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## INTERFACIAL LAYERS AND THE SHEAR ELASTICITY OF THE COLLAGEN–WATER SYSTEM

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*A hypothesis has been put forward that, in collagen hydrogels, there exist interfacial water layers surrounding the collagen helices. A model of interfacial water layer is proposed which does not contradict experimental data on the shear elasticity of collagen hydrogels. Such layers can exist in a loose connective tissue and prove its supporting function.*

*Keywords:* collagen, shear modulus, polymer mesh, chain configuration.

### 1. Introduction

The research of collagen-water systems has a long history of nearly a century (see, e.g., works [1–6]). Such a permanent interest in those systems is explained by the fact that both collagen and water are constituents of human body. By interacting with each other, they participate in complicated processes that take place in organism. The importance of researches of collagen-water systems consists in that they allow the character of their interaction to be studied *in vitro*. In particular, they were studied using various methods: viscometric [1, 6], radiographic [2], calorimetric [3], and others.

In this work, we will study the shear elasticity of the indicated systems. We did not find works where their shear modulus had been measured. Therefore, we developed a method that allows this parameter for those systems to be measured [7–9].

It is known (see, e.g., work [10]) that a straightened collagen molecule has the length  $L = 3000 \text{ \AA}$  and the diameter  $d = 15 \text{ \AA}$ . It is composed of three polypeptide chains twisted into a single helix. Its molecular weight equals  $M = 350000$ .

Collagen is also known (see, e.g., work [11]) to be the main component of connective tissue. There are cartilaginous and loose connective tissues. The former is known to contain 70–80% of water. Collagen comprises from 50 to 70% of its dry substance. Collagen molecules form a fibrillar structure: the molecules unite into fibrils 20–100 nm in diameter, the fibrils aggregate to form fibers 1–10  $\mu\text{m}$  in diameter, and the fibers compose bundles up to 150  $\mu\text{m}$  in diameter.

The loose connective tissue, like any other connective tissue, consists of cells and an intercellular substance. The latter is a jelly substance including collagen, elastin, and an amorphous matrix containing water. The amount of collagen in the loose connective tissue is considerably smaller than that in the cartilaginous one, and the formation of fibrils is not observed. Instead, collagen molecules form a mesh.

In works [12, 13], the structure of a water layer at the interface with collagen in the cartilaginous tissue was calculated. As was already mentioned, collagen chains form a fibrillar structure in this tissue. Due to a high rigidity of this structure, a model of absolutely hard medium was adopted for it. Accordingly, water was assumed to contact with an absolutely hard wall.

It was found that water molecules are so oriented in the layers parallel to the wall that the unit vector

$\vec{n}$  directed normally to the plane of a water molecule is directed perpendicularly to this wall (Fig. 1). The distance between the layers is  $2 \text{ \AA}$ .

In the loose tissue, as was said, the fibrillar structure is absent. What are the interfacial layers in this case? In this paper, we will try to answer this question.

For an experimental study of the problem concerned, it is necessary to have an object that could serve as a model of intercellular substance. As was already indicated, this substance is a gel. Therefore, the collagen hydrogel was chosen as its model. Of course, this is a simplified model. But it preserves the main crucial feature of the intercellular substance: the presence of a collagen mesh.

## 2. Experimental Part

Collagen hydrogels with the concentrations  $c = 50, 70, 100,$  and  $200 \text{ g/l}$  were studied. Hydrogels were obtained from gelatin solutions. The process of hydrogel formation was monitored by measuring the refractive index  $p$  of the solution. Figure 2 demonstrates a typical dependence of the refractive index  $p$  on the time  $t$ . As one can see, the gel formation took an interval of about 10 min after the solution preparation.

The real (the dynamic modulus  $G'$ ) and imaginary (the loss modulus  $G''$ ) parts of the complex shear modulus of hydrogel were measured. The experimental technique is described in works [7–9]. According to it, cylindrical polyethylene cuvettes filled with a gelatine solution served as specimens. The measurements were made in 10 min after the cuvettes had been filled, which, in accordance with Fig. 2, guaranteed the formation of hydrogel in them.

In Fig. 3, the dependence of the dynamic modulus  $G'$  on the concentration  $c$  obtained at the temperature  $T = 293 \text{ K}$  is shown. The temperature dependences of the dynamic and loss moduli of the frozen gelatin gel with a concentration of  $50 \text{ g/l}$ , which approximately corresponded to the concentration of collagen in the intercellular substance of the loose tissue, were also measured (Fig. 4). For comparison, similar dependences for ice are presented as well (Fig. 5).

## 3. Discussion of Experimental Results

The polymer mesh is known (see, e.g., work [14]) to arise owing to the formation of nodes, i.e. links between the chains. The location of nodes in space

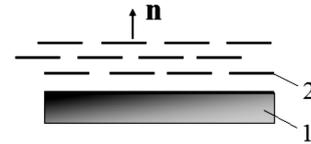


Fig. 1. Schematic diagram of the arrangement of water molecules in the interfacial layer of cartilage tissue: fibrillary structure (wall) (1), water molecule (2)

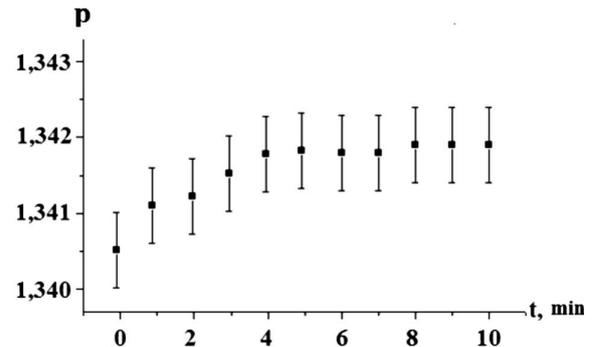


Fig. 2. Time dependence of the solution refractive index ( $c = 70 \text{ g/l}$ )

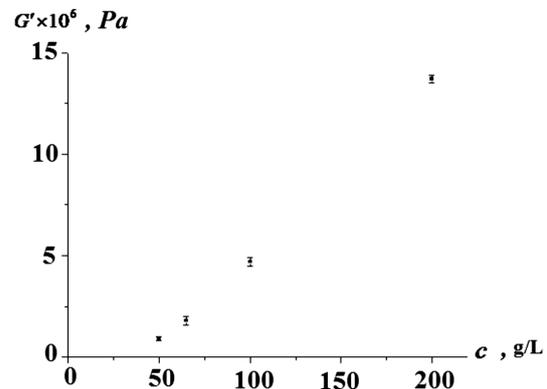
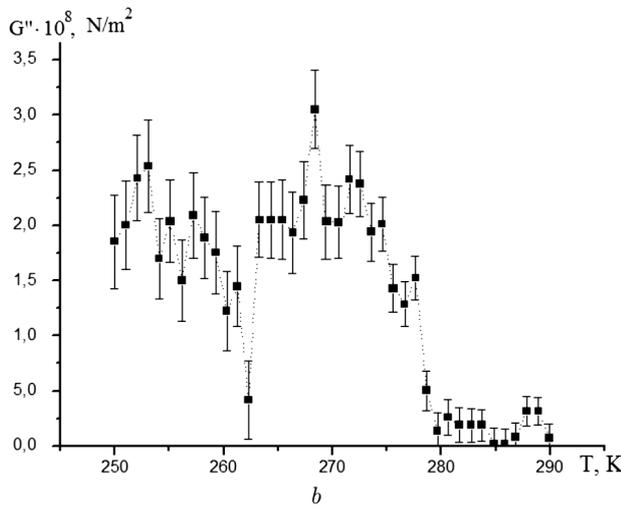
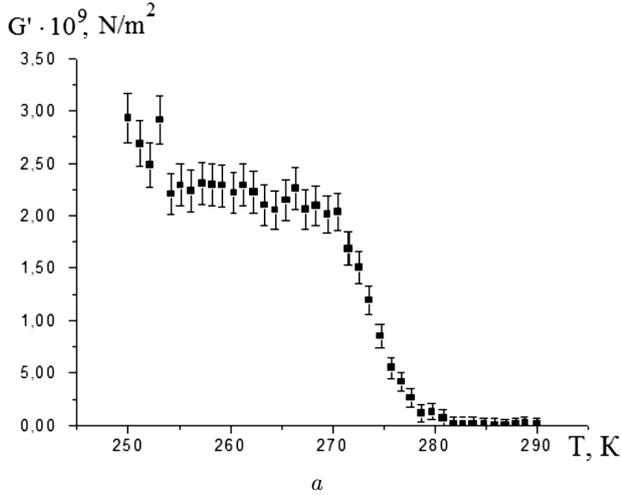


Fig. 3. Dependence of the dynamic modulus  $G'$  of hydrogel on the collagen concentration  $c$  ( $T = 293 \text{ K}$ )

is stochastic, and the chains themselves can acquire different configurations with certain probabilities (Fig. 6, a).

To simplify the picture, let us introduce a model of ideal mesh in which the chains are completely straightened to form a lattice. The lattice sites are simultaneously the mesh nodes. The lattice cell is cubic. This model is supported by the fact that collagen belongs to the class of rigid-chain polymers. The latter are known (see, e.g., [14]) to have a tendency to form straightened configurations.



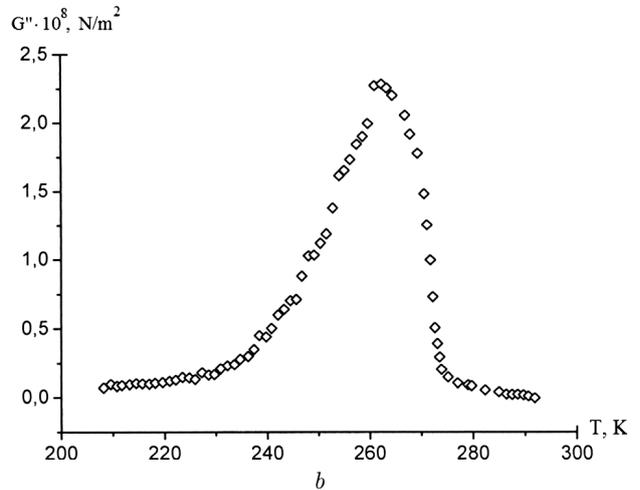
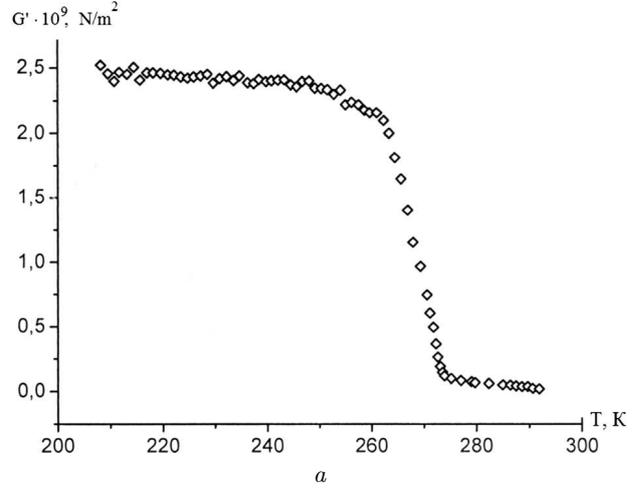
**Fig. 4.** Temperature dependences of the dynamic modulus  $G'$  (a) and the loss modulus  $G''$  for the frozen collagen gel ( $c' = 50$  g/l (b))

Let us determine the dependence of the edge length  $\ell$  of the unit cubic cell on the concentration  $c$ . The area of the cube edge cross-section  $S = \pi d^2/4$ . Each edge has a volume equal to  $S \times \ell$ . The edge is a part of four adjacent cells, so that only  $1/4$  of the calculated volume is related to one cell. The cube is surrounded with 12 edges. Therefore, the volume  $V_p$  of the polymer in a cell equals

$$V_p = 3S\ell. \quad (1)$$

Since the cell volume is  $V_c = \ell^3$ , the expression for the volume fraction  $\varphi$  of the polymer looks like

$$\varphi_p = \frac{V_p}{V_c} = \frac{3S}{\ell^2}. \quad (2)$$



**Fig. 5.** Temperature dependences of the dynamic modulus  $G'$  (a) and the loss modulus  $G''$  for ice [8] (b)

By definition,

$$\varphi_p = \frac{c}{\rho_p}, \quad (3)$$

where  $\rho_p$  is the polymer density, which is calculated by the formula

$$\rho_p = \frac{M}{SL}. \quad (4)$$

Using formulas (2)–(4), we obtain

$$\ell = \left( \frac{3M}{cL} \right)^{1/2}. \quad (5)$$

Then, according to formula (5), if the concentration  $c = 50$  g/l, the edge length equals  $100 \text{ \AA}$ .

Following the approximation adopted in works [12, 13], where the collagen structure was considered as an absolutely hard medium, let us model a collagen helix by an absolutely hard rod. Then, by analogy with the case analyzed in the cited works, water molecules in vicinities of this rod have to be so oriented that their vectors  $\mathbf{n}$  should be directed along the normal to the rod surface (Fig. 7). Hence, the calculations made in works [12, 13] suggest that every collagen molecule in hydrogel is surrounded by an interfacial layer, whose structure differs from the structure of water in bulk. This layer looks like a hollow cylinder and can be composed of a few elementary layers.

Let us estimate the number of water molecules that can be arranged along the circle in the layer adjacent to the rod (collagen helix) surface. As was already mentioned, the rod radius equals  $\frac{d}{2} = 7.5 \text{ \AA}$ . According to calculations of works [12, 13], the layer closest to the interface is located at a distance of  $2 \text{ \AA}$  from it. Therefore, the relevant circle has a radius of  $9.5 \text{ \AA}$ . Assuming that the distance between water molecules equals  $2.76 \text{ \AA}$ , as it is in the ice lattice, we find that 22 water molecules are contained in the considered elementary layer.

As was indicated above, the proposed model of interfacial layer is based on the assumption that the collagen helix is an absolutely hard rod. However, a situation can arise, in principle, when water molecules penetrate into the helix and separate its polypeptide chains from one another. Then the model shown in Fig. 7 becomes invalid. Let us demonstrate that such a situation does not take place in our case.

The moduli  $G'$  and  $G''$  characterize the deformation properties of a physically infinitesimal volume [15, 16]. By definition, the size  $\Delta r$  of this volume must be substantially larger than the microscopic scale used to describe the arrangement of particles in this volume. In our case, this condition looks like

$$\Delta r \gg d.\ell. \quad (6)$$

When measuring the moduli  $G'$  and  $G''$  in a physically infinitesimal volume, a pure shear state is created on the average. At the same time, deformations of various types – stretching, bending, and so forth – can arise at the microscopic level. To describe those deformations, microscopic elastic constants are introduced. They include, e.g., Young's  $E$ -modulus for the helix, which describes the stretching of a helix

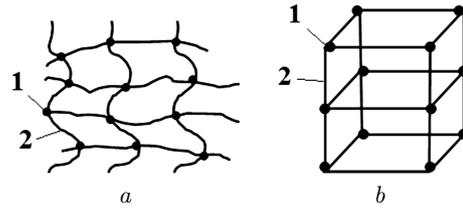


Fig. 6. Schematic diagram the polymer mesh structure: random mesh (a), model of perfect mesh (b): nodes (1), chains (2)

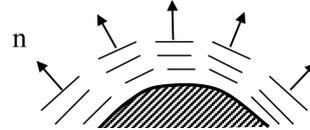


Fig. 7. Schematic diagram of the water molecule arrangement near the collagen helix surface

along its axis. The values of  $E$  for polymeric helices lie within an interval of  $10^{10}$ – $10^{11}$  Pa.

Let us determine Young's modulus  $E$  on the basis of experimental data depicted in Fig. 2. If the obtained  $E$ -value falls within the indicated interval, this will mean that the helix retains its integrity in hydrogel.

First of all, we should elucidate how the helix deforms, when the modulus  $G'$  is measured. The inequality  $d \ll \ell$  means that the helix under an external load tends to bending. Let us check whether the helix bending really takes place during a measurement of the dynamic modulus. The ability of a rod (helix) to bend is characterized by the bending stiffness  $Q$ , which is defined by the formula

$$Q = EI, \quad (7)$$

where  $I = \frac{\pi d^4}{64}$  is the axial moment of inertia of the rod (helix) cross-section. Therefore, if the helices become bent at a measurement of the dynamic modulus, then the modulus  $G'$  has to depend on  $Q$ .

The applied model of perfect mesh has two characteristic sizes:  $d$  and  $\ell$ . The dynamic modulus  $G'$  must depend on both those quantities. The size  $d$  is already engaged in formula (7). Therefore, a conclusion is drawn that the modulus  $G'$  is a function of two quantities,  $Q$  and  $\ell$ :

$$G' = f(Q, \ell). \quad (8)$$

The form of this function can be found using dimensional considerations. The only combination of two quantities  $Q$  and  $\ell$  that has the dimensionality of

pascal is  $Q/\ell^4$ . Therefore, expression (8) should be rewritten in the form

$$G' \approx \frac{\pi}{64} E \frac{d^4}{\ell^4}. \quad (9)$$

From formulas (1)–(5), we obtain the relation

$$c \sim \frac{d^2}{\ell^2}, \quad (10)$$

which leads, in turn, to the expression

$$G' \sim c^2. \quad (11)$$

According to it, if the helices become bent at the measurement of the modulus  $G'$ , the obtained concentration dependences of this parameter must be quadratic. This conclusion is in agreement with the experimental data exhibited in Fig. 3. Hence, the bending of helices really takes place, and formula (9) can be used to calculate  $E$ . Substituting the required numerical values into this formula, we obtain the value  $E = 4 \times 10^{10}$  Pa for Young's modulus. Thus, the helix retains its integrity in hydrogel, which agrees with the model of helix as a completely rigid rod, which the model of interfacial layer is based on.

The aim of this experiment, as well as the experiment whose results are presented in Figs. 4 and 5, was to prove the existence of a layer surrounding every helix in the collagen mesh. As one can see from Fig. 5, *b*, there is a peak in the dependence  $G''(T)$  for ice in the temperature interval 240–273 K. Let us call it the A-peak. According to Fig. 4, *b*, the dependence  $G''(T)$  for the frozen hydrogel has two peaks: in intervals from 250 to 260 K and from 260 to 273 K. The latter peak precedes the melting, so that it evidently has the same nature as the A-peak for ice, and we preserve the same term for it. The former peak will be called the B-peak.

The temperature dependences  $G'(T)$  in Figs. 4, *a* and 5, *a* were used to determine the melting point. Expectedly, the melting point of ice was found to equal 273 K. For frozen gel, this temperature was 283 K. Hence, according to Figs. 4 and 5, the following differences were observed in the behavior of frozen hydrogel and ice:

- a new peak (B) appeared in the dependence  $G'(T)$  for frozen hydrogel in comparison with that for ice;

- the intensity of the A-peak for hydrogel was lower than the intensity of the same peak for ice;
- the melting point of hydrogel was higher than that of ice.

In comparison with ice, the frozen hydrogel contained a new component, collagen. Therefore, it is reasonable to assume that the B-peak appeared due to a relaxation process in the collagen helices. However, in this case, the intensity of A-peak for frozen hydrogel should have remained practically the same as for ice, because the collagen concentration was low. Moreover, the melting point also should have been equal to 273 K as for ice. But this was not the case. So, we arrive at a conclusion that there was another structure, besides ice, which was formed by water molecules.

The results of calculations [12, 13] allow us to assert that this structure consisted of interfacial layers around the collagen helices. An additional argument in favor of this conclusion is the fact that the melting point of frozen gel was higher than that of ice. It testifies that the density of a new structure was higher than that of ice, which corresponds to calculation data [12, 13].

Let us calculate the thickness of the interfacial layer on the basis of data exhibited in Figs. 4, *b* and 5, *b*. By definition, the area under the peak in the dependence  $G''(T)$  is proportional to the volume occupied by the structure responsible for this peak. Denoting the areas under the A-peaks of frozen gel and ice as  $I_g$  and  $I_e$ , respectively, and the corresponding volumes as  $V_g$  and  $V_e$ , respectively, we obtain

$$\frac{I_e - I_g}{I_e} = \frac{V_e - V_g}{V_e} = \varphi_n, \quad (12)$$

where  $\varphi_n$  is the relative volume occupied by non-ice structures, namely, collagen and interfacial layers. Accordingly,

$$\varphi_n = \varphi_p + \varphi_s, \quad (13)$$

where  $\varphi_s$  is the relative volume occupied by interfacial layers.

Using formulas (3) and (4), we obtain  $\varphi_p = 0.05$  for  $c = 50$  g/l. Calculating the values of  $I_e$  and  $I_g$  from Figs. 4, *b* and 5, *b*, we obtain from formula (12) that  $\varphi_n = 0.43$ . Hence, for the relative volume  $\varphi_s$ , we have a value of 0.38.

As was already mentioned above, in the perfect mesh model, an interfacial layer looks like a hollow

cylinder surrounding a collagen helix. Let  $D$  denote the external diameter of this cylinder. Within a unit cell, the length of this cylinder is equal to  $\ell$ . Its cross-section area amounts to  $\frac{\pi}{4}(D^2 - d^2)$ . By analogy with formula (3), we may write

$$\varphi_s = \frac{3\pi}{4} \frac{(D^2 - d^2)}{\ell^2}. \quad (14)$$

Substituting the numeric values of quantities into Eq. (8), we obtain  $D = 40 \text{ \AA}$ . Accordingly, for the thickness of the interfacial layer, we have  $\frac{D-d}{2} = 12.5 \text{ \AA}$ .

The existence of interfacial layers in the collagen-water systems is also confirmed by literature data. In work [3], a transition associated with the collagen denaturation was considered. In particular, the dependence of the transition heat on the content of aminoacid residues (Pro, OPro) was studied. It was found that the transition heat increases with the content of those groups. This growth cannot be explained in the framework of the model, where the collagen helices are stabilized only by intra-molecular hydrogen bonds, because aminoacid residues do not form them. Hence, a conclusion was drawn that the helices are stabilized by an ordered water structure that exists around them.

The interfacial layer is an ordered structure of water molecules. Therefore, we may assert that the conclusion about the existence of such structures in collagen-water systems agrees with the results of previous studies [3, 4]. But our conclusion was made on the basis of different experimental data, namely, the parameters of shear elasticity. Furthermore, in this work, we propose a model for such an ordered structure.

It was already mentioned that the considered collagen hydrogel can serve as a model of the intercellular substance in the loose connective tissue. Therefore, the fact of the formation of interfacial layers in the collagen hydrogel gives us ground to assert that the same layers are formed in the intercellular substance as well. Owing to the interfacial layer, the thermal motion cannot bring the molecule into a folded configuration. As a result, the intercellular substance, unlike water, can withstand the action of shear deformations, and this circumstance makes it gel. This property means an ability to preserve the form, which is conventionally associated with the supportive (biomechanical) function of connective tissue.

#### 4. Conclusions

Our experimental and calculation results obtained in this work, as well as the literature data, enable us to assert that collagen helices in the collagen-water system are surrounded by an interfacial water layer having the shape of a hollow cylinder with a wall thickness of  $12.5 \text{ \AA}$ . This layer consists of a few elementary layers. In each of them, water molecules are so arranged that unit vectors that are perpendicular to the molecule plane are directed normally to the cylinder surface. The interfacial layer stabilizes the helix, a straightened configuration of collagen molecule, and prevents the molecule from acquiring a folded configuration by means of thermal motions. This circumstance can be important for the functioning of loose connective tissue in human body by providing its supportive (biomechanical) function.

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#### ПРИМЕЖОВІ ШАРИ ТА ЗСУВНА ПРУЖНІСТЬ СИСТЕМИ "КОЛАГЕН-ВОДА"

#### Резюме

Висунуто гіпотезу про існування в гідрогелях колагену при-  
межових водних шарів, що оточують колагенові спіралі.  
Пропонується модель примежового водного шару. Встанов-  
лено, що ця модель не суперечить отриманим експеримен-  
тальним даним по зсувній пружності гідрогелів колагену.  
Стверджується, що такі примежові шари можуть існувати  
в пухкій з'єднувальній тканині, забезпечуючи її опорну  
функцію.