these concentrations of EDTA, the integrity of the cells was already disturbed. Complete suppression of repair of double-stranded DNA breaks also was observed when arabinofuranosyl adenine, a specific inhibitor of DNA-polymerases, was used in a suspension of irradiated ascite cells; the concentration in this case was 400 μM and the duration of irradiation reached 1 h. Unlike the polypeptides used, arabinofuranosyl adenine has a much stronger toxic action on cells [8]. The nontoxicity of strongly acid polypeptides, and the total inhibition of repair of double-stranded DNA breaks which they produce, as demonstrated on eukaryotic cells, make the search for ways of using them in tumor radiotherapy promising.

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EFFECT OF DISORGANIZATION OF CARBOHYDRATE–PROTEIN COMPLEXES OF THE GROUND SUBSTANCE ON STRUCTURE AND PHYSICOCHEMICAL PROPERTIES OF HUMAN HYALINE CARTILAGE

V. A. Dubinskaya, S. S. Nikolaeva, Yu. A. Khoroshkov, and O. A. Koroleva

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Diseases accompanied by changes in cartilage tissue occupy an important place in human pathology [4, 14]. Cartilage tissue, being a complex composite system, contains cartilage cells, a fibrous carcass, and ground substance, represented mainly by proteoglycans consisting of several different glycosaminoglycans (GAG) [7, 9]. In this system the fibrous carcass performs a reinforcing function, whereas the ground substance is a unique filling agent and performs integrative and buffering functions. The ground substance is also responsible for binding and transport of water into the tissues, and it is also involved in the formation of its elastic properties [3-5]. Pathological changes in cartilage tissue are often based on autoimmune processes, accompanied by enzymic disorganization of the carbohydrate protein complexes of the ground substance [4, 12].

The aim of this investigation was an experimental study of the character of the possible effect of disorganization of the carbohydrate–protein complexes of the ground substance on the structure, water-capacity, and biomechanical properties of cartilage.

Research Laboratory of Biological Structures, Ministry of Health of the USSR, Moscow (Presented by Academician of the Academy of Medical Sciences of the USSR S. S. Debov.)

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TABLE 1. Composition of Hyaline Cartilage

Cartilage	Water content, %			Biochemical component, g/100 g dry tissue		
	total	bound	free	collagen	hexuronic acids	hexosamines
Patella	71,1±0,7	51,8±1,2	19,3±1,4	58,7±2,3	4,83±0,20	4,78±0,51
Trachea	65,8±0,6	34,8±1,5	31,0±2,0	33,7±3,2	4,96±0,31	5,06±0,49
Costal	57,4±0,6	34,5±0,7	22,9±1,4	56,0±1,3	4,30±0,10	5,18±0,19
Costal after removal of GAG	61,0±1,1	29,2±1,2	31,8±1,7	54,8±2,5	3,21±0,27	3,90±0,19

EXPERIMENTAL METHOD

Samples of hyaline cartilage (from the patella, trachea, and rib), obtained from autopsy material within 24 h of death, from subjects aged 35-55 years dying from trauma, were used as the test objects. To remove the proteoglycans of the ground substance, costal cartilage was treated with 4 M guanidine hydrochloride, and then with papain (1:20) in phosphate buffer (pH 6.2) with the addition of 9.6 mg/mole of cysteine and hyaluronidase (1:50) in acetate–citrate buffer (pH 5.0) at 37°C for 24 h. The biomechanical characteristics of cartilage were studied on a special apparatus capable of determining deformation during tangential loading of the sample under dynamic conditions followed by unloading. The coefficient of rigidity (γ, N/m) and the residual deformation (h_{res}, %) were calculated from the resulting hysteresis curves:

$$\gamma = P_{max}/h_{total},$$

where P_{max} denotes the load, in N; h_{total} deformation at P_{max}, in m; h_{res} was calculated as a percentage of h_{total}. The aquametric analysis was carried out with Fischer's reagent [2]. The quantity of bound water was determined after expression of free water from the tissue twice under a pressure of 800 atm. Isotherms of water vapor adsorption by costal cartilage were obtained under static conditions; when calculating the parameters of adsorption (a_m, a_{max}) the Brunauer–Emmet–Teller equation [1] was used. The degree of swelling in water (W, %) was determined from the change in weight of the samples. The content of hydroxyproline [13], hexosamines [5], and hexuronic acids [6] was determined in the test tissues. The conversion factor for hydroxyproline to collagen is 14 [8]. Control samples of costal cartilage and samples after treatment with enzymes were studied by scanning electron microscopy (SEM). Specimens were prepared for SEM by the freeze–drying method on the FDU-010 apparatus, and sections sprayed with copper were examined in the SEM 515 microscope (Philips).

EXPERIMENTAL RESULTS

Data on the biochemical composition of the different types of human hyaline cartilage, given in Table 1, demonstrate that the tissue with the highest water capacity is cartilage from the patella. It contained the largest quantity of both total (71.1%) on bound water (51.8%). Tracheal cartilage differed from the other tissues studied in having the highest content of the free water fraction, whereas its content in patellar cartilage was the lowest (19.3%).

Much of the water in cartilage tissue is considered to be bound and retained by proteoglycans. However, despite differences in their water content, all three types of cartilage had about equal amounts of hexosamines and hexuronic acids. Meanwhile the samples of cartilage tissue studied differed in their collagen content the higher the collagen content in the cartilage, the greater the content of bound water. It is perhaps the collagen fibers which retain the greater part of the bound water, whereas proteoglycan aggregates are responsible for mobile, free water.

Taking costal cartilage as the example, the effect of disorganization of the ground substance on the structure, water content, and mechanical properties of cartilage tissue can be examined. The structural organization of the fibrous carcass of costal cartilage (Fig. 1) is represented by a system of branched collagen fibers, with complex organization, running in different directions, and forming a fine-mesh network. This forms distinctive capsules around the cartilage cells and is buried in the ground substance filling the spaces between the collagen fibers and masking them. Accordingly, in control preparations for investigation by SEM, the cartilage tissue appeared to be quite homogeneous. Its surface relief is smooth and slightly undulating, sometimes with contours of collagen fibrils barely perceptible above the surface, as a result of masking of the structural details by amorphous material (Fig. 1a). As a result of consecutive treatment of the costal cartilage with guanidine hydrochloride,

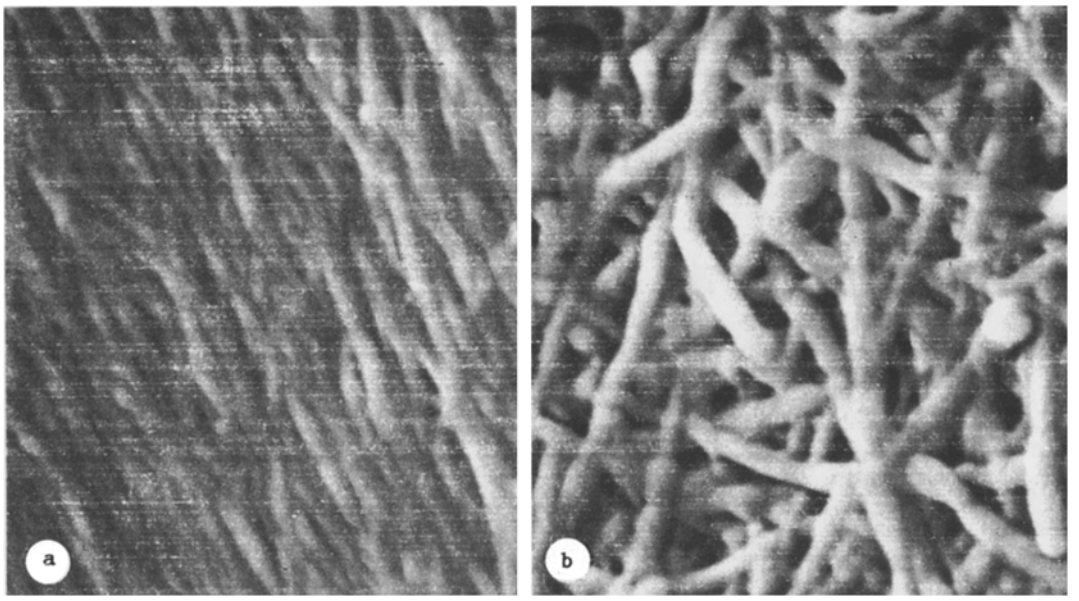

Fig. 1. Structure of costal cartilage before (a) and after disorganization of ground substance of cartilage tissue (b). a) Control preparation of cartilage. Cartilage tissue has appearance of a relatively homogeneous material. Contours of fibrillar carcass, buried in ground substance, can be seen on surface of preparation SEM, 21,200×. b) Structure of cartilage after experimental treatment. Cartilage tissue has become cellular in structure due to destruction of ground substance between elements of fibrillar carcass, and some degree of their disintegration. SEM 21,200×.

TABLE 2. Effect of Disorganization of Ground Substance of Costal Cartilage on Water Metabolism and Mechanical Properties of Tissue

Sample	Adsorption of water vapor		Swelling in liquid water, W, %	Mechanical characteristics	
	a_m	a_{max} at P/P_s ~ 96%		rigidity, 10^3 N/m	residual deformation, %
Costal cartilage	14,55	44,1±0,4	62,9±1,2	19,4	10,5
The same after removal of GAG	12,75	51,3±0,2	67,3±1,2	14,8	18,0

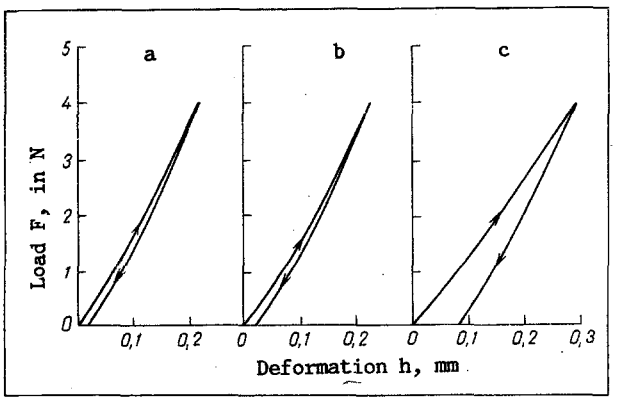

Fig. 2. Deformation of costal cartilage as a function of load during tangential compression followed by unloading: a) control preparation, b) 12 days after death, c) after experimental treatment.

papain, and hyaluronidase, a certain proportion (24.7%) of hexosamines and of hexuronic acids (25.3%) was removed from the tissue (Table 1). Components of the fibrous carcass thereby become unmasked, exposing the system of collagen fibrils (Fig. 1b). Destruction and subsequent removal of the amorphous material of the cartilage tissue leads to the formation of free spaces, in the form of cells and cavities between the structural elements of the fibrous carcass, i.e., it increases the porosity of the tissue. No appreciable disturbances of the architectonics of the collagen fibrils, characteristic of cartilage, or of their structure could be observed under these circumstances.

The above changes in cartilage tissue were accompanied by disturbance of its water metabolism (Tables 1 and 2).

After destruction of the proteoglycan complex, values of the effective capacity of the monolayer a_m, characterizing the state of hydration when each hydrophilic group of the biopolymer binds with one molecule of water, decreased. This is evidence of a decrease in the number of active functional groups capable of binding water. However, the ability of cartilage to adsorb water vapor at maximal water content (a_{max}) and to take up liquid water (W) after partial removal of the GAG was increased. Thus the increase in the total water content in the cartilage took place despite substantial disturbance of carbohydrate–protein complexes of the ground substance, capable of binding with and retaining a large quantity of water. The formation of free spaces and cavities in the tissue evidently overlaps the effect of removal of GAG as hydrophilic substrates. An increased water content also was found in degenerating particular cartilage with a reduced GAG content [10].

The virtual absence of residual deformation and of the hysteresis loop after unloading (Fig. 2) indicates that cartilage behaves as a nonlinear elastic material. Under these circumstances, for a long time after death (up to 12 days) cartilage preserves its viscoelastic properties which it possesses in vivo. As a result of removal of the ground substance from the matrix of the cartilage its rigidity is reduced, the area of the hysteresis loop is increased, and residual deformation appears after unloading. These changes are evidently connected with disturbance of the composition of the cartilage tissue. In particular, the reduction of rigidity can be explained by increased mobility of the structural elements of the fibrillar carcass due to destruction of the ground substance. One cause of the appearance of residual deformation of cartilage after loading is evidently disturbance of the structure and architectonics of the proteoglycan complexes located between collagen fibrils, and playing the role of elastic spacers.

Thus disorganization of the carbohydrate–protein complexes of ground substance is accompanied by a series of changes involving the structural organization and physicochemical and biomechanical properties of the cartilage tissue.

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