STUDY OF WATER DIFFUSION IN LYMPHOCYTE MEMBRANES FROM THYMUS GLAND AND SPLEEN OF RATS BY METHOD OF QUASI-ELASTIC SCATTERING OF SLOW NEUTRONS

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Abstract

The aqueous suspensions of lymphocyte membranes from the rat thymus gland and spleen cells were studied by the method of a quasi-elastic neutron scattering. Effect of X-ray irradiation on dynamics of water molecules in suspension of such membranes was examined. It was shown that a diffusion coefficient of water molecules decreases with increasing of a dose of X-ray irradiation animals (rats) from 0.5 up to 1.0 Gray.

Introduction

Investigation of water molecules diffusion in aqueous biological solutions allows us to understand the influence of the dissolved biological materials on the state of water molecules, and thus on the structure of dissolved biological objects, because the interaction of biomolecules with water influences essentially their spatial structure. For such investigation the method of quasi-elastic scattering of slow neutrons (QESSN) is very useful. It allows us to study the structure of investigated biological object from the analysis of QESSN spectra. The authors of the paper [1] initiated this direction of research.

The purpose of this paper was to research by means of the QESSN method the diffusion of water molecules in aqueous suspensions of lymphocyte membranes from the rat thymus gland and spleen cells. We investigated 3 various samples: a control sample, obtained from animals (rats) not exposed X-rays and other two samples, obtained from rats exposed to a X-ray irradiation by doses 0.5 and 1.0 Grays, accordingly. The investigation was performed at the nuclear reactor of the Institute for Nuclear Research of the National Academy of Sciences of Ukraine on the special equipment for testing the self-diffusion in water systems.

Theory

Recently due to the quick development of neutron engineering for research of self-diffusion and diffusion of water molecules in aqueous and water suspensions of biological objects by the method of quasi-elastic scattering of slow neutrons (QESSN) has been applied [1-3]. Taking into consideration novelty and originality of QESSN, it is reasonable to describe briefly the fundamentals of this method.

Within the framework of the time-space correlation functions in the Van-Hove formalism, that is usually applied for the description of scattering of slow neutrons in liquids, the double differential cross-sections of coherent (c) and non-coherent (nc) neutron scattering on the system of N atoms are Furrier-images of the full time-space correlation function $G(\vec{r},t)$ and its autocorrelated part $G_a(\vec{r},t)$, accordingly:

$$\left(\frac{d^2\sigma}{d\Omega d\varepsilon}\right)^c = \frac{N\langle b^2\rangle k}{2\pi\hbar k_0}\iint d\vec{r}dte^{i\vec{q}\vec{r}-i\omega t}G(\vec{r},t), \quad (1)$$

$$\left(\frac{d^2\sigma}{d\Omega d\varepsilon}\right)^{nc} = \frac{N\left(\langle b^2\rangle - \langle b\rangle^2\right)k}{2\pi\hbar k_0}\iint d\vec{r}dte^{i\vec{q}\vec{r}-i\omega t}G_a(\vec{r},t),. \quad (2)$$

where b is the amplitude of neutron scattering; $\vec{k}$ and $\vec{k}_0$ are initial and final wave vectors of a neutron; $\vec{q}=\vec{k}-\vec{k}_0$ is the momentum transfer of a neutron at scattering; The analysis of experimental data is often made in the Gaussian approximation for the autocorrelation function $G_a(\vec{r},t)$:

$$G_a(\vec{r},t) = [2\pi\Gamma(t)]^{-\frac{3}{2}}\exp\left[-\frac{r^2}{2\Gamma(t)}\right], \quad (3)$$

where $\Gamma(t)$ is a function, which describes dynamics of the system, physical sense of which is mean root-square distance of the particle from the origin during the time t:

$$\Gamma(t) = \frac{1}{3}\int d\vec{r}r^2G_a(\vec{r},t). \quad (4)$$

In theory of neutron scatterings it is shown, that the Gaussian approximation is always valid in the limit of large ($t >> \hbar/2kT$) and small ($t << \hbar/2kT$) time units [4]. Thus

$$\Gamma(t) = \begin{cases} -i\hbar t/M, & t\to 0 \\ 2Dt + const, & t\to\infty \end{cases}$$

where *D* is the self-diffusion coefficient; *M* is the particle mass.

The experimental data about $d^2\sigma^{nc}/d\Omega d\varepsilon$ can be analyzed within the framework of some models. Let us consider that the diffusion of liquid molecules corresponds to a macroscopic level of diffusion (continuous model of diffusion), so it is possible to write

$$\frac{\partial}{\partial t}G_a(\vec{r},t) = D\nabla^2G_a(\vec{r},t), \quad (5)$$

where $G_a(\vec{r},t)$ is a density of probability to find a molecule during time *t* in a point with coordinate *r*, if at *t=0* it is in the origin. The solution of the equation (5) is a function

$$G_a(\vec{r},t) = (4\pi Dt)^{-\frac{3}{2}}\exp\left[-r^2/4Dt\right], \quad (6)$$

Thus double differential cross-section of a non-coherent neutron scattering or intensity of a non-coherent neutron scattering in water systems, as a function of scattering neutrons energy, which is observed in the experiment, looks like a Lorenz function with a half-width

$$\Delta E = 2\hbar q^2 D. \quad (7)$$

Thus the widening of the quasi-elastic peak of non-coherent scattering of slow neutrons on liquid molecules, which is set by a function of Lorenz, looks like:

$$\Delta E = 2\hbar P(q), \tag{8}$$

and for jumping model of diffusion

$$P(q) = \frac{1}{\tau_0}\left[1 - \frac{\exp\{-2W\}}{1 + Dq^2\tau_0^{-1}}\right]. \tag{9}$$

Here τ_0 is a time of no migratory life of molecules in a final equilibrium position.

The simultaneous taking into account the activation and inactivation diffusion mechanisms based on the count of continuous diffusion of a final equilibrium position of molecules, which oscillate:

$$P(q) = \frac{1}{\tau_0}\left[1 + D_0 q_0^2 \tau_0 - \frac{\exp\{-2W\}}{1 + (D - D_0)q^2\tau_0}\right], \tag{10}$$

where D_0 is a diffusion coefficient of the oscillation center.

Thus, investigating of the experimental widening of the quasi-elastic peak ΔE, it is possible to determine a self-diffusion coefficient D of molecules and using the experimental data to analyze adequacy of the different diffusion models.

Experimental

In this paper we investigated a diffusion of water molecules in aqueous suspension of biological membranes of lymphoid cells from the rat thymus gland and spleen by the QESSN method. The process of lymphoid cells extraction consists in several stages:

1. Animals (white rats of a Vistar line (130-150 g) are exposed by the X-ray irradiation dose of 0.5 and 1.0 Gray on the installation RUN-17 under following conditions: dose rate – 24.5 Gr/min, filters of 1 mm Cu + 0.5 mm Al, voltage on the tube is 200 kV, current intensity is 5 mA, focal distance – 50 cm. In 12 hours after irradiation animals were decapitated and their spleen and thymus gland were removed and disintegrated. As a result the suspension of cells was obtained. The buffer medium (pH=7.4) and the solution used for cells extraction contained NaCl, KCl, Na_2HPO_4, HEPES, glucose, $NaHCO_3$, $CaCl_2$, $MgSO_4$.
2. A suspension of cells was left in the rest in 10 min and then the residue was disposed, lymphoid cells (lymphocytes) were extracted from the mixture by centrifugation in a gradient of Ficoll-Paque (density 1.077). For this purpose in tubes the mixture of cells was underplayed in the Ficoll solution with density ρ =1.077. Then the tubes were centrifuged at the rate of 1500 r.p.m for 40 min. Finally on the interface of the Ficoll and mixture of cells the "ring" was observed. It was the lymphocytes that have to be extracted. The “ring” collected by a syringe, washed, placed in the tube, added in a buffer and centrifuged within 10 min at the rate of 1500 r.p.m. The washed residue was solved in 1 ml of the buffer solution. The obtained lymphoid cells (lymphocytes) were frozen in liquid nitrogen to obtain disintegrated cellular membranes.
3. The samples were taken from liquid nitrogen, melted, homogenized and centrifuged for 30 min at the rate of 14000 r.p.m. The residue includes the plasma membranes. It was placed in 1 mm tris-HCl buffer solution at pH=7.0 and homogenized ones more.

The samples of the lymphocyte plasma membranes of the rat thymus gland and spleen cells, obtained by such method, were investigated by the QESSN method. In this section we shortly describe the special installation for the research of self-diffusions in water systems (the scheme is shown in Fig. 1).

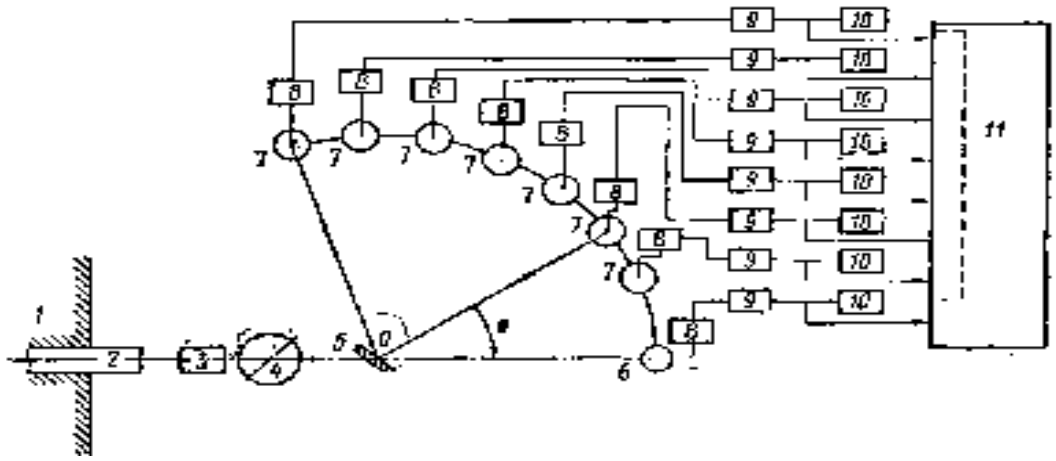

Fig. 1. Flowchart of the installation for study of a self-diffusion in water systems by QESSN method.

The "white" beam of the neutrons from the reactor *1*, formed by the collimator *2*, falls on the crystal-monochromator *3*. As the monochromator the Zinc monocrystal is used. For creation of pulse flow of monochromatic neutrons the neutron beam with energy *E* reflected from Zinc monocrystal fell on the mechanical interrupter *4*. The pulse flow of monochromatic neutrons falls on the tested sample, located in the pod *5*. The possible changes of intensity of the monochromatic neutrons beam were fixed using the screen monitor *6*. The neutrons scattered by the sample under different angles were detected by the system of detectors *7*. The power spectra of scattering neutrons were measured by the flyover time simultaneously under several angles. The momentums from the registered neutrons strengthened by the preamplifier *8* and went through cathode followers on the inputs of vibrator units *9*. The formed impulses from the outputs *9* are given onto inputs of the hour analyzers *11* (analyzer ANDI-4096), and also on the units of common inventory *10*, that allows counting the full number of impulses from each detector.

The information on self-diffusion of water molecules can be received on investigating a widening of quasi-elastic peaks at different transferred momentums of a neutron, i.e. at different scattering angles. As in a spectrum of scattering neutrons there is no clear difference between a non-elastic part, related to collective motions of fragments in liquid, and quasi-elastic, related to individual diffusive motions, it is apparent, that the observed half-width of peaks ΔE should differ from real one. At large angles of scattering because of decreasing intensity of quasi-elastic peak the contribution of scattering, which is superimposed on quasi-elastic one, results in the increase of a half-width of a quasi-elastic peak.

The modern views of the self-diffusion process are based on hierarchy of time scales of molecular motion. During the processes of non-equilibrium system evolution it is possible to mention two main stages: quick – kinetic one, dealing with the quasi-equilibrium distribution of particles on coordinates and slow one - hydrodynamic, that is characterized by mean locally equilibrium values of the pulse energy density. In accordance to this it is possible to divide the self-diffusion coefficient into two parts: one-particle (Frencel) part and collective (Lagrange) part, which are formed accordingly at small $(\tau < \tau_0)$ and large $(\tau > \tau_0)$ observation times of a diffusing molecule (τ_0-the time of the same order as the shift viscosity relaxation one). At slow neutrons scattering in liquid the quasi-elastic dissipation, related to the energy exchange

of a neutron with diffusive degrees of freedom of a molecule, which results in the widening of monochromatic line that is a decreasing. The neutron during interaction process changes the momentum on $\Delta p = \hbar|q|$. From indeterminacy principle it follows, that the area, which one is sounded by a neutron, is characterized by the size

$$\Delta x \approx \frac{h}{\Delta p} = \frac{1}{|q|} \tag{11}$$

Selecting definitely conditions of neutron experiment, it is possible to see diffusive motions both at large, and at small spacing intervals according with the expression (11). Considering, that the shift of a molecule δx behind a temporary interval of observation t_0 is set accordance to stochastic Einstein formula $\left\langle \delta q^2 \right\rangle = 6Dt_н$, and identifying Δx with δ, we obtained:

$$t_0 = \frac{1}{6Dq^2}$$

Change of $\left(Dq^2\right)^{-2}$ corresponds to the time, which is necessary for a neutron to "experience" diffusion of a particle at the distance δx. Thus, for large values of q the time of overseeing for a diffusing molecule in a method of quasi-elastic scattering of slow neutrons is small and we observed basically a vibration motion of liquid molecules. At decreasing κ the time of overseeing for a molecule increase and neutron “feels” asymptotically diffuse behavior, as well as motion of molecules, similar to thus motion in a crystal.

Outgoing from hierarchy of temporary scales of fast one-particle motion and more slow collective motions, the widening of a quasi-elastic peak can be is shown as

$$\Delta E = \Delta E^f + \Delta E^l \tag{12}$$

where ΔE^f, ΔE^l are one-particle and collective the contribution in a widening of a quasi-elastic peak, respectively. Really, in the Oskotski model [4] for a widening of a quasi-elastic peak is obtained:

$$\Delta E = 2\pi D^l q^2 + \frac{2\hbar}{\tau_0}\left[1 - \frac{\exp\{-2W\}}{1+\left(D-D^l\right)q^2\tau_0}\right], \tag{13}$$

where first term is possible conformity diffusion of a “Lagrangian” particle, and second "Frencel"; τ_0 is a lifetime in an equilibrium state; $D - D^l = D^f$ is the “Frencel” contribution into a self-diffusion coefficient.

The separation of the one-particle and collective contributions in a self-diffusion coefficient on the basis of using of a method of a quasi-elastic neutron scattering is possible from the analysis of $\Delta E = \Delta E\left(q^2\right)$. At large values of a neutron transferred momentum from (13) it follows

$$\Delta E \approx 2hD^l q^2 + \frac{2\hbar}{\tau_0}\cdot \tag{14}$$

Thus, analyzing the relation $\Delta E = \Delta E\left(q^2\right)$ at large values of q^2, it is possible to determine the collective contribution to a self-diffusion coefficient and time of “no migratory” life τ_0. The total value of a self-diffusion coefficient *D* at small values q^2 can be retrieved from the formula

$$\Delta E = \frac{2\hbar}{\tau_0}\left[1 + D^l q^2\tau_0 - \frac{\exp(-2W)}{1+\left(D-D^f\right)q^2\tau_0}\right], \tag{15}$$

where $\exp(-2W)$ - the Debye-Waller factor, which one images oscillating motions of atoms (molecules) and is connected to intensity I_0 as follows

$$\exp(-2W) \sim I_0 \sim \exp(-x^2 q^2). \quad (16)$$

Here x^2 - mean square deviation of atoms from a final equilibrium position because of oscillations. At $q^2 \to \infty$ the formula (15) becomes

$$\Delta E(q^2) = \frac{2\hbar}{\tau_0} + 2\hbar D^l q^2 \quad (17)$$

whence

$$D^l = \frac{1}{2\hbar}\frac{\partial(\Delta E)}{\partial(q^2)} \quad . \quad (18)$$

At $q^2 \to 0$ from the ratio (17) it follows

$$\Delta E(q^2) = 2\hbar D q^2 \quad (19)$$

From which one follows

$$D = \frac{1}{2\hbar}\frac{\partial(\Delta E)}{\partial(q^2)} \quad (20)$$

Thus, the diffusion coefficients D, D^l, and D^f can be counted after the formulas (17), (18) and (19), if the experimental relation $\Delta E(q^2)$ is known.

The outcomes of experimental measurements of QESSN spectra of biological solution of membranes of lymphocytes of rat's thymus gland and spleen cells are represented in the Fig. 2. On the experimentally measured QESSN spectra we constructed relation $\Delta E(q^2)$, as shown in the Fig. 3.

Results and discussion

About usage of the obtained experimental relations $\Delta E(q^2)$ after the formulas (17)-(19) a coefficients D, D^l, and D^f and lifetime of atoms (molecules) were calculated in a vibratory state τ_0. Further these values were substituted in the formula (17) and by matching the computational relation $\Delta E(q^2)$, obtained from the experimentally obtained values in all the range of a neutron momentums, their corrected values are obtained and listed in the Table.

The purpose of this article was to research by means of the QESSN method the diffusion of water molecules in aqueous suspensions of lymphocyte membranes from the rat thymus gland and spleen cells. We investigated 3 various samples: a control sample, obtained from animals (rats) not exposed X-rays (Sample 1) and other two samples, obtained from rats exposed to a X-ray irradiation by doses 0.5 (Sample 2) and 1.0 Gray (Sample 3).

From Table it is visible that the diffusion constants in Samples 1-3 are less than the relevant values obtained for water. It is possible to explain this result as following. During the process of micelle formation the hydrophobic parts of lymphocyte membranes destroy the water structure and it results in a rupture of hydrogen bonds between water molecules. It results into decreasing a diffusion coefficient of water molecules in solution keeping the biological objects. It is possible to judge intensity of hydrogen bonds on quantity τ_0. The

decreasing τ_0 for Samples 2 and 3 demonstrates that in the result of influence of X-ray irradiation a collapse of lipid stratum of lymphocyte membranes takes place.

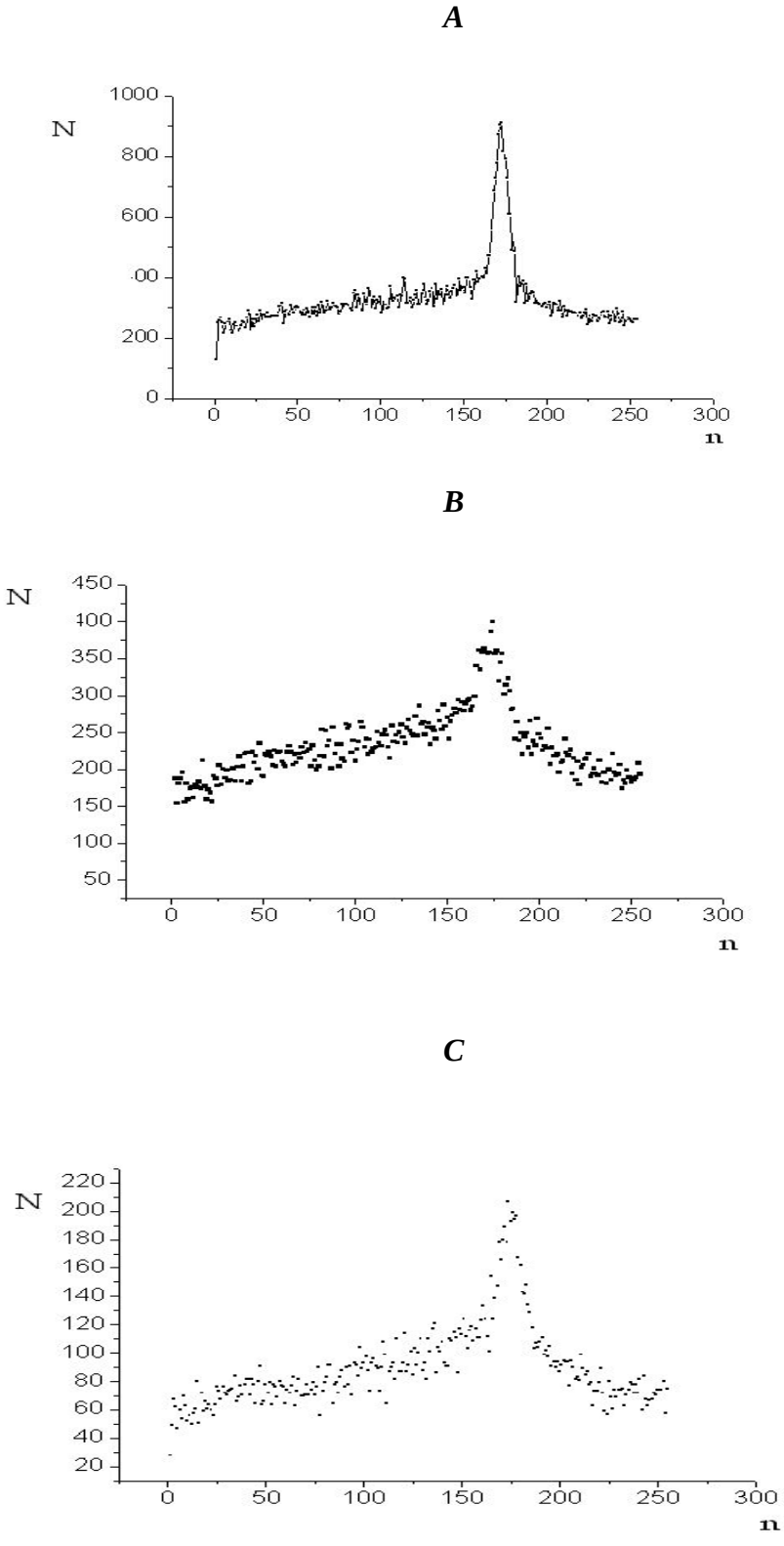

Fig. 2. Spectra of quasi-elastic scattering of slow neutrons for aqueous suspensions of lymphocytes: (*A*) - Sample 1, (*B*) – Sample 2, and (*C*) – Sample 3.

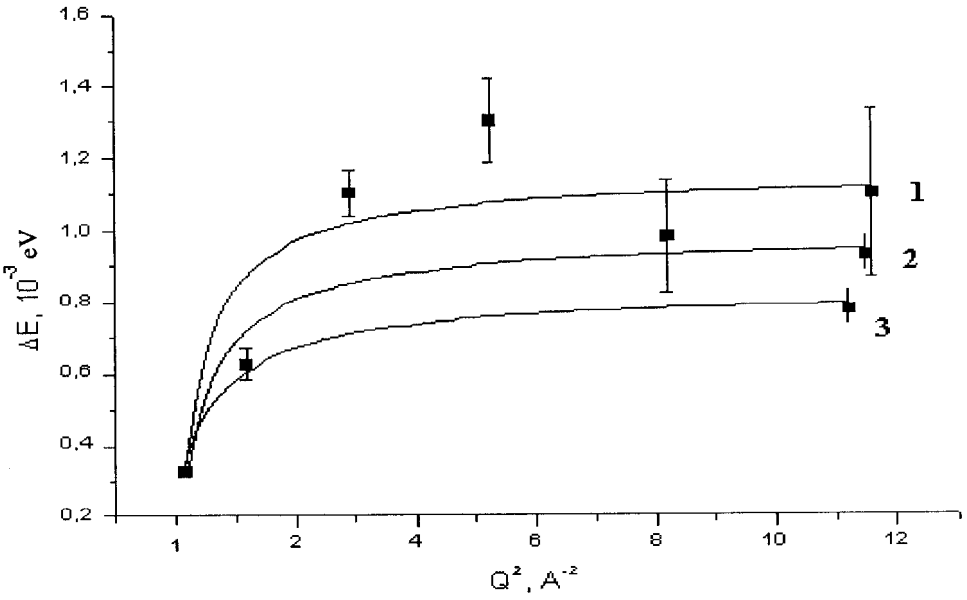

Fig. 3. $E\left(q^2\right)$-Dependencies for aqueous suspensions of lymphocyte membranes: (■) - experimental values, continuous lines (*1-3*) - calculated with the formula (19) for Samples 1, 2, and 3, respectively.

Table. Values of diffusion coefficients.

Sample	Diffusion coefficients, $\times 10^5$ cm^2/s			τ_0, $\times 10^{12}$ s
	D	D^l	D^f	
1	1.71404	0.00029	1.714	1.52904
2	1.47390	0.03979	1.438	1.45050
3	1.37130	0.01330	1.358	1.34870
H_2O	2.23	0.46	1.770	2.8

Thus, we can make a conclusion that under influence of X-ray irradiation of animals (rats) a partial destruction of lymphocyte membranes takes place. It is primarily true for lipid shell of membranes. At small doses (0.5 Gray and less) irradiation may has an appreciable effect, but as the dose increases (0.5 Gray and more) its influence becomes irreversible.

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