopedics, Albert Einstein College of Medicine, New York, N. Y.)

From bovine nasal cartilage two products were isolated, each consisting mainly of chondroitin sulfate and protein (1). These were called proteinpolysaccharides (PP), and the two kinds were distinguished as PP-H and PP-L. Human costal cartilage also yielded two products which, in the extraction and fractionation procedures, behaved rather like the two products from bovine nasal cartilage and were also called PP-H and PP-L (2), although they differed from the corresponding products from bovine cartilage in yield and composition. Isolation and fractionation of the products from human cartilage was far more difficult and gave results more variable than with bovine cartilage.

The PP of bovine and human cartilages contain components other than chondroitin sulfate and protein. Partridge and his co-workers (3, 4) found glucosamine and galactose among degradation products of PP from cartilage. Gregory and Rodén found keratan sulfate after digestion of bovine nasal PP-L with hyaluronidase and papain (5). Alkaline degradation of bovine nasal PP-L also gave keratan sulfate in an amount 5% of the weight of the PP-L (6). Meyer and his co-workers (7, 8) reported obtaining keratan sulfate from whole human costal cartilage and found this component to increase with age. Anderson (9, 10)

reported sialic acid in some fractions isolated from mixtures of human cartilages. Gregory, Laurent, and Rodén (11) showed that xylose was present in bovine nasal PP-L.

We have made a more extensive study of human cartilages; our work includes a simplification of the method of isolation of PP-H and PP-L from human cartilage; a wider set of analytical data on these products to provide information on their contents of protein, chondroitin sulfate, keratan sulfate, sialate, and the extra hexose usually found present; a comparison of the products obtained from articular and costal cartilages; a comparison of products from individuals of different ages from 0 to 70 years; and a method using lanthanum, which makes fractionation of the human PP-L into three distinct products possible.

### Methods and Results

Cartilage samples were collected, generally within 24 hours after death, cleaned, diced, and weighed. Articular cartilage from adults was taken from the patella and distal femur, excluding any cartilage that showed evidence of degenerative change. Articular cartilage from newborns was pooled from humeral head, femoral head, and distal femur. Some samples were extracted in the fresh wet condition immediately after cleaning; others were dried by shredding in ethanol in a Waring blender, washing with ether, and drying in a vacuum, and were extracted later. The latter procedure gave data on the ratio of dry weight to wet weight of the whole cartilage. In earlier work (2) it was shown that both fresh and dried human cartilage give the same yields of PP.

Human cartilage (4 g dry) was homogenized with water (250 ml) in a Virtis model 45 for 30 minutes in an ice bath. Ethanol (500 ml) was added and the mixture centrifuged ( $3,000 \times g$ , 30

\* Submitted for publication April 22, 1965; accepted June 24, 1965.

This work was supported by U. S. Public Health Service grant AM 00028-13 from the National Institute of Arthritis and Metabolic Diseases and Research Career Program Award 5-K6-AM-18,434-03.

† Part of this work was done during a traineeship of the National Institute of Arthritis and Metabolic Diseases, graduate training grant T1 AM-5082. At present a fellow of the Helen Hay Whitney Foundation.

‡ Address requests for reprints to Dr. Maxwell Schubert, Dept. of Medicine, New York University School of Medicine, 550 First Avenue, New York, N. Y. 10016.

TABLE I  
Yields of crude PP, and of PP-L and PP-H from human costal cartilage\*

Age	Dry wt/ wet wt	Crude PP/ dry cartilage	Yields		PP-L/PP-H
			PP-L/ crude PP	PP-H/ crude PP	
<i>years</i>		<i>g/g</i>		<i>g/g</i>	
.1	.243	.271	.537	.333	1.6
.2	.233	.204	.606	.290	2.1
.3	.236	.202	.625	.282	2.2
.3	.237	.179	.521	.326	1.5
2			.625	.247	2.5
10		.187	.617	.272	2.3
12	.343	.164	.646	.201	3.2
16			.640	.230	2.8
24	.406	.113	.399	.323	1.23
26			.266	.633	.42
26	.427	.231	.184	.718	.26
29		.177	.440	.408	1.08
36	.408	.223	.242	.638	.38
36	.406	.197	.385	.399	.96
38	.458	.149	.217	.676	.32
40		.283	.266	.372	.71
50		.175	.090	.617	.15
55		.182	.354	.408	.87
60		.143	.148	.762	.19
62	.484	.144	.219	.573	.38
63	.487	.231	.183	.623	.29
Averages					
0-16	.258	.201	.602	.273	2.2
24-63	.439	.187	.261	.550	.56

\* PP = proteinpolysaccharides; PP-L and PP-H = types of PP.

minutes) giving a residue (R, about 90 ml) and to remove stray floccules. Addition of potassium acetate (6 g) precipitated the crude PP, which was passed through a glass wool plug in a funnel was separated by centrifugation, washed, and

TABLE II  
Yields of crude PP, and of PP-L and PP-H from human articular cartilage

Age	Dry wt/ wet wt	Crude PP/ dry cartilage	Yields		PP-L/PP-H
			PP-L/ crude PP	PP-H/ crude PP	
<i>years</i>		<i>g/g</i>		<i>g/g</i>	
Premature	.138	.386	.381	.361	1.1
0	.179	.346	.514	.171	3.0
17	.245	.250	.286	.669	.43
21	.269	.161	.237	.685	.35
24		.198	.311	.501	.62
26	.254	.291	.277	.521	.53
29	.266	.239	.411	.428	.96
36	.203	.169	.413	.434	.95
36	.272	.155	.338	.582	.59
38	.257	.142	.234	.604	.39
50		.149	.194	.550	.35
55		.192	.464	.364	1.31
60		.188	.273	.638	.43
61	.245	.284			
62		.319	.385	.451	.85
64		.219	.514	.385	1.33
73	.263	.275	.179	.558	.32
Averages					
0	.158	.366	.447	.266	1.8
17-73	.253	.217	.323	.526	.67

dried. The crude PP was dissolved in KCl solution (.15 M, 125 ml) by stirring for an hour. The opalescent solution was centrifuged at  $78,000 \times g$  (1 hour) giving a residue, crude PP-H, and a clear supernatant solution from which PP-L was precipitated by addition of ethanol (250 ml). The crude PP-H was dissolved in water (300 ml) by stirring for an hour with the Virtis model 45 at low speed, and after addition of KCl (3 g) the solution was centrifuged at  $78,000 \times g$ . The resi-

due, washed and dried, is PP-H. Additional amounts of PP-H and PP-L may be isolated from the cartilage residue (R, above) by adding water (180 ml) to it, homogenizing again, and continuing the steps as above. After the second extraction, only slight (1 to 2%) amounts of PP can be extracted with water from the human cartilage residue although it retains 10 to 15% of its weight as PP. A similar situation exists in the case of the residue of bovine nasal cartilage left after two

TABLE III  
*Analytical data on PP-L from human costal (C) and articular (A) cartilage*

Cartilage	Age	Protein (biuret method)	Hexosamine	Hexuronate	Hexose	Sialate
	years	%	%	%	%	%
A	0	17.7		16.6	5.7	1.3
C	.1	19.5		16.9	7.3	
C	.2	23.2		18.7	7.4	
C	.3	20.3		17.7	7.3	
C	.3	25.3		17.4	7.3	1.8
C	3.	21.9		9.4	7.2	2.2
C	12.	23.6		13.1	10.5	2.9
A	17.	23.6	17.5	13.1	9.0	
A	21.	22.9	15.3	12.0	10.1	
A	24.	27.0	15.8	7.9	11.7	
C	24.	31.4	14.7	10.2	11.4	2.1
A	26.	30.4	15.1	10.9	10.7	2.9
C	26.	22.1		11.6	12.1	2.3
A	29.	31.8		11.6	12.3	3.8
C	29.	30.8	17.5	10.0	12.7	2.9
A	36.	30.2	15.5	10.0	11.6	3.4
C	36.	29.4	17.6	10.1	12.2	2.7
A	36.		15.5	8.6	12.0	
C	36.	30.4		10.0	11.2	
A	38.	31.5		8.4	11.8	3.1
C	38.	29.6		6.6	12.7	2.7
A	50.	28.6		10.6	11.6	3.0
C	50.		14.6			3.0
A	55.	32.5	15.1	8.9	12.4	
C	55.	35.1	14.9	8.0	11.5	2.6
A	60.	29.7	14.8		10.1	2.9
C	60.	38.7	14.6	9.1	10.9	2.4
A	62.	39.3	15.1	7.9	11.4	
C	62.	35.9	15.7	8.1	11.8	
A	64.	38.2	14.1	7.4	11.7	3.4
A	73.	27.2	14.6	7.1	10.4	3.1
Averages						
A	17-73	30.2	15.3	9.6	11.2	3.2
C	24-62	31.5	15.7	9.3	11.8	2.6
A + C	0-12	21.6		15.7	7.5	2.0
A + C	17-38	28.6	16.1	10.1	11.5	2.9
A + C	50-73	33.9	14.8	8.4	11.3	2.9

extractions. A recent study (12) describes a simple method to extract this PP from bovine nasal cartilage residue with hydroxylamine, and preliminary experiments indicate that the method is also applicable to human cartilage residue.

Tables I and II show some results for articular and costal cartilage. In the adult range (17 to 74 years) the average ratio of dry weight to wet weight is higher for costal cartilage (.44) than for articular cartilage (.25). The average yield of crude PP per gram of dry cartilage is the same for costal cartilage (.19) as for articular cartilage (.22). Too few samples of cartilage of children were secured to allow any detailed comparisons. There were seven samples of costal cartilage, unevenly covering the range of 1 month to 12 years, and two samples of articular cartilage from one premature and one stillbirth. In the age range of up to 1 year the ratios of dry weight to wet weight of both cartilages are much lower than for adults. The yields of crude PP per gram of dry articular cartilage are much higher for fetal or newborn than for adult cartilage, but this is not true of costal cartilage. The most striking change

with age is the drop in the ratio of PP-L to PP-H that occurs in costal cartilage. In children (0 to 12 years) the ratio of PP-L to PP-H averages 2.2; in adults (20 to 73 years) it averages about .6. The change from the first ratio to the second seems to occur in the age range between 12 and 20, the same age range in which there is a marked change in the ratio of dry weight to wet weight of the whole cartilage. Only two cases occur within this age range; the 16 year old was included in the children's group, the 17 year old in the adult group, in each case because his ratio fit that of the group to which he was assigned. Within each group there is wide fluctuation in the ratios in individual cases, yet there is almost no overlap in values between the two groups.

Analytical methods have been described in earlier works (6, 13) and include methods for protein, hexosamine, hexuronate, hexose, sialate, and hydroxyproline. In a few cases the method of Cessi and Piliego (14) was used for galactosamine. Analytical results on PP-L from articular and costal cartilage are so similar that they are both included in Table III. The same is true of results

TABLE IV  
*Analytical data on PP-H from human costal and articular cartilage*

Cartilage	Age	Protein (biuret method)	Protein (Folin method)	Hexos- amine	Hexur- onate	Hexose	Sialate
	years	%	%	%	%	%	%
C	.1	64.4			4.7	7.2	.8
C	.2	53.4			9.9	8.8	
C	.3	60.0			8.6	8.1	
C	.3	54.8	59.7		6.9	10.7	1.0
C	3.	47.2	50.6		8.0	11.4	1.7
C	12.	38.2	45.0		7.5	14.2	2.4
A	24.	67.8		11.1	4.9	10.4	
C	24.	72.3		10.7	4.4	9.3	
A	29.	66.6		11.4	3.9	9.9	
C	29.	57.7		12.2	5.0	10.6	
A	36.	73.0		9.0	2.8	7.8	
C	36.	75.0	61.2	9.5	2.9	9.8	
A	38.	62.5	58.0		3.2	8.8	1.3
C	38.	78.0	66.2		2.4	8.4	1.6
A	50.	70.0		9.7	3.6	8.2	
A	55.	63.8		12.0	5.2	11.4	
C	55.	68.8	61.1		2.8	8.3	1.3
A	60.	64.3			3.9	7.4	
A	62.	71.6	64.5	10.2	3.8	9.8	2.1
C	62.				2.3	7.4	1.3
Averages							
A	24-62	67.5		10.6	3.9	9.3	1.7
C	24-62	70.3		10.8	3.3	9.0	1.4
C	0-12	53.0			7.6	10.1	1.5
A + C	24-38	69.1		10.6	3.7	9.4	1.4
A + C	50-62	67.7		10.6	3.6	8.8	1.6

TABLE V  
Yields and analytical data on PP-L and PP-H from two successive extractions of human costal and articular cartilage

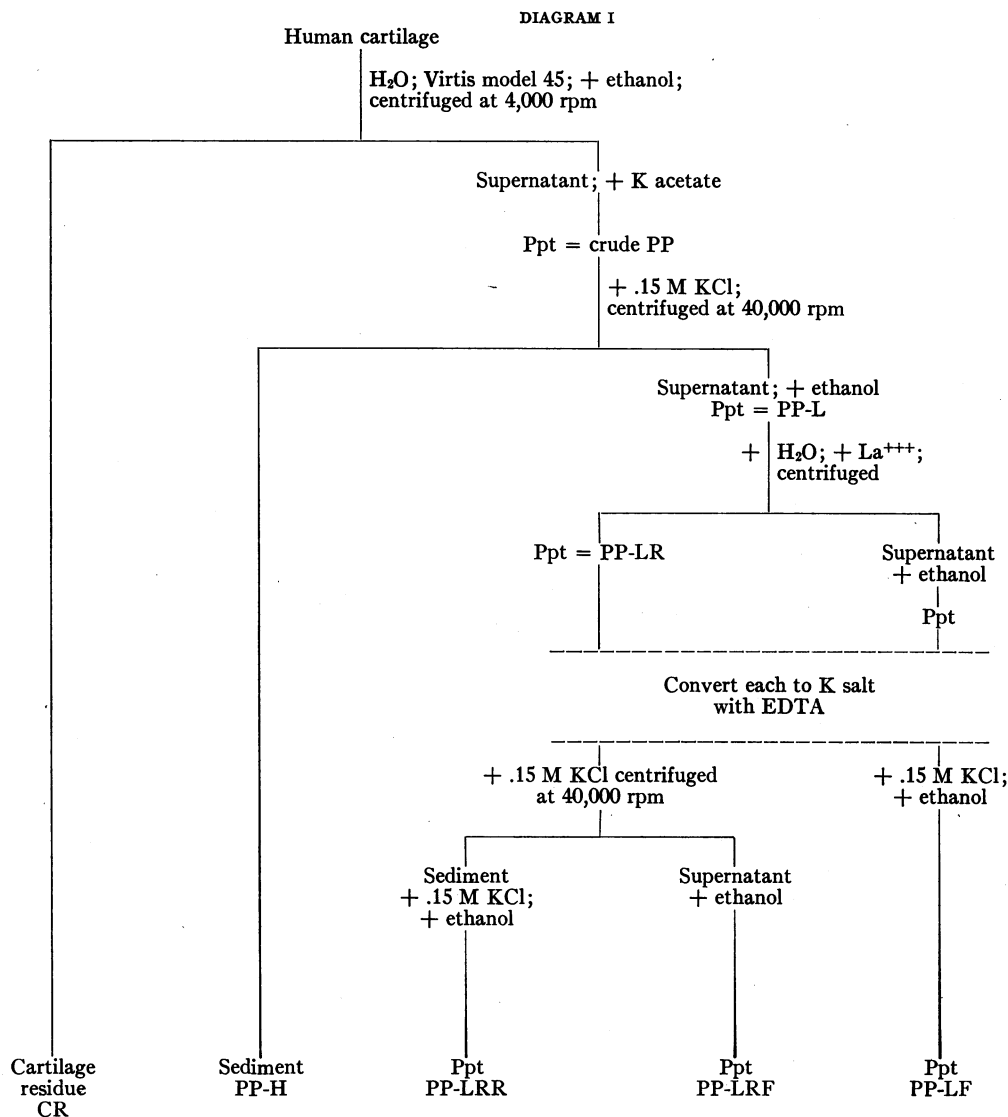
Cartilage	Age	Extraction	Yield/ dry cartilage	Protein	Hexo- amine	Hexur- onate	Hexose	Sialate	Hydroxy- proline
	years		g/g	%	%	%	%	%	%
PP-L									
C	24	1	.050	19.0	17.4	12.5	11.6	2.8	.02
		2	.034	28.7	17.6	12.8	12.0	2.8	.07
C	46	1	.042	26.0	16.8	11.9	11.0	3.0	.10
		2	.016	27.0	15.0	10.4	11.6	2.5	.03
A	Adult	1	.070	45.6	13.4	9.7	12.4	2.1	
		2	.013	39.0	15.4	10.7	13.6	1.7	
Average				30.9	15.9	11.3	12.0	2.5	.05
PP-H									
C	24	1	.073	55.0	9.2	5.9	7.5	2.3	1.3
		2	.070	58.0	8.7	4.9	7.9	1.5	2.1
C	46	1	.094	81.5	8.2	3.7	6.0	1.4	1.5
		2	.061	77.9	7.3	3.5	6.9	1.2	.7
A	Adult	1	.094	80.3	9.0	4.5	5.9	1.1	
		2	.042	90.0	8.2		9.3	1.3	
Average				73.8	8.4	4.5	7.2	1.5	1.4

on PP-H that are included in Table IV. In both Tables separate averages are calculated for articular and costal cartilages. Differences between children and adults are also apparent in the analytical data on PP-L and PP-H. The line of separation is less sharp than it is in Tables I and II. For consistency, the same line is drawn as in Tables I and II: children, 0 to 12 years; adults, 17 to 73 years.

In the case of PP-L, the highest hexuronate values (17.1%), and the lowest protein values were found in infants less than 1 year old. With increasing age, hexuronate decreased, to average 8% in the seventh decade. This indicates a chondroitin sulfate content in infant PP-L more than double that of the oldest subjects studied. Hexose increased from 7.0% in infant PP-L to 11.5% in the third decade, after which there was no further change. Concurrently, average protein concentration increased with age. Hexuronate concentration in infant PP-H was also more than double that of the oldest subjects studied. Analytical data of Tables III and IV have been averaged separately for children (0 to 12 years), young adults (17 to 38 years), and old adults (50 to 73 years). Analytical data on hydroxyproline are not included in the Tables; for PP-L these values were always less than .1%; for PP-H they averaged .6%.

In a few cases cartilage samples were extracted by two successive treatments with water in the Virtis model 45, and the crude PP of each extract was separately fractionated into PP-H and PP-L. Table V gives yields and analytical data on the products of such successive extractions. Analytical data of successive samples of PP-L or of PP-H fall in the same range as the figures of Tables III and IV. The ratio of PP-L to PP-H for the first extraction averages .62, whereas the ratio for the second extraction averages .35. This fall in PP-L to PP-H ratio with successive extractions (2) has been consistently observed with all cartilages examined and probably reflects the fact that PP-L is more readily soluble in water. This also probably accounts for the greater ease of extraction of PP from children's cartilage, which has a higher proportion of PP-L.

PP-L and PP-H of human cartilage are not individual chemical substances. Attempts to fractionate or to purify them by the use of columns have not been clearly successful so far, but a method has been worked out by which human costal PP-L can be separated into three fractions. This method is included in the following diagram, which shows the whole procedure by which human cartilage can be made to yield four PP fractions, three of which are derived from PP-L.



The beginning of the fractionation of human costal PP-L, HC-PP-L, depends on the earlier finding (13) that bovine nasal PP-L, BN-PP-L, is almost completely precipitated from cold dilute aqueous solution by lanthanum. HC-PP-L is not completely precipitated; 12 to 40% remained in solution after addition of the lanthanum and removal of the fraction precipitated. The fraction that remained in solution was precipitated with ethanol (2 vol). Both fractions were reconverted to potassium salts by the methods described (13).<sup>1</sup>

<sup>1</sup> In the preparation of the EDTA stock solution given in this reference the amount of water used should be 1,000 ml instead of 100 ml.

The fraction precipitated with lanthanum was called PP-LR; the fraction remaining in solution was called PP-LF. It was then found that PP-LR dissolved in KCl solution (.15 M) and centrifuged at 40,000 rpm for one-half hour yielded a considerable sediment, which in four cases lay between 15 and 35% of the weight of the PP-LR used. In one case, however, this fraction amounted to 70% for no apparent reason. The sediment dissolved in KCl solution (.15 M) gave a clear viscous solution from which, by addition of ethanol, the product was precipitated and called PP-LRR. The clear supernatant solution containing the fraction of PP-LR that did not sediment, on addi-

tion of ethanol, yielded the fraction called PP-LRF. Thus HC-PP-L gave the three fractions called PP-LRR, PP-LRF, and PP-LF. Yields and analytical data are in Table VI, which describes the results for five randomly selected cases. Efforts are being made to simplify the method and apply it to more cases. This is particularly important since there may be trends in the proportions of the fractions with age. Two of these fractions, PP-LF and PP-LRF, are of low viscosity and may lend themselves to chromatographic or electrophoretic methods to establish their homogeneity or heterogeneity.

### Discussion

Perhaps the most striking new information in Table I is the sharp change in the ratio of PP-L to PP-H of the products extracted from costal cartilage from an average of 2.2 in children to an

average of .6 in adults. There is no evidence of a progressive variation within either group, but individual variations are so large they may obscure trends. The large individual variations could be real, but they could also be due to the complexity of the mixture or to the crude state of the method. Yet, the distinction between children and adults seems clear. Parallel with the drop in the ratio of PP-L to PP-H, there is a drop in the water content of the native cartilage shown by the rise in the ratio of dry weight to wet weight, which also occurs with the change from the child's to the adult's cartilage. This ratio fluctuates in a much narrower range at each of its two levels. In adult articular cartilage, Table II, the ratio of PP-L to PP-H is similar to that of adult costal cartilage, but lack of articular cartilage from children makes it impossible to decide whether a sharp change in this ratio also occurs with the change from child to adult cartilage.

TABLE VI  
*Yields and analytical data on the four fractions isolated from five samples of human costal cartilage by the procedure outlined in Diagram I\**

Yield or component†	Fraction	10 year‡	16 year	23 year	26 year	63 year
Yield	PP-H	% .051	% .046	% .098	% .187	% .144
	PP-LRR	.010	.024	.019	.045	.009
	PP-LRF	.050	.063	.060	.011	.015
	PP-LF	.046	.016	.021	.009	.009
Protein	PP-H	57.	46.	69.	70.	92.
	PP-LRR	36.	30.	50.	47.	66.
	PP-LRF	17.	33.	33.	46.	50.
	PP-LF	13.	23.	34.	27.	34.
Hexos-amine	PP-H	13.2	12.5	12.8		8.4
	PP-LRR	15.6	18.3	17.6	13.2	11.6
	PP-LRF	24.0	20.5	18.3	21.6	17.9
	PP-LF	20.8	19.7	18.3	18.4	15.8
Galactos-amine	PP-H	5.6	8.2	8.2	5.8	1.2
	PP-LRR	8.4	10.2	12.0	9.2	4.8
	PP-LRF	14.6	14.4	17.4	16.0	8.4
	PP-LF	10.8	14.8	9.0	8.0	7.6
Hexuron-ate	PP-H	8.1	9.2	8.2	7.2	2.5
	PP-LRR	10.7	14.0	12.1	13.2	4.1
	PP-LRF	16.7	16.4	13.3	13.4	11.1
	PP-LF	11.3	14.8	11.8	6.0	6.4
Hexose	PP-H	12.9	13.0	12.0	9.5	8.0
	PP-LRR	10.1	9.5	11.0	10.8	9.8
	PP-LRF	9.7	8.4	9.9	9.6	11.5
	PP-LF	9.6	18.2	15.4	8.4	16.5

\* See Methods and Results section of text for explanation of fractions of PP.

† Values give yields as gram per gram of dry cartilage, or per cent of each component in each fraction isolated from each sample of cartilage.

‡ Age of subject from which sample was taken.



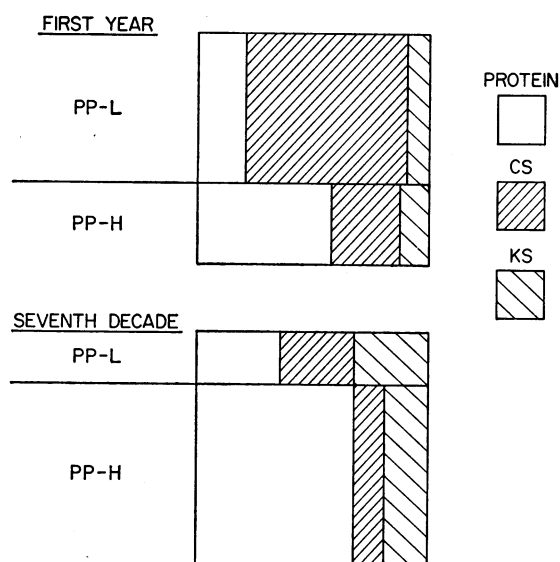


FIG. 1. COMPARISON OF COMPOSITIONS OF THE TOTAL PROTEINPOLYSACCHARIDES (PP) EXTRACTED FROM COSTAL CARTILAGE OF INFANTS (1 YEAR) AND ADULTS (SEVENTH DECADE). The total amount of PP extracted is nearly the same, represented by the equal sizes of the large squares. Ordinate distances represent the proportions of the two types of PP, PP-L, and PP-H; abscissa distances represent proportions of protein, chondroitin sulfate (CS), and keratan sulfate (KS).

Changes in the PP of human cartilage during aging are of three kinds: a) change in composition of PP-L; b) change in composition of PP-H; and c) change in the ratio of PP-L to PP-H. These changes are shown in Figure 1, in which a comparison is made of the proportions and compositions of PP-L and PP-H in infants and the oldest subjects studied. Although the data of Figure 1 are contained in Tables I to IV, they become more immediately apparent in the Figure. The great decrease in the amount of chondroitin sulfate (CS) in human cartilage with aging is the result of several effects: the decrease in the content of CS in both PP-L and PP-H (abscissa), and the great decrease in the ratio of PP-L to PP-H (ordinate) with aging. The data of the Tables show that this does not occur uniformly at all ages, but that most of the change occurs in the transition of the child to the adult.

The fractionation method outlined in Diagram I applied to the costal cartilage of six individuals (10 to 63 years) gave the yields recorded in Table VI. These figures, like the yields of PP-L and

PP-H of Tables I and II, show not a progressive tendency of individual fractions to change with age, but a difference between children and adults. Adults give higher yields of PP-H, and lower yields of PP-LF and PP-LRF, but there was no clear difference in PP-LRR.

The analytical data of Table VI on these fractions show a number of fairly marked regularities. The fractions are arranged in the same order (from top to bottom) as in Diagram I (from left to right) with the most readily sedimentable fractions highest, and the least precipitable with lanthanum lowest. Protein content, in this order, generally decreases from top to bottom. The fraction PP-LRF has, in practically all cases, the highest content of total hexosamine, galactosamine, and hexuronate. In no one of the four fractions is there any marked tendency for either chondroitin sulfate (CS) or keratan sulfate (KS) to become the dominant polysaccharide. This has been investigated by a study of the ratio of CS to (CS + KS), calculated on the assumption that uronic acid is present only in CS, and that hexosamine is present only in CS and KS. Then, the molar ratios of hexuronate to hexosamine and galactosamine to hexosamine both equal CS to (CS + KS). Table VII shows both ratios calculated for all fractions. In only a few cases does the value of this ratio deviate widely from its average of .6. The fractionation method, which separates fractions differing greatly in sedimentability and precipitability with lanthanum, does not seem to separate

TABLE VII

Calculated ratios of chondroitin sulfate (CS) to total polysaccharide, CS + keratan sulfate (KS), in each of the fractions of Table VI

Fraction	Age*				
	10	16	23	26	63
Molar ratio, hexuronate/hexosamine					
PP-H	.57	.68	.59		.29
PP-LRR	.63	.70	.64	.92	.32
PP-LRF	.65	.74	.51	.57	.57
PP-LF	.50	.67	.63	.30	.38
Molar ratio, galactosamine/hexosamine					
PP-H	.42	.65	.64		.15
PP-LRR	.54	.61	.68	.70	.41
PP-LRF	.61	.70	.95	.76	.47
PP-LF	.52	.75	.49	.44	.48

\* Age, in years, of subject from whom sample was taken.

products rich in either CS or KS. Most fractions contain CS and KS in a weight ratio of CS to KS close to 1.5. Eichhorn and Butzow (15) studied the degradative effect of lanthanum at 64° C on polyribonucleotides, which involves splitting of ester phosphate bonds. In the fractionation of human PP-L with lanthanum no evidence of degradation has yet been observed.

The work of Hass (16) and of Leppelmann (17) is cited as evidence of decreasing content of CS in human cartilage with age, but the methods used were not specific for CS in the presence of KS. Shetlar and Masters (18) measured the uronic acid content of human cartilage over a wide age range and reported a progressive drop with age. The greatest drop, about 50%, occurred between infants and adults. Only two cases between 4 months and 25 years were included, both of which resembled adults in their uronic acid contents. Kaplan and Meyer (8) were the first to be aware of the need to distinguish CS and KS in cartilage. By isolation of the polysaccharides of human costal cartilage they showed a progressive decrease in CS from 0 to 75 years and a constant level of KS in adults from 25 to 75 years. In the present study these aging changes are described in terms of the alterations in the proportions and compositions of PP-L and PP-H, the major extractable protein-polysaccharides of native cartilage.

### Summary

Using a simplified procedure for isolating the proteinpolysaccharides (PP) from human cartilage and separating them into two fractions, PP-H and PP-L, we have made a comparative study of these fractions from human articular (knee joint) and costal cartilages, from individuals ranging in age from 0 to 70 years. Between children (0 to 12 years) and adults (17 to 73 years) there is a sharp drop in water content and a sharp drop in the ratio of PP-L to PP-H of products isolated from costal cartilage. The same may be true of articular cartilage. The composition of PP-L is the same from articular and costal cartilage, and so is the composition of PP-H. There is a clear difference in the composition of PP-L from children and adults, with the latter having higher protein, hexose, and sialate contents, and a lower uronate content. Between young adults (17 to 38) and

old adults (50 to 73) there are similar but smaller changes in protein and uronate content. There is a parallel but less marked difference in the composition of PP-H from children and adults. Both human PP-H and PP-L differ from the corresponding products from bovine nasal cartilage in their much higher content of protein and keratan sulfate. Human PP-L has been separated into three fractions by the use of lanthanum. In most of these fractions the ratio of chondroitin sulfate to keratan sulfate does not deviate widely from 1.5.

### References

1. Gerber, B. R., E. C. Franklin, and M. Schubert. Ultracentrifugal fractionation of bovine nasal chondromucoprotein. *J. biol. Chem.* 1960, **235**, 2870.
2. Johnson, B., and M. Schubert. Proteinpolysaccharides of human costal cartilage. *J. clin. Invest.* 1960, **39**, 1752.
3. Partridge, S. M., H. F. Davis, and G. S. Adair. The chemistry of connective tissues. 6. The constitution of the chondroitin sulfate-protein complex in cartilage. *Biochem. J.* 1961, **79**, 15.
4. Partridge, S. M., and D. F. Elsdon. The chemistry of connective tissues. 7. Dissociation of the chondroitin sulfate-protein complex of cartilage with alkali. *Biochem. J.* 1961, **79**, 26.
5. Gregory, J. D., and L. Rodén. Isolation of kerato-sulfate from chondromucoprotein of bovine nasal septa. *Biochem. biophys. Res. Commun.* 1961, **5**, 430.
6. Scheinthal, B. M., and M. Schubert. Fractionation of the degradation products of compounds of protein and polysaccharide from cartilage. *J. biol. Chem.* 1963, **238**, 1935.
7. Meyer, K., P. Hoffman, and A. Linker. Mucopolysaccharides of costal cartilage. *Science* 1958, **128**, 896.
8. Kaplan, D., and K. Meyer. Aging of human cartilage. *Nature (Lond.)* 1959, **183**, 1267.
9. Anderson, A. J. Some studies on the occurrence of sialic acid in human cartilage. *Biochem. J.* 1961, **78**, 399.
10. Anderson, A. J. Some studies on the relationship between sialic acid and the mucopolysaccharide-protein complexes in human cartilage. *Biochem. J.* 1962, **82**, 372.
11. Gregory, J. D., T. C. Laurent, and L. Rodén. Enzymatic degradation of chondromucoprotein. *J. biol. Chem.* 1964, **239**, 3312.
12. Pal, S., and M. Schubert. The action of hydroxylamine on the proteinpolysaccharides of cartilage. *J. biol. Chem.* 1965, in press.
13. Doganges, P. T., and M. Schubert. The use of lanthanum to study the degradation of a proteinpoly-

- saccharide from cartilage. *J. biol. Chem.* 1964, **239**, 1498.
14. Cessi, C., and F. Piliego. The determination of amino sugars in the presence of amino acids and glucose. *Biochem. J.* 1960, **77**, 508.
15. Eichhorn, G. L., and J. J. Butzow. Interaction of metal ions with polynucleotides and related compounds. III. Degradation of polyribonucleotides by lanthanum ions. *Biopolymers* 1965, **3**, 79.
16. Hass, G. M. Studies of cartilage. IV. A morphologic and chemical analysis of aging human costal cartilage. *Arch. Path.* 1943, **35**, 275.
17. Leppelmann, H. J. Der Mukopolysaccharidgehalt des Knorpels in Abhängigkeit vom Lebensalter. *Z. Rheumaforsch.* 1959, **18**, 348.
18. Shetlar, M. R., and Y. F. Masters. Effect of age on polysaccharide composition of cartilage. *Proc. Soc. exp. Biol. (N. Y.)* 1955, **90**, 31.