Introduction Melafen is a plant growth regulator - heterocyclic organic phosphor compound, synthesized at the A. E. Arbuzov Institute of organic and physical chemistry of RAS. Melafen is the melamine salt bis (oximethyl) phosphinic acid. It was acquired by one stage with high stepping out of industrially available products [1]. Melafen is a hydrophilic poly functional substance. Melafen raises the plants stress-resistance under the bed environment - in the conditions of overcooling and drought. Melafen increases the effectiveness of energy metabolism. At these cases Melafen changes of the fatty acid composition and the microviscosity of microsome and mitochondrial membranes in vegetable cell [2, 3]. But there are the great concentration limitations, when Melafen application. Large concentrations of Melafen cause the immediate death of seeds and plants. And low and ultra small Melafen concentrations activate seed germination and raise the plant resistance in stress conditions overcooling and drought. However, for organisms of animal origin such influence may have another vector that was depended from BAS concentrations. This is why the meaning of the detection of patterns of exposure of Melafen gains the great significance. The applications of Melafen over a wide range of concentrations on the primary targets of animal organisms were our paramount tasks. First exterior target for BAS is membranes and its components, always. Because these reasons the explorations of several model objects, which imitated the real objects of different degree of organisation were used. As was earlier we shown the influences of Melafen, at the wide concentration range, on the structural properties of lipid membranes with different composition [4]. The lipid-Melafen interactions were tested with aid of differential scanning microcalorymetry and small-angle X-ray diffraction. We did not reveal by X-ray diffraction any noticeable structural changes of the egg lecithin membranes at concentrations of Melafen that used at crop production. But on the basis of data, obtained by using of differential scanning microcalorymetry, we concluded that the domain structure of dimyristoilphosphatidylcholine membrane was changed greatly by Melafen. The next model objects for our investigations were the cellular membranes, as which were isolated from the animal’s cells, as the native cells which were insolated from animal body. These membranes consist of lipids, proteins, glycoproteins and other components. Now we tested the Melafen influences to the membrane bounded proteins and lipids-proteins interrelationships. Melafen is the strong and dangerous regulator of plants growth. Aqueous solutions of Melafen at concentration 10-11 - -10 M intensified the plant development and the stress tolerance under the bed environment But under the concentration of Melafen to 10-8, 10-7 M plant’s seeds dyed. Therefore, our studies were carried out in a wide range of concentrations (10-21M - 10-2M). Taking into account the close interdependence of vegetation and animal bodies in nature, it was be necessary to investigate the action of plant growth regulator at any objects of animal origin. The primary targets for BAS in animal’s cells are membrane and their components. When BAS appeared into the blood-vascular system the first targets become as blood cells. This is why for carrying out the tests of

Melafen actions as of simple models of primary targets, erythrocytes, which were isolated as of whole cells, and insulated ghosts that were the erythrocyte's membrane, emancipated from hemoglobin, were selected and were used. The main purpose was to determine how the aqueous solutions of Melafen in a wide range of concentrations influence to the structure of proteins with animals originated. Due to of the present work the Melafen action as on membrane proteins, as well as on protein-lipid interactions has been examined. Structural studies when influences of aqueous solutions of Melafen over a wide range of concentrations (10-21 - 10-2 M) were held on two models: erythrocyte's ghosts and insolated native erythrocytes. Conformational rearrangements of protein's domains in membranes of erythrocyte ghosts examined method of adiabatic differential scanning microcalorimetry. The structural organization of whole membrane taking into account and lipid, and protein's phase characterized, gauging by the registrations of the membrane microviscosity. The functional changes of native cells were provided by testing of Melafen actions to the erythrocyte's hemolysis degree. The degrees of, spontaneous and induced hemolysis were registered by spectral method. In order to verify our assumption that Melafen influencing to the cellular membranes may be mediated by the actions to membrane-bounded protein components, microcalorymetry scanning of erythrocyte membranes was held. The testing of Melafen actions on the thermostability of protein microdomains in erythrocyte membrane was performed by using of erythrocyte ghosts. The erythrocyte ghosts are the plasmatic erythrocyte membranes with all elements of cytoskeleton. These erythrocyte ghosts are fully emancipated from hemoglobin by means of hypo osmotic hemolysis [5, 6]. At fig. 1 (A) the scheme is submitted, where revealing the erythrocyte conversion to the ghost when hemoglobin was exited. The main protein elements of erythrocyte's cytoskeleton and the external plasmalemma are shown. Erythrocyte membrane raised its permeability to hemoglobin when hypo-osmotic medium. The maintaining of cold thermal regime (4o C), without freezing, when centrifugation permits to receive the colorless draft of ghost membranes. Ghost membranes were suspended at phosphate buffer easily. It should be noted that erythrocyte ghost are the fine model for studying of protein-lipid interactions at cellular membrane and cellular cytoskeleton. The main components are typical for the most of cells of animal body (fig.1 A). A B Fig. 1 - Scheme of erythrocyte ghost structure after hemoglobin releasing due to of hypo-osmotic hemolysis - (A). Thermograms of erythrocyte ghosts were registered by means of DASM-4. 5 identified endothermic phase transitions of protein's microdomains (A, B1, B2, C, D) in erythrocyte ghosts when registered by the DSC method are noted - (B) For explorations on such model comfortable practice a method of adiabatic differential scanning microcalorimeter (DSC) by means of high-sensitive calorimeter DASM-4 [7]. The microcalorymetry research at isotonic conditions discovers five structural thermo denaturation transitions into ghost membranes (fig 1 B), called A- transition, B1-transition, B2-transition, C- transition and D-transitions [8, 9]. The A- transition is

determined by the microdomains denaturation of cytoskeleton, set up of complex α - and βspectrin and actin. The denaturation of spectrin-actin complex microdomain results in disappearance of A-transition. That is followed by the total loss of erythrocytes deformability [10] and ghost membranes [11]. B1-the transition is linked to denaturation of membranous microdomain, set up of ancyrin and proteins of bands 4.1, 4.2 and demantin. B2transition is linked with denaturation of cytoplasm fragment of protein band-3 microdomains. C-transition is linked with denaturation of membrane fragment 55 κDa of proteins band-3, which are ion-channels microdomains. D-transition is linked with unidentified proteins denaturation and membrane bubbling microdomains [8, 9]. It should be noted that pathology damaged erythrocytes (for example - oxidized, etc.) in blood stream are lacking, since utilized by macrophages. However, we used the native erythrocytes isolated from healthy organism. All treatments were provided without macrophages in experimental test glass. The model of erythrocyte ghosts for DSC studies of cell membranes is quite adequately. Because the same changes are occur in membranes and cytoskeletons of other cells. The proteins composition of cytoskeletons: spectrin, ancyrin, bands proteins 4, 1 and 4, 2 are developing and band protein 3 is submitted consisting of membranous skeletal in practice all cells of organism. The DSC method together with polyacrylamide protein electrophoresis allow to define arising when change pathologies in cell membrane and erythrocyte cytoskeleton [12-15]. Materials and methods The materials: Melafen (melamine salt bis (oximethyl) phosphinic acid) was synthesized at Arbuzov A.E. Institute of Organic and Physical Chemistry of RAS Kazan. Melafen was used as the aqua solutions at the wide concentration range (10-21 M - 10-3 M). The erythrocytes and its ghost were prepared by method [12]. The Melafen interactions with membrane bounded proteins at the erythrocyte’s ghost were tested by the differential scanning microcalorymetry (DSC) method [7]. The alcoholic solutions of probes were inputted to 5%-suspensions of erythrocytes as 30 min before up to experimental objects measurements. Registrations were occurred by the ESR spectrometer ER-200D SRC of company Bruker (Germany). On obtained the electron paramagnetic resonance spectrums, employing formula for high turning probes, calculated the time of rotational correlation of probe τc10-10c, which is the reorientation time of probe at the angle ~p/2 of and describes the lipids microviscosity in membrane [16]. The results were expressed in relative units. Control served the membranes models without addition of Melafen solutions. The hemolysis was being conducted during 45 min at room temperature. The erythrocytes stability to spontaneous hemolysis studied by the method of Jager, based on photometry (λ=540 nm) ectoglobular hemoglobin, which incoming on medium owing to snap lyses of erythrocyte membranes, induced by the lipid oxidation by the air oxygen [17]. To operate we used the 5%-suspension of erythrocytes on medium, containing 0.1 M Tris-HCl buffer (pH 7,4) and the saline solution in correlation 1: 1. All examining held with fresh, just insulated erythrocytes. The hemolysis level identified on optical absorption of supernatant at the wavelength

540 nm by spectrophotometer “Specord M40”. For 100 % the hemolysis level supernatant absorption after hemolysis, caused by 0,1 % of Triton X100 is elected. Results and discussion For investigations of effects of Melafen aqueous solutions to the protein’s microdomains organizations at cellular membrane we used ghost of rat erythrocytes. This model of fairly simple to be used, and on thermograms are clearly mirrored all peaks of thermo inducted denaturation transitions of cytoskeleton’s proteins [18]. At fig. 1 (B) five temperature inducted transitions of protein microdomains in ghost membranes of rat erythrocytes were submitted in rate and after preliminary incubation with testable the aqueous solutions of Melafen. A B Fig. 2 - The Melafen influence to the thermodenaturation of protein’s microdomains at erythrocyte ghost membrane at the first day (A) and second day (B) following the receiving of ghost At fig 2 (A and B) ghost’s thermograms in the presence of Melafen aqueous solutions at first and second days following receive ghost from rat erythrocytes are submitted. As it is seen from, the structure of ghost in keeping (ageing) was changed essentially, but the addition of Melafen solution before scanning was not affecting of curves outlines outwardly. Any dislocations of thermo denaturation peaks and any essential changes of peak amplitudes do not happen. It indicates that the aqueous solution of Melafen was not affected directly on the structural organization of protein components of membrane. But the Melafen aqueous solutions caused some restructuring of protein’s microdomains as on freshly-isolated preparations (3-9%), and in the process of ageing of the erythrocytes ghost. In calculation of relative enthalpy has been received the data, presented at table. 1. Table 1 - Melafen influence on temperature dependence of relative enthalpy (thermograms - peak A) of membranes suspensions of erythrocyte ghosts at the first day of after receiving of erythrocyte ghosts Erythrocyte ghosts DCp* D (%)** without Melafen 17,1+_0,01 - 10-5 M Melafen 17,6+_0,01 +3% 10-3 M Melafen 18.6+_ 0.01 +9% * Δ Cp - change of relative heat capacity (J/K) in peak maximum of heat absorption (transition intensity), ** Δ (%) - the different quantity, where 100% - the quantity of control answer. As it can be seen from data of table 1, the Melafen, aqueous solutions when concentrations 10-5 M and 10-3 M, did not cause of important changes in relative heat capacity (J/K) in peak maximum of heat absorption and (the A- transition intensity) (3-9%). A-transition is the characteristic of induced endothermic denaturation of two most important protein’s cytoskeleton components in practice of all cells of animal origin - spectrin and related actin (refer to fig. 1. A and B). Other endothermic transitions, which being the characteristics thermo denaturation properties of cytoskeleton components and channel fragments also, in practice didn't change of its amplitude. The same the data have been received and for thermograms, registered in the second day following the ghost precipitation, i. e. on “aged” ghosts. It is results allude to the fact that in the presence of Melafen aqueous solutions of high concentrations the microdomains organization of cytoskeleton proteins and plasma membrane were not suffering the major changes. So that, as this within sensibility of

DSC method was occurred, the protein structure of plasma membrane (and channels in its composition) has been identified, and also the cytoskeleton elements in the presence of Melafen aqueous solutions under large concentrations was not suffering some major changes. It indicates that Melafen not made any considerable affects on protein components thermo denaturation properties at cellular membrane and cytoskeleton directly. Melafen not causes a change, leading to proteins loss, or to restructuring of protein's domains. The structural research in influencing of Melafen aqueous solutions of high concentration to animal's cellular membranes had been continued on insulated entries erythrocytes by the measurements of microviscosity by means of electron spin resonance method (ESR). Lipids surrounding the proteins play a major role in the structure organization and in the manifestation of activity of the membrane protein components. This was why lipid's microviscosity in area of lipid-lipid and lipid-protein's interactions at membrane has the great significance. The microviscosity of erythrocyte membranes estimated on rotational correlation time included in lipid phase of membranes of two of spin probes: 2,2,6,6-tetramethyl-4-capriloil-oxipiperidin-1-oxil (probe 1) and 5,6-benzo-2,2,6,6- tetramethyl-1,2,3,4- tetra hydro-g-carbolin-3-oxil (probe 2) [19]. Two spin probes vary in its hydrophobic properties. Their structural formulas are introduced on fig. 3. It is known that the probe 1 primarily localizes in surface regions of lipid membrane in the area of lipids (τCI) to a distance 2-4 Å, and probe 2 - in the zone of near protein of annular lipids (τCII) at a depth of 6-8 Å from surface [20]. Fig. 3 - Structural formulas of nitroxyl radicals - spin-labeled probes 1 and 2 The influences of Melafen aqueous solutions to membranes microviscosity of insulated erythrocytes were measured by using of two probes (table.2). Table 2 - Melafen influence on relative change of microviscosity of erythrocyte membrane Melafen concentration (M) Probe 1 (arb. un.) Probe2 (arb. un.) Without Melafen Melafen 4 x 10-5 Melafen 4 x 10-3 1.0 0.98 0.74 1.0 0.93 0.90 From data table.2 it is possible draw conclusion that Melafen, when the biggest doses, reduced the microviscosity of both regions of bilayer: and at cursory lipid region at a depth of 2-4 Å, and at the area of lipid in near protein's regions - 6-8 Å. The microviscosity of both regions of erythrocyte membrane were tapered off, when large concentration 10-3 M of Melafen aqueous solutions. In lipid region at a depth of 2-4 Å was tapered off maximally - up to 25%. Earlier the works [21] by using of aqueous solutions of Melafen, when smaller concentrations (10-10 - 10-7 M), was also shown the microviscosity change of erythrocyte membranes at near protein's regions on 7%, in lipid - surface regions low concentrations of Melafen in practice unaltered the bilayer microviscosity. Melafen aqueous solutions when higher concentrations influenced on lipid-lipid interactions in surface regions of membranes essentially (up to 25%). And the aqueous solutions of Melafen acted the insignificantly influence (up to 10%) on lipid-protein interactions in deeper areas of membrane of erythrocytes, when very large, and low concentrations of Melafen. When test the action of Melafen aqueous solutions on cellular membranes functioning was some important changes in

membrane permeability were found. This part of work was accomplished to insulated entries erythrocytes. For these tests we used the model of hemolysis of erythrocytes in hypo and hyper osmotic medium in contrast with spontaneous hemolysis. Such model reflects the probability of Melafen influence on degree of stability of membrane to damaging factors of bed environment. The hemolysis when immediate by addition of small amounts of Melafen aqueous solutions with different concentrations to erythrocytes suspension, will be describe the damaging action of plasmalemma by the Melafen. As it was disclosed that Melafen in all range of concentrations (10-13 - 10-3 M) do not cause additional hemolysis and do not protect against spontaneous hemolysis. So, if 100% - to count the hemolysis, in which the hemoglobin from erythrocytes when addition of triton x-100 fully is released, then the spontaneous hemolysis compiled 3%, and the hemolysis in the presence of Melafen - 3%+-0, 3%. The hemolysis measurement in hypo and hyper osmotic conditions (0 - 4 mM NaCl) [22] we held because we want to answer to the next question: what the explored preparation Melafen can render some protective effect on erythrocyte's membrane? Or also Melafen can speed up erythrocyte's membrane destruction when local unfavorable factors of medium. Obtained data are given at fig. 4. Fig. 4 - Melafen influences on hyper osmotic hemolysis of erythrocytes. The hemolysis level identified on optical absorption of supernatant at the wavelength 540 nm. For 100 % the hemolysis level supernatant absorption after hemolysis, caused by 0,1 % of Triton X100 is elected As it can be seen from data fig. 4, the general trend of increasing of erythrocytes hemolysis degree varied significantly by Melafen presence over a wide range of concentrations (10-9 - 10-3 M). And these trends become so bigger under the increasing of environment osmolarity. But the Melafen addition in concentration 10-12 M and 10-11 M don't change of general picture of hemolysis of erythrocytes (data were not shown). Conclusion In conclusion of this part of our investigations that were devoted to influence of plant growth regulator Melafen on organisms of animal origin let us mention that as of test object the erythrocytes were chosen. Erythrocytes are the one of the first targets when some material appeared in blood river-bed. This model is very simple for preparation and fairly it is stable for provided somebody measurements. At the same time the erythrocytes have the cytoskeleton protein composition similar of most animal's cells to a considerable extent. By this the investigations that were provided with this experimental object had the essential significance. The composition and the structure of structural elements of erythrocyte membrane are representative for many cells of organism. The obtained data about measurements of proteins thermostability, their relationships with lipids, the changing of membrane permeability indicated that Melafen didn't exerted the great destructive actions to the insolated erythrocytes ant its isolated membranes (with cytoskeleton) under the concentrations that activate the plant growth. But Melafen influencing was essential to the structure and functioning of membrane-bounded proteins, when the bigger Melafen concentrations.