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NEW ASPECTS FOR THE ESTIMATION OF THE STATE OF THE NATURAL WATER

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Abstract. The hydrochemical composition and physicochemical properties of natural water samples from various sources in the Voronezh and Moscow regions have been studied. The highest mineralization of water was found in the snow collected near the highway, and the highest content of *N*-containing compounds in the water of the Usman River in the Voronezh reserve. Two model systems are proposed for assessing the state of the aquatic environment UV spectroscopy with spectrum decomposition by the Gauss method and spontaneous aggregation of lecithin in a polar medium. The presence of various organic, *N*- and *P*-containing compounds, even at low concentrations, leads to significant changes in the lecithin ability to form nanosized aggregates and change their electrophoretic properties. Based on the performed investigation, it was determined that the size of lecithin aggregates decreases, and the value of their zeta potential increases with an increase in the content of hydrophobic compounds in natural water.

Keywords: lecithin, hydrochemical index, water quality, UV-Vis spectroscopy, Gauss method.

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Introduction

The natural aquatic medium is a complex multicomponent system determined by the chemical composition of the boundary phases [1]. The pronounced self-organizing ability of water due to the hydrogen bonds formation has led to the expansion of studies on the liquid water structure. To the beginning of the 21st century many experimental data were obtained proving both the influence of low doses of inorganic salts and aminoacids on the nanostructured state of water, and peculiarities in the action of the biologically active substances and the low intensity physical factors on systems of varying complexity [2,3]. Also, ultra-low concentrations of the biologically active substances in systems are enough to be interrelated with a change of the physicochemical properties of water [4-6]. The formation of the supramolecular complexes with the linear sizes of 1-100 μm is also revealed in the water prepared from melted snow [7].

The normal functioning of the complex system is associated with oxidative processes, which play an important role not only in evaluating water quality, but also in metabolism regulation of the biological objects of varying complexity [8,9]. Hydrogen peroxide present in the natural aquatic medium is one of the necessary participants to support the balance of its redox reactions [1,8,10] and an initiator of the lipid peroxidation (LP) in the biological system [9]. Upon entry into the body, all biologically active substances take part in the regulation of oxidative processes in tissues and significantly affect the structural state of biological membranes [11] at doses close enough to the background [3,11,12]. Similarity, the LP processes in the membrane, cellular and organ levels [13] whose stationary is supposed by the physicochemical regulatory system allows to propose new aspects to evaluate the effect of aquatic medium of components on the state of the LP processes in biological objects.

The aim of this work was to study the physicochemical properties of natural water samples from different sources, and to evaluate their influence on the biological membranes using the ability of the natural phospholipids (some of the biologically active substances components of membranes) to form nanosized particles in the polar medium.

Experimental

Materials

Chemically pure salts of ammonium chloride, sodium nitrite, potassium nitrate, potassium dihydrogen phosphate pre-dried for 2 hours at 105°C were used as standards for the determination of biogenic elements. Reagents used for spectrophotometric measurements (Griess reagent, Nessler reagent, Nitrover 5 nitrate reagent (HACH Company), ammonium molybdate, ascorbic acid) were of "chemically pure" and "pure for analysis" grades.

Sampling points description

The samples were collected from the natural water from Usman (Voronezh nature reserve) and Don (Voronezh region), Dubna and Sestra rivers (near settlment Ustie-Strelka, Moscow region). Samples of snow from the ice of the Usman river, in the pine forest and the grove of leafy trees, the water from the well (Voronezh nature reserve) and snow near the motor road in Rylsk city were also analysed. All samples were collected between March 10 and 21, 2021. The geographic location of samples in Voronezh and Moscow regions are presented in Table 1.

Determination of the hydrochemical indices

Water samples were filtered prior to analysis through a "blue ribbon" paper with pores of 3-5 µm. The quantitative content of the NH_4^+, NO_2^-, NO_3^- and $(PO_4)^{3-}$ ions and the mineralization index were determined by photometric methods according to the method described elsewhere [14,15].

Isolation of lipids and analysis of their phospholipid composition

A 10% solution of the soy bean lecithin (BIOLEK, Ukraine) was used as analogue of the natural phospholipids. The fractional composition of its phospholipids was determined by thin layer chromatography according to the standard procedure [16]. Glass plates 90×120 mm, type G silica gel (Sigma, USA) and the solvent mixture of chloroform:methanol:glacial acetic acid:distilled water as a mobile phase in a volume ratio of 12.5:7.5:2:1 were used. Five chromatographic lines were analysed according to procedure described elsewhere [16]. Methodological details on analysis of the fractional phospholipid composition was reported previously [17].

UV-Vis spectroscopy

The UV-Vis spectroscopy measurements of solutions of lecithin in distilled and natural water were carried out in 10 mm quartz cuvettes using a Shimadzu UV-1700 PharmaSpec (Japan) spectrophotometer. The obtained UV spectra were mathematically processed by the Gauss method using the Excel solver, by minimizing the sum of the squares of the difference between the experimental and calculated spectra under the following conditions: coincidence of the contour of the original spectrum with the calculated one after approximation at the level of 1×10^{-3} - 1×10^{-4}.

Determination of the particle size and ξ-potential by dynamic light scattering method

The aggregation of lecithin in aquatic medium was studied by dynamic light scattering measurements using a Malvern Zetasizer Nano-ZS analyzer (Malvern Instruments Ltd., UK) equipped with a He – Ne laser (4 mW and wavelength 633 nm) and automated program for data processing. The solution was put into an optic cell (10×10 mm). The measurements were carried out at 25°C and a 173° fixed scattering angle. The data were processed with Zetasizer Software 6.20. The particle size was presented as the hydrodynamic diameter *d*. The *ξ*-potential measurements of the lecithin nanoparticles in aquatic medium were also performed using dynamic light scattering.

Table 1

The geographic location of samples in Voronezh and Moscow regions.

Sampling point no.	*Sampling location*	*Date of sampling*	*Coordinates*	
			Latitude, N	*Longitude, E*
1	Usman river, Voronezh nature reserve	10.03.21	51.87924	39.65948
2	Snow from ice, Usman river, Voronezh nature reserve	10.03.21	51.87924	39.65948
3	Snow in the grove of leafy trees, Voronezh nature reserve	10.03.21	51.87924	39.65948
4	Snow in the pine forest, Voronezh nature reserve	10.03.21	51.88088	39.65217
5	Snow near the bridge over the river Seim on the Rylsk - Lgov motor road	13.03.21	51.94973	36.30433
6	Don river near the Novozhivotinnoe village	11.03.21	51.88958	39.16353
7	Usman river, near Orlovo village	14.03.21	51.76638	39.60188
8	Usman river, near Usman workers' settlement	14.03.21	52.01232	39.72948
9	Well in floodplain of the Usman river, 70 m from the coastline	14.03.21	52.01232	39.72948
10	Dubna river, near settlment Ustie-Strelka	21.03.21	56.71014	37.22877
11	Sestra river, near settlment Ustie-Strelka	21.03.21	56.71324	37.22524

It is defined as the electrostatic potential at the slipping (shear) plane outside a charged particle moving in an electric field, where the viscous forces and the electrostatic forces are balancing each other [18]. The velocity of a particle in an electric field is commonly referred to as its electrophoretic mobility, which can be used to obtain the zeta potential of the particle by application of the Henry equation (Eq.(1)).

$$U_E = 2\varepsilon\, \xi\, f(ka) / 3\eta \qquad (1)$$

where, ξ - zeta potential;
U_E - electrophoretic mobility;
ε - dielectric constant;
η – viscosity, *f(ka)* - Henry's function.

Electrophoretic determinations of zeta potential are most commonly made in aqueous media and moderate electrolyte concentration, *f(ka)* in this case is 1.5, and is referred to as the Smoluchowski approximation. Therefore, the calculation of zeta potential from mobility is straightforward for systems that fit the Smoluchowski model, *i.e.* particles larger than about 0.2 μm dispersed in electrolytes containing more that 10^{-3} M salt.

Smoluchowski's equation was used to calculate the value of the ξ-potential [19] from the electrophoretic mobility. In all experiments the lecithin concentration was 30 μg/mL (4.3×10^{-5} mol/L). Measurements were proceeded during 2 – 2.5 hours after preparation of solutions to reach the dynamic balance. The measurement of each sample was repeated for at least five times.

Statistical procedure

The data were processed with a standard statistic method using MS Excel product, and by KINS program [20]. The significance of differences was evaluated using the *t*-Student's distribution. The experimental data are presented in the form of the average arithmetic means with their mean square errors (M±m).

Results and discussion

In March 2021, expedition surveys of water bodies in the Voronezh and Moscow regions were carried out. For research, samples were collected of both water and snow in adjacent areas with different vegetation cover. Data regarding the concentrations of the different ions and pH of samples from Voronezh region are presented in Table 2.

Table 2

Hydrochemical indices of studied samples from Voronezh region.

Point no.	*Point of sampling*	*pH*	*Mineralization, mg/L*	*[N-NH_4^+], mg/L*	*[N-NO_2^-], mg/L*	*[N-NO_3^-], mg/L*	*[P-PO_4^{3-}], mg/L*
1	Usman river, Voronezh nature reserve	8.22	389	3.92 ±0.55	0.84 ±0.21	0.70 ±0.20	0.47 ±0.17
2	Snow from ice, Usman river - Voronezh nature reserve	6.85	29.4	2.18 ±0.31	0.017 ±0.008	6.0 ±1.7	0.466 ±0.004
3	Snow in the grove of leafy trees, Voronezh nature reserve	6.38	15.0	1.24 ±0.17	0.001±0.0	0.094 ±0.026	0.001 ±0.0
4	Snow in the pine forest, Voronezh nature reserve	6.36	10.1	1.00 ±0.14	0.0	0.040 ±0.011	0.0
5	Snow near the bridge over the river Seim on the Rylsk - Lgov motor road	6.86	4670	3.03 ±0.42	0.051±0.026	0.0	0.064 ±0.029
6	Don river near the Novozhivotinnoe village	8.10	227	0.459 ±0.064	0.011 ±0.005	0.51 ±0.14	0.057 ±0.034
7	Usman river, Orlovo village	8.14	341	2.97 ±0.42	0.019 ±0.009	0.42 ±0.12	0.37 ±0.17
8	Usman river, Usman workers settlement	8.56	349	3.43 ±0.48	0.22 ±0.08	0.69 ±0.19	0.99 ±0.36
9	Well in floodplain of the Usman river, 70 m from the coastline	8.71	343	0.81 ±0.11	0.014 ±0.007	0.040 ±0.011	0.066 ±0.030

As may be observed from the data, there are substantial differences between values of these indices depending on the location of sampling. Thus, snow is characterized by pH values which are close to neutral, while all natural water samples had alkaline pH. Besides, both the level of mineralization and concentration of all studied ions had the lowest values in samples of snow from the pine forest and the grove of leafy tress, and the highest ones were identified in the snow collected near the motor road. The lowest values for both pH and concentrations of NH_4^+, NO_2^-, NO_3^- and $(PO_4)^{3-}$ ions were determined in the sample of the Don water, and the well water had the lowest value of nitrate ions.

It was shown on model systems previously that aquatic medium components have an impact on the changes of the state of the oxidative processes in the medium, as well as in the biological objects present in that medium [12,21]. To evaluate these changes, two model systems were used: UV-Vis spectroscopy with the mathematical analysis spectra by the Gauss method and the spontaneous aggregation of lecithin in distilled water and in the presence of investigated samples of the aquatic medium.

The analysis of UV-spectra revealed that samples of natural water from different sources can be divided in three groups: *group 1*: water from Dubna and Sestra rivers, Moscow region, and Don river, Voronezh region; *group 2*: water from the snow samples (with ice of Usman river, in the pine forest and in the grove of leafy tress, Voronezh nature reserve; snow near the motor road, Voronezh region), *group 3*: water of Usman river (points 1, 7, 8 and 9 (Table 1).

UV-spectra of the natural water samples of group 1 have only two absorption bands, one of which is presented in all three samples at λ= 208-210 nm. It is suggested that absorption bands are typical for the carbonyl and *N*-containing organic compounds and phenols in water. The second absorption band is situated at λ= 227.2, 238.6 and 245.0 nm for water from the Dubna, Sestra and Don rivers, correspondingly. Presumably, this band is due to the presence of organic acids, compounds with conjugated double bonds and compounds with carbonyl groups, respectively. As an example, UV-spectrum of the Don water is presented in Figure 1.

As follows from the UV-spectra of the group 2 samples, the content of chemical substances is according to the sampling location. In particular, the snow in the pine forest with the lowest values of all hydrochemical indices (Table 1) has only three absorption bands (λ= 236.4 nm, 254.0 nm and 291.9 nm) with a low absorbance (A< 0.020). This suggests the presence of organic substances with conjugated double bonds and carbonyl groups. Although snow in the grove of leafy trees has four absorption bands (λ= 197.6 nm, 218.3 nm, 241.3 nm and 260.7 nm), their absorbance is low. Thus, the most intense band (λ= 197.6 nm) has A= 0.0535. In the snow samples from the ice of the Usman river and near the motor road, there are also four absorption bands in the UV-spectra. The most intense absorption band has the maximum λ in the region 195 - 197 nm due to the presence of the hydrophobic compounds such as esters and compounds with unconjugated double bonds. However, organic acids and compounds with carbonyl groups were also identified in these samples, and polycyclic compounds - in the snow sample collected near the motor road (Figure 2). It was expected that the sample of snow near the motor road would have the highest values of both the hydrochemical indices (Table 2) and intensity of the absorption band in UV-spectra.

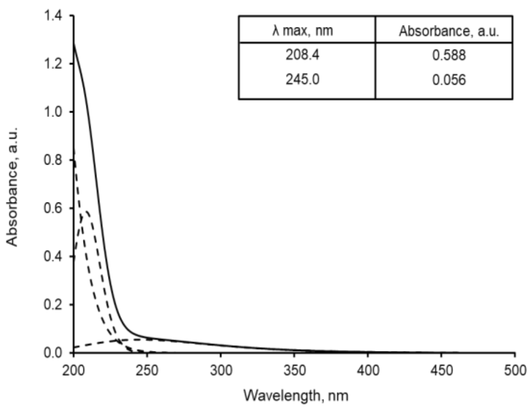


Figure 1. UV-spectrum of water from the Don river and its Gaussian. (here and further: solid curve is initial and calculate spectra, dotted lines are its Gaussian)

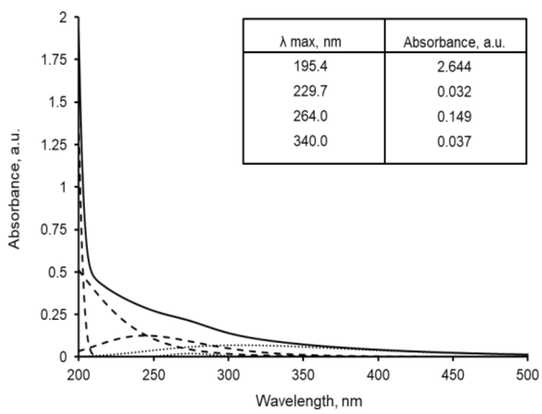


Figure 2. UV-spectrum of snow from the motor road and its Gaussian.

The number of the absorption bands and their intensity in the UV-Vis spectra of group 3 water samples are due to an inhabitation of people near the sampling location. Thus, two absorption bands are found only in the water from the Usman river (point 8, Table 1) characterized by a high content of hydrophobic compounds (λ= 201.2 nm, *A*= 2.203) and the presence of compounds with a conjugated double bonds (λ= 230.2 nm, *A*= 0.370). In the sample of the well water were identified organic acids and *N*- and *P*-containing compounds (λ= 221.3 nm, *A*= 0.135; λ= 254.7 nm; *A*= 0.1345; λ= 332.3 nm, *A*= 0.018) which were detected in accordance with a high content of NH_4^+, NO_2^- and NO_3^-, ions (Table 2). Samples of the Usman river water from Voronezh natural reserve (point no. 1) and near Orlovo village (point no. 7) contain a large number of hydrophobic compounds and other substances (as an example, the UV-Vis spectrum of sample no. 7 is presented in Figure 3).

The above presented analysis of the literature data allows us to conclude that the substantial changes in the chemical composition of the aquatic medium should affect the structural state and physicochemical properties of membranes of the biological objects. Indeed, the determination of the hydrodynamic diameters of the nanosized particles from lecithin and their ξ-potential in the presence of the natural aquatic samples supports this conclusion.

It is well-known that lecithin is a mixture of natural lipids and the proportion of phospholipids (PL) in its total lipid composition is at least 50%. Among PL, phosphatidylcholine is the main fraction. Earlier it was shown that the aqueous solution of lecithin is the adequate model system of the evaluation both presence of toxicants in the water medium and their influence on biological objects [12,21]. It should be noted that samples of the natural objects of different batches differ in terms of the quantitative ratio of PL fractions and share of PL in the total lipid composition [17]. The PL composition of the lecithin batch used is shown in Table 3. The share of PL of the lecithin batch used in the total lipid composition was 65.5±5.0% (*n*= 8).

The substantial differences in values of the hydrochemical indices and chemical composition of studied samples of the natural water cause changes in the ability of lecithin to aggregate (Figure 4). As seen, the main traction of lecithin (87.0±0.85%) consists of particles with an average size of 995±115 nm, whereas 13.0±0.5% belong to nanosized particles with 109±12 nm in *d*. In the presence of the Don and Usman rivers water samples (points no 1 and 6, Table 1) the lecithin aggregates consist of practically one fraction,1.2–1.3 times smaller in size.

Table 3

The fractional composition of phospholipids in the used lecithin.

Fraction of phospholipids	*Share of fraction (%P)*
Lysoforms of phospholipids	3.03±0.26
Sphingolipids	3.47±0.42
Phosphatidylcholine	84.1±1.4
Phosphatidylinozitol+ phosphatidylserine	2.67±0.32
Phosphatidylethanolamine	1.89±0.38
Cardiolipin	3.19±0.45
Phosphatidic acid	1.64±0.54

Number of chromatographic lines n= 5.

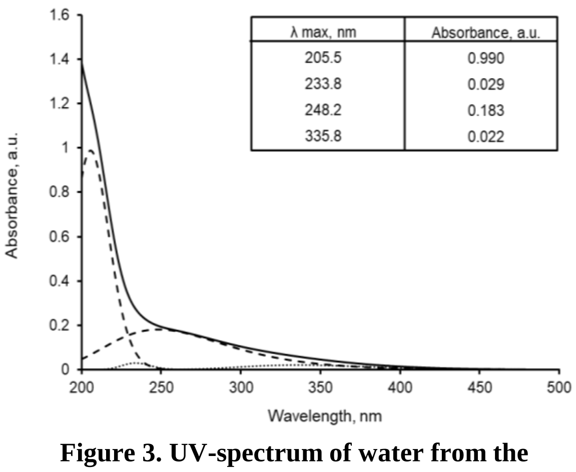


Figure 3. UV-spectrum of water from the Usman river near Orlovo village. (here and further: solid curve is initial and calculate spectra, dotted lines are its Gaussian)

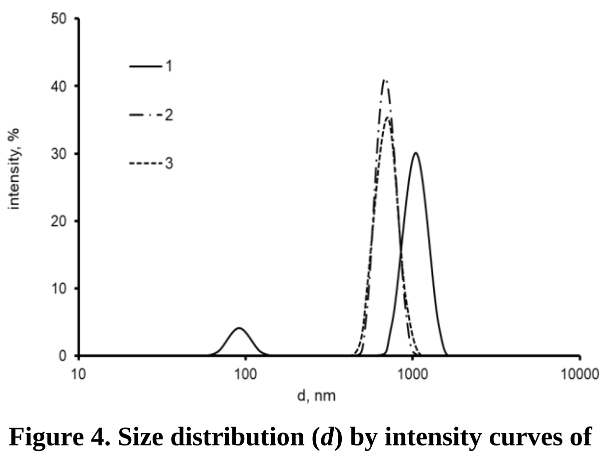
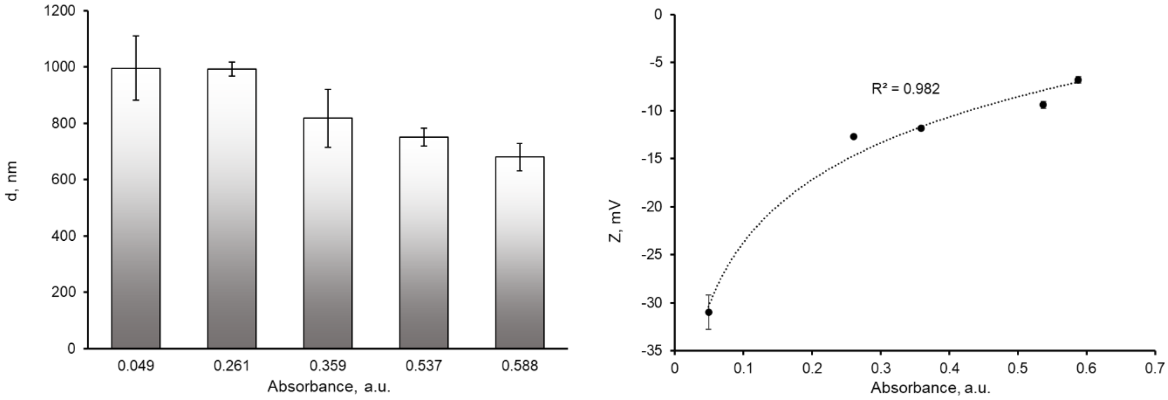


Figure 4. Size distribution (*d*) by intensity curves of lecithin aggregates in distilled water (*1*) and in the presence of the natural water from Don (*2*) and Usman (*3*) rivers (Voronezh nature reserve).



(a) ***(b)***

Figure 5. Size (*d*) particles of lecithin in the distilled water and in the presence of the natural water (*a*) and ξ-potential of the lecithin aggregates in the presence of the natural water (*b*) from Dubna (point no 10), Sestra (point no 11), Don (point no 6) and Usman (point no 1) rivers *vs* the contain of the hydrophobic compounds in the medium.

Table 4

Values of *ξ*-potential of the lecithin aggregates in the presence of natural water samples.

Samples	*ξ-potential, mV*
Dubna (point no 10)	−11.83 ± 0.05
Sestra (point no 11)	−12.72 ± 0.07
Usman (point no 1)	−9.41 ± 0.36
Usman (point no 7)	−12.97 ±0.17
Usman (point no 8)	−14.7 ±0.22
Usman (point no 9)	−7.98 ±0.28
Don (point no 6)	−6.78 ± 0.33
Snow (point no 4)	−25.5 ± 0.66

Besides, in samples from the natural water from Dubna, Sestra, Don and Usman (points no. 1, 6, 10 and 11 rivers, an increase of the size of hydrodynamic diameters *(d)* is attended by the reduction in content of the hydrophobic compounds (Figure 5*(a)*). However, the most substantial influence of the presence of different additives in the natural water are revealed for magnitudes of *ξ*-potential of the lecithin aggregates (Table 4). In distilled water, the *ξ*-potential value is -31.0±1.8 mV (*n*=8).

Noteworthy, the decrease in the negative value of the *ξ*-potential of the lecithin nanosized particles in the presence of the studied water samples is accompanied by an increase of the hydrophobic compounds in the natural aquatic medium in accordance with the parabolic function (Figure 5*(b)*).

Conclusions

From the obtained hydrochemical and spectrophotometric data, three groups of water bodies can be distinguished, the properties of which are probably associated with different watersheds and ecological conditions. In particular, the snow near the road, compared to the snow in the background areas, is subject to significant pollution with the content of predominantly reduced forms of biogenic elements (3.03 mg $N\text{-}NH_4^+$/L, 0.051 mg $N\text{-}NO_2^-$/L) and high mineralization (4670 mg/L).

The presented two model systems (mathematical analysis of the UV spectra of natural water using the Gauss method and spontaneous aggregation of lecithin in distilled water and in the studied samples of natural water) showed that the presence of various organic, *N*- and *P*-containing compounds, even at low concentrations, leads to significant changes in the ability of lecithin to form nanosized aggregates in an aqueous medium (a decrease in size by 1.2–1.3 times, with an increase in the content of hydrophobic compounds in natural water), as well as to change the electrophoretic mobility of biological membranes in biological objects. A significant increase in the *ξ*-potential was noted in all samples of natural waters. The greatest increase in the *ξ*-potential, in particular, is shown in a sample of the natural water of the Don River, up to -6.88 mV compared to distilled water (control) -31.0 mV. This indicates the possible influence of the composition of natural water on the functioning of biomembranes in the cells of living organisms. The structure of biomembranes and their functions are closely interrelated, and this plays an important role in the normal functioning of biological objects. This effect still needs to be studied in detail.

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