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Changes Of Hydration Level In Type I Collagen And Glycosaminoglycans Synthesized In The Rat's Skin Under The Mechanical Stress

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Abstract. Changes of Hydratation Level Of Type I Collagen And Glycosaminoglycans That Are Synthesized In The Rat's Skin Under The Mechanical Stress. The effect of the mechanical stress on the levels of hydratation of type I collagen and glycosaminoglycans that are synthesized in it, has been studied in vitro using the rats' skin. The measured hydration of isotherms has shown that mechanical stress in the skin increases and decreases the amount of absorbed water in glycosaminoglycans and in collagen, respectively. Calculated the average amounts of water molecules in collagen tripeptide and glycosaminoglycans disaccharide unit in the inside and outside layers of their hydrate shells.

Keywords: collagen; glycosaminoglycans; hydration; mechanical stress.

Introduction. Mechanical stress in the connective tissue is the permanent factor which causes structural changes in the matrix and matrix's components – biopolymers. These structural changes are usually considered as an adaptation to the stress' changes in the tissue. Level and nature of hydration is an important feature of biopolymers that significantly determines their structural stability [1-3].

Nowadays, however, it is unknown whether the hydration properties of major biopolymers – collagen and glycosaminoglycans (GAG), which are part of supramolecular complexes of extracellular matrix, are changed under the action of mechanical stress.

Materials and methods. With regards to the above, the effect of skin's stretching on the levels of hydration in type I collagen and GAG, and distribution of water molecules under the mechanical stress, has been studied in vitro by measuring the hydration isotherms.

The study used a skin's layer of a 3 month old male rat, line Wistar (animal weight – 200-250g) which was taken from animal's back and cleared from fur and subcutaneous fat.

In order to obtain de novo synthesized type I collagen and GAG the skin samples were incubated in Krebs-Ringer solution for 6 hours on the device [4] in the absence and with the action of the static stretching tension σ = 0,025; 0,05 and 0,15 MN/m^2

Incubated samples were degreased in acetone for 48 hours, dried off and ground in liquid nitrogen until powdered.

Type I collagen was extracted from the powder of the solution of 1 M NaCl and dialyzed by phosphate buffer pH = 7.0 for 48 hours [2].

Total glycosaminoglycan (Σ GAG) - a mixture of hyaluronic acid heparan, chondroitin-4 chondroitin-6-sulfates, dermatan, keratan – has been extracted after the enzymatic hydrolysis of powder by papain in acetate buffer for 24 hours at T = 65^0 C, and after the precipitation from their hydrolyzate with cetylpyridinium chloride and subsequent reprecipitation with ethanol [5].

Hydration isotherms of type I collagen and ΣGAG at different values of relative humidity (RH) have been received by piezogravimetry method described in [6], by determining the dependence of resonance frequency changes Δf of the quartz crystal mass biopolymers m, which were deposited on the surface of the quartz plate [6,7].

$$\Delta f = \frac{-f_0^2 \cdot K \cdot m}{N \cdot q \cdot A} = G \cdot mf \quad (1)$$

where, f_0, q, A - resonant frequency, density and area of quartz; N, K - constant of quartz. With the small values of m and at $\Delta f/f_0 << 0{,}01$ formula (1) gives a good linear approximation, which can be used to determine m.

Equal thickness of collagen and ΣGAG membranes has been achieved by evaporating water from 1 μl biopolymers solutions that were put on platinum contacts of quartz crystal. Samples desiccation has been carried out in a vacuum chamber at 20^{0}C and with the pressure 10 $^{-3}$ torr.

Membranes have been moistened by dispensed supply of water's vapor to the chamber from thermostatic cylinder with twice distilled and degasified water. Thermostating quartz sensors have been carried out with accuracy to ± 0,5^{0}C. Measuring error of the frequency of quartz resonators was less than ± 1 Hz.

The experimental hydration isotherms were approximated using modified in [8, 9] D'Arcy and Watt equation [10], which includes the heterogeneity of the adsorbent - type I collagen and a mixture of five glycosaminoglycans with different chemical composition and molecular weight.

$$V = \frac{V_m \cdot a_l x}{1 + a_l \cdot x} + a_H \cdot x + \frac{b}{1-b} \quad (2)$$

where, Vx - relative humidity (RH), 1st component of the formula's right side (2) that describes the stage of adsorption of water molecules by the Langmuir law; 2nd component - the adsorption of water molecules by the Henry law, 3 rd component corresponds to multilayer absorption where the hydration shell is fully formed by loosely bound water molecules; Vm - monolayer capacity, a_l, a_H and b - water activity respectively, at the stage of adsorption according to the Langmuir law, to the Henry law and to the multilayer absorption that are proportional to the adsorption equilibrium constants (K_A , K_H, K_b).

The number of water molecules that correspond Langmuir, Henry and multilayer adsorption, has been calculated against average weight of tripeptide Gly-X-Y for collagen and disaccharide unit for ΣGAG respectively.

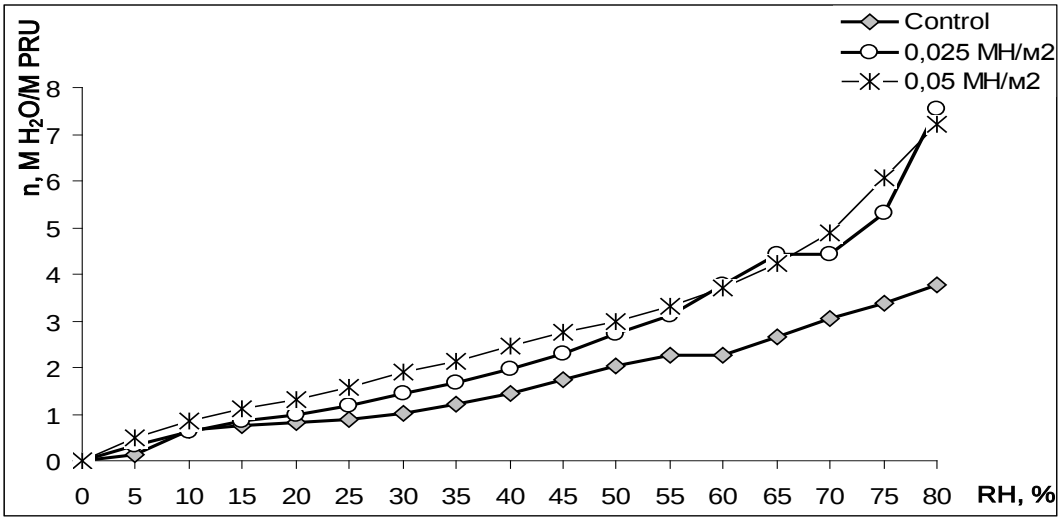
Parameters of equation (2) Vm, a_l, a_H and b are generally unknown. For the measured experimental hydration curves they have been determined by the selection of the optimization algorithm using method of deformed polyhedron. The discrepancy between the experimental and calculated isotherms [11, 12] has been an objective task.

Program of multivariable function minimization has been used for the approximation of hydration isotherms. Mean square deviation, calculated from experimental isotherms hydration was no more than 0.05 g of water per g of both biopolymers.

The presented experimental curves were performed by averaging of at least three independent experiments.

Results and discussion. Fig.1.shows the measured isotherm of hydration Σ GAG and type I collagen, averaged over all samples, which have been synthesized in the rat's skin under various mechanical stresses. As can be seen, changes occur in the processes of hydration both in the collagen and GAG molecules that are synthesized under the mechanical stress, compared with both biopolymers extracted from a tissue that haven't experienced mechanical stress.

A



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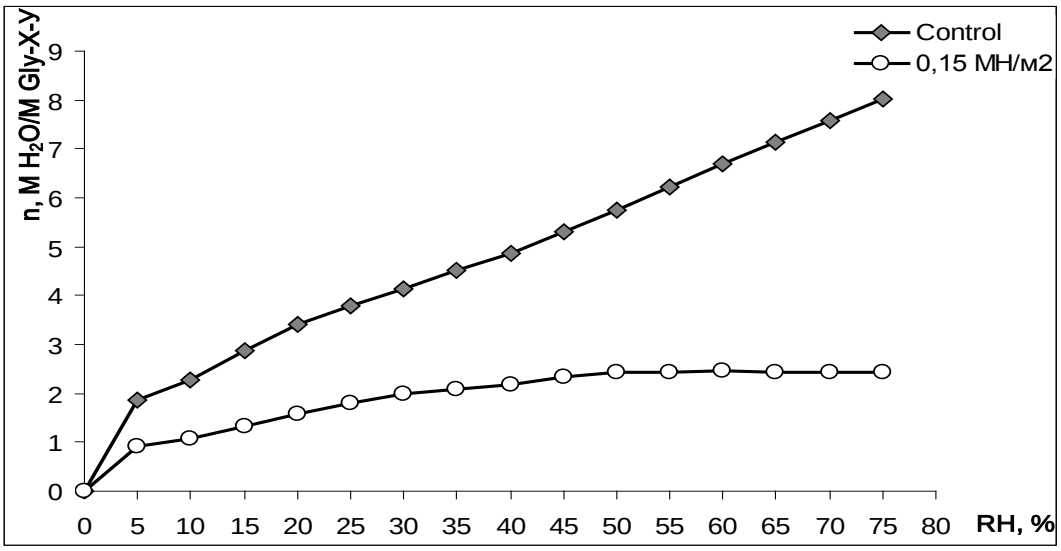


Fig. 1. Hydration isotherms of Σ GAG (A) and type I collagen (B) that are synthesized in the rat's skin under various mechanical stresses. (The number of adsorbed water molecules per Gly-X-Y for collagen and per polymer repeat unit (PRU) is given as a function of the relative humidity (RH).)

However, these changes are multidirectional. Under the stress overall level of hydration in Σ GAG increases, and collagen decreases.

High increase in the adsorption of water is observed for both biopolymers in the presence and in the absence of mechanical stress in the skin, in a range of RH. For Σ GAG it is 60-80%, and for the type I collagen - 5-10%.

This may indicate structural transitions of molecules; followed by activation of the hydration system in general. On the isotherms hydration of ΣGAG that synthesized by the action of mechanical stress, structural transition occurs at lower values RH and has a larger parameter increment in comparison with samples that are synthesized without mechanical action.

Saturation of the collagen hydration shell, *de novo* synthesized with the absence of mechanical stress, occurs at 80% RH, this value is 40% RH under the action of stress, against reducing the adsorption capacity of 3.2 times compared with the control.

Parameters of D'Arcy and Watt equation have been calculated using hydration isotherms to study changes in hydration and type I collagen ΣGAG and in different layers under tension (Table 1).

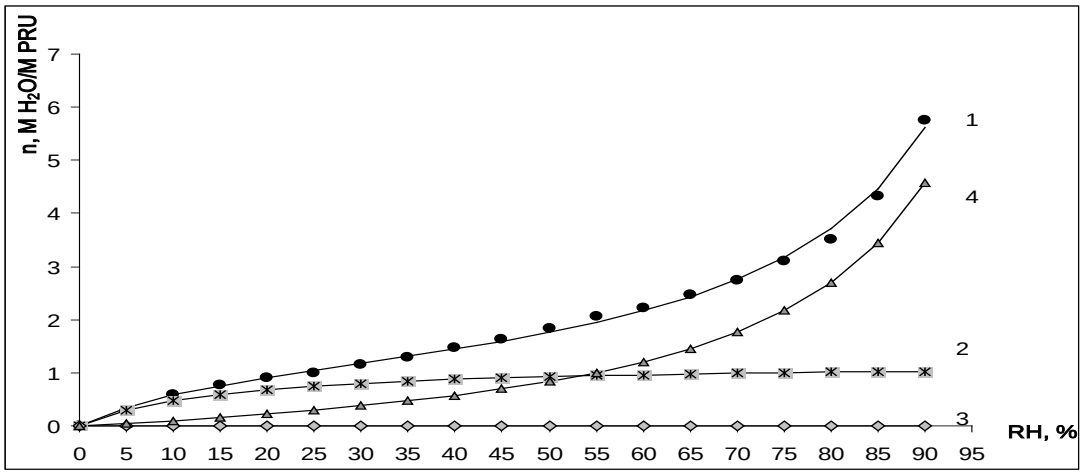
Table 1.

Parameters of D'Arcy and Watt equation for Σ GAG and type I collagen, which are synthesized in the rat's skin with the presence or absence of mechanical stress.

Biopolymers	σ, MN/m²	Type of adsorption			
		Langmuir		Henry	Multilayer
		Vm	a_l	$a_н$	*b*
Σ GAG	0	1,33	5,35	0	0,90
	0,025	1,13	0	2,98	1,08
	0,05	2,45	0	4,85	0,94
Type I collagen	0	3,45	15,21	2,20	1,04
	0,15	2,54	7,50	0,01	0,30

Hydratation isotherms for both types of biopolymers were decomposed to the curves using the calculated parameters that correspond Langmuir, Henry, multilayer adsorption. Resulted decomposition of isotherms for ΣGAG and type I collagen are shown in Fig. 2. and Fig. 3., respectively.

A



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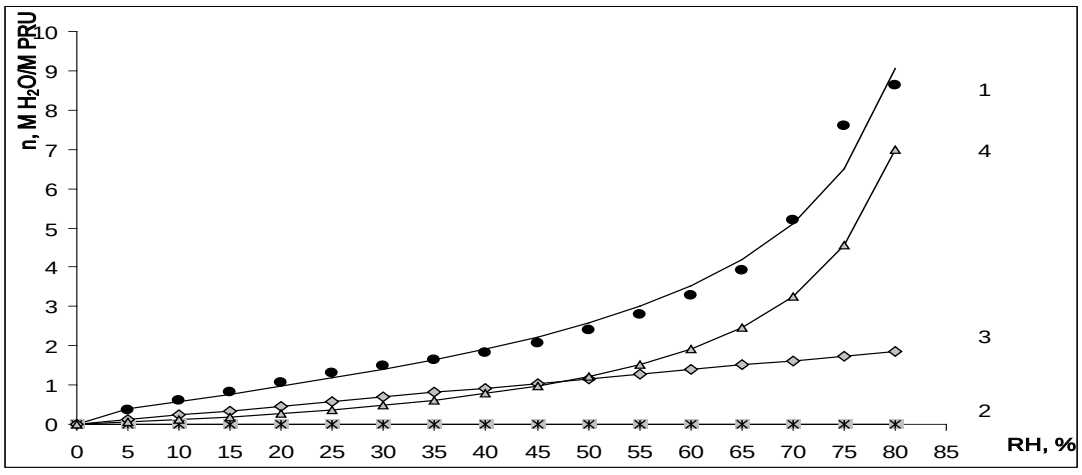

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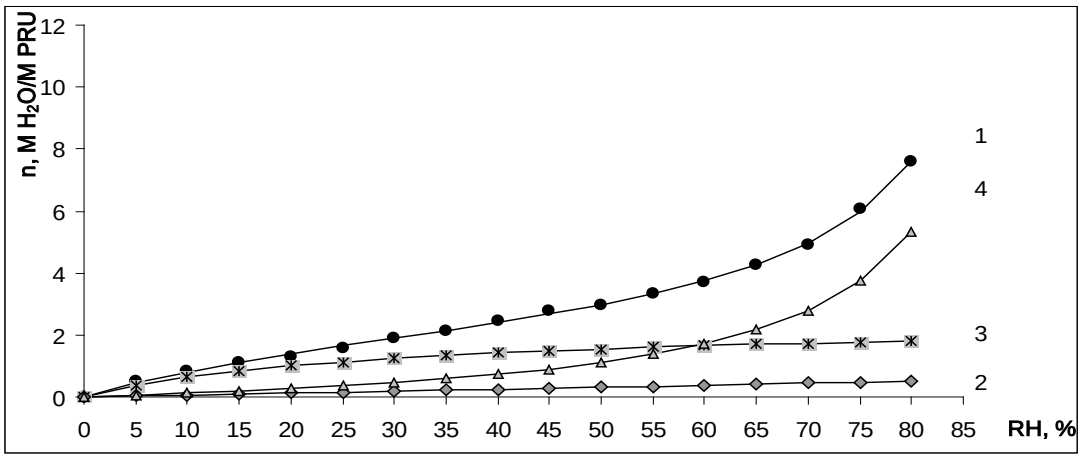
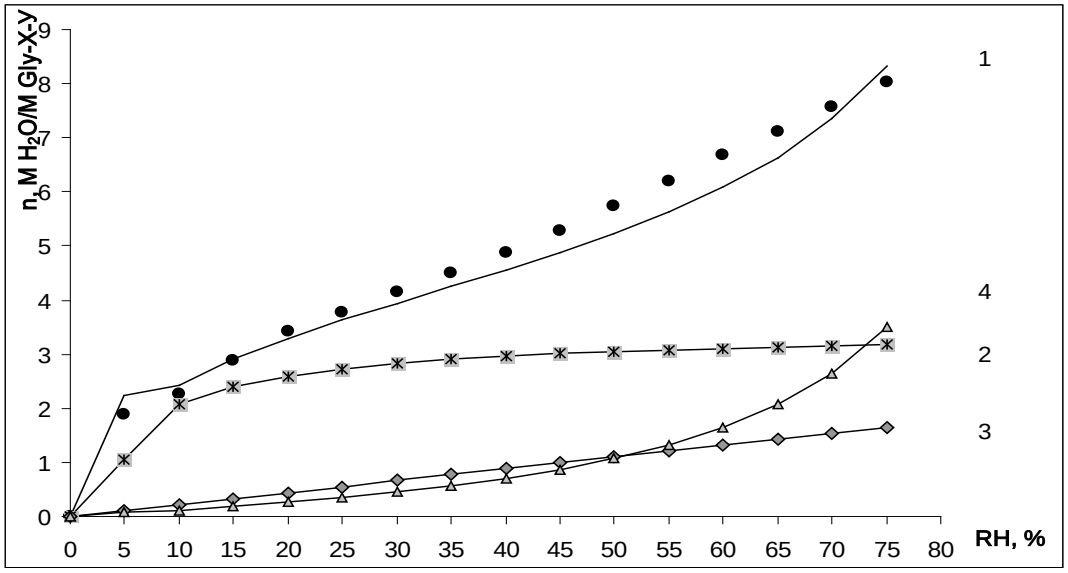


Fig.2. Isotherms of hydration (1) and their decomposition curves for adsorption by the Langmuir (2), and Henry laws (3) and multilayer adsorption (4) for Σ GAG that are synthesized in the skin when σ = 0 MN/m² (A), σ = 0,025 MN/m² (B), σ = 0,05 MN/m² (C).

A



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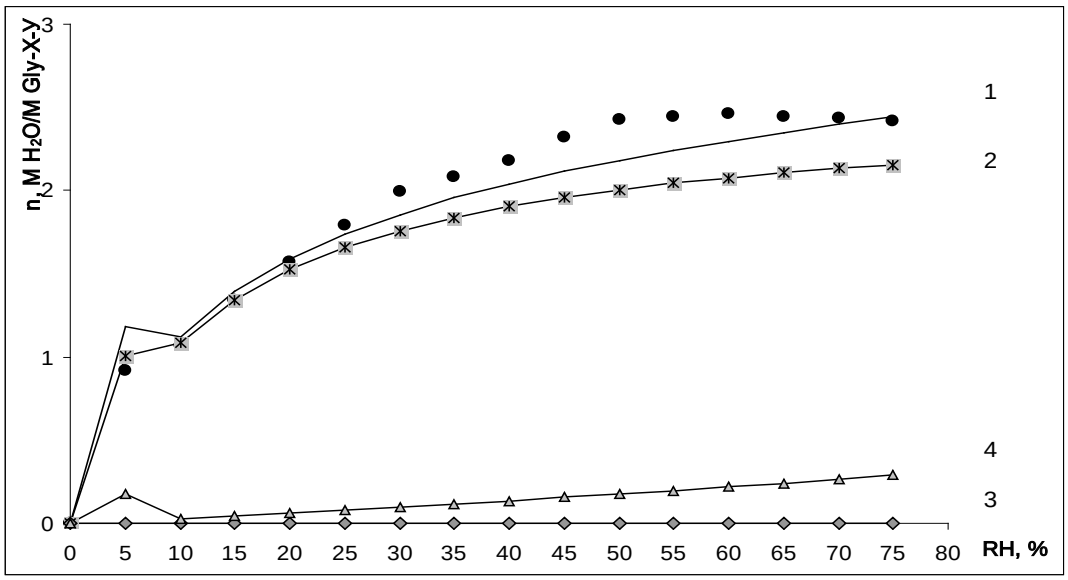

Fig.3. Isotherms of hydration (1) and their decomposition into curves for adsorption by the Langmuir (2), and Henry laws and multilayer adsorption (4) to collagen type I that are synthesized under mechanical stress σ = 0 MN/m² (A), σ = 0,15 MN / m² (B).

Table 2 shows calculated the number of water molecules in the inner and outer layers of the hydration shell tripeptide Gly-X-Y in type I collagen and disaccharide unit ΣGAG that are synthesized in the skin of rats in the presence or in the absence of mechanical stress, according to the hydration isotherms Fig. 2 and Figure 3.

Table 2.

The number of water molecules in the inner and outer layers of the tripeptide Gly-X-Y in collagen type I and disaccharide unit Σ GAG respectively, are synthesized in the skin of rats in the presence or absence of mechanical stress.

Biopolymer	σ, MN/м^2	RH, %	Inner layer	Outer layer
Σ GAG	0	90	1,1	4,17
	0,025	80	2,4	6,3
	0,05	90	4,36	5,6
Type I Collagen	0	90	4,83	3,50
	0,15	80	2,16	0,29

As we can see, the internal hydration level makes the main contribution to the overall increase of adsorbed water in the GAG under the mechanical stress, compared to controls.

Perhaps the deformation of tissue under mechanical stress leads to the fact that GAG molecules that are synthesized in these conditions have less organized and more expanded conformation. This may be a cause of penetration of a large number of water molecules into the interior of the molecules.

As for collagen, the decrease of the amount of water makes more specific contribution to the outer layer amid a general decline of hydration. Obviously this is likely to occur due to the decreasing level of hydroxylation of proline in collagen, which is synthesized in the tissue under the mechanical stress [11, 12], since the OH group of hydroxyproline, which forms hydrogen bonds with water, is located outside of collagen molecules.

Therefore, the obtained effects may be adaptive, as the increasing hydration of GAG in the skin under the mechanical stress has to lead to an intensification of metabolic processes in the extracellular matrix and has to level the reduction of collagen hydration.

Conclusions. Under the tensile mechanical stress in the rat's skin, the overall hydration level of synthesized glycosaminoglycans increases and type I collagen decreases.

These effects of glycosaminoglycans occur due to the absorbed water in the inside layer, and in collagen – due to the outside layer. The multidirectional changes of GAG and collagen hydratation compensate each other to some extent and are important for maintaining constant physical and chemical properties of the matrix during the changing course of mechanical stress in the tissue.

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Изменения уровня гидратации коллагена типа I и гликозаминогликанов, синтезируемых в коже крыс при действии механического напряжения

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Аннотация. In vitro изучено влияние растягивающего механического напряжения в коже крыс на степень гидратации коллагена типа I и гликозаминогликанов, которые в ней синтезируются. На основании измеренных изотерм гидратации обнаружено повышение и снижение величины сорбции воды гликозаминогликанами и колагеном, соответственно. Рассчитаны количества молекул воды, приходящихся в среднем на трипептид в коллагене и дисахаридную единицу в гликозаминогликанах во внутреннем и внешнем слоях их гидратных оболочек.

Ключевые слова: коллаген; гликозаминогликаны; гидратация; механическое напряжение.