Reports

Mucopolysaccharides of Costal Cartilage

Probably no other connective tissue has been investigated more frequently than hyaline cartilage. From the time of Boedecker in 1854 to the present, this tissue has been used for the preparation of chondroitin sulfate. In recent years it has been used in studies of the native protein complexes of mucopolysaccharides (*1*) and on biosynthesis of the carbon chain and the incorporation of ester sulfate (*2*). Meyer *et al.* have previously reported on the isolation of chondroitin sulfates from bovine tracheal cartilage and costal and sternal cartilage of newborn children (3). On the basis of optical rotation and solubility of the Ca salts, the mucopolysaccharide fractions appeared to be mainly A (4-sulfate) with a minor C (6-sulfate) component (*4*).

In this report (*5*), attention is called to the isolation of keratosulfate from costal cartilage in two cases of Marfan's syndrome (for literature, see *6*) and from two 23-year-old males with apparently normal skeletal development.

Costal cartilage was obtained at autopsy from two males age 33 and 44 with typical Marfan's syndrome (*7*) who had died of ruptured aneurysm of the aorta and one 23-year-old male who had died suddenly after operation for removal of a brain tumor. The other died after an overdose of a drug. The cartilage was dissected free of extraneous tissue and of perichondrium and dehydrated in acetone. The air-dried tissue was homogenized in 0.1*N* HCl in a high-speed blender, digested with pepsin at *p*H 1.5 in the presence of toluene followed by tryptic digestion at *p*H 7.5. After deproteinization, the mucopolysaccharide fractions were obtained as calcium salts as described previously (*3*).

The total mucopolysaccharide fractions isolated represented 11 and 12 percent of the dry weight of the cartilage in the two cases of Marfan's syndrome and 16 to 18 percent for the controls (*8*). The former consisted of approximately equal quantities of chondroitin sulfate which was characterized as chondroitin sulfate C by analysis, solubility, and infrared spectra and of keratosulfate which was identical in analysis, rotation, and infrared spectrum with that isolated from bovine cornea (*9*). The hexosamine of the keratosulfate fraction was identified by paper chromatography as glucosamine and isolated from one fraction as the crystalline hydrochloride ($[\alpha]_D$ + 72°), and galactose was identified by paper chromatography, and as the crystalline α-methylphenyl hydrazone by melting point and infrared spectrum. The controls yielded chondroitin sulfate and keratosulfate in a ratio of 2.5 to 1 instead of the 1 to 1 ratios in the Marfan's syndrome (*8*).

Thus far keratosulfate, a polymer of unknown structure composed of equimolar amounts of N-acetyl glucosamine, galactose, and sulfate, had been isolated from bovine cornea (*9*) and nucleus pulposus (*10*) where it constitutes approximately one-half of the total mucopolysaccharides, and from the end pieces of the long bones of calf where it was only 1 percent of the total mucopolysaccharide (*3*). In cornea the other half of the mucopolysaccharides is made up of chondroitin sulfate A and chondroitin, while in nucleus pulposus the remaining half appears to be chondroitin sulfate C. Thus the mucopolysaccharide pattern of the costal cartilage in Marfan's syndrome resembles that of nucleus pulposus.

It appears possible that keratosulfate occurs more widely in connective tissue than believed heretofore and that it has been missed because of the greater alcohol solubility of its salts. The occurrence of glucosamine in articular cartilage has been reported by Kuhn and Leppelmann (*11*), who noted a large decrease in galactosamine and a small increase of glucosamine with increasing age. Similarly Shetlar and Masters (*12*) noted in costal cartilage a large decrease in uronic acid with increasing age, while the total hexosamine increased only slightly. These results were interpreted as indicating an increase of a "neutral mucopolysaccharide" in aging, but are readily explained by the absence of detectable amounts of keratosulfate in costal cartilage of newborn infants and the relatively large amounts found in mature costal cartilage. The indication from the rotation and solubility data that the chondroitin sulfate of the newborn infant costal cartilage was mainly A and of the mature costal cartilage mainly C was verified by the infrared spectra (*4*).

It appears from these data that the mucopolysaccharide pattern of hyaline cartilage is more complex than believed heretofore. The distribution of keratosulfate and of the types of chondroitin sulfate will have to be reinvestigated, with regard to age, species, and the source of the cartilage.

KARL MEYER
PHILIP HOFFMAN
ALFRED LINKER*

Department of Medicine, Columbia University College of Physicians and Surgeons, and *Edward Daniels Faulkner Arthritis Clinic of Presbyterian Hospital, New York*

References and Notes

1. T. Malawista and M. Schubert, *J. Biol. Chem.* 230, 535 (1958).
2. F. D'Abramo and F. Lipmann, *Biochim. et Biophys. Acta* 25, 211 (1957).
3. K. Meyer, E. Davidson, A. Linker, P. Hoffman, *ibid.* 21, 506 (1956).
4. P. Hoffman, A. Linker, K. Meyer, *ibid.*, in press.
5. This work was supported in part by a grant from the U.S. Public Health Service.
6. V. A. McKusick, *Heritable Disorders of Connective Tissue* (Mosby, St. Louis, Mo., 1956).
7. Obtained through the courtesy of the department of pathology of this institution.
8. The differences in yields are not considered significant, but the differences in the ratios of polysaccharide are valid.
9. K. Meyer, A. Linker, E. A. Davidson, B. Weissmann, *J. Biol. Chem.* 205, 611 (1953).
10. S. Gardell, *Acta Chem. Scand.* 9, 1035 (1955).
11. R. Kuhn and H. J. Leppelmann, *Ann. Chem. Liebigs* 611, 254 (1958).
12. M. R. Shetlar and Y. F. Masters, *Proc. Soc. Exptl. Biol. Med.* 90, 31 (1955).

* Established investigator of the American Heart Association.

13 June 1958

Possible Role for Vitamin K in Electron Transport

Coupled oxidative phosphorylation in extracts of *Mycobacterium phlei* is dependent on a particulate and a soluble fraction (*1*). In an attempt to elucidate their respective roles, a study of electron-transport pathways in these fractions was undertaken (*2*). The characterization of some of the soluble electron-transport enzymes has been described in an earlier report (*3*).

Cells grown with continuous aeration

Instructions for preparing reports. Begin the report with an abstract of from 45 to 55 words. The abstract should *not* repeat phrases employed in the title. It should work with the title to give the reader a summary of the results presented in the report proper. (Since this requirement has only recently gone into effect, not all reports that are now being published as yet observe it.)

Type manuscripts double-spaced and submit one ribbon copy and one carbon copy.

Limit the report proper to the equivalent of 1200 words. This space includes that occupied by illustrative material as well as by the references and notes.

Limit illustrative material to *one* 2-column figure (that is, a figure whose width equals two columns of text) or to *one* 2-column table or to *two* 1-column illustrations, which may consist of two figures or two tables or one of each.

For further details see "Suggestions to Contributors" [*Science* 125, 16 (1957)].