BIOPHYSICAL AND PHYSIOLOGICAL INVESTIGATIONS ON CARTILAGE AND OTHER MESENCHYMAL TISSUES

V. IDENTIFICATION OF THE POLYSACCHARIDE OF BOVINE *NUCLEI PULPOSI**

by

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The intervertebral *Nucleus Pulposus*** may be regarded as a three-dimensional lattice gel[9,11,12]. About one fifth of its organic matter is made up of a previously non-identified polysaccharide, suggested to be hyaluronic acid[4,5]. Our preliminary data[9,11,12] contrarily stressed the occurrence of a sulphated polysaccharide, containing hexosamine and uronic acid, presenting a higher electrophoretic mobility than hyaluronic acid. Additional data on the isolation and identification of the N.P. polysaccharide will be briefly reported in this paper.

Batches of N.P. from the intervertebral discs of young calves were dissected free from the tendonous *Annulus Fibrosus*, and stored in the refrigerator for 8 to 10 weeks. After this time the pooled material contained about 13.5% d.w. A total of 150 g was subjected to fractional extraction and purification largely according to EINBINDER AND SCHUBERT[1].

TABLE I

FRACTIONAL EXTRACTION OF N.P. AT INCREASING ALKALINITY

	Extractions during 2 days in aqueous solution containing	*Yield of protein-free purified[1] polysaccharide contained in the supernatant*	
First step	KCl 30% + K_2CO_3 1%; pH about 7–8; Sediment treated further	3.2 g	"N.P. I"
Second step	KCl 30% + KOH until a pH of about 10; Sediment treated further	0.65 g	"N.P. II"
Third step	KOH 2%; temperature 0° C	0.15 g	"N.P. III"
Dissolved proteins + tissue residues	11.6 g d.w.		
Total yield calculated per 19.5 g d.w.		4 g (20%)	

The analytical data (Table II) are compared with data on hyaluronic acid and chondroitin sulphuric acid. The three polysaccharide samples N.P. I–III were all protein-free, and showed similar uniform one-component patterns during three hours' run in the electrophoresis. This may, however, not exclude the presence of traces of other polysaccharides (hyaluronic acid?).

* This is the fifth report of a program of joint investigations on the physiology and nutrition of some mesenchymal tissues contracted between the Caroline Institute (Depts. of Orthopedic Surgery and Cancer Research Division of Radiumhemmet) and the Institute of Biochemistry at the University of Uppsala. The work was supported by research grants from Konung Gustaf V:s 80-årsfond, and from Eli Lilly and Co., Indianapolis, U.S.A.

** Abbreviated N.P.

TABLE II

	Protein-free polysaccharide from bovine Nucleus Pulposus			*Hyaluronic acid*[13]	*Chondroitin sulphuric acid*	*Total tissue residues*
	N.P. I	*N.P. II*	*N.P. III*			
Nitrogen (Kjeldahl) in per cent d.w.	2.53	2.47	2.83	2.75	2.9	17.3
Total S content[10] in per cent d.w.	5.29	5.88	5.89	< 0.02	6.0	0.07
Molar ratio N:S	1.10	0.96	1.10			
El. mobility $\times 10^5$ cm^2/volt sec at pH 4.2, ionic strength 0.1, temp. $+0.5°$; acetate buffer	12.0	13.6	13.0	9	12	—
$[\alpha]_D^{20}$	$-17°$	$-15°$	$-11°$	-35 to $-40°$ ref. 6		
Ash content	32.7	32.6	32	—	32.5*	

* Calculated on potassium salt.

Following acid hydrolysis and conversion of amino-hexose to pentose according to GARDELL *et al.*[2], the subsequent paper chromatography revealed the presence of lyxose but not arabinose. It is, therefore, concluded that the amino-sugar in this polysaccharide material would be galactosamine and not glucosamine.

The infra-red spectra of all three samples of the N.P. polysaccharide were measured by Dr S. F. D. ORR[8]. These were very similar, especially from 3–10 μ, to the spectrum of chondroitin sulphate[7] derived from cartilage; they did, however, show bands at 770 and 820 cm^{-1} not present in the reference sample. The absorption in this region is due to the molecular skeleton and, since differences in the hexosamine types present are excluded by chromatography evidence, these bands probably indicate a difference in the position of the sulphate group in the N.P. polysaccharide fractions from that of the reference sample of chondroitin sulphate obtained from cartilage.

Available data thus indicate that the bulk of the N.P. polysaccharide apparently is chondroitin sulphate or sulphate isomers. No appreciable amount of either hyaluronic acid or hyaluronic sulphate was found.

The identification of chondroitin sulphate will also explain the marked alcohol-resistant metachromasia and the fact that digestion with testicular hyaluronidase occurs at a slower rate than in the case of hyaluronic acid[14,15] (*cf.* LILLIE[3]).

REFERENCES

1 J. EINBINDER AND M. SCHUBERT, *J. Biol. Chem.*, 185 (1950) 725.
2 S. GARDELL, F. HEIJKENSKJÖLD AND A. ROCH-NORLUND, *Acta Chem. Scand.*, 4 (1950) 970.
3 R. D. LILLIE, E. W. EMMART AND A. M. LASKEY, *A.M.A. Arch. Pathol.*, 52 (1951) 363.
4 K. MEYER, in *Modern trends in Ophthalmology*, London 1948, p. 71.
5 K. MEYER, *Connective Tissues*, Trans. of the First Conf. April 24–25, 1950. Josiah Macy, Jr. Foundation. New York, p. 95.
6 K. MEYER AND M. M. RAPPORT, *Science*, 113 (1951) 596.
7 S. F. D. ORR, R. J. C. HARRIS AND B. SYLVÉN, *Nature*, 169 (1952) 544.
8 S. F. D. ORR, Infra-red data to be published.
9 S. PAULSON, B. SYLVÉN, C. HIRSCH AND O. SNELLMAN, *Biochim. Biophys. Acta*, 7 (1951) 207.
10 S. PAULSON, *Acta Chem. Scand.* (1953) (in press).
11 B. SYLVÉN, *Acta Orthopaedica Scand.* 20 (1951) 275.
12 B. SYLVÉN, S. PAULSON, C. HIRSCH AND O. SNELLMAN, *J. Bone and Joint Surg.*, 33 (1951) 333.
13 B. SYLVÉN AND H. MALMGREN, *J. Lab. Invest.*, (in press).
14 B. SYLVÉN, Unpublished observations.
15 G. B. WISLOCKI, H. BUNTING AND E. W. DEMPSEY, *Am. J. Anat.*, 81 (1947) 1.

Received October 14th, 1952