WATER AND EFFECT OF BIOLOGICALY ACTIVE SUBSTANCES IN ULTRA LOW CONCENTRATIONS.

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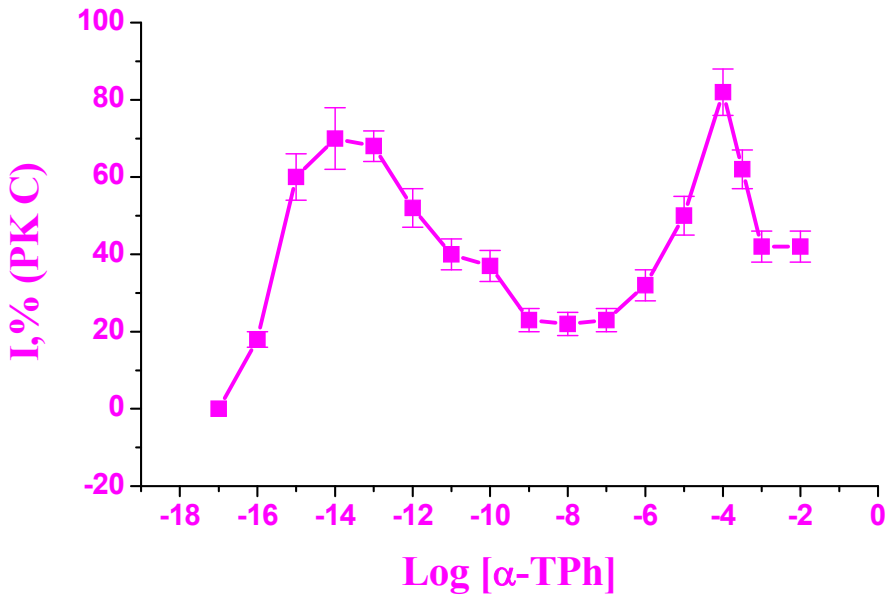
INTRODUCTION

- **Observation of biologically active substances (BAS) effect in ultra - low concentrations (10^{-22}-10^{-15}M) on the living systems with different complicity is one of the most impressive discoveries of the last decades.**

- **The observed effect was investigated using a number of BAS: antioxidants and anti-metastatic agents, radioprotectors, inhibitors and stimulators of the growth of plants, neurotropic compounds of different classes, hormones, adaptogens, immunomodulators, detoxicants, peptides, etc. At the moment more than 100 compounds have demonstrated this property.**

THE CHARACTERISTIC FEATURES IN THE EFFECT OF BAC IN ULTRALOW CONCENTRATIONS.



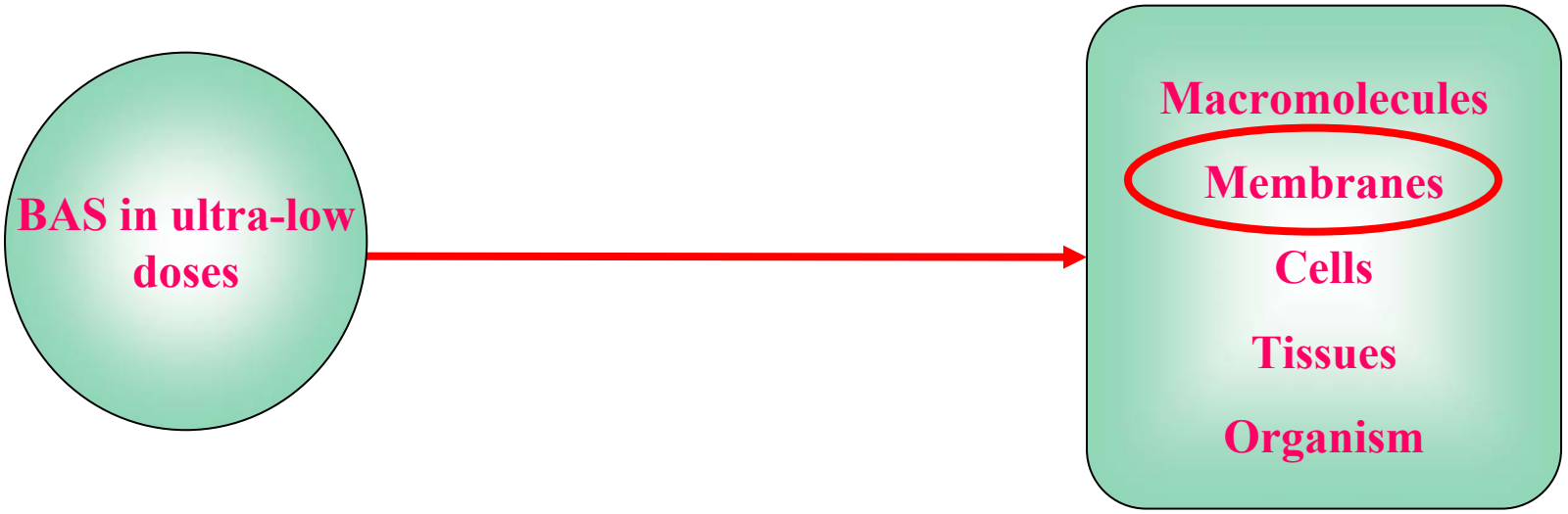
Inhibition of Protein Kinase C Activity in Dependence on α-Tocopherol Concentration.

Pal'mina N., Mal'tseva E., Kurnakova N.,Burlakova E. Biochemistry, 1994

1. **Nonmonotonous, nonlinear polymodal concentration-effect dependence. In most cases, the maximal activity is observed at certain concentration ranges, separated from one another by so-called "dead area", where the effect is not expressed.**
2. **Changes in the sensitivity (usually an increase) of biological objects to various agents (both endo- and exogeneous).**
3. **Manifestation of kinetic paradoxes, i.e. the possibility of detecting the effect of BAC at ultralow concentrations when the same compound is present in the cell or organism at the concentrations that exeed the ultralow concentration by several orders of magnitude.**
4. **"Segregation" of properties of BACs upon a decrease in its concentration, when its activity is still retained, but side effects disappear.**

At the first stage, the main task of the researchers was to prove the truth of having the effect of ultra-low doses in various biological systems.

• At the moment the level of biological organization on which the effect of ultra-low doses (ULD) of BAS became evident, also greatly different – from macromolecules, cells, organs and tissues to animals, growing organisms and even populations.



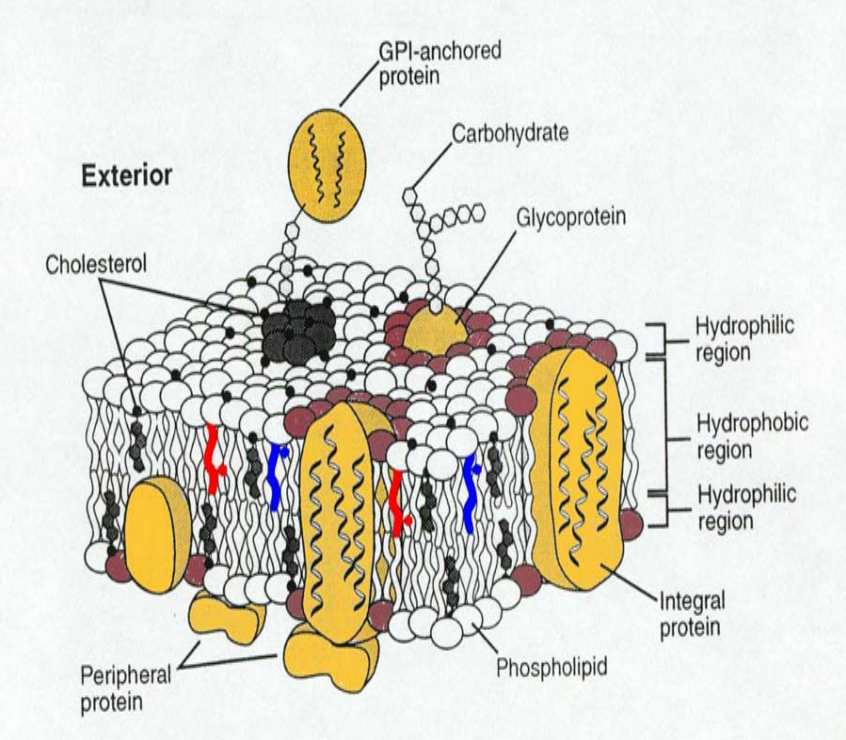
Ashmarin et all, 1999; Burlakova et all, 2003;2004

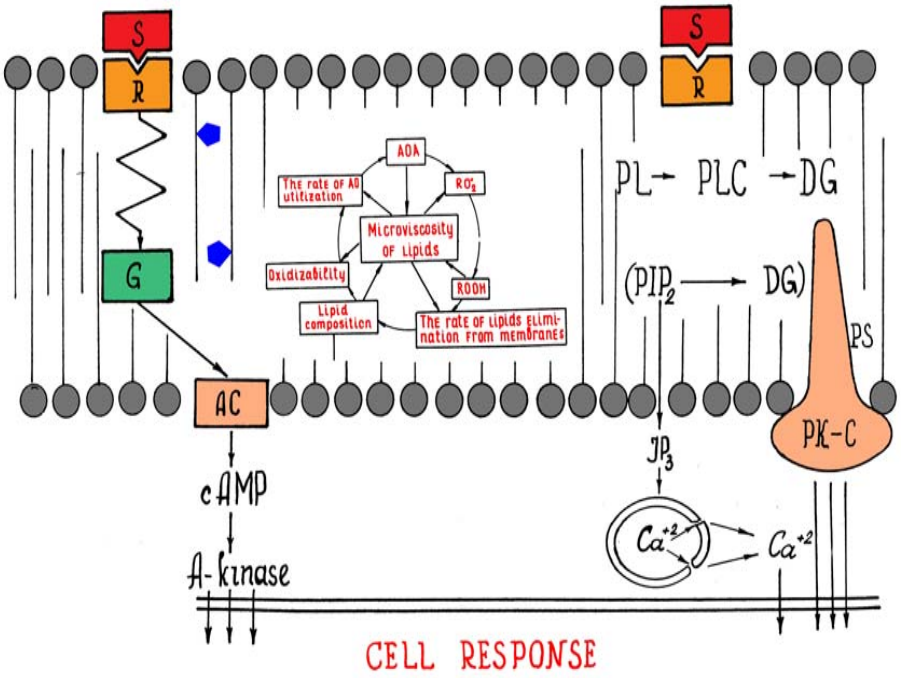
From our point of view the study of this phenomena on relatively simple models is the most of interest because in these cases we can exclude the complicated interconnection and influence of different metabolic ways and reactions on each other.

We have used as a model for our research isolated cell membranes and carried out our experiments *IN VITRO* .

WHY ?

CELL MEMBRANES AS A TARGET OF BIOLOGICALY ACTIVE SUBSTANCES IN ULTRA-LOW DOSES.





THREE THE MOST IMPORTANT CONTROL SYSTEMS ARE LOCALIZED IN THE CELL MEMBRANES : CYCLIC NUCLEOTIDES, PHOSPHOINOSITOL CYCLE AND LIPID PEROXIDATION. CROSS-TALKS TAKE PLACE BETWEEN THEM.

LIPID PEROXIDATION,

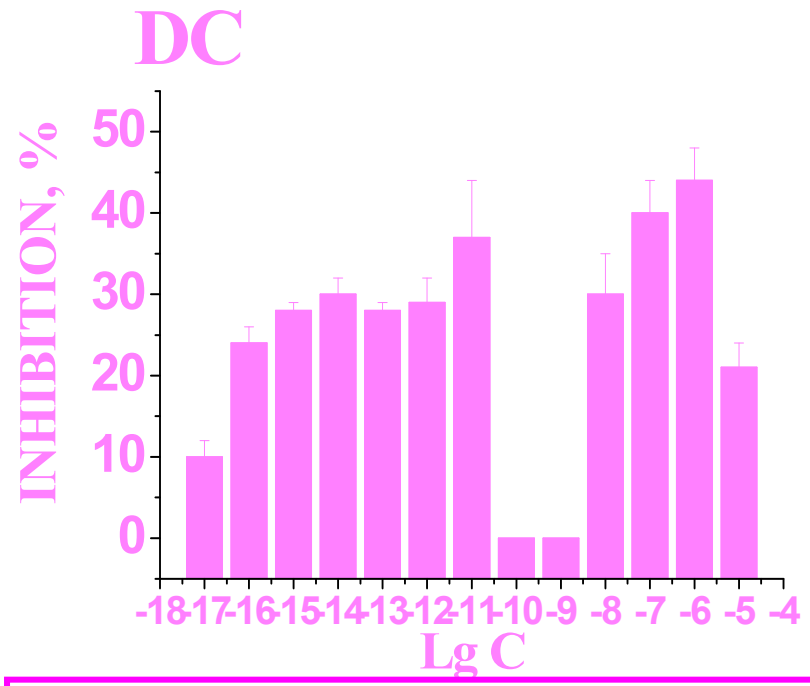
STRUCTURE OF DIFFERENT LIPID REGIONS OF CELL MEMBRANES,

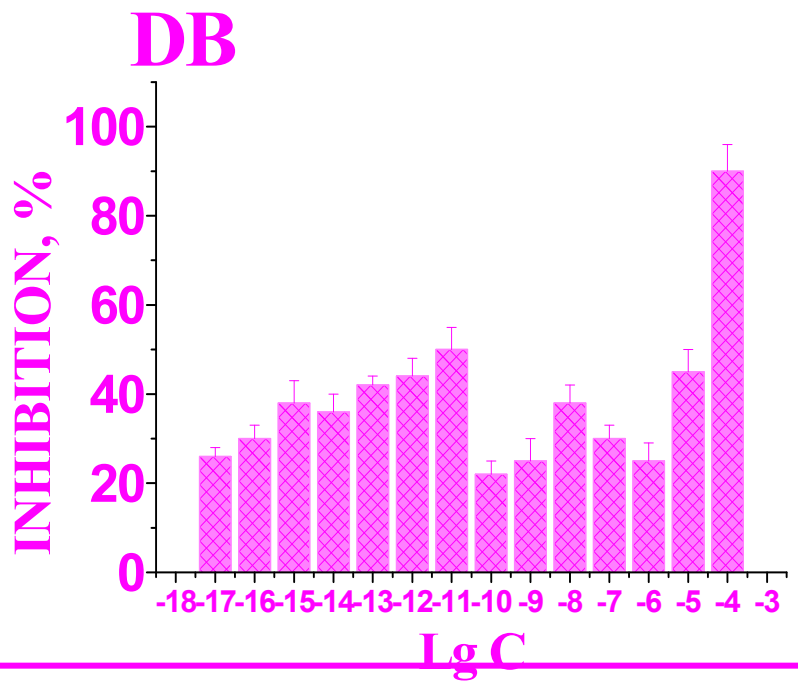
ACTIVITY OF MEMBRANE-BOUND ENZYMES

HAVE BEEN STUDIED UNDER THE EFFECT OF DIFFERENT BAS IN A WIDE RANGE OF CONCENTRATIONS.

(α-tocopherol; potassium salt of β-4-hydroxy-3,5-di-tret-butylphenyl-propionic acid; thyroliberin; phorbol esters)

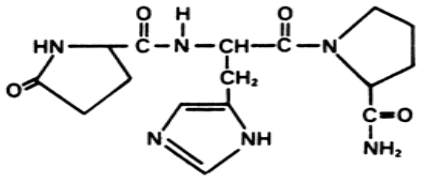
EFFECT OF SYNTHETIC ANTIOXIDANT (PHENOZAN) ON INITIATED LPO IN MICROSOMAL MEMBRANES.

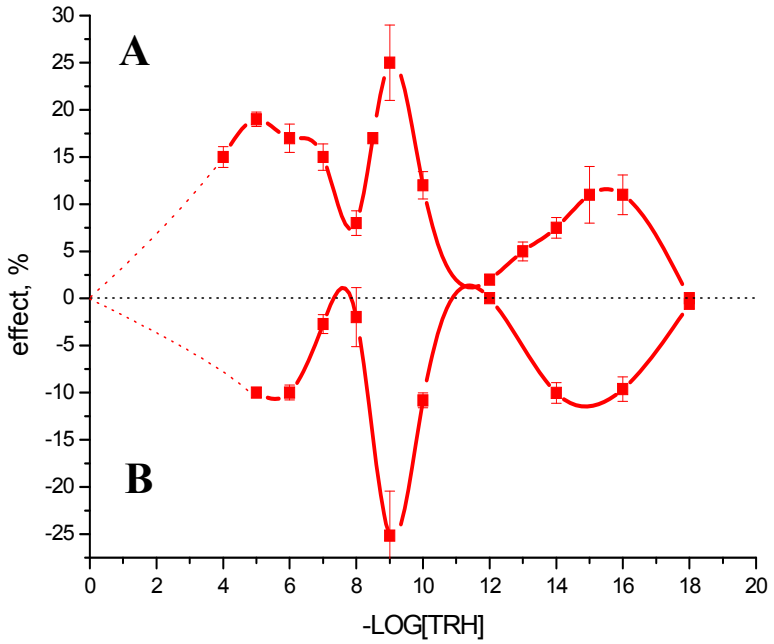




- PENOZAN shows antioxidative effect on the model of initiated LPO estimated on the basis of dienic conjugates (DC) formation and total content of double bonds (DB) decreasing.
- DEPENDENCE CONCENTRATION - EFFECT HAS NONLINEAR POLYMODAL CHARACTER.
- Pal'mina N. et all, 2004

Effect of thyroliberin (TRH), a small neuropeptide (p-Glu-His-Pro-NH_2 in a wide range of concentrations on lipid microviscosity of plasmatic membranes *IN VITRO*.





Change in the of rotary correlation time τ_C (% to control) of spin probe 16-DSA in plasmatic membranes of liver (A) and brain (B) cells in dependence on TRH concentration. Probe concentration of $5x10^{-5}$ M.

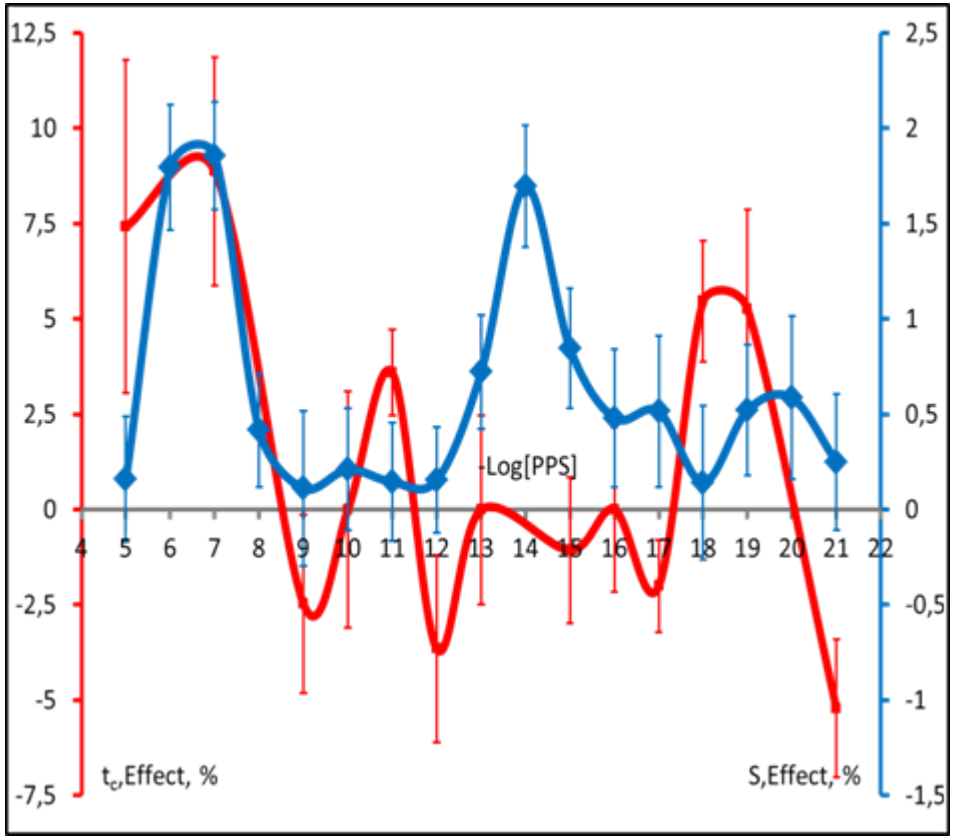
Zhernovkov V., Pal'mina N., Bull ExpBiolMed, 2006

· TRH in the 10^{-4}-10^{-18}M dosage interval results in unspecific total change in the structural properties of the lipids in the plasmatic membranes isolated from the liver and brain cells. The TRH concentration – effect dependence is nonlinear and polymodal.

· TRH causes increasing the of the lipid microviscosity in the area of probe C_{16} (20-22Å) localization in the liver plasmatic membranes and decreasing of τ_C in the brain membranes.

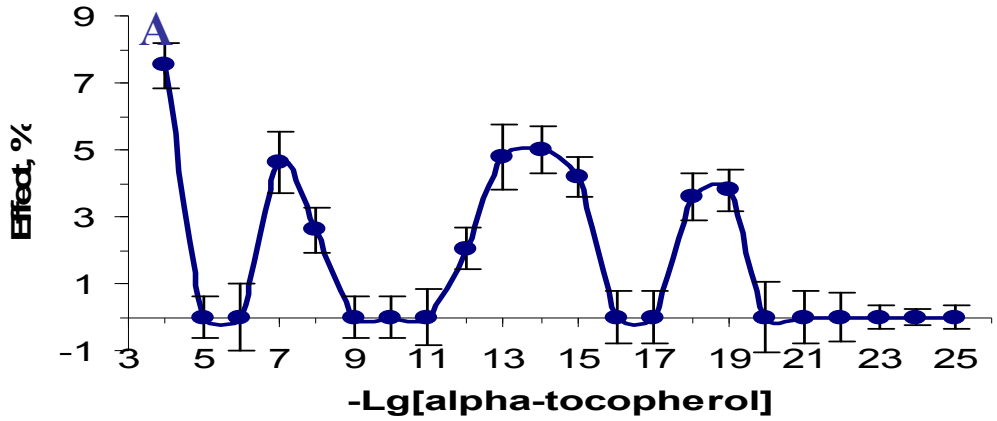
· Used in ultra – low concentrations (10^{-14}-10^{16}M) TRH had the same effect on plasmatic membranes as after introduction in "physiological" doses (10^{-4}-10^{-6} M).

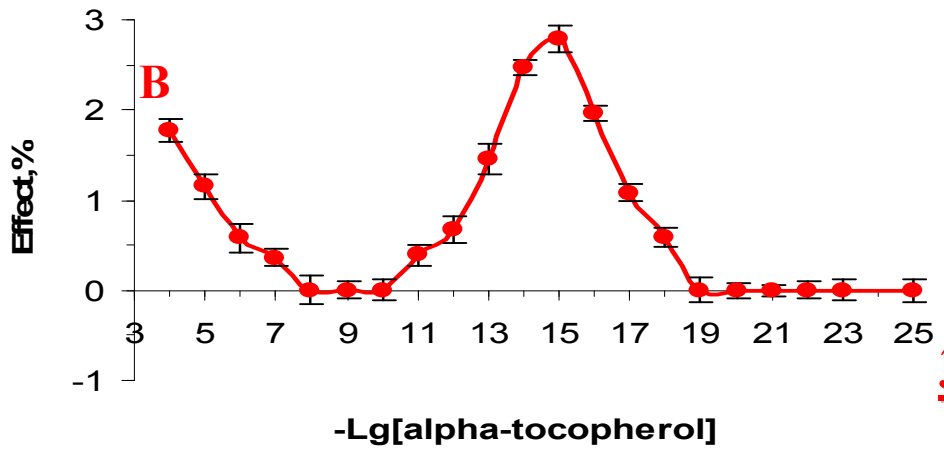
Dose dependences of the effect of PPS on the microviscosity (tc, red curve) of deep areas and rigidity (S, blue curve) of surface areas of plasmatic membranes.



Chasovskaya T., Mal'tseva E., Palmina N., Biophysics, 2013

EFFECT OF α-tocopherol ON LIPID BILAYER STRUCTURE IN PLASMATIC MEMBRANES.





Belov V., Mal'tseva E., Pal'mina N., Biophysics, 2011

MECHANISMS OF α-tp EFFECTS:

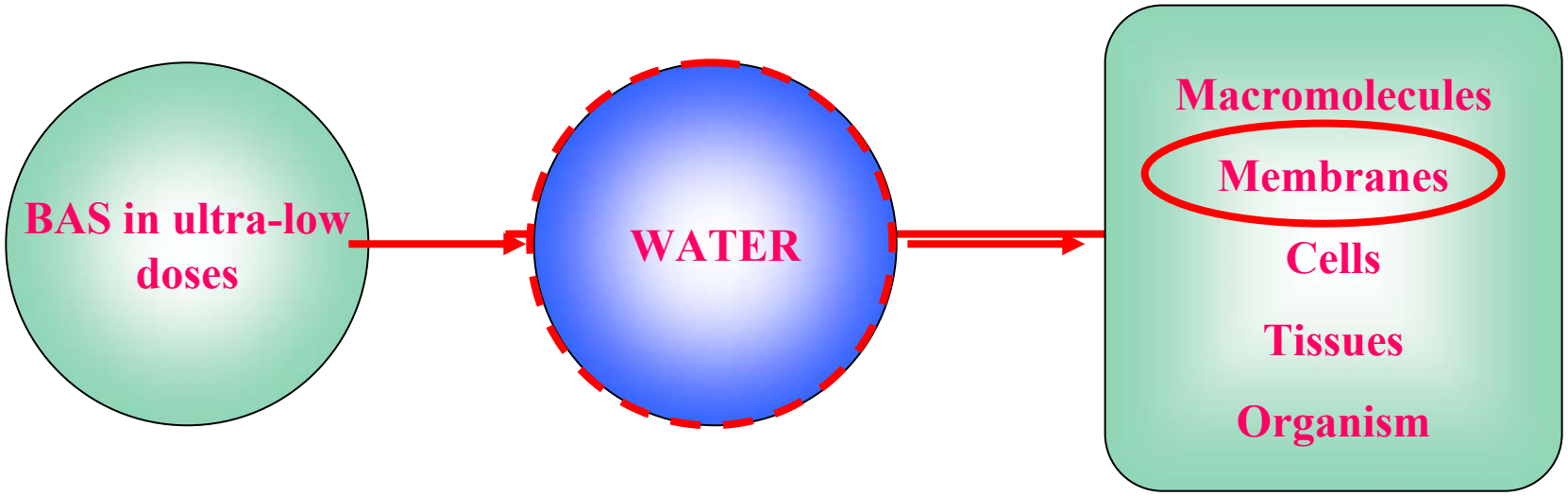
1. **The area of physiological concentrations (10^{-4}M-10^{-9}M)** – restriction of conformational mobility of lipids as a result of α-tp incorporation into the membrane;

2. **The area of ultra low doses(10^{9}M-10^{17}M)** – specific interaction with binding sites on the membrane (e.g. protein kinase-C (PKC), or inducing by α-tp formation of microdomains in the membrane (e.g. rafts, indirect evidence is the appearance of additional transition at physiological temperature);

3. **The area of "apparent" concentrations (<10^{-17}M)-**

?

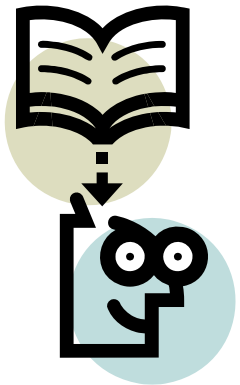
STRUCTURE OF WATER AS A TARGET OF ULD EFFECTS.



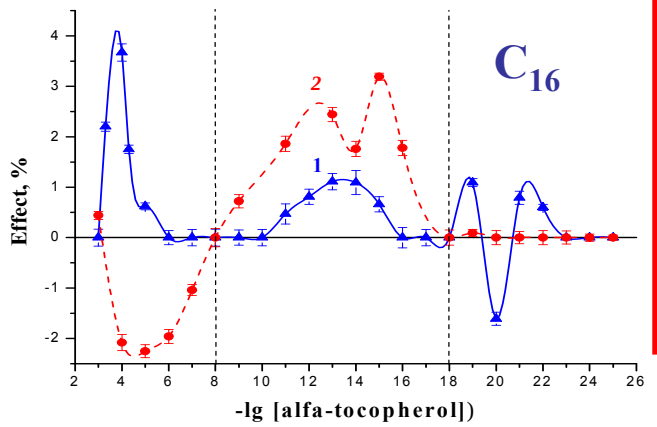
✓ A set of investigations of diluted BAS aques solution properties using IR-spectroscopy methods was carried out [Zubareva, Kargapolov,2003; Fesenko, Terpugov, 1999; Yamskov, Yamskova, 1999; Havinson, 2003]. All of them have observed the instability of the spectrum parameters of water thin layers during the time, for example, transmittance fluctuations in IR spectrum of water.

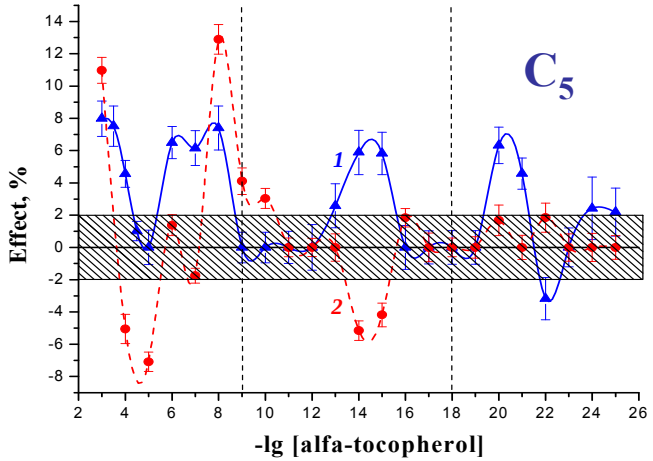
✓ The physical basis of the process can be connected with formation of a huge regular clusters (~1 mkm), [Smirnov, Siroeshkin,2003]. Such kind of fluctuations can be one of the characteristic feature of water dynamics and structure under the certain conditions and different effects, for example BAS.

IS IMPORTANT THE SOLVENT POLARITY IN EFFECT OF ULD?



ROLE of SOLVENT POLARITY in the MECHANISM of ACTION of BAS in ULTRALOW CONCENTRATIONS.





The effect of α-TP in polar (1) and nonpolar (2) solvents on the order parameter – S of spin probe C5 and microviscosity value - τc of spin probe C16 localized in the surface lipids (a) and hydrophobic lipid regions (b) of membranes correspondingly.

THERE ARE 3 AREAS OF CONCENTRATIONS ON THE CURVES:

PHYSIOLOGICAL CONCENTRATIONS (10^{-4}-10^{-9}M);

ULTRA-LOW DOSES (10^{-9}-10^{-18}M);

"APPARENT" CONCENTRATIONS > 10^{-18}M

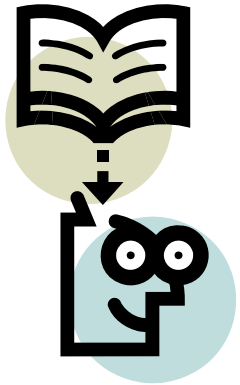
CONCLUSIONS:

1). α-TL exhibits its effect using both as in polar as in nonpolar solusions in the areas of physiological and ultra low concentrations.

2) only aqueous solutions of α-TL were effective at the area of "apparent" concentrations and shown a significant effect on the structural parameters of membrane lipids.

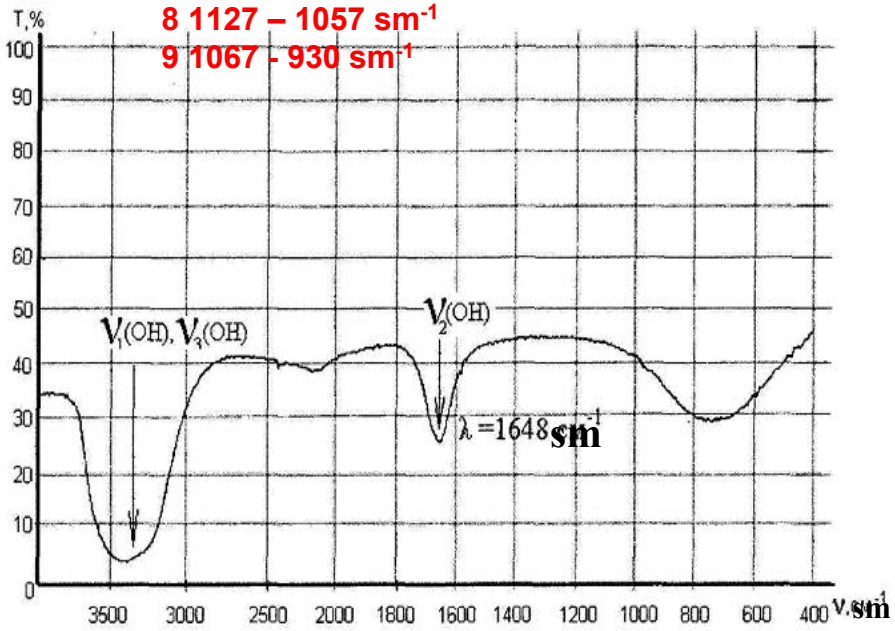
Belov V., Mal'tseva E., Pal'mina N., Burlakova E., DAN 2004

SOLVENT POLARITY HAS A PRINSIPAL IMPORTANCE IN MECHANISM OF TRANSMISSION OF "INFORMATION" ABOUT COMPOUNDS.



The Study of BAS solutions in the middle IR-spectrum range (800-3500sm^{-1})

1 3500 - 3200 sm^{-1}
2 3085 – 2832 sm^{-1}
3 2120 – 1880 sm^{-1}
4 1710 – 1610 sm^{-1}
5 1600 – 1535 sm^{-1}
6 1543 – 1425 sm^{-1}
7 1430 – 1210 sm^{-1}
8 1127 – 1057 sm^{-1}
9 1067 - 930 sm^{-1}

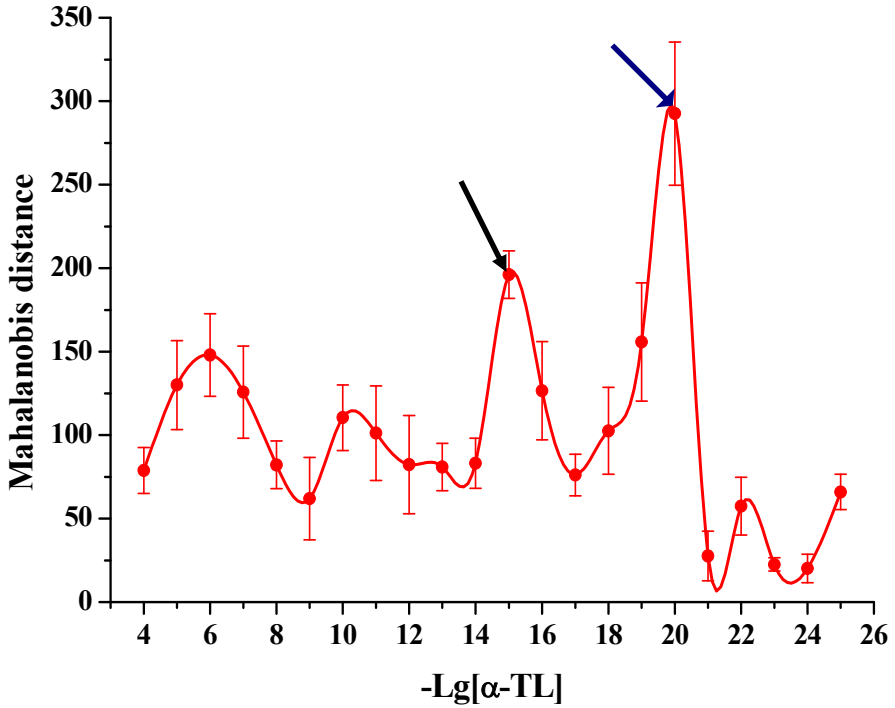


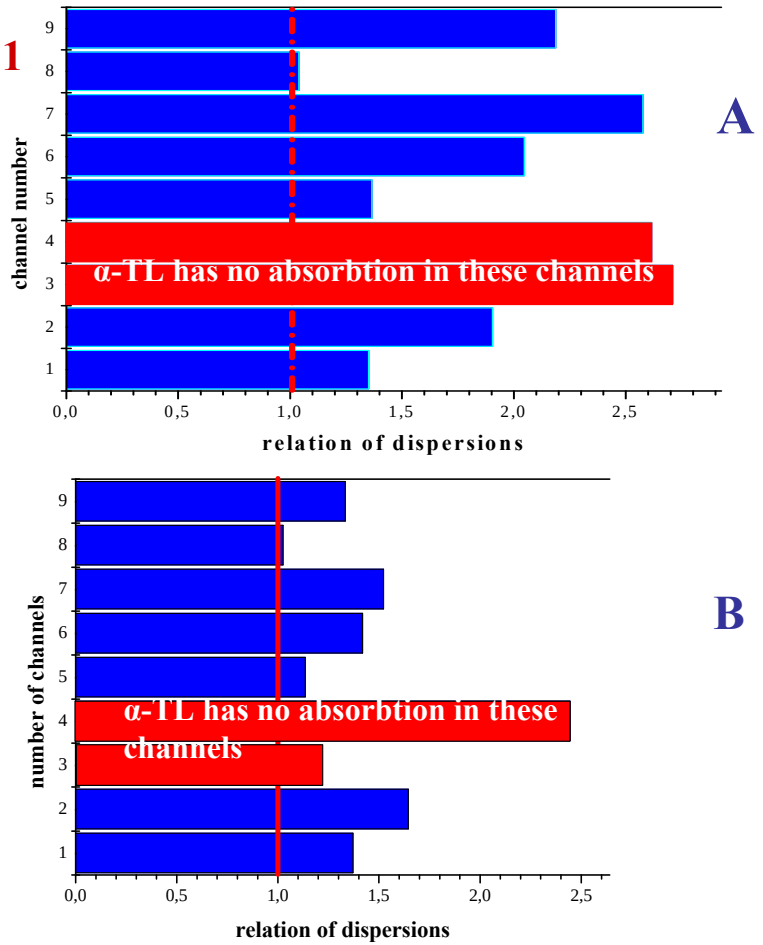
The spectrum of water in the middle range of IR – irradiation.

We have used for our research IR-spectrometer IKAR, new programmed hardware set designed in Tver Medical Academy. This set allows quick quantitative determination of transmittence fluctuantions in nine ranges of IR spectrum. The record rate is 1 measurment by 9 channels per second. The range width was defined by optical parameters of the appropriate interference filter. The measurement was carried out in cuvettes from KRS-5 20 μm thick. For IKAR system, the measurement error of transmittance is below ±0.3%.Experimental conditions gave us possibility to determine the transmittence fluctuations which are connected in accordance with literature data with the specific changes in water clusters of studied solutions.

The influence of α-TL in different concentrations (10^{-4}M – 10^{25}M) on the structure of water estimated by Mahalonobis criterion.

Belov V.,Belyaeva I., Zubareva G.,Pal'mina N., DAN, 2011



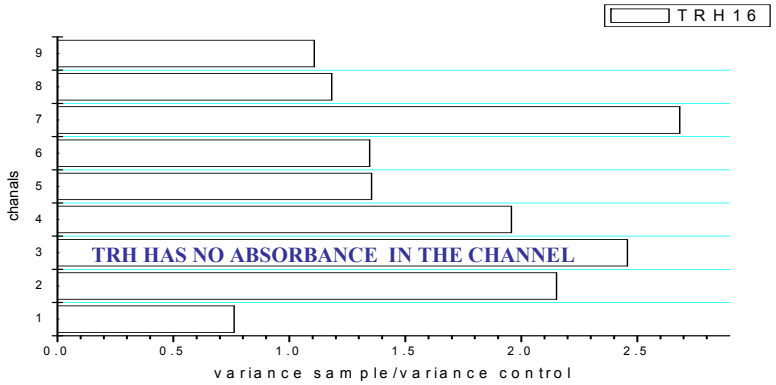


The significant changes in the structural parameters of water have been observed under the effect both as ULD(10^{-14}-10^{-16}M) as "apparent"(10^{-18}-10^{-21}M) α-TL concentrations which induced the great deviations in cell membrane structure.

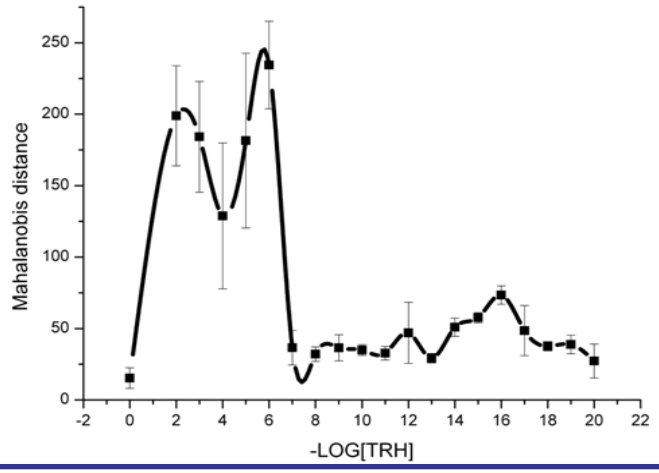
<u>These data confirmed our suggestion concerning the role of water in ULD effect.</u>

Dispersions of transmission coefficients of α-TL solutions : 10^{-15} M (A) and 10^{-20}M (B) in relation to etalon in 9 ranges of spectrum.

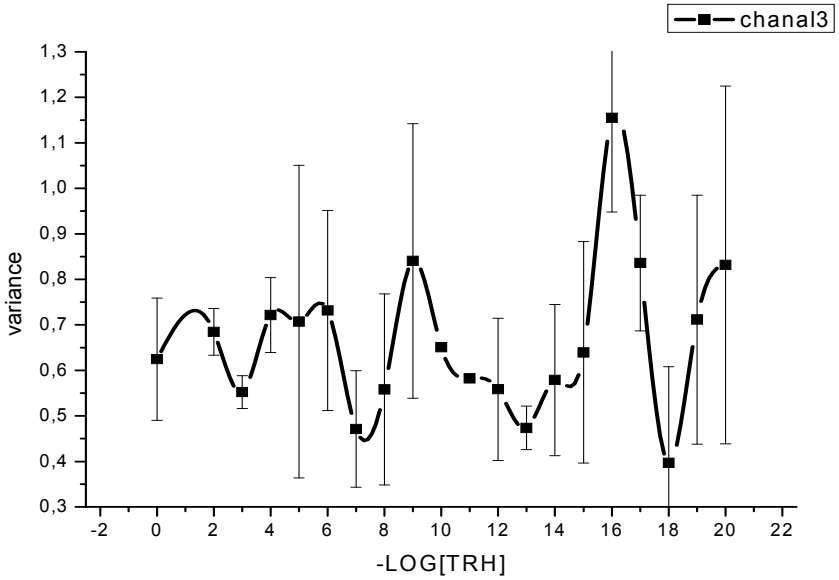
THE STUDY OF ULTRA –LOW TRH CONCENTRATION.



Ratios of dispersions for TRH solution in concentration 10^{-16}M.



Changes in Mahalanobis distance in dependence on TRH concentrations.

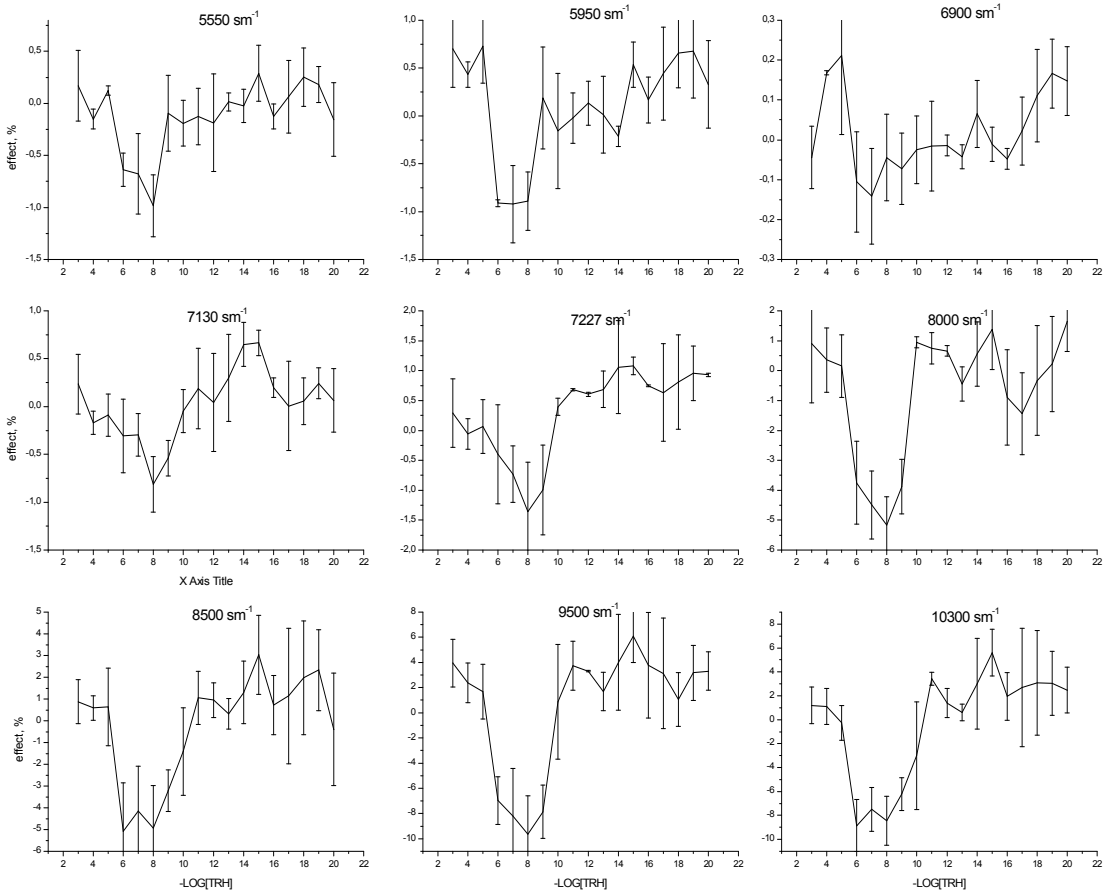


The changes of dispersion relatios for channel 3.

ULD of TRH (10^{-16}M) induced a big changes in Mahalanobis distance, ratios of transmittance coefficient in channel 3 and its dispersions

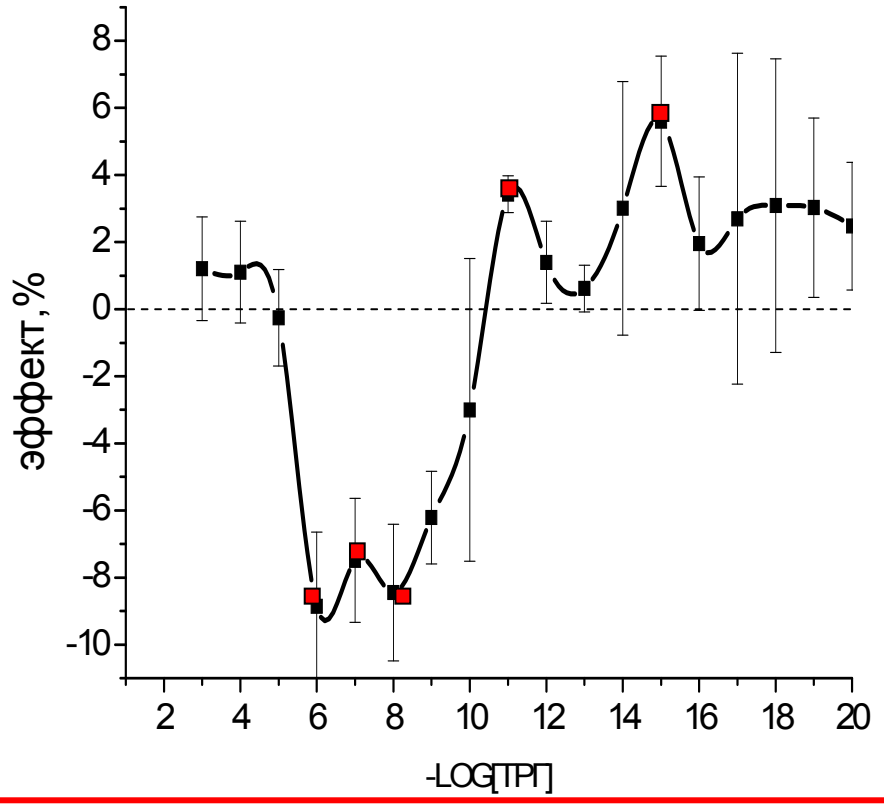
Zhernovkov V., Roschina I.,Zubareva G.,Pal'mina N., Water,2010

The changes of absorbance coefficient in dependence on TRH concentration in water solution for 9 chosen regions 5550, 5950, 6900, 7130, 7227, 8000, 8500, 9500, 10300 см-1, where the maximal deviations have been found. The effect was calculated by the formula:[(absorption index for TRH solution) / (absorption index for control sample – 1)] x 100.



Zhernovkov V., Lokshin B., Pal'mina N., in press

The changes of absorption coefficient in dependence on TRH concentration for the frequency 9500 $см^{-1}$



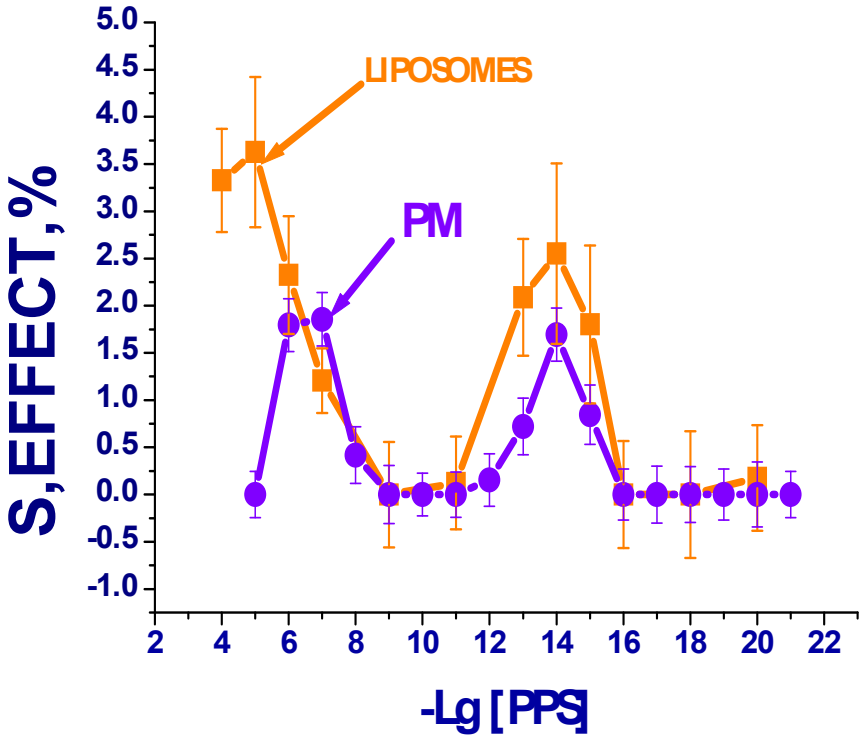
TRH itself had no absorption in the near IR-irradiation. We can conclude that observed effect is due to the structure of water under TRH action.

For all spectrum regions, the effect dependence on TRH dose is nonlinear with the minimum for high doses and general return to the control level for low ones, except of TRH concentration of 10^{-15}M, which is statistically different from the effect of higher doses. For high TRH concentrations (10^{-6}-10^{-9}M) causing a strong impact on absorbtion index and reducing it down to 10%, the greatest quantitative changes are observed.

By amplitude of changes, 2 bands are separated: 9 500 and 10 300 sm-1, where the absorbtion index changed from minus 10% for high TRH doses (10^{-6}-10^{-9}M) to 5% for 10^{-15}M.

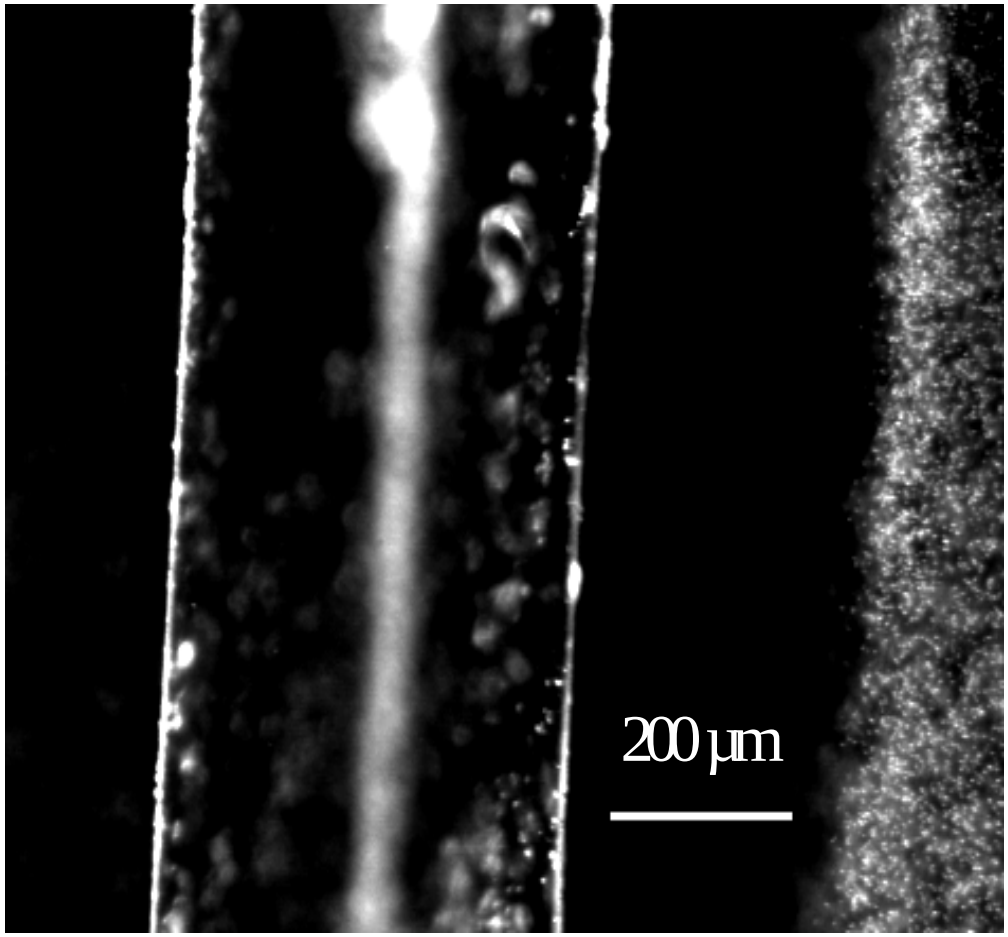
Effect of PPS on the surface lipid area of the PM and liposomes (probe C5, the parameter S)

Chasovskaya T., Plaschina I., Pal'mina N., DAN, 2013



There are the next special features of the curve for the liposomes compared with the curve for the PM: the absolute value of the effect of PPS on liposomes was or equal to the effect on the PM (in the range of SMD), or superior to its more than 1.5 times (in the range of high concentrations), a maximum of "physiological" concentrations shifted to their increase, which may be associated with PPS interaction with other components of the membrane, which is not in the liposomes. In the range of ULD maxima of the parameter S in the PM and the liposomes almost completely coincide. In our view, correspondence received by the dose dependency for the effects of PPS on the PM and the liposomes, especially the coincidence of maximum in the range of SMD shows that PPS acts directly on the lipid component of the membrane.

Lipids are the primary target of the PPS.

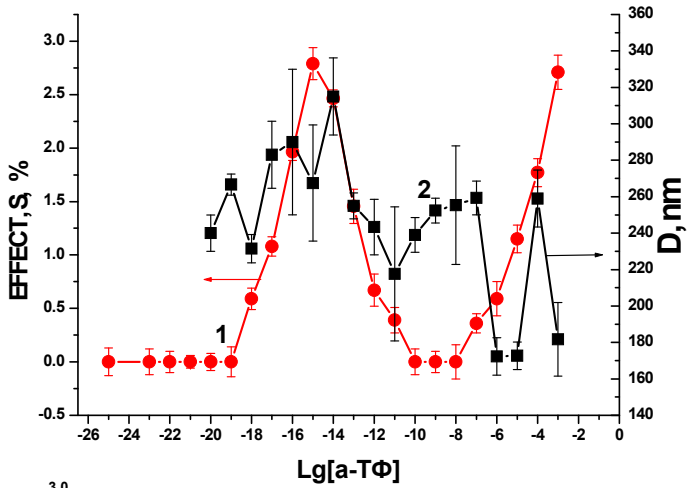


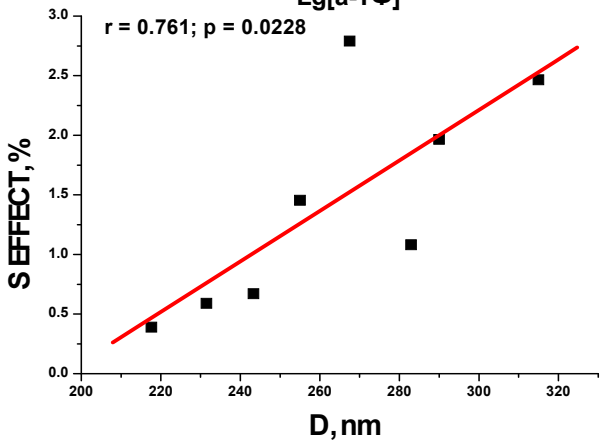
As one of the mechanisms of the effect of BAS in the ULD, some authors consider the transfer of information through the water system [Zubareva, Fesenko, Yamskov, Lobyshev, Voeikov]. Previously, we also found that the effect on biological membranes of certain active substances (tocopherol, thyrotropin-releasing hormone) in the ULD was correlated with the properties of aqueous solutions of biologically active substances in the infra-red spectral region, using solutions of tocopherol in vaseline oil effect of the drug in the ULD would disappear. In the studies Pollack develop a view of the presence of hydrophilic surfaces and particles, which can be considered as a membrane, and "surface" layers of water, differing from the "bulk" water on the viscosity, density, dielectric constant and conductivity.

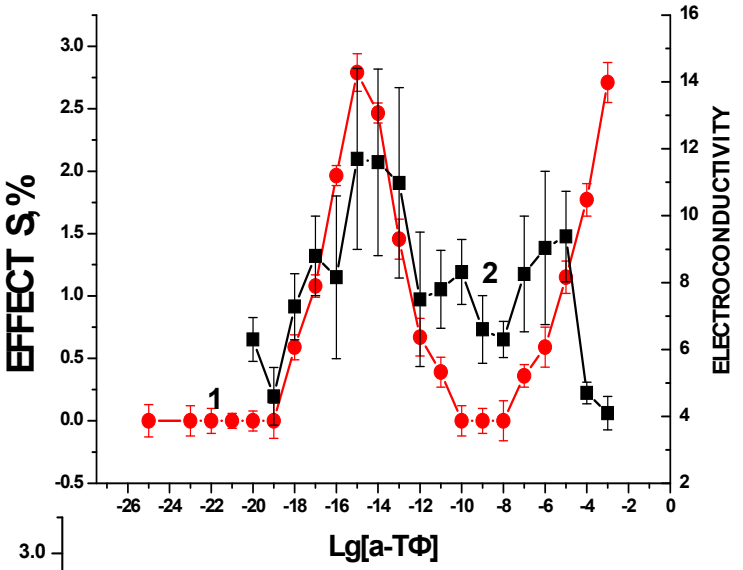
Academician AI Konovalov and colleagues found that many biologically active substances formed in aqueous solutions nanoassociates of about 200 nm, and the concentration dependence of the size and nanoassociate conductivity solutions are polymodal and interconnected.

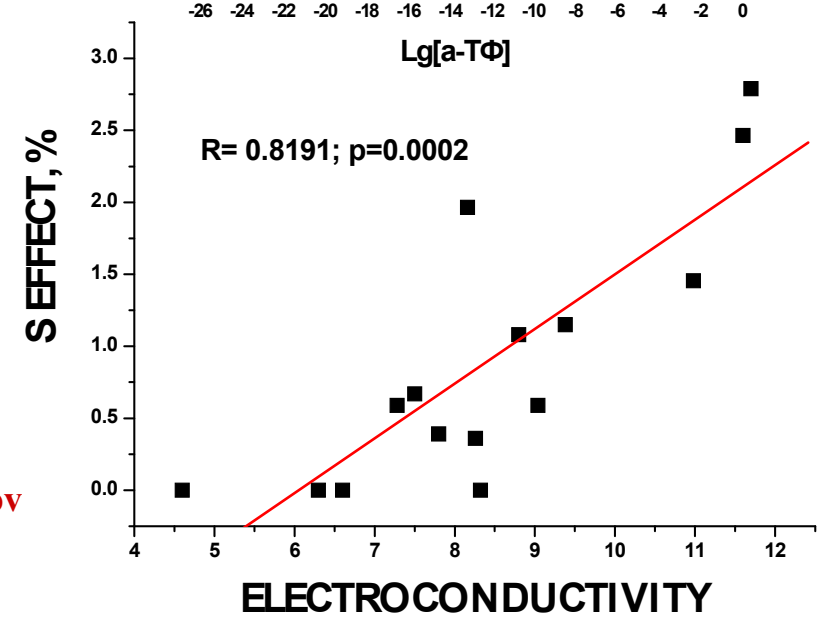
Experiments with dilute solutions of PPS carried out under the direction of A.I.Konovalov and I.S. Ryzhkina showed that, indeed, an antioxidant creates with the water molecules nanoassociates. Size and their charge, as well as the electrical conductivity of solutions have nonlinear change depending on the concentration of solutions.

Relationship between the change in the order parameter (*S*) of lipids in the surface areas of the lipid bilayer of plasma membranes vs. diameter of nanoassociates and specific electrical conductivity of α-tocopherol solutions in the concentrationrange of 1×10^{-3}–1×10^{-20} mol/L.



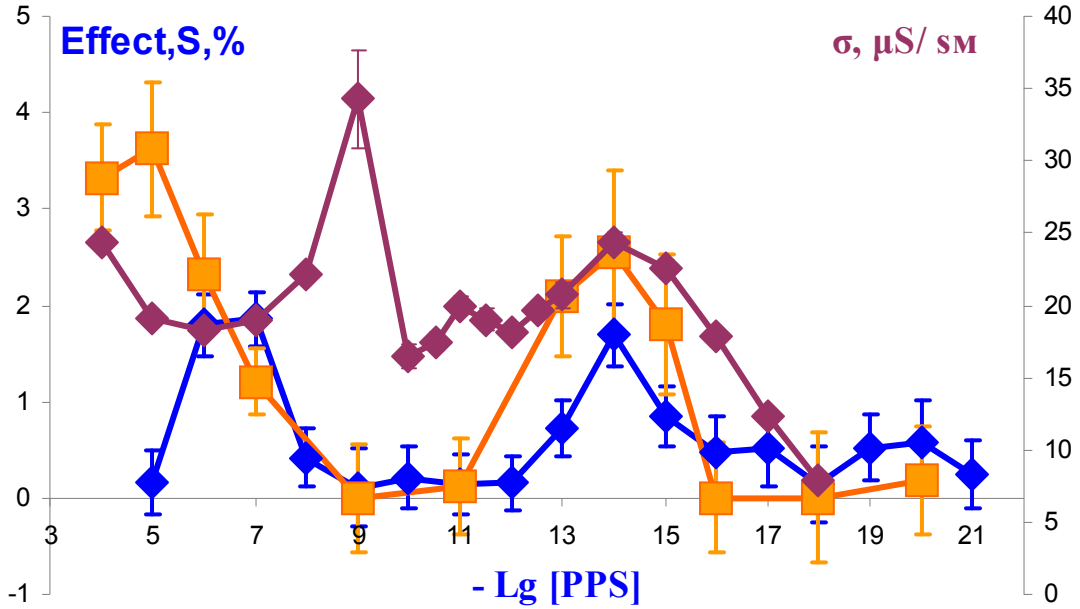


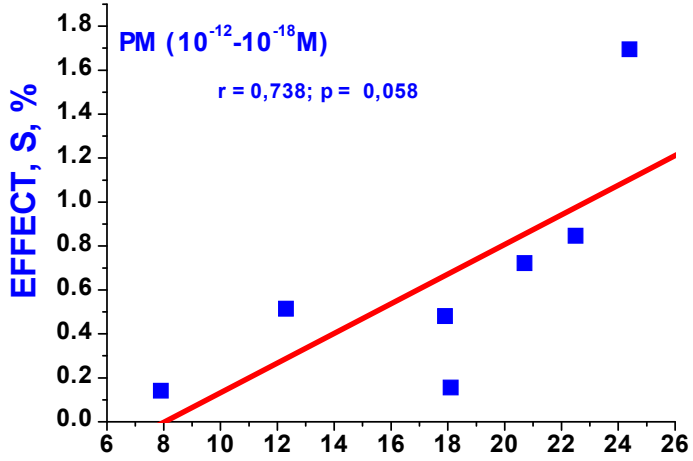


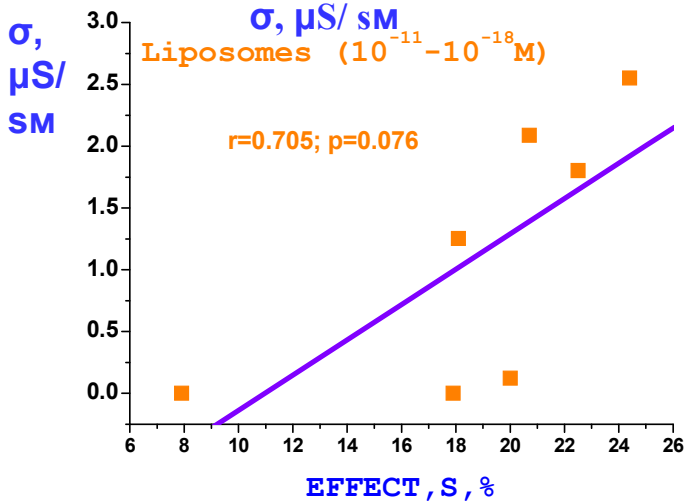


Ryzhkina I., Kiseleva Yu, Murtazina L., Pal'mina N, Belov V., Mal'tseva L.,Sherman E., Timosheva A., and Konovalov A., DAN, 2011

Comparison of data on the electrical conductivity of PPS dilute solutions and their influence on the structure PM and liposomes.



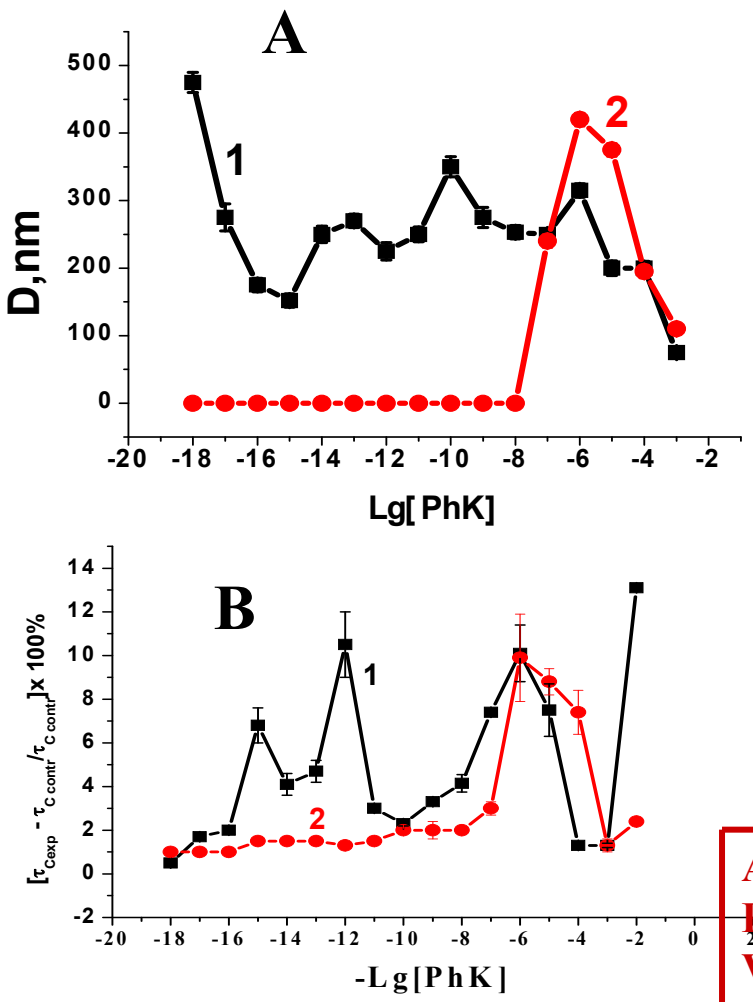




In the range of SMD found a statistically significant correlation between changes in the conductivity of dilute solutions of PPS and their influence on the surface layers of lipids in the PM and in liposomes.

N. P. Palmina, T. E. Chasovskaya, I. S. Ryzhkina, L. I. Murtasina, and A. I. Konovalov, DAN, 2009

Influence of External Electromagnetic Field on the Self-organization of Solutions with Low Concentrations of the Antioxidant of Potassium Phenozan and their Effect on the Microviscosity of the Lipid Membranes in Vitro



NO MAGNETIC FIELD –

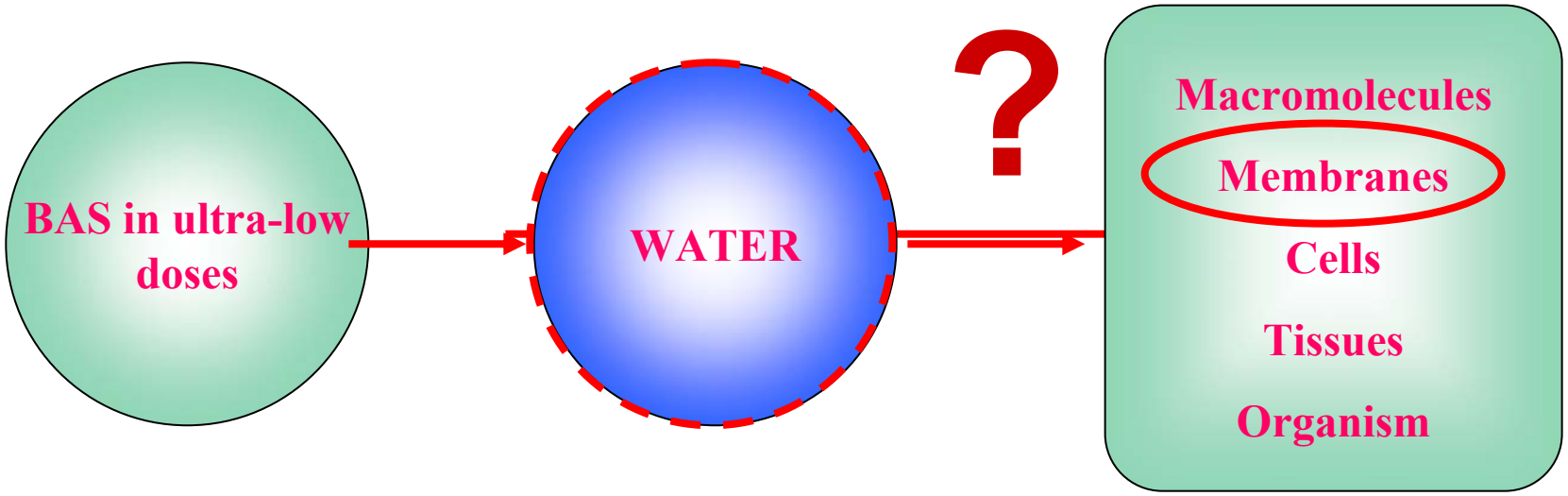
NO NANOASSOCIATES –

NO BIOLOGICAL EFFECTS.

Alexander I. Konovalov, Elena L.Mal'tseva , Irina S. Ryzhkina*, Lyaisan I.Murtazina, Yuliya V. Kiseleva, Valery V. Kasparov, Nadezhda P. Pal'mina, in press

The concentration dependence of changes in the size (D) nanoassociates formed in the PP solutions (A) and the membrane lipid microviscosity of the synaptosomes (B) using dilute the PP prepared under common conditions (curves 1) and kept in the container permalloy (curve 2) for 24 hours.

ULD EFFECT.



1. We know what happens in cell membranes.
2. We know what happens in diluted solutions.
3. We don’t know the way of nanoaccociates interaction with cell membranes. We shall do it.