# GROWTH AND FUNCTIONAL REGULARITIES OF UNICELLULAR ORGANISMS UNDER ALTERED GRAVITY CONDITIONS.

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**Abstract**

A great number of space flight experiments was carried out on unicellular organisms **Ciliata** and **Flagellata.** Their main morphological and physiological characteristics: form, size, ultrastructure, division rate, metabolic activity and motility , protein and nuclear acids content, mineral balance in hyper-, hypo- and microgravity are well studied. On the basis of experimental data analysis and theoretical conclusions regularities of growth and function of unicellular organisms in altered gravity are revealed. It is demonstrated that cell sensibility to gravity depends not only on its form and size but is mainly determined by its metabolism level and motility. A working hypothesis is suggested which explains the cause of variability of gravity effects on individual cells and unicellular organisms differing in their morphological and ecological characteristics. The data adduced are of great theoretical as well as of practical significance while they might be used by constructing biological life support systems and also for improving biotechnological processes.

**Introduction**

Free- living unicellular organisms are a suitable subject for studying particularities of cell structure and function and its behavioural characteristics by altered gravity. Analysis of experimental results obtained at the last years and taking into account some theoretical theses let us suppose an existence of at least three levels of gravity effects on unicellular organism. The first level- molecular- is bounded with the particularities of intracellular processes organization and is the field of interest and competence of cell biomechanics and thermodynamics. Theoretical and experimental aspects of possible mechanisms of gravity stimulus realization on this level are in detail discussed in a number of mordern analytical publications /l,2,3,4,5/.The second level- morpho-functional- is defined by the fact that an individual cell, as a rule, is an independent free- living unicellular organism which behavioural characteristics are determined by geo-, chemo- and sometimes by phototaxis. By studying the mechanisms of gravity effect on this level traditional methods of cell biology and physiology are used/6,7,8,9,10/. The third level- physio-ecological - is a consequence of cell population organization and is mainly bounded with the principals of two-component system interactions: cell population - environment. In this case besides morpho-physiological particularities of the cells one should take into consideration phisico- chemical and reological characteristics of the environment. Results of these investigations published in a number of original works are summarised by us/4,11,12/.

At the same time one discerns direct and indirect gravity effects on unicellular organism. According to theoretical theses direct influence of this factor on a cell as on a biomechanical construction is determined by the difference in intracellular organells densities and by the weight of the cell

itself.There are certain requirements from biomechanics of intracellular structures interactions and energetical "cost" of their spatial disposition in cell by altered value and direction of gravity. And besides, the consequences of the shift in rythmics and organisation of intracellular processes caused by direct effects of gravity may be of a certain significance for the whole population.

Indirect effects of gravity are mainly connected with the variations of environmental physico-chemical parameters and, at first, with concentration gradients of nutrition and metabolism substrata of the cells functioning for a long time in a population under unstable gravity conditions. The mechanisms of indirect effects of altered gravity on a cell are mainly realised on intercellular interactions level. In both cases apart from the primary cause of gravity -depended vibrations in a biological system realization of the mechanism affected by this factor may initiate a chain reaction according to a sceme: environment - cell population- cell- intracellular processes- cell- cell population- environment. Thus, it might be difficult to calculate a specific contribution of gravity in the processes taking place on this or that level of this interaction, also on the cellular one.The question of the priority of direct or indirect gravity effects on a cell and of the mechanisms of gravity stimulus realization is not solved up to now.

**Results and Discussion**

The subjects of our investigations were representatives of various types of free- living Protozoa: *CiEuta(Tetrahymena pyriformis, Bursaria truncaiella, Dilepius anser. Loxodes strial us),* **Flagellata** *(Euglena gracilis, Chlamydomonas reinhardtii), §амсодлла(АтоеЬа proteus).* These unicellulars have different metabolic levels, differ in their oxygen consumption which determine their inhabitation in water strata. Levels of distribution of the enumerated types of Protozoa according to their inhabitations in nature are scematically represented on figure 1. Their general biological characteristics are summarized in table 1.

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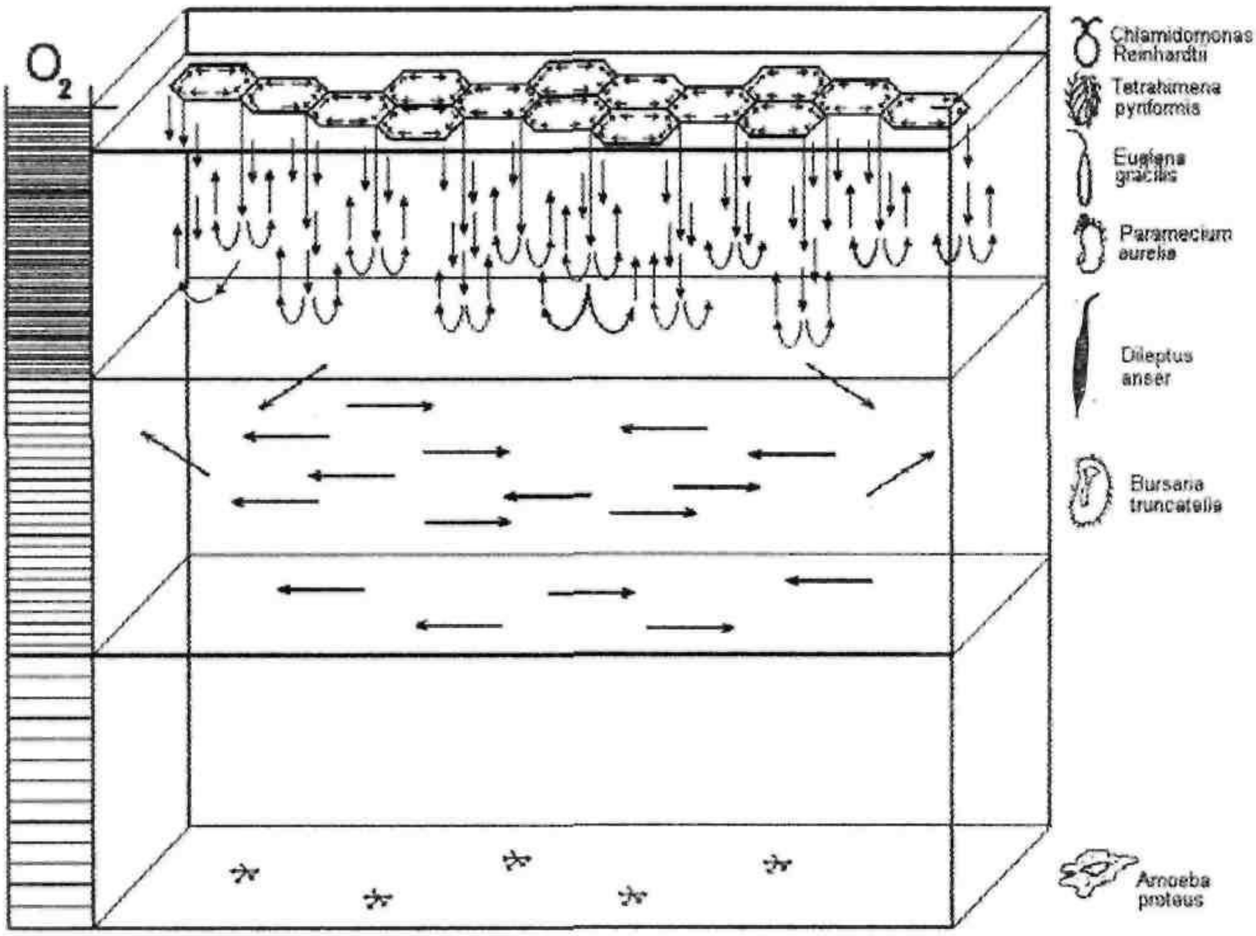
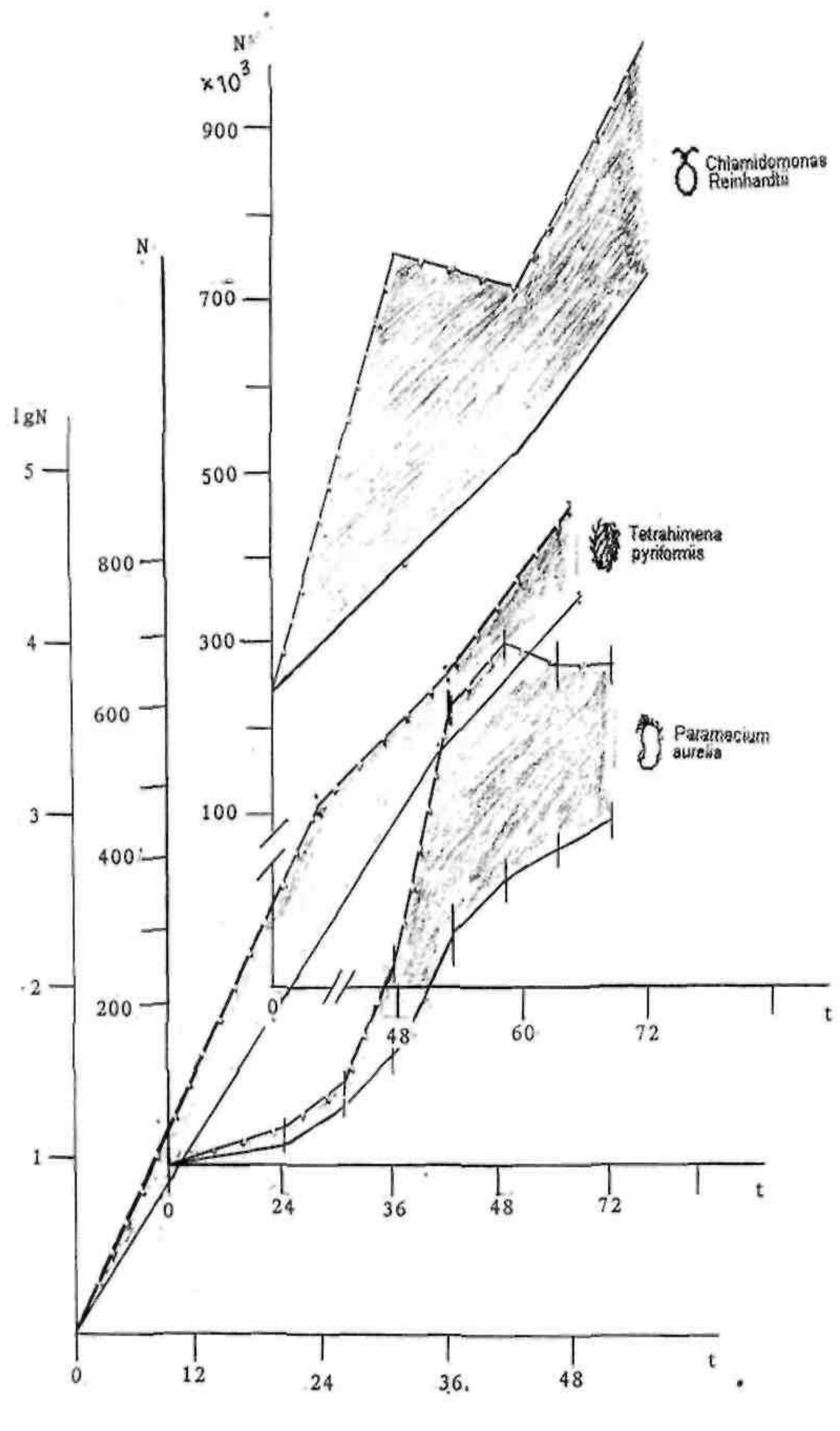



**Figure 1. Distribution of unicellular organisms in an experimental vessel according to their natural inhabitation (a sceme).**

Experiments under microgravity conditions on board space flight apparatuses - biosatellites "Cosmos" were carried out in different time periods on ciliates *T. pyriformis:* on board "Cosmos-1887" in 1987 and "Cosmos- 2044" in 1989. In one case the culture was in logarithmic, in the other- in stationary growth phase /13/. According to the results of these experiments one can imagine the culture development in weightlessness by the following way: stimulation of culture growth rate in a logarithmic growth phase which cause an exceeding of culture density in weightlessness comparing with the same in control. As a result an early exhaustion of the limited nutrition resources takes place and, consequently, an acceleration of culture "depression" processes. These data are in line with the results of investigations earlier conducted by French specialists on *Paramecium aure/ia* /14/. In the experiment carried out on board biosatellite "Cosmos- 2044" on developing *Ch.reinhardtii* culture it was shown that microgravity cause an enlargement in cell size by constant relative sizes of intracellular organells /15/ . Moreover, the latest data obtained by Dutsh specialists on the same culture in **the** experiment carried out on board biosatellite "Foton-10" provide evidences about an increase in cell division rate under microgravity conditions , are in line with the results obtained on *T.pyriformis* and support the conclusion about stimulating effect of weightlessness on metabolic activity of growing culture by the presence of enough nutrition resources in medium /10/. Growth dynamics of the investigated types of unicellular organisms under space flight conditions (in microgravity) are represented on figure 2.




---- experiment —— control t- time in hrs N- cell number

**Figure 2. Culture growth dynamics of different types of unicellular organisms in microgravity.**

It is known that clinostat conditions model one aspect of weightlessness- the absence of orientation to gravity direction. In this case the factor gathering cells in a certain place of experimental vessel is absent and under conditions of sufficient oxygen provision (this condition was fulfilled in all conducted experiments) cells' "inhabitation space" may be enlarged and this may promote the culture growth stimulation. However, nutrition resources are limited, medium's toxity increases while cell number is growing and it should cause an earlier delay of culture growth rate. As experimental data showed, in microgravity (for *T.pyriformis)* and by clinostating (8 rpm) (for *B.truncatella)* the above-mentioned regularity was really observed/16/.

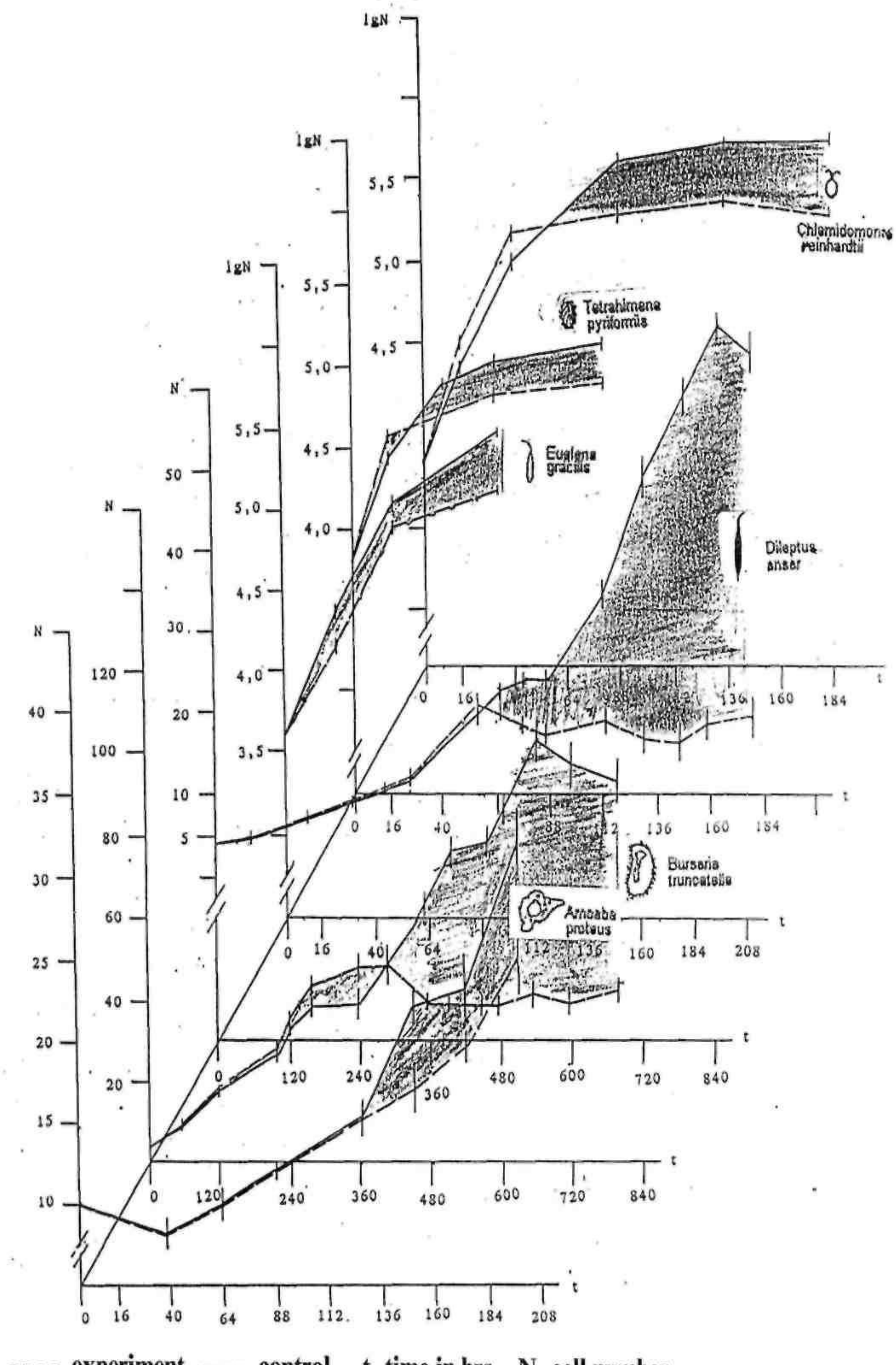
Investigations carried out by means of centrifuge (hypergravity) on *T.pyriformis, Ch.reinhardtii* and *E. gracilis* revealed the same changes at the initial period of culture development /17/. In logarithmic growth phase by *T.pyriformis, Ch.reinhardtii* and *E.gracilis* at 2g, 3g and 5g a short period of growth rate stimulation was observed by constant cell sizes. However, an increase of gravity value from 2 up to 5g caused a reduction of stimulation period. The increase of gravity value caused an earlier approach of the stationary growth phase by lower population density than in control and what's more, by further growing the difference between control and experiment remained the same or became more obvious. At the last stage of experiment under the influece of hypergravity the cell sizes of *T.pyriformis* and *E.gracilis* diminished and *Ch.reinhardtii* lost its motility and sank to the bottom. Results of space flight and hypergravity experiments show that *T.pyroformis, Ch.reinhardtii* and *E.gracilis* populations are adapted to "normal" gravity. Altering of these conditions at the beginning of culture growth cause at first stimulation but further an increase of biomass delays, signs of earlier "depression" in general approach in population though nutrition medium is not exhausted. Modification of parameters describing population physiological state gives evidences about the fact that it becomes more unfavourable by altered gravity .

Experiments conducted by hypergravity on *B.truncatella* also revealed growth rate stimulation as it was in cases of *Ch.reinhardtii, T.pyriformis* and *E.gracilis* /18/. Under certain conditions a process of intraclonal conjugation was observed by *B.truncatella.* It was noted that in hypergravity conjugation began earlier than in control and a number of conjugating "pairs" was greater. That provides a clear evidence about an activation of adaptation processes in *B.truncatella* culture under hypergravity conditions. However, in spite of an obvious tendency to adapt these conditions a complete adaptation had not been archieved and by further growth in stationary phase the culture density remained lower than in control. The same decrease of culture density was observed in case of *D.anser.* At the end of experiments the cells of *B.truncatella* and *D.anser* underwent the changes of some morpho-functional characteristics /16,18/.

For the cells with an obvious oxytaxis gathering in upper strata of water *(Ch.reinhardtii, T.pyriformis, E.gracilis)* hypergravity cause an earlier (by lower culture density) drawing of culture into bioconvection process; that may cause an early growth stimulation. In further, because of the lower cell number remaining in surface stratum and diminishing of the depth of cells falling bioconvection becomes not so effective growth stimulator as by "normal" gravity. In other words, after a certain period of growth stimulation a period of of reduction of cell "inhabitation space" comes which probably cause a decrease of culture density in stationary growth phase.

For the bottom organisms *{B.truncatella, D.anser)* hypergravity may also be an unfavourable factor because of some events, the main of which might be the fact that heavy, slowly swimming cells under hypergravity unfluence are pressed to the bottom while their more active food *{P.caudatum* and *T.pyriformis)* having obvious oxy- and geotaxis are still swimming upward. Thus, growth decrease of "slowly swimming" ciliates *(B.truncatella. D.anser)* may also be explained by reduction of their "inhabitation space". But this explanation is not suitable for the culture growth stimulation at the beginning of experiments.

As for *A.proteus*, its "inhabitation space" does not reduce by altered gravity. But an increase of gravity value up to 5g in some time cause a delay of its growth rate /17/. Consequently, in this case at least one more factor exept "inhabitation space" reduction influences on culture growth. We may suppose it to be a direct influence of gravity on intracellular processes. While in compare with Protozoa having a constant form, *A.proteus* supports its positional homeostasis in a small degree and have a slightly expressed cytoskeleton structure (exept the periods when a cell prepaires itself for division and becomes round in shape touching the surface only in one site). It resists stress with a small mechanichal force and can obviously response altered gravity; that causes changes in its growth rate in spite of the absence of redistribution of cells in medium. Growth dynamics of the enumerated types of cultures under hypergravity conditions are represented on figure 3.




---- experiment —— control t- time in hrs N- cell number

**Figure 3. Culture growth dynamics of different types of unicellular organisms in hypergravitation (5g).**

## Conclusion

On the basis of the obtained results we suggested a hypothesis about priority of ecological and physiological characteristics of unicellular organisms (inhabitation conditions of unicellulars , their metabolic and motile activity) over their morphological parameters (mass, size and form) in the processes of acception and realization of gravity stimulus. In other words, it was shown that the degree of sensibility to gravity of unicellular organisms could be a function of their general metabolism level and motility . By this way an essentual correction was introduced into one of the basic statements of gravitational biology about the existance of direct correlation between sizes of living organisms and their sensibility to gravity force.Thus, our investigations show that the main factors determing a degree of sensibility (tolerance) to gravity of free-living unicellular organisms are not their size and mass, exactly, not only their morphological characteristics, but, at first, the particularities of their inhabitation and their metabolism level, i.e. ecological and physiological conditions of inhabitation of cell and cell population as a whole.

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