On the Mucopolysaccharides of *Nucleus Pulposus*

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In 1948 Karl Meyer [1] assumed that *nucleus pulposus* must contain hyaluronic acid. Evidence for the presence of a polysaccharide was presented by Malmgren and Sylvén [2] in 1952. They isolated chondroitin sulfuric acid using the method of Einbinder and Schubert [3]. Since they obtained a fairly good yield of the polysaccharide with an almost theoretical composition they considered the bulk of the polysaccharide material in *nucleus pulposus* to consist of chondroitin sulfuric acid.

In a study of the distribution of the aminohexoses in different parts of the animal body we found that glucosamine and galactosamine occurred together and in almost equal amounts in the human *nucleus pulposus*.

Human intervertebral discs were collected from autopsy material, the lumbar and the lower thoracal discs being taken. After cleaning from surrounding tissue the *nuclei pulposi* were frozen at −20° C. When sufficient material had been collected the mass was minced in a meat chopper in the frozen state. It was then freeze-dried and further pulverized in a Turmix blendor.

Samples of this dry powder were hydrolysed with 6 *N* hydrochloric acid for 8 hours on a boiling water bath. The aminosugars were then determined colorimetrically applying the chromatographic technique of Gardell [4]. The results of this analysis together with the analysis of ester sulfate, nitrogen, ash, and moisture is given in Table 1.

Table 1.

Moisture	12.0 %
Ash	7.8 »
Nitrogen (Micro-Kjeldahl)	10.8 »
Sulfur in ester sulfates	1.2 »
Glucosamine-HCl	5.4 »
Galactosamine-HCl	5.1 »

This analysis clearly indicates the presence of a glucosamine containing polysaccharide together with chondrotinsulfuric acid. Preliminary experiments have also been made for the preparation of the polysaccharides.

Twentyfive g of the freeze-dried powder was coagulated by pouring it in small portions into 500 ml of boiling water. The material was then digested with pancreatic and intestinal glycerol extracts as described by Gardell [5]. After ten days of digestion the solution was concentrated by evaporation *in vacuo* to a syrup and precipitated with several volumes of alcohol at pH 8. The precipitate was dissolved in about 50 ml of water and the pH adjusted to 2.5 with hydrochloric acid. From this solution the crude polysaccharide was precipitated with ten volumes of alcohol. It contained 7.1 % nitrogen, 11.0 % glucosamine-HCl and 11.4 % galactosamine-HCl. The yield was 8.35 g.

This material was dissolved in about 40 ml of water, digested and precipitated as above. An almost white preparation weighing 6.18 g with a nitrogen content of 5.5 % was obtained. The digestion and precipitation procedure was repeated once more. The last precipitate was dissolved in water, neutralized, and freeze-dried. The freeze-dried preparation was extracted with 50 ml of 90 % phenol over night and centrifuged at 25 000 g. The residue was dissolved in faintly alkaline water the solution acidified to pH 2.5 and alcohol added. The polysaccharide precipitated weighed 2.69 g and contained 14 % glucosamine-HCl and 17.5 % galactosamine-HCl; the nitrogen content was 4.9 %.

Of this preparation 1.93 g was dissolved in water, freeze-dried and submitted to repeated phenol extraction. In this way 1.28 g of a white polysaccharide fraction relatively low in nitrogen and high in aminosugar and sulfur content was obtained. The analysis of this fraction is given in Table 2.

Table 2.

Moisture	12.8 %
Ash	19.9 »
Nitrogen (Micro-Kjeldahl)	2.84 »
Sulfur in ester sulfates	4.32 »
Uronic acid (Tracey [6])	13.7 »
Glucosamine-HCl	9.2 »
Galactosamine-HCl	15.6 »
Galactose	7.0 »

Galactose was determined according to Gardell [7]. The polysaccharide was hydrolysed with 1 *N* sulfuric acid 14 hours on a boiling water bath. After hydrolysis the

mixture was passed through a "Zeo-carb 225" 4.5 % crosslinked cation-exchanger in the hydrogen form, neutralized with barium hydroxide, evaporated to dryness in a desiccator and finally taken up in the solvent used in the chromatography.

Uronic acid and galactosamine are present in equimolar proportions thus there is strong evidence for the presence of chondroitin sulfuric acid. However, the sulfur content is much to high. Another interesting feature is the presence of glucosamine and galactose in equimolar proportions. This, together with the high sulfur content, strongly suggests the presence of a compound similar to the keratosulfuric acid recently described by Meyer *et al.*[8] to occur in the *cornea*. The sulfur content is sufficient to account for both these polysaccharides.

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On the Mechanism of the Heparin-induced Lipemia Clearing Reaction

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The clearing of alimentary lipemia by injection of heparin, first discovered by Hahn [1], is due to the production *in vivo* of an active substance (Anderson & Fawcett [2]) called the clearing factor. For the formation of this substance a factor from tissues and another from plasma are needed in addition to heparin (Anfinsen *et al.*[3]). By the action of clearing factor on (lipemic) serum the concentration of lipoprotein particles of the lowest density decreases, while a concomitant increase

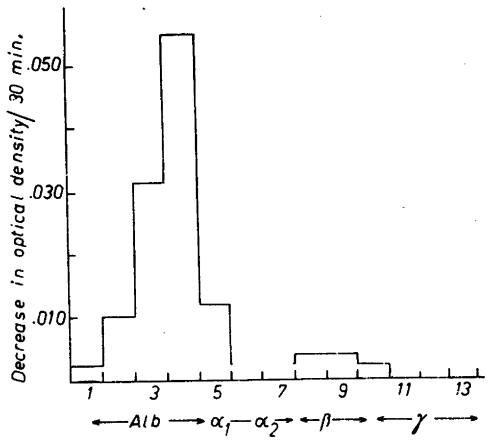

Fig. 1. Ability of electrophoretically purified serum protein fractions to clear a purified clearing factor — olive oil emulsion mixture (= electrophoretic pattern of the lipid acceptor-protein). Clearing/30 minutes/mg protein : albumin 0.010, β globulin 0.032.

occurs in the more dense low-density lipoproteins and alpha lipoproteins (Graham *et al.*[4], Anfinsen *et al.*[3], Boyle *et al.*[5], Nikkilä [6]). This means that new protein molecules enter into combination with lipids. The lipid acceptor-protein, if identical with the "coprotein" of Anfinsen *et al.*[3], is claimed to be localized in Fraction III−0 of the conventional ethanol fractionation scheme (Anfinsen *et al.*[3]). Recently, serum albumin has been reported to increase the clearing rate, probably by binding the fatty acids, which are known to be released under the action of clearing factor (Shore *et al.*[7]) and which inhibit the clearing reaction (Gordon *et al.*[8]). This paper reports briefly on some experiments carried out to throw more light on the mechanism of the clearing reaction.

As is to be expected in view of the necessity of an acceptor-protein, a tween-stabilized olive oil emulsion is not cleared upon the addition of purified (Nikkilä [9]) clearing factor. When normal serum is added to this system the clearing rate increases directly proportionally to the serum concentration up to a certain limit (20−30 % of the total volume of the mixture) but declines on the further increase of the amount of added serum, thus suggesting the presence of some slightly inhibiting substance(s) in serum. To elucidate