FROM THE CANCER RESEARCH DIVISION OF RADIUMHEMMET, STOCKHOLM, SWEDEN

ON THE BIOLOGY OF NUCLEUS PULPOSUS

By

BENGT SYLVÉN

This is a progress report about joint investigations on the biophysics of avascular mesenchymal tissues with particular reference to the nucleus pulposus of the intervertebral disc. Some years ago when this programme was planned (1, 2), it was quite evident that previous static conceptions based on the morphology of such tissues as cartilage and nucleus pulposus had to be supplemented by new data pertaining to the physiology of these tissues. The present work has been accelerated by progress in various other fields of research, such as the biophysics of collagen and connective tissue (3), and further, the recent development of the chemistry of acid polysaccharides. Clinical investigations on the genesis of low back pain, in particular the work performed at the Orthopaedic Clinic of the Karolinska Institutet, has stimulated interest in the fundamental biological problems of connective tissue diseases of orthopaedic significance.

Since the biological problems relating to nucleus pulposus are largely the same as those in cartilage and in other avascular mesenchymal tissues rich in polysaccharides, the reader will be referred to previous reviews for more complete references (1, 2, 3). This paper will only cover some results and tentative speculations derived from recent investigations which will be published elsewhere in more detail.

Pertinent Problems.—When considering avascular tissues such as cartilage, cornea, and nucleus pulposus, the investigator will be surprised to find many similarities in structure, suggesting the existence of a common pattern. They seem to have a regular and uniform submicroscopic structure with reference to the architecture of collagen and the high content of acid polysaccharides and water. The biophysical significance of these structural entities has not been clearly understood. Cartilage and nucleus pulposus are also subjected to

early "degenerative" changes, which have been described so far only in terms of morphology.

Our first task was to study the ultrastructure of healthy nucleus pulposus by means of electron microscopy and by various fractionation procedures for the segregation of tissue constituents. Later on, attention was focused on various possibilities for the transport of water and metabolites through a jelly tissue of this type. Would for instance diffusion be sufficient to maintain the nutrition of nucleus pulposus as previously suggested? Was there a functional relationship between molecular structure and the transport mechanisms? Comparison had also to be made between normal nucleus and specimens subjected to degenerative changes, in order to gain some additional insight into the functional sequences of such changes.

The reader will surely understand that while it would be of value to bring down the discussion to a molecular level, this would involve great experimental difficulties.

The Ultrastructure of Nucleus Pulposus.—The following structural elements have to be distinguished in healthy nucleus pulposus from young calves and human beings:

Chondrocyte-like cell bodies. In freeze-vacuum dehydrated specimens the cytology of their cytoplasm and nuclei could be studied under the light microscope and thus the disadvantage of common mordanting procedures was avoided (4).

The intercellular matrix. By means of physical fractionation procedures (unpublished) the matrix was segregated into (*a*) collagen fibrils, (*b*) a polysaccharide component, (*c*) a protein component attached to the polysaccharide, and (*d*) water. In their native condition these elements form a three-dimensional lattice gel system (5) with a water content of about 83 per cent. The interlacing collagen fibrils form a dense net-work. Their surface is coated by the polysaccharide-protein complex. The net contains meshes or "pores" of varying diameters, which, however, cannot be measured by electron microscopy.

All fractions obtained are now being analysed, and have given the following results so far: the extractable polysaccharide-protein complex contains about 5.5-6 per cent nitrogen per dry weight; it shows a very marked alcohol-resistant metachromasia with Azure A and Pinacyanol; it contains large amounts of etheral sulphate. A rough estimation suggests that the complex contains about three times as much polysaccharide as protein. The electrophoretic mobility of the former is of the same magnitude as that of chondroitin sulphuric acid. To all appearances this polysaccharide is not hyaluronic acid, as previously

stated by Meyer (6), but most likely chondroitin sulphuric acid. The protein moiety (c) has not yet been studied in detail.

The very high water-binding capacity is explained in particular by the polar (—OH) groups of the polysaccharide.

These constituents seem to be linked into a regular molecular pattern in the following way. The polysaccharide molecules are presumably attached to the surface of the collagen fibrils by chemical linkage, and the free protein component (c) is linked to the other end of the polysaccharide, thus being directed outwards to the pores. Most of the polysaccharides and the protein (c) can easily be extracted by different methods, but the last carbohydrate residues are more difficult to separate from the collagen fibrils (unpublished). The polysaccharide coating renders the collagen fibrils hydrophilic. Since the distribution of available electric charges along the surface of the collagen fibrils is unknown, further speculation as to the mode of linkage between the collagen fibrils and the polysaccharide in question seems unjustified.

Nutritional Pathways.—In order to gain some information as to the actual rôle diffusion plays in the transportation of nutrients, an interferometer apparatus (7) was constructed for absolute determinations of the diffusion constants in sections of fresh nucleus pulposus. It was found that salts, such as NaCl, $CaCl_2$, and Na_2SO_4, and organic compounds like urea, amino acids, and simple sugars (glucose, sucrose) diffuse only about half as fast in nucleus pulposus as in water (8). The diffusion constants were of the same order of magnitude as in gelatin gels of the same water content as nucleus pulposus. The measurements indicate that the average "effective pore size" in nucleus pulposus is only about 15 Å (1 Å = one tenth of a millimicron); this means that smaller molecules than 15 Å can enter and diffuse through the intercellular matrix. Larger molecules are prevented from diffusing.

It was thus concluded (8) that nucleus pulposus presented a gel structure with a variety of interfibrillar pores of different sizes. The investigation indicates moreover that in the case of such a "passive" *in vitro* system of normal nucleus the nutritional requirements cannot be fulfilled by diffusion alone, which usually is a very slow process of transportation. Some other transport mechanism(s) must consequently play a rôle, and the interrelationship between molecular structure and nutritional transport has thus to be studied from other aspects.

Changes During Aging.—The nucleus pulposus of man retains in general the normal regular ultrastructure until the third decade of life, when progressive changes appear which are believed to be of

"degenerative" nature. The changes afflict both the cells and the intercellular matrix. A gradual decrease in the number of cells is noted, parallel with a patchy loss of mucoid material. Large collagen fibre bundles apppear and the water content diminishes. The partial disappearance of the mucoid has been ascertained by means of the metachromatic staining technique, by electron microscopy (5), and by a new micro-method for sulphur assay (unpublished). In advanced stages of "degeneration" large collagen bundles completely devoid of mucoid material may be seen. In the course of aging, these changes will naturally lead to impaired nutrition, but no objective evaluation of the impairment could be made with the interferometric method since such specimens were optically too inhomogeneous (7, 8).

These changes imply very marked alterations of the previously regular lattice gel, alterations leading to loss of gel structure. The responsible factors are unknown, but it may be assumed that all organic components of nucleus pulposus are involved.

SUMMARY

A progress report on the biophysics and physiology of nucleus pulposus is presented. This tissue represents in its healthy state a three-dimensional lattice gel system with a regular molecular pattern. The results indicate that nutrition cannot be accomplished only by diffusion from the surrounding tissues. The so-called degenerative changes during aging imply loss of gel structure. The results reviewed here are published elsewhere in more detail.

ZUSAMMENFASSUNG

Ein Bericht über den Fortschritt bezüglich der Biophysik und Physiologie des Nucleus Pulposus wird vorgelegt. Dies Gewebe stellt in gesundem Zustande ein dreidimensionales Gitter-Gelsystem mit regelmässigem molekulärem Aufbau dar. Die Ergebnisse zeigen, dass Ernährung nicht allein durch Diffusion von den umgebenden Geweben aus durchgeführt werden kann. Die sogenannten degenerativen Veränderungen während des Alterns bedeuten Verlust von Gel-Struktur. Die in der Arbeit zusammenfassend besprochenen Ergebnisse sind an anderer Stelle eingehender veröffentlicht.

RESUME

Il est donné un exposé sur la biophysique et la physiologie du nucleus pulposus. Ce tissu apparaît à l'état sain comme un treillis gélatineux à trois dimensions avec un dessin moléculaire régulier. Les résultats indiquent que la nutrition ne peut s'accomplir que par diffusion des tissus environnants. Les modifications dites dégénératives qui apparaissent dans la vieillesse impliquent la perte de la structure gélatineuse. Ces résultats révisés ont publiés ailleurs avec plus de détails.

LITERATURE

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