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Hexosamine and Ester Sulphate Content of the Human *Nucleus Pulposus* at Different Ages

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The hexosamine and sulphate content of adult human *nucleus pulposus* (central part of the intervertebral disc) has been determined. Equimolar quantities of hexosamine and ester sulphate were found. An increase could be noted in the ratio of glucosamine/galactosamine, from about 0.5 at 15 years of age to 1.5 at 90 years. It is concluded that, with rising age, a change takes place in the mucopolysaccharide pattern, in the form of an increase in the ratio of keratosulphate/chondroitin sulphate.

The *nucleus pulposus* (*N.P.*), the central part of the intervertebral disc, is a form of connective tissue that contains unusually large quantities of carbohydrate. It has been shown to contain chondroitin sulphate by Malmgren and Sylvén [1], and keratosulphate by Gardell and Rastgeldi [2]. A method for partial separation of the two polysaccharides has been described by Gardell [3].

The object of the present investigation was to determine whether there are any age differences in the mucopolysaccharide pattern of *N.P.*, and whether it has a high hyaluronic acid content, as claimed by Meyer [4] and by Hall *et al.*[5] Since keratosulphate contains equimolar quantities of glucosamine and ester sulphate, chondroitin sulphate equimolar quantities of galactosamine and ester sulphate, and hyaluronic acid hexosamine but no sulphate, this can be done by comparing the quantities of the hexosamines and ester sulphates at different ages.

EXPERIMENTAL

Non-pathologic lumbar and lower thoracic intervertebral discs were taken from autopsy cases. The *N. P.* was dissected free from the surrounding *annulus fibrosus*, freeze-dried and ground in a mortar. About 4 mg were analyzed for amino sugars according to Gardell [6]. Hydrolysis was performed in sealed tubes with 0.5 ml of 6 N hydrochloric acid for 8 h in a boiling water bath. After desiccation the hydrolysate was put on the top of a Dowex-50 column; the hexosamines were eluted with 0.3 N hydrochloric acid, and determined with the Elson-Morgan method.

About 50—100 mg of the *N. P.* powder were taken for determination of the sulphate content. The powder was hydrolyzed in 4 ml of 6 N hydrochloric acid for 4 h in a boiling

Table 1. Hexosamine and sulphate-sulphur content of freeze-dried *N. P.* from subjects of different ages.

Age, years	% Hexosamine	% Sulphur	Mole hexosamine / Mole sulphur
19	11.3	2.85	0.7
22	10.5	2.48	0.7
27	12.2	2.72	0.8
38	10.0	2.44	0.7
43	8.1	1.13	1.3
50	12.0	2.07	1.0
60	8.5	1.84	0.8
68	7.0	0.98	1.3
74	5.5	1.01	1.0
79	5.5	1.77	0.6
86	6.4	1.07	1.1

water bath, and filtered. Barium sulphate was precipitated from hot solution by the addition of barium chloride. On the following morning, the precipitate was collected on a platinum-iridium Gooch filter, dried and weighed.

To remove free sulphate ions, which might have interfered to some extent with the ester sulphate determinations, the *N. P.* was digested with proteolytic enzymes according to Gardell [7], and run down an anion exchange column according to Boström and Månsson [8]. One g of *N. P.*, both from subjects aged 20—60 years and aged 70 years, was denatured in boiling water; the pH was adjusted to 8.5, and glycerol extracts from pig pancreas and intestinal mucosa were added. The mixtures were digested at 40°C for 2—3 weeks, the pH being controlled and, when necessary, adjusted by the addition of dilute sodium hydroxide. After some particles had been centrifuged off and discarded, the solutions were allowed to pass Dowex-2×8 20—40 mesh columns in chloride form. No barium sulphate opalescence could then be detected on addition of barium chloride. The materials were freeze-dried, and about 10 mg were taken for hexosamine determination and 100—150 mg for sulphate determination.

RESULTS

The results of the analyses are presented in Tables 1—3. As can be seen in Table 1, the hexosamine and sulphate content of dry *N.P.* decreases with rising age. The values were only roughly reproducible, since the powders were not sufficiently homogenized. The values are, however, of the order of magnitude expected for equimolar quantities of hexosamine and ester sulphate being present

Table 2. Hexosamine and sulphate-sulphur content of *N. P.* digested with proteolytic enzymes and devoid of free sulphate ions.

N. P.	% Hexosamine *	% Sulphur	Mole hexosamine / Mole sulphur
20—60 years	6.1	1.24	0.9
70 years	3.5	0.72	0.9

* The ratio of glucosamine/galactosamine remained essentially unchanged during digestion; 0.9 for subjects aged 20—60 years, and 1.2 for 70 years.

Table 3. Hexosamine content and ratio of glucosamine/galactosamine of freeze-dried *N. P.* from subjects of different ages.

Age years	No. of cases	% Hexosamine Mean	Range	% Glucosamine Mean	Range	% Galactosamine Mean	Range	Glucosamine / Galactosamine Mean	Range
11–20	6	14.7	11.3–16.8	4.8	4.3–5.6	9.9	6.9–12.0	0.50	0.41–0.64
21–30	6	11.2	10.3–12.7	4.5	3.2–5.3	6.4	5.4– 8.0	0.75	0.60–0.90
31–40	4	10.6	9.5–11.9	5.2	4.6–6.3	5.4	4.9– 5.9	0.96	0.89–1.13
41–50	4	9.5	7.6–12.0	4.4	3.8–5.5	5.1	3.8– 6.5	0.89	0.66–1.05
51–60	12	8.3	6.1–11.0	4.0	2.8–5.5	4.3	2.8– 5.6	0.96	0.69–1.33
61–70	9	7.8	4.2–11.5	4.1	2.4–5.9	3.7	1.7– 6.1	1.13	0.90–1.38
71–80	5	6.7	5.5– 8.1	3.7	3.1–4.2	3.0	2.4– 3.8	1.25	1.10–1.45
81–90	3	6.6	6.4– 6.8	3.8	3.6–3.9	2.9	2.5– 3.1	1.42	1.17–1.53
91–	1	3.3	–	2.1	–	1.2	–	1.79	–

Table 2 shows the hexosamine and sulphate content of freeze-dried *N.P.*, digested with proteolytic enzymes and devoid of free sulphates. Within errors of method equimolar quantities of hexosamine and ester sulphate were found with no excess of hexosamine. The figures are lower than could be expected from the values in Table 1, probably depending on a higher water content.

It can be inferred from Table 3 that a fairly consistent change in the hexosamine pattern of *N.P.* takes place during aging. Not only does the hexosamine content decrease from about 14 % of dry weight at 15 years of age to 6 % at 90 years, but the ratio of glycosamine/galactosamine increases from about 0.5 to 1.5.

DISCUSSION

The presence of equimolar quantities of hexosamine and ester sulphate suggests that hyaluronic acid contributes very little to the amount of the human *N.P.* mucopolysaccharides. This is in agreement with the findings of Gardell and Rastgeldi [2], who concluded that the glucosamine in *N.P.* belongs to keratosulphate and the galactosamine to chondroitin sulphate. The changes observed in the hexosamine pattern of *N.P.* with age thus reflect an increase in the ratio keratosulphate/chondroitin sulphate.

Hirsch *et al.*[9] studied the sulphate content of human *N.P.* They found that although in the dry substance it diminished with age, as also observed in the present investigation, it was practically constant per volume native tissue.

It is probable that a similar change in the mucopolysaccharide pattern with rising age occurs in other tissues as well. Hass [10] studied the reducing power and sulphate content of dried costal cartilage, and noted a slight increase up to the third and fourth decades of life, and a slight decrease thereafter. Loewi [11] found equimolar amounts of hexosamine, as determined with the Elson-Morgan method, and ester sulphate. With age the values slightly decreased. Shetlar and Masters [12], on the other hand, who determined uronic acid with the carbazole method of Dische, and hexosamine with the Elson-Morgan

method, found that uronic acid had a maximal value at birth and then diminished markedly. Moreover, with increasing age, there was much more hexosamine than could be expected from the uronic acid content. Kuhn and Leppelmann [13] studied the hexosamines of joint cartilage, using paper chromatography for the separation of glucosamine and galactosamine, and found that the ratio of glucosamine/galactosamine increased with rising age, in a similar manner as found for *N.P.* in the present investigation.

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