BIOPHYSICAL AND PHYSIOLOGICAL INVESTIGATIONS ON CARTILAGE AND OTHER MESENCHYMAL TISSUES

VI. *Characteristics of Human Nuclei Pulposi during Aging*[1].

By

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Previous electron microscopic (1), diffusion (2), and fractionation studies (3; partly unpublished) indicate that the healthy intervertebral Nucleus Pulposus[2] in young subjects represents a three-dimensional lattice gel, in which cell bodies and intercelluar material can be distinguished. The latter contains a dense collagen-reticulin feltwork embedded in a matrix or "ground substance", which holds large amounts of chondroitin sulphate (4), water and salts. These components seem to be arranged in some orderly pattern outlined in a previous paper (3). Electron microscopic and fractionation data further suggest a very close relationship between the collagen and polysaccharide moieties (1, 3). Since the structural changes of N.P. during aging attract considerable attention from the clinical and pathological aspects (5) it became of interest to obtain more insight into the quantitative relations between the protein and polysaccharide constituents in various ages.

In so far as gross morphology and light microscopy are concerned such age changes are fairly well known (5, 11, 13, 15, 16). *Püschel* registered the decrease in water content of N.P. during aging, but did not correlate this with changes in relative amounts of constituents.

As previously mentioned (1) we were not able to draw a sharp

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[2] Abbreviated N.P.

distinction between age changes "in the normal range" and those more excessive changes generally labelled as "nuclear degeneration". From a qualitative point of view, pertinent data emphasize that criteria other than chemical have to be introduced. It should further be stressed that pertinent age changes in N.P. are illustrative of similar changes in many other mesenchymal tissues, such as articular cartilage (6, 14, 16), tendons, connective tissue proper, etc.

In order to supply a basis for discussion some analytical data on healthy N.P. from young calves are presented in Table 1.

TABLE 1

Average Composition of Pooled Nuclei from Young Calves.

Water content	83.5	per cent	
Dry weight (solid material)	16.5	"	
Kjeldahl nitrogen	11	"	d. w.
Protein content, calculated	50–60	"	"
Polysaccharide content, yield after extraction	20[1]	"	"
" " , calculated from S value	30–36	"	"
Total sulphur content, less methionine	2– 2.2	"	"
Ash content	6.8[2]	"	"

[1] Acc. to Malmgren and Sylvén (4).
[2] Cp. ash content of cartilage 9.7 per cent acc. to Winter.

MATERIAL AND METHODS

A total number of 65 lumbar and lower thoracic discs were collected from 10 autopsy cases of various ages, from birth until 73 years of age. Data on the gross anatomy and microscopy of the N.P. were registered, together with wet and dry weight determinations and the percentage sulphur values.

The samples were first subjected to wet weight determination, then dried in vacuum over silica gel at $+60°$ centigrade until constant dry weight. They were then combusted with nitric acid in the presence of an oxidizing catalyst for four hours at ordinary pressure. The sulphate ions were determined by a new conductometric semi-micro method developed by Paulson (7). During this procedure all sulphur present as polysaccharide ester sulphate is converted quantitatively to sulphate. Of the protein sulphur, cystine-cysteine is simultaneously converted to sulphate, but the methionine sulphur is only converted to a small extent. For details and evaluation of the method the reader is referred to the original paper (7).

Considering other sources of sulphur in N.P. the content of cystine-cysteine sulphur in collagen is very low (8, 9)–about 0.02 per cent (0.0–0.1 g-residues 100 g)–and consequently negligible. Even so a two- or three-fold increase in the amount of collagen would not contribute very much to our sulphur values. The sulphur content of the small amount of other proteins present in N.P. (cp. 3) is not known, but cannot be expected to interfere. The sulphur values obtained are consequently considered to represent the amounts of polysaccharide ester sulphate present in N.P. The contribution derived from collagen sulphur is calculated to the order of 0.02 per cent.

The analytical errors are largely found in the sulphur analyses, and amount to about 1 per cent (7).

RESULTS

Our dry weight and total sulphur determinations are shown in *Tables 2* and *3*. The events in human nuclei during aging may be compiled as follows.

There is a marked increase in mass per unit tissue volume simultaneously with a corresponding decrease in the percentage water content. The increase in mass is largely explained by increased amount of collagen material per unit tissue volume. The percentage amount of total sulphur undergoes a gradual decrease if measured per dry weight, but the absolute amount per unit tissue volume remains largely on the same level. The ratio between sulphur and collagen contents is decreasing with aging. In other words, the amount of collagen material per unit tissue volume increases, while the absolute amount of polysaccharide largely remains on a stationary level or undergoes a slight decrease with high age.

Comparison between sulphate and dry weight values in the thoracic and lumbar region indicates that the typical "degenerative" changes appear at an earlier age preferably in the lower lumbar segments. As to the operative mechanism underlying this fact, attention is paid to the mechanical conditions supposed to play an important role in the lower lumbar segments.

In nuclei presenting considerable gross morphological changes (degenerated) with ruptures of the *Annulus* still greater decrease in sulphur values down to 1.0 per cent per d.w. was observed (Table 3). No apparent correlation was found between the amount of total sulphur and the occurrence of marginal osteophytes.

The figures so far presented on human nuclei are to be regarded as average values. Determinations (not reported in detail) on small samples cut out from different parts of same nuclei demonstrate that

TABLE 2

Mean Values and Calculated Ratios in Nuclei Pulposi of Various Ages Presenting Normal Gross and Microscopic Morphology.

Discs with herniation and rupture of annulus are marked × and reported in Table 3.

		Full-term fetus ♂	2 months ♂	13 years ♂	17 years ♂	28 years ♂	37 years ♂	40 years ♀	52 years ♂	56 years ♂
Dry weights	Thoracic and LI–LII	7.5	10.2	10.9	13.8	16.5	—	17.11[1]	31.3	24.0
	Lower lumbar LIII–LV	5.4	8.2	9.5	12.3	×	—	×	26.1	20.2
Total sulphur content in per cent (less methionine)	Thoracic and LI–LII	2.4	2.7	3.0	3.4	3.2	2.7	3.31[1]	2.9	2.4
	Lower lumbar LIII–LV	2.5	2.6	3.3	3.3	×	2.6	×	2.9	2.2
Sulphur/mass ratio × 100		38	28	31	26	19	—	19	10	10

1 Cf. Fig. 1.

TABLE 3

Dry Weight and Sulphur Content of Nuclei in cases of "Degeneration" Accompanied by Rupture of Annulus Fibrosus.

Sex	Age	Disc No.	Percentage dry weight	Percentage sulphur content	Calculated Ratio x 100	Morphology of nucleus
Male	28	L IV	17.11[1]	1.81[1]	10.5	Moderately fibrous
		L V	17.81[1]	2.01[1]	11	„ „
„	37	L V	—	1.2	—	Fibrous, partly necrotic
Female	40	L II	23.2	1.6	7	Moderately fibrous (Fig. 1)
		L III	28.0	1.2	4	Very fibrous, necrotic (Fig. 1)
		L IV	24.51[1]	1.61[1]	6.5	Moderately fibrous (Fig. 1)
		L V	16.1	1.0	6	Necrotic (Fig. 1)
Male	52	L II	28.9	2.2	7.5	Moderately fibrous
		L III	31.1	1.7	5.5	Markedly „
„	56	L V	20.5	1.9	9	Moderately „
„	67[2]	Th X	26.4	1.6	6	Markedly „
		Th XI	27.5	1.4	5	„ „
		Th XII	27.0	1.4	5	„ „
		L I	24.4	1.7	7	„ „
		L II	24.0	2.0	8.5	Moderately „
		L III	24.8	1.9	7.5	Markedly „
		L IV	19.3	2.2	11.5	Moderately „ , haemorrhagic
		L V	26.0	1.5	6	Markedly „
„	73	L V	28.0	1.1	4	From central part; dry, disintegrating
		L V	27.5	1.2	4.5	„ „ „ „ „
		L V	27.0	1.4	5	„ peripheral part; fibrous
		L V	27.2	1.4	5	„ „ „ „

1 Mean of 5 determinations on small samples from same disc.

2 All discs except L II showed great changes with marginal osteophytes.

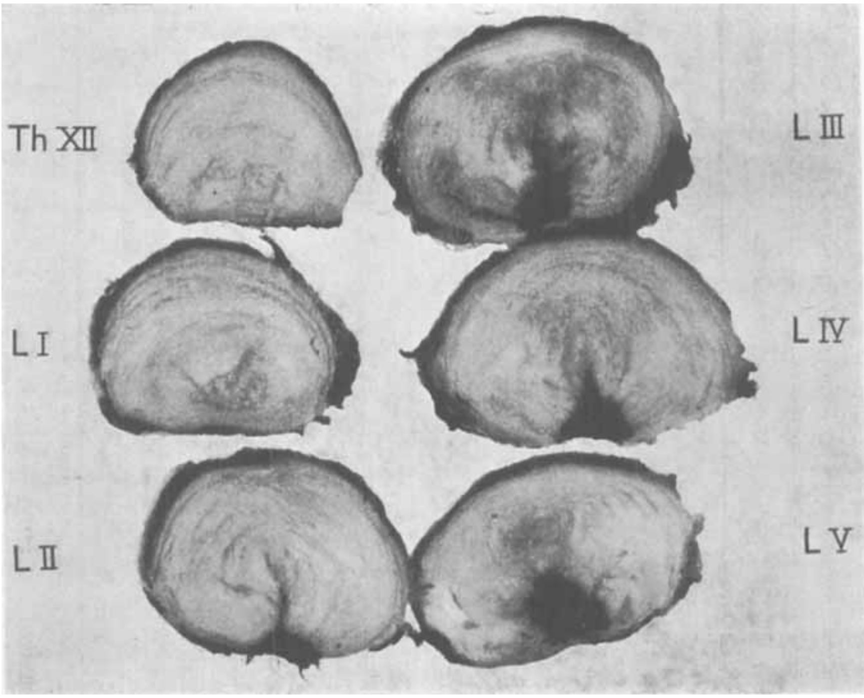

Fig. 1.
Intervertebral discs from a woman aged 40, dead from cerebral haemorrhage. Dry weight and sulphur determinations were performed on small samples of the Nuclei Pulposi. Those presenting normal morphology (Th XII and L I) are recorded in Table 2; the pathological ones in Table 3.

considerable local variations in mass and sulphur content do occur during aging. This tallies well with previous morphological data (1).

In conformity with previous data on tendons (10), the fibrous *Annulus* contains larger amounts of mass and considerably lower sulphur content than the nucleus (Table 4).

DISCUSSION

These findings illustrate what could be inferred from previous gross anatomical and microscopical data (11, 13, 15). The apparent increase in collagen per tissue section was demonstrated long ago, and the increasing "dryness" of nuclei and decrease in water-binding capacity (swelling) was noted.

The point is that the amount of collagen increases while the ratio between the amounts of polysaccharide and collagen per unit tissue volume has decreased during aging. This implies that the protein

TABLE 4

Determinations on samples of normal lumbar Annuli Fibrosi.

(Autopsy specimens from a 34 year old female with cerebral tumour).

Disc	Dry weight in per cent	Sulphur Content in per cent d. w.
L I	19.9	1.35
L II	24.6	0.80
L III	22.8	0.70
L IV	26.2	0.63
L V	29.3	0.52
Mean	24.5	0.8

moiety will occupy a larger number of polar groups of the polysaccharide in the interfibrillar ground substance. A smaller number of polar groups will consequently be available for other linkages and for the binding of water. Since therefore the free electro-negative charge density is decreasing with age the water and dye binding capacities will undergo a gradual decrease in spite of the fact that the absolute amount of polysaccharide per unit tissue volume is probably unchanged. This provides an explanation of the previously observed decrease of metachromasia in aging nuclei (1).

During aging, this avascular tissue thus increases its amount of protein material and keeps the original content of carbohydrate. In a biological sense, this can hardly support the idea of "degeneration" of the nucleus with age (cp. 14). Let us assume that the events outlined above may continue to a certain point, e.g. a two-fold increase in mass, the water content will have decreased so far that the nuclei become more solid, presenting less elasticity, etc. This would correspond to a condition when the function of the disc as a whole is beginning to deteriorate, usually simultaneously with ruptures of the Annulus. Then, from a clinical point of view, it would be advisable to apply the term "disc degeneration". The decrease in the amount of ground substance with aging will further render the collagen fibrils less hydrophilic (cp. 3).

These postulations also open new speculations into the wide field of mesenchymal pathology. Qualitative and quantitative changes in collagen as well as polysaccharide content have to be considered separately in order to explain the biophysical characteristics of aged and diseased mesenchymal tissues. Detailed analysis of mass relations and quantitative ratios of various tissue constituents may guide future classification of those disorders.

SUMMARY

Dry weight and sulphur levels were determined on human Nuclei Pulposi of various ages, and the findings were correlated with the gross appearance and microscopical characteristics of same material. Pertinent changes in physical characteristics of nuclei during aging are primarily ascribed to alterations of the ratio between the amounts of collagen and polysaccharide present, leading to decreased water content and impairment of function. The histochemical and biological implications are briefly discussed.

RÉSUMÉ

On a déterminé le poids net et les niveaux de souffre dans des nuclei pulposi humains de différents âges en rapprochant ces trouvailles de l'aspect de la masse et des caractéristiques microscopiques. Les changements qui se manifestent dans les caractéristiques physiques des nuclei vieillissant sont primairement imputables à une modification du rapport entre les éléments collagènes et polysaccharides qui engendre une diminution de la teneur en eau et un affaiblissement de la fonction. Les faits histochimiques et biologiques sont brièvement discutés.

ZUSAMMENFASSUNG

Trockengewicht und Schwefelgehalt von menschlichen Nuclei pulposi verschiedenen Alters wurden festgestellt und die Befunde wurden mit dem makroskopischen Aussehen und der mikroskopischen Eigenart des gleichen Materiales verglichen. Entsprechende Veränderungen in der stofflichen Eigenart der nuclei während des Alterns werden vor allem den Veränderungen des Verhältnisses zwischen der vorhandenen Menge Kollagen und Polysaccharid, die zu herabgesetztem Wassergehalt und Herabsetzung der Funktion führen, zugeschrieben. Die histochemischen und biologischen Folgerungen werden kurz besprochen.

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