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The effect of osmotic stress on the microrelief of the medicinal leech hemocytes

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Relief or cellular surface topography is highly mobile characteristics: it varies depending on the functional state of the cell. Surface roughness is a set of irregularities forming microrelief. Quantitative evaluation of membrane surface roughness is of practical importance, since it allows identifying the effect of surface homogeneity and heterogeneity on the processes of capture of foreign objects and resistance to hypo- and hyperosmotic stress.

Hirudo medicinalis (Linnaeus, 1758) hemocytes were used as subjects of research, previously classified into four types by morphofunctional features. The hemolymph was divided into three parts: 10 mcl of NaCl solution of a certain concentration (hypotonic solution – 0.4% NaCl, isotonic solution – 0.8% NaCl, hypertonic solution – 1.2%) was added to each part of hemolymph. Incubation was conducted for 1 minute. The research was conducted using Integra Vita NT-MDT scanning probe microscope in atomic force spectroscopy mode. We have analyzed the following amplitude average surface roughness parameters in accordance with the international standards. Changing under the influence of environmental factors, cellular surface microrelief reflects the features of their functional status. Using atomic force microscope images allowed estimating the behavior of *H. medicinalis* hemocyte surface microrelief after incubation in solutions of different concentrations. The reduction in osmotic pressure leads to a significant volume increase of all cell types. On scanning images, hemocytes

have a spherical shape and smooth membrane without folds. Micro-elevations of the surface dominate, and large elevations and depressions were not detected. In a hypertonic environment, surface of the blood cells of medicinal leech varies considerably. Protrusion of cytoplasmic granules, and fibrils of the cytoskeleton through plasma membrane were detected; micro-elevations are absent. In hypotonic environment, heights of the peaks and depths of the depressions increase; in case of the increase in osmotic pressure, relief smoothing is observed. Changes in the symmetry of the distribution of various relief structures when changing the osmotic pressure were not observed. The entire cellular surface is uniformly transformed under the effect of uncharacteristic salinity.

Roughness coefficient of all cell types in this environment increases, however this is not always associated with an increase in the number of micro elevations – deepening of depressions and increase in the height of micro-relief elements also plays a significant role. The changes in the surface topography of hemocytes are described in contact interactions with solid substrate, and when exposed to environments other than physiologically normal. An increase/decrease in the thickness of the disturbed layer in hypotonic and hypertonic environment respectively is characteristic for cells that perform phagocytic function, with maintenance or increase in the number of micro elevations per unit area. The prevalence of invaginations in hemocytes with abundant content of granules is observed when exposed to conditions with increased osmotic pressure.