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Morphometric Parameters and Microrelief of Hemocytes of *Blaberuscraniifer* in the Conditions of Osmotic Loading

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Abstract

The purpose of this work is studying of influence hypo and hyperosmotic load of morphometric parameters of uniform elements of hemolymph of *Blaberuscraniifer*. Application of methods of nuclear and power microscopy has allowed to estimate such parameters of hemocytes as microrelief of the cell surface, the linear sizes of cages, including height. Changes of topography of surface of hemocytes at contact interactions with solid substrate are described and at the influence of the environments other than the physiologically normal.

Keywords: Hemocytes, Hypoosmotic and Hyperosmotic Loading, Microrelief of Surface

1. Introduction

The relief or topography of the cell surface, is very mobile characteristic: It changes depending on functional condition of cage[1–3]. The roughness of surface represents set of the roughness forming microrelief[4]. The quantitative assessment of roughness of the surface of membranes has important practical value as allowed to reveal the influence of homogeneity or heterogeneity of surface on processes of capture of foreign objects and stability to hypo to hyperosmotic loadings[5,6].

2. Materials and Methods

As objects of research *Blaberuscraniifer* hemocytes which are previously classified by morfofunktsionalny features on 7 types[7] served. The received hemolymph was divided into three parts, each of which was placed in separate cup of Petri. Added to each part of human lymph 10 mil NaCl solution of certain concentration (the hypotonic – 0.45% of NaCl, isotonic solution – 0.9% of NaCl, hypertensive – 1.35%). The incubation was carried out within 1 minute. The drop of hemolymph was placed on the glass and did dabs. Researches are conducted with the use of the scanning probe microscope Integra Vita NT-MDT in the mode of nuclear and power spectroscopy when imposing loading in 25 local sites of cellular surface[3–6,8]. Processing of the received ASM-images was carried out by means of the software of Image analysis 3.5[9]. The analysis of the following amplitude average parameters of roughness of surface according to the international standards has been carried out: Average quadratic roughness of Sq (nm); Height of the highest peak of Sp (nm); Depth of the deepest hollow of Sv (nm); Asymmetry of Ssk – characterizes of skewness of distribution of profile, when one recession abrupt and another – flat; the excess of Sku characterizes the extent of distribution of profile; Sz – the parameter characterizing thickness of the superficial, indignant layer which is not completely filled with material in which there is change of relief. Also values of one of the functional parameters characterizing the relief in the local area and degree of smoothness of surface – density of tops (peaks) of seeds have been defined $(1/\mu m)^1$. This indicator shows quantity of tops per acre[5,10,11].

3. Results and Discussion

On a surface of native prohemocytes the microeminences

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which are not exceeding 187 NM prevail. Decrease in osmotic pressure leads to insignificant increase in volume of cages. On scanning image of hemocytes have close to spherical form and smooth, almost deprived of folds, membrane.

In hypertensive condition indicators of mean square roughness of surface race with 53.7 to 68.8, but the distance between eminences decreases. There is increase in height of the peaks and depth of hollows. In the conditions of lowering of osmotic pressure smoothing of relief is noted. Changes in symmetry of distribution of different structures of relief at osmotic loading were not observed.

Native plasmocytes differ in the form close to the fiscal. Height of cages about 1.2 μm. The outer plasma membrane creates pectineal folds on the periphery. In the conditions of the lowered osmotic pressure observed considerable spreading of hemocytes, extreme differences of profile on height are absent, then the value of the indicator of skew equal 2.88 speaks. On the periphery the wide realm of lamelloplazma forms. The relief of membrane has got a bigger quantity of microeminences and microhollows in the hypertensive environment to what the increase in the indicator of the density of peaks at surface unit testifies. The incubation of hemocytes in the hypotonic environment has not caused essential change in this parameter.

Thickness of the indignant layer (size) of native cages has made 546, 52±0, 4 nm. At the hemocytes incubated in the environment with the increased osmolarity, this indicator has increased to 562, 32±0, 8 nm and in the hypotonic has decreased to 409, 36±1, 2 nm. Thus, there is a reduction of the thickness of the indignant layer in the conditions of the lowered osmotic pressure to preservation or increase in number of microeminences per acre.

Native granulocytes are characterized by the correct roundish or oval form. Surface rough, protrusion of granules is noted. The profile of hemocytes of this type is deepening in the nuclear area. Height of cages about 1.5 μm, in the field of kernel this parameter can be lower – about 1.1-1.2 μm. In the hypotonic environment the microrelief of surface accepts more homogeneous character, roughness indicators for this purpose like hemocytes have considerably decreased. In the conditions of the increased osmotic pressure of microeminence on surface of hemocytes get angular outlines.

Sferulotsita – spherical or ovoid hemocytes. The kernel is well noticeable on scanning image, has the correct round form, is located in the center or can be a little displaced to edge. Radial tyazh with the cross shoots creating cells depart from the kernel to the periphery in the form of beams – there is accumulation of spherical inclusions. In the field of the kernel height of the cage does not exceed 1.0 μm while the profile of another part of honest reaches 1.8-2 μm.

On the environment, excellent from physiologically normal, cages show stability is concerns both form and the parameters characterizing features of microrelief of surface. Sometimes the incubation in hypertensive conditions leads to stronger invagination of sites of membrane between spherical inclusions.

Character of surface of spindle-shaped plasmocytes differs in smaller morphological variety, in comparison with simple plasmocytes. This type of cages keeps form, including at incubation in the environments other than the isotonic better. The large oval kernel located is central, hardly considerably. Height of hemocytes has no reliable differences in the field of kernel and on the periphery of cage.

The membrane creates hemispherical microeminences which after incubation of hemocytes in the hypertensive environment get angular outlines. Increase and lowering of osmolarity of the environment has reliable inversely proportional impact on change of the linear sizes of cages only on short axis. Influence of hypotonic solution involves small reduction of thickness of the indignant layer. In general, osmotic loading has not led to essential deformations of surface of hemocytes of this type.

The membrane of koagulotsit creates set of folds that defines ability of these cages to keep integrity at incubation in the hypotonic environment, using membrane reserve. The central raised kernel region is accurately distinguishable. The thin layer of cytoplasm in the direction from perinuclear space to the periphery of hemocyte is in any conditions practically level with substrate. Thus, height of cages in the field of kernel makes 1.6 μm and other part of cage does not exceed 0.9 μm. In the conditions of hypertension the surface gets the maximum distribution density of furrows and crests and also shows high value of average quadratic roughness.

The surface of enotsitoid is characterized by availability of deep invagination of membrane in peripheral area that is confirmed by negative value of excess (-0.81). The size of this parameter has decreased to -0.76 after incubation of cages in the environment with the increased salinity. Thus the frequency of occurrence of microemi-

nences on unit of area, especially in the field of kernel has increased. On scans the crests with cross branches reaching from the center for edges of cage are noticeable. The area of kernel is raised rather other part of hemocyte. In general the microrelief of surface of enotsitoid differs in the greatest variety that was reflected in roughness indicators.

At all types of uniform elements, at incubation in environments with different osmolarity, values of excess (sku) are in the range from 0 to 3 that speaks about lack of extreme peaks and hollows. The visual analysis of scans has shown that for microrelief of koagulotsit and enotsitoids dominance of invagination is characteristic are confirm also negative ssk values (-0.48 and -0.81 respectively for two types of types of cages). This parameter at other types of hemocytes accepts the values close to zero that is connected, with approximately equal ratio of hollows and ledges of plasma membrane.

4. Conclusion

Changes of topography of surface of hemocytes at contact interactions with solid substrate are described and at influence of the environments other than the physiologically normal. For the cages which are carrying out phagocytic function the reduction/increase in thickness of the indignant layer in hypotonic and hypertensive environments according to preservation or increase in number of microeminences per acre is characteristic. At hemocytes with the plentiful maintenance of granules dominance of invagination at hit in conditions with the increased osmotic pressure is noted. The roughness coefficient at all types of cages in this environment increases, however it is not always connected with increase in number of microeminences – the significant role is also played by deepening of hollows and increase in height of elements of microrelief.

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