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Influence of a dispergent on the cell mebrane potential

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INTRODUCTION

In recent decades the effects of different chemicals, heavy metals and complex wastes of effluents on freshwater organisms of aquatic exosystems have been described *(8, 9)*. The injury of several organisms structures, and disturbances of physiological processes, particularly photosynthesis in freshwater algaes were described specifically *(7, 6)*.

The membrane potential of cell is very sensytive to the physical and chemical effect of the surroundings *(5, 1)*.

The purpose of this work was to investigate the influence of the dispergent, as the possible pollutant of aquatic ecosystems on the bipotential of algal cells.

MATERIAL AND METHODS

In the experiments described herein, the dispergent HEMISOL, manufactured in Modriča, was used. Hemisol belongs to the medium toxicity group of dispergents.

Experiments were performed on freshwater algae *Nitella* belonging to the *Characeae* family. The algae were cultured at the room temperature in glass containers filled with tap water. All experiments were done on cells constituting the second internodus from the top. The lenght of cells varied from 10 to 15 mm, and the diametar from 0.30 mm to 0.45 mm. After isolation, the cells were kept for one hour in a medium containing equal parts of 0.1 mM KCl and 1.0 mM NaCl with the aim to obtain the balance of ions (basal solution).

The membrane potentials were measured by standard microelectrode technique, using glass microcapilares filled with 3 M KCl solution. A microelectrode was inserted into the vacuola of the cells, and the references electrode was immersed into the surrounding solution. The potentials measurements were carried out in a grounded metallic box to prevent the influence of electromagnetic fields from outside. All measurements were done at the room temperature and the constant white light intensity. The apparatus used has been already described *(3)*.

The membrane potentials of the cells were measured continuously, first in the basal solution, and then in basal solution contaminated with various concentrations of a dispergent. The bathing solution was contaminated with increasing doses of the dispergent ranging from 0.001‰ to 100‰. The dispergent was added each time when the stable membrane potential was reached. The membrane potentials of contaminated cells were measured after contamination until cell death. For investigations in post treatment period the cells were kept in basal solution at the daily illumination and room temperature.

The results were evaluated by using the methods of testing the differences of arithmetical means and proportions for small indipendent samples, and analysis of variance *(4)*.

RESULTS

The changes of resting potentials during contamination with increasing doses of a dispergent are illustrated in Figure 1. The values of potentials changes are indicated by ΔM and expressed in precentages of initial resting potentials of non-contaminated basal solution. Each point represents the mean value of 9-6 measurements. Error bars represent 95% confidance interval. It is evident that the dispergent caused decrease of resting potentials, i.e. the depolarisation of the cells membrane. The value of depolarisation increased with the dispergent concentration.

During the experiments the polarisation in some cells at the concentrations of 0.1‰ and 1.0‰ of the dispergent occured. In Table 1 the observed number of such cells are indicated by N_p, whereas in the bars the total number of treated cells by N.

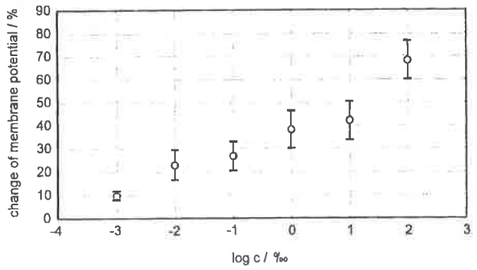

FIGURE 1. *The changes of resting potentials as a function of dispergent concentration.*

The potentials changes seen in both of the experiments are compared in the last column of Table 1. Significant differences were found for dispergent concentration of 0.1‰.

The resting potential is an indicator of cell activity; when the cell dies, the biopotential disappears. After the contamination the membrane potentials were measured at intervals of two days until the cell death. Three different dispergent concentrations were used. Cells survivals are represented in Table 2. Each value presents the means of 7-6 observations (± mean error). The differences of cells survival was tested by the analysis of variance. The difference of survivals between contaminated and non-contaminated cells is statistically significant at the dispergent concentration of 0.1‰ and 100‰.

In treated (experimental) and control cells, the oscillations of membrane potentials was obserwed in certain sets of cells. In Table 3 the number of these cells is expressed in percentage. The oscillations in experimental cells are lower than in control cells, where differences were not statistically significant.

TABLE 1

The changes of resting potentials between successive and directly dispergent contaminations

	successive contamination		direct contamination		difference (significancy)
concentration per thousand	N_p (N)	Δ PM %	N_{HP} (N)	Δ PM %	$(\Delta PM\ \%)_{succ} - (\Delta PM\ \%)_{direc}$
0.1	4 (8)	– 26.4 ± 6.2	4 (7)	1.3 ± 1.8	27.7 ± 6.5 ($P < 0.05$)
1	2 (7)	– 37.9 ± 8.2	0 (6)	– 36.6 ± 9.1	1.3 ± 12.2 ($P > 0.05$)

To investigate how each of these concentrations influences the potential changes, the basal solution was contaminated directly with 0.1‰ or 1.0‰ of dispergent concentrations. The results of both experiments are shown in Table 1. The number of hyperpolarised cells is indicated by N_{HP}. The hyperpolarisation was found only at the dispergent concentration of 0.1‰.

TABLE 2

Survival of the cells at different concentrations of dispergent.

	experimental cells			control cells
concentration per thousand	0.1	1	100	0
N	7	6	6	6
survival of the cells / days	11 ± 2.5	15.6 ± 3.5	12.5 ± 1.7	25.8 ± 3.2

TABLE 3

Oscillations of membrane potential in contaminated and not contaminated cells.

	experimental cells	control cells
N	22	17
Osc %	27.3	58.8
Δ p %	31.5 ± 15.9	
significancy	$0.05 < P < 0.055$	

DISCUSSION

Comparing the potentials changes in cells contaminated with the increasing doses of dispergent concentrations from 0.001‰ to 0.1‰ and potential changes in cells contaminated with a single dose of a dispergent, we found that contamination with the dispergent at the concentration of about 0.1‰ resulted in hyperpolarisation. Namely, the potentials changes in successively contaminated cells suggested that the membrane permeability was effected by previous contaminations.

The dispergent decreased the life time of cells. This conclusion is not valid for concetration of 1symbol 1‰ because of a high variability of cells survival. To achieve a reasonable conclussion, the number of cells in this sample should be higher.

The oscillations of membrane potentials in a certain number of the cells are present continuously. It is believed that these are generated spontaneously, but their origin is not clear yet *(2)*.

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ABSTRACT

Influence of a dispergent on the cell membrane potential

Background and purpose: Many investigations of the influence of temperature, ultrasound, electromagnetic waves, heavy metals, and ionic solutions on the cell membrane potentials have confirmed that the membrane potenital is very sensitive to physical and chemical effect of the surroundings. The purpose of this work was to investigate the influence of a dispergent, as the possible pollutant of aquatic ecosystems, upon the membrane potential of algal cells.

Material and methods: Experiments were performed on freshwater algae Nitella *belonging to the* Characeae *family. The potentials were measured using the microelectrode technique. A microelectrode was inserted into the cell vacuole and the reference electrode was immersed into the basal solution containing equal parts of 0.1 mM KCl and 1.0 mM NaCl. This solution was contaminated with various concentrations of a dispergent (from 0.0001% to 10%).*

Results: The observed changes of resting membrane potentials showed that the dispergent had caused the depolarisation of the membrane, proportionally with concentration if the dispergent was successively increased. If the basal solution was contaminated directly with dispergent concetration of 0. 01%, the hyperpolarization of the membrane occurred. Conrol cells lived longer than experimental ones that were contamined with 0.01% and 10% concentrations of the dispergent. Control cells had more oscillations than experimental cells, but the observed difference is not statistically significant.

Conclusion: The observed changes of resting membrane potentials showed that dispergent caused the depolarisation of the membrane, proportionally with its concentration.

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