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THE COLLAGENOUS CHANGES IN THE INTERVERTEBRAL DISK WITH AGE AND THEIR EFFECT ON ITS ELASTICITY

AN X-RAY CRYSTALLOGRAPHIC STUDY

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The histological changes in the structure of the intervertebral disk throughout life have been well described by Coventry, Ghormley, and Kernohan (1945). These indicate that an integration process occurs during the first and second decades of life, but that in the third decade evidence of degenerative change appears. Little attention has been paid to the collagenous structure in the disk, and our work has been directed to this in an endeavour to elucidate changes in the collagen/mucopolysaccharide complex in the disk. The bearing of this work upon the aetiology of disk herniation is being studied at the present time and will be described in a later publication.

Our studies were made on fresh human intervertebral disks removed at operation or necropsy, using an x-ray

spectrometer with a comparatively high intensity x-ray beam. This allowed x-ray diffraction photographs of the specimens excised from the disks to be examined rapidly, so that little change in the structure of the material was possible during the exposure of approximately half an hour.

Experiments with Air-dried Specimens

The annulus fibrosus was peeled from the disk and cut into long strips which could be held in the clamps of a stretching frame for the investigation of the elasticity of the disk wall. The specimens of both nucleus and annulus were allowed to dry for ten hours to eliminate most of the unbound water, which, if present, produces an intense diffuse ring on the x-ray diffraction photographs of the material and so masks, to some extent, the x-ray diagram of the collagen or collagen/polysaccharide complex. If this process was allowed to continue too long, irreversible changes appeared in the structure of the collagen of the disk. In particular, specimens allowed to stand over several days showed changes in orientation, and the original structure could not be restored on wetting the sample again. Thus we think the specimens examined had a uniform moisture content during the course of the examination, and changes in crystallinity and orientation of the collagen were definitely related to the conditions of the material *in vivo*.

Our later experiments were made using a modified technique in that the disk was frozen and thin sections were then cut and mounted in the x-ray spectrometer. By this means the samples retained their swollen condition, as little water was dried out during the examination.

The collagen of the rat-tail tendon was used for comparison, and the following effect on its x-ray diagram was found on stretching: (a) increase in orientation of the fibrils, and (b) a reversible increase in the spacings associated with distances along the fibre axis comparable to the lattice extension of α keratin before the main $\alpha \rightarrow \beta$ transformation occurs (Table I).

TABLE I.—Reflections in X-ray Diagram of Rat-tail Tendon

Equatorial Reflections		Meridional Reflections		Other Reflections	
Unstretched	Stretched	Unstretched	Stretched	Unstretched	Stretched
11.8 Å	11.8 Å	2.86 Å	2.95 Å	7.2 Å	7.4 Å
5.7 Å	5.7 Å	3.95 Å	4.04 Å		
4.6 Å	4.6 Å				

A. Nuclear Changes with Age

Many specimens from subjects whose ages ranged from 3 weeks to 79 years were examined. The results of the x-ray diagrams are summarized in Table II (see Figs. 1-3).

The equatorial reflection at 11.8Å is associated with the side-chain spacing—that is, the average distance between the chains. It can be seen that this remains approximately constant in the air-dried specimens first investigated, except in those of an early age in which the reflection was absent.

FIG. 1

FIG. 2

FIG. 3

FIG. 4

FIG. 1.—Nucleus pulposus. Age 3 weeks (x-ray beam parallel to spinal axis). FIG. 2.—Nucleus pulposus. Age 48 years (x-ray beam parallel to spinal axis). FIG. 3.—Nucleus pulposus. Age 70 years (x-ray beam parallel to spinal axis). FIG. 4.—Annulus fibrosus. Age 2½ years (x-ray beam parallel to radial direction of disk).

This, coupled with the fact that the $2.85\text{\AA}$ spacing is most probably associated with the amino-acid repeat distance along the fibre axis, would suggest that the gels of the nucleus pulposus are made up of aligned collagen molecules separated by a distance much greater than the normal $11.8\text{\AA}$. This structure is probably a collagen/poly-saccharide/water system giving a deformable gel in which the structure is retained by the aligned but widely separated collagen chains of the complex. The $2\text{\AA}$ reflection is more difficult to identify, the two possibilities which seem most feasible being that it is either (a) the second order of $\sim 4\text{\AA}$ polar arc of the standard collagen diagram, or (b) a reflection due to sodium chloride. More information is required before any definite conclusions can be formed, and this aspect is being investigated.

The other spacings shown in Table II seem to agree with those found for the rat-tail tendon, but it is difficult to identify them with any specific measurement in the same manner as those of the $11.8\text{\AA}$ and $2.85\text{\AA}$ spacings, except for the $4.7\text{\AA}$ reflection, which would appear to be due to a disorientated β structure superimposed on that of the orientated collagen. In the freeze-dried specimens there is an increase from $11.8\text{\AA}$ to a minimum of $14.5\text{\AA}$ in the side-chain spacing. A careful study of the spreading of these arcs on the x-ray diagram would suggest a mean separation of chains of about $20\text{--}30\text{\AA}$. It is interesting to note this agreement with the measurements of the pore-size given by Sylvén *et al.* (1951) in their electron microscope studies of the nucleus pulposus.

It can be seen from the results recorded that with advancing age the collagen of the nucleus pulposus becomes more orientated and a degenerative process is at play until the stage of crystallization and fibrillation of the collagen is reached in the disks of very old subjects. It is probable that this process occurs in relation to the absorption of mucopolysaccharide from the nucleus. Thus the nucleus loses its gel structure with advancing age. The presence of the disorientated diagram typical of a β protein (identified by a strong ring at $4.7\text{\AA}$ and a more diffuse reflection at about $3.9\text{\AA}$ in the specimens from subjects aged 70 and 78 years) is significant. It may be due to the denaturation of the folded and soluble proteins into a fully extended form to give a disorientated and tightly hydrogen-bonded β crystalline lattice which would inhibit any elasticity in the nucleus. These diagrams of specimens from subjects aged 70 and 78 suggest, therefore, that the nucleus pulposus is no longer an isotropic medium and cannot be capable of distributing stresses uniformly throughout its structure.

FIG. 5

FIG. 6

FIG. 7

FIG. 5.—Annulus fibrosus. Age 48 years (x-ray beam parallel to radial direction of disk).
FIG. 6.—Annulus fibrosus. Age 78 years (x-ray beam parallel to radial direction of disk).
FIG. 7.—Annulus fibrosus. Age 45 years (x-ray beam parallel to radial direction of disk).
Photographs taken immediately after excision from the disk—that is, in a "wet" state).

FIG. 8.—Biaxial orientation of annulus fibrosus—dry specimens. (a) Aged 44. Beam perpendicular to spinal axis. (b) Aged 35. Beam parallel to spinal axis. (c) Aged 48. Unstretched. (d) Aged 48. Stretched. (e) Aged 53. (f) Aged 70.

FIG. 9.—Biaxial orientation of annulus fibrosus—wet specimens. (a) Aged 45. (b) Aged 51.

B. Changes in the Annulus Fibrosus with Age

Table III shows the reflections given by specimens of annulus fibrosus (Figs. 4–7 are representative x-ray diagrams).

As the crystallites in the fibrous system such as collagen are in general not parallel, its diffraction picture will not consist of individual spots, but rather of arcs due to the dispersed orientation of the crystallites about an axis. This distortion of the internal structure causes the angular spread

TABLE II
Nucleus Pulposus. Air-dried Specimens

Age	Spacings of Reflections			Remarks
	Equatorial	Meridional	Other Reflections	
3 weeks		2.85 Å	2.0 Å	Completely disorientated. Structure. Side-chain spacing absent
1½ years	11.8 Å	2.85 Å	2.0 Å 3.2 Å	Still disorientated. 3.2 Å and 4.3 Å are diffuse
2½ "	11.8 Å 4.6 Å	2.85 Å	4.3 Å	2.0 Å ring has disappeared. Side-chain spacing at 11.8 Å now resolved
25 years to 28 years. Diagram still disorientated, but shows increasing definition				
48 years	{ 11.8 Å 4.7 Å	{ 2.85 Å 3.20 Å	4.3 Å	} Collagen phase well orientated—i.e., becoming crystalline
70 "	{ 11.8 Å 4.7 Å	{ 2.85 Å 3.90 Å		
78 "	{ 11.8 Å 4.7 Å	{ 2.85 Å 3.90 Å		
Newly Excised Freeze-dried Specimens				
51 years	14.5 Å	{ 2.85 Å 4.00 Å		Disorientated liquid phase

TABLE III
Annulus Fibrosus—Air-dried Specimens

Age	Spacings of Annulus Fibrosus			Remarks
	Equatorial	Meridional	Other Reflections	
3 weeks	11.8 Å	2.85 Å		Disorientated structure with other very diffuse reflections
1½ years	11.8 Å	2.85 Å		Still almost completely disorientated
2½ "	11.8 Å	{ 2.85 Å 3.92 Å	4.7 Å	Slight orientation; uniaxial dispersion
From 2½ to 36 years the orientation increases, with the reflections becoming sharper				
44 years	11.8 Å	{ 2.85 Å 3.94 Å	{ 4.7 Å 5.8 Å 7.3 Å	Orientated biaxial structure
48 years to 70 years, the spacings varied only slightly about values given for age 44. The main differences are in the increasing orientation with increase in age. Biaxial dispersed system becoming uniaxial				
78 years				
<i>Freeze-dried Specimens</i>				
45 years	14.5 Å	{ 2.85 Å 4.00 Å	{ 7.0 Å 3.3 Å	Disorientated liquid phase

of the spots, and from the geometry of the diffraction diagram and measurements of this angular spread of the arcs on the x-ray diagram the dispersion of the crystallites around the fibre axis can be calculated (Astbury, 1947).

Before the age of 35 years, the crystallites of the collagen of the annulus fibrosus show a uniaxial dispersion, but older specimens show an increasing biaxial character (see Figs. 8 and 9, dispersions shown diagrammatically). With increasing age beyond 48 years, a preferred orientation appears to develop in the biaxial system, so that there is a tendency to revert to the uniaxial fibrillar orientation. X-ray photographs of specimens from subjects aged 35 years were taken with the x-ray beam parallel to the spinal axis (Fig. 8,b) and perpendicular to the radial direction of the disk. In the latter case the dispersion is approximately twice that of the former. Thus it would appear that the fibrils are laid down in approximately concentric cylinders around the radius in the plane of a cylinder.

Table IV shows the dispersions of the fibres at varying ages.

TABLE IV
Annulus Fibrosus. Air-dried Specimens

Age (Years)	Uniaxial Semi-angle ϕ	Biaxial		Ω	Remarks
		$2\phi_1$	$2\phi_2$		
2½	60°				Beam parallel to spinal axis
35	56°				
35	28°				
44		50°	40°	45°	Approximately equal intensity of arcs
48		60°*	70°	65°	Unstretched (see Fig. 8,c)
48		40°	40°	40°	Stretched approximately 15% (see Fig. 8,a)
53		50°	60°	55°	See Fig. 8,c
67	42°				
70		50°*	60°	55°	See Fig. 8,f
78	45°				
<i>Freeze-dried Specimen</i>					
45		25°	25°	70°	See Fig. 9
51		30°	25°	65°	" " 9

ϕ = Semi-angle of dispersion in uniaxial system. ϕ_1 and ϕ_2 = Semi-angles of dispersion about the two axes of the biaxially dispersed system. Ω = Angle between the two axes.

* Arcs of increasing higher intensity with age, showing the development of a preferred orientation and the eventual reversion from the biaxial to a uniaxial system. This change, however, occurs at ages varying between 65 and 75 years.

Discussion

The reversible elasticity of the keratin-epidermin-fibrinogen group of proteins has been shown to be associated (at least in part) with a molecular transformation of the $\alpha_2\beta$ type. It is known that the collagen molecule is an extended form of a particular configuration of the amino-acid residue in the polypeptide chain. Apart, therefore, from a small lattice stretch of about 2%, and at the most 5%, the fibrils of collagen are inextensible. Thus any elongation is clearly connected with the orientation of the collagen fibrils in the wall as shown by the results of the x-ray diffraction diagrams of the stretched annulus fibrosus. From the angles of dispersion of the reflections produced before and during stretching of the specimens of annulus fibrosus, it can be seen that elongation is clearly connected with changes in orientation of the collagen fibrils in the wall. Figs. 8,c and 8,d show diagrammatically the effects of stretching upon the disk wall, demonstrating that extension is mainly achieved by alignment of the fibrils—that is, by change of orientation in the wall. A calculation for the case of a specimen from a subject aged 40 years, using the measured dispersions, shows an elongation of about 13% (Happley, Macrae, and Naylor, 1953). This is in reasonable agreement with the measured stretching of the actual specimen (about 15%), and gives an indication of the order of extension which can be obtained by such a source of elasticity. Further, as this extension seems readily reversible, it is reasonable to assume that the increase in orientation of the collagen fibrils is associated with the elasticity of the annulus fibrosus.

It would appear, therefore, that the elasticity and efficient function of an intervertebral disk are dependent upon at least two major factors: (1) The gel properties of the nucleus pulposus. The nucleus should be readily deformed but incompressible, and so convert any vertical pressure into a horizontal and radial thrust on the annulus. A diagrammatic representation of this mechanism is given in Fig. 10. (2) The annulus fibrosus must act as an elastic ring to dissipate the forces applied. This is possible only because of the arrangement of the collagen fibres in their surrounding

FIG. 10.—Elastic mechanism of the intervertebral disk.

matrix, which is flexible enough to allow the fibrils to show their elasticity due to changes in orientation.

The experiments undertaken show that with advancing age, beginning at the end of the third decade, there are changes in the collagen structure of both nucleus pulposus and annulus fibrosus which affect both factors.

The nucleus loses its gel properties, as seen by the increased orientation and crystallization of the collagen with advancing age, whilst the collagen of annulus fibrosus undergoes similar changes, the fibrils being laid down in a uniaxial arrangement which inhibits its elastic mechanism. In these circumstances the intervertebral disk loses its normal physiological function, with secondary effects upon its associated spinal joints, particularly impaired mobility.

Summary

The collagenous changes in the human intervertebral disks with age have been studied, using x-ray crystallographic methods.

The x-ray diffraction diagrams so obtained prove that the elasticity of the annulus fibrosus is dependent upon the orientation and mobility of the collagen fibrils, properties which decrease with advancing age after the third decade.

The x-ray diffraction diagrams of the nucleus pulposus show that increased orientation and ultimate crystallization of the collagen occurs with advancing age, indicating loss of gel structure and hence impaired elasticity.

The combination of these factors will explain the reduced elasticity of the intervertebral disk with age.

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SERUM ANTITHROMBIN CONTENT IN PREGNANCY

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In a follow-up examination of adult cases of coeliac disease the serum antithrombin content estimation was used as a screening test to exclude fibrocystic disease of the pancreas as a possible alternative diagnosis. The results were normal in all cases but one—that of a woman three months gravid. Sera from six other pregnant women were then examined, and since in each case the antithrombin content was found to be much lower than that of normal serum the investigation now reported was undertaken.

Sera were obtained from 120 women whose pregnancy varied from four weeks to full term; from 41 women whose delivery had been from one to twelve weeks previously; from cord blood at 30 births; and from 15

babies aged 1 to 14 days. All pregnancies were normal and the sera were obtained from routine laboratory collections. The babies from whom blood was obtained were likewise normal and full-term. The Table shows the distribution of these sera. The antithrombin content was estimated in every case. The technique

Details of the Sera Examined and the Results Obtained, Expressed as a Percentage of the Normal Mean

Sera	No.	Antithrombin Content			Sera	No.	Antithrombin Content		
		Max.	Min.	Mean			Max.	Min.	Mean
Pregnancy:					Post Partum:				
4-7 weeks	3	72	60	67	1-4 weeks	28	62	18	35
8-11 "	9	66	26	50	5-8 "	4	106	65	90
12-15 "	24	58	22	35	9-12 "	9	107	89	100
16-19 "	9	45	22	32					
20-23 "	10	48	23	34	Total	41			
24-27 "	11	41	22	33					
28-31 "	23	52	22	35	Babies:				
32-35 "	11	48	20	36	Cord blood	30	58	20	32
36-39 "	16	41	18	29	Newborn				
Full term	4	41	22	32	(1-14 days)	15	64	26	40
Total	120								

employed has already been detailed by one of us (MacFarlane, 1952) and is a modification of the original method described by Innerfield *et al.* (1951). Briefly, the test consists of the incubation in a water-bath at 37° C. of a standard solution of thrombin mixed with the serum to be tested, and the timing of the clotting of standard amounts of normal citrated plasma by this thrombin-serum mixture after 5, 10, 15, and 20 minutes' incubation. The clotting times of each sample are expressed as a percentage of those obtained using normal serum as a control, normal and abnormal being tested concurrently.

Results

In the assessment of the results obtained the percentages of the normal of the 20-minute sample of the thrombin-serum mixture only are considered, for in this sample any deviation from the normal is most marked. We have used the same technique over a period of more than two years: the variation in clotting time of some 800 "normal" sera in this 20-minute sample was from 90 to 110% of the mean of each batch of normals tested, and we have come to regard any figure of less than 80% as being pathological. The Chart shows the scatter of the results obtained on examination of the 120 sera taken during pregnancy and of the 41 sera obtained post partum, while the Table also lists the highest, lowest, and mean result obtained in each four-weeks period. It will be seen that from the fourth week of pregnancy onwards the serum antithrombin content is reduced to an abnormal degree. The mean of the three sera tested in the fourth to seventh week of pregnancy is 67%; that of the nine sera in the eighth to eleventh week is 50%; and the means of the sera belonging to the succeeding four-weeks periods to full term all lie around 35% of normal. After delivery the antithrombin content remains low for several weeks, certainly for as long as four weeks, for the highest figure of this group of 28 sera was 62%, with a mean of 35%. Somewhere between five and eight weeks post partum the results return to normal in the majority of cases, three of the four sera in this period tested being normal, while from the ninth week onwards all nine sera tested gave normal results.

All 30 sera obtained from cord blood gave abnormally low results, varying from 20 to 58% of normal, with a mean of 32%. The sera of the babies 1 to 14 days old also showed an antithrombin content much lower than that of normal serum, the mean here being 40%. In the routine investigation to exclude fibrocystic disease of the pancreas the antithrombin content has been within the normal limits in the serum of all the 2-months-old normal babies tested.