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INFLUENCE OF ALUMINUM ON THE LIPID COMPOSITION OF POND SNAILS PRESENT IN MEDIA WITH DIFFERENT ACIDITIES

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It has been established that the toxic action of aluminum on the hydrobiont Lymnaea stagnalis *L. increases in an acid medium. The dual action of acidification and a toxicant lowers the survival rate. The lipid and phospholipid compositions of pond snails of control and experimental groups at various concentrations of toxicant and various pH values of the water have been investigated.*

The acidification of water bodies caused by anthropogenic action is an urgent problem of the environment in many northern industrially developed countries [1, 2]. According to known concepts, the toxic action of aluminum on the organism is connected with the presence in the aqueous medium of some soluble form of the metal [3]. A rise in the acidity of the water leads to an increase in the passage of aluminum into a biologically active labile form [4]. A clear interrelationship has been established between the decrease in the number of species of fish, the decrease in the density and biomass of fish populations, and the increase in the mortality of fish larvae when water bodies are acidified to pH 5.0 [5, 6]. The above-mentioned negative processes become more serious with an increase in the concentration of aluminum in the water bodies [7, 8]. It is assumed that the action of aluminum at the cell level amounts to the possibility of changing the charge of the membrane [9]. Since the main components of cell membranes are lipids [10], the task of the investigation was to determine the change in their concentration in the organisms of hydrobionts experiencing a dual toxicological load (acidification of the medium and influence of aluminum), which will provide the possibility of tracing the development of adaptation processes from the point of view of the biochemical response of the organism at the cell level.

Analysis of the lipid compositions of the pond snails showed that phospholipids (PLs) are the main lipid component of their organism, amounting in the norm to 70% and more (Fig. 1). The addition of an aluminum salt in a neutral medium (experiments I and II) led to a fall in the total amount of PLs by a factor of 1.2 as compared with the control, and the fall in the concentration of PLs increased with a rise in the dose of toxicant. We tend to consider that this is connected with the hydrolysis of the PLs, an indirect proof of which may be the rise in the level of lyso-PLs that we have observed (Fig. 2). Since the pH of water falls on the addition of aluminum salts (Table 1), the hypothesis has been expressed of an activation in mollusk tissues, connected with the hydrolysis of the PLs, of lysosomal enzymes, a common property of which is a fairly low optimum pH for their activity (phospholipases and phosphatidate phosphatase) [15]. A fall in the concentration of PLs leads to a breakdown in the main foundation of the membrane — the phospholipid bilayer — as a result of which it becomes more porous and its permeability is disturbed [10]. In the same cases, a 1.2-fold increase in the concentration of cholesterol (Chs) was observed in the experiment as compared with the controls (see Fig. 1). Here the Chs/PL ratio increased in proportion to the rise in the concentration of toxicants (0.07, 0.09, 0.1). It may be assumed that this feature has an adaptation nature. It is known that the densifying action of Chs is shown only if the phospholipid components are disordered [10]. This situation can be understood not only as a change in the state of the hydrocarbon chains of the fatty acids of the PLs but also as the appearance of empty space in the membrane at the position of the hydrolyzed phospholipid component. Such a reaction (a fall in the level of PLs with an increase in the amount of Chs) on the addition of a lead toxicant has been shown for the fresh-water mollusk *Viviparus* [11]. In experiments on the toxicity of aluminum performed on fish eggs a change in the structure of the membrane

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TABLE 1. Experimental Scheme (n^* = 10).

Experiment (group)	Al concentration, mg/liter	pH
I (control)	–	7.7
II	2	6.0
III	5	6.0
IV	Acidification	5.5
V	2	5.0
VI	5	5.0

*n — Number of organisms.

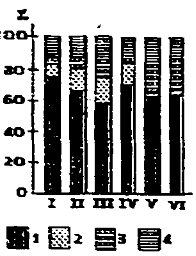

Fig. 1

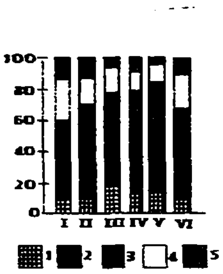

Fig. 2

Fig. 1. Levels of lipids in pond snails (% of the total): I-VI) experimental groups according to the scheme of the experiment; 1) PLs; 2) TAGs; 3) Chs; 4) Chs esters.

Fig. 2. Levels of individual phospholipids in pond snails (% of the total): I-VI) experimental groups according to the scheme of the experiment: 1) lyso-PLs; 2) Spm; 3) PS; 4) PE; 5) PAs.

was detected. The surface of the outer layer of the eggs — chorion — appeared more friable in the experiments than in the control [12].

It has been established that in a neutral medium (experiments I and II) aluminum causes an increase in the total amount of choline-PLs: sphingomyelin (Spm), phosphatidylcholine (PC), and lyso-PC — on a background of a fall in the concentration of phosphatidylethanolamine (PE) (see Fig. 2). A result of this is a unidirectional rise in their ratio (2.5, 4.0, 5.3), with an increase in the dose of toxicant. It has been established that a replacement of the functional groups of PLs (ethanolamine by choline) leads to a change in the temperature of the phase transition in the phospholipid biolayer of a membrane and affects its permeability [13]. This adaptation reaction permits the retention of, or even an increase in, the density of the membrane, particularly its outer side (PC and Spm) in addition to the rise in the concentration of Chs.

On the basis of the results of the experiment it may be concluded that the resistance of pond snails to the action of aluminum in a neutral medium depends directly on the enhancement of processes leading to the additional synthesis of lipids, which is necessary as an alternative to the hydrolysis of the PLs under the action of the toxicant. We have previously observed an analogous reaction of hydrobionts in experiments with nickel [14]. In the present experiment a 1.4-fold rise in the amount of lipids on the dry weight and a 1.2-fold rise on the crude weight was found (Table 2), which to some degree levels out the concentrations of the PLs in the experimental and control groups when calculated to the weight of the sample. However, in spite of this, the increase in the amount of lyso-PLs in the experimental groups II and III, calculated to the weight of the sample (2-fold, on an average, as compared with the control) particularly with an increased dose of aluminum, confirmed the hypothesis of an intensification of PL-destroying processes as a result of the activation of, in particular, phospholipase A_2. The result of the action of just this enzyme is a fall in the amounts of PC and PE and a rise in the concentration of lyso-PLs [15].

TABLE 2. Some Experimental Indices for the Experimental Groups

Group	Survival rate	Amount of total lipids, %	
		dry weight	crude weight
I	100	6.48±0.6	1.48±0.04
II	100	7.82±0.6	1.50±0.01
III	100	8.88±0.6	1.75±0.04
IV	100	6.64±0.04	1.35±0.005
V	60	11.11±1.4	1.78±0.1
VI	30	7.62±0.9	1.37±0.2

Observations of the animals for 48 h under the conditions of the experiment did not reveal appreciable differences between groups I, II, and III (the survival rate was 100% in all cases). However, biochemical investigations, in fact, showed that the experimental animals (groups I and II) were present at the second stage of the stress reaction — adaptation or resistance [16]. It may be assumed that at this stage the vital activity of the organism can be fully restored with the cessation of the action of aluminum or a fall in its concentration. In the opposite case, an exhaustion of the adaptation mechanism is possible. Thus, in spite of the 100% survival rate we found a toxicity of aluminum for pond snails present in a neutral medium.

In an acid medium (experiment IV) the level of lipids in the pond snail organism (see Table 2) and also the concentration of PLs as a whole was close to the control (see Fig. 1). However, a change was observed in the distribution of the individual phospholipid components (see Fig. 2). A considerable increase was found in the amount of choline-containingPLs against a background of a retention of the level of PE, so that their ratio increased by a factor of 3 as compared with the control. Apparently, the additional hydrogen cations attracted to the membrane and freely penetrating through it are accepted by the negatively charged PL components — PE and PA — located mainly on the inner side of the membrane [13]. It is possible that the adaptation reaction connected with an increase in the proportion of choline-containing PLs, as a factor densifying the outer layer of the membrane and lowering its negative charge, is a protection against the external agent and leads to a preservation of the internal medium of the cell from acidification. The ratio of neutrally charged PLs to those having a negative charge increased by a factor of 2.3 as compared with the control. Thus, it may be concluded that the adaptation mechanisms at the level of a change in some components of the pond snail organism that develop when the aqueous media is acidified to pH 5.5 for 48 h ensure the retention of their viability (the survival rate in this group was 100%).

The addition to the acid medium of a toxicant, as well — aluminum at a concentration of 2 mg/liter (group V) — led to an intensification of all the above-mentioned adaptation processes. In the first stage, the total synthesis of lipids increased considerably (see Table 2), which stabilized to some extent the total amount of PLs calculated to the weight of the sample. However, the amount of PLs as a percentage of the total lipid content fell, with a clear and considerable rise in the concentration of Chs (4-fold as compared with the control) (see Fig. 1), which, in its turn, served as the cause of a disturbance in the rate of metabolic processes as a whole and a fall in the survival rate of the pond snails to 60% 48 h after the beginning of the experiment (see Table 2). Here, changes were observed in the concentrations of individual PLs (see Fig. 2). Thus, the ratio of choline-PLs to PE was 7.6, which was three times higher than in the control. This fact showed a considerable additional densification of the outer layer of the membrane due to the neutral PLs. The total proportion of negatively charged PLs (PE and PAs) fell to 15.8%, as compared with 38.6% in the control, 21.2% in the acid medium without aluminum, and 29.9% in a neutral medium at a similar concentration of aluminum.

An increase in the dose of aluminum caused a fall in the survival rate of the pond snails of group VI to 30%. The animals of this group were present in a state of increased stress at the "exhaustion" stage, during which the development of adaptation processes ceases and the resistance of the organism falls [16]. This was also confirmed by some biochemical indices. It was established that the total amount of lipids in the organism of the pond snails of this group remained unchanged when calculated to the dry mass of the sample and even increased somewhat when calculated on the crude mass (see Table 2), which makes possible the stabilization of the level of PLs to some degree. However, at the same time, no appreciable changes whatever took place in the concentrations of individual PL components, with the exception of the Spm and PA. Apparently, aluminum added to an acid medium, in contrast to aluminum in a neutral medium, blocks a number of enzymatic reactions to different degrees as a function of its concentration. As a result of this, there was no definite regularity in the change in the amounts of individual phospholipid components. Thus, in the pond snails of group VI, compared with the control, the amount of Spm increased 1.3-fold with a similar fall in the concentration of PA (see Fig. 2). This may correspond to a strengthening of the outer PL layer of the membranes and to a fall in the negative charge of the internal PL layer through its binding with hydrogen and metal cations. The disturbance in the permeability of the cell membranes also led to a considerable increase in the Chs — more than 4-fold relative to the control.

Thus, the investigations of the lipids of pond snails present under the action of acidification of the medium and of aluminum showed that:

1) under the action of the toxicant both in acid and neutral media the total amount of PLs falls with a rise in the proportion of ChS and an increase in the Chs/PL ratio;

2) the concentration of lyso-PLs increases, which shows hydrolysis of the PLs;

3) the action of aluminum causes a rise in the amount of choline-PLs with a fall in the amount of PE; and

4) the toxicity of aluminum may be due to the fact that the metal cation changes the charge of the membrane, thus disturbing the transport of ions through it, which is shown additionally by an increase in the proportion of neutral PLs (lyso-PLs, Spm, Chs) as compared with negatively charged ones (PE and PA) located on the inner side of the membrane.

In addition, it has been established that a rise in the concentration of toxicants in a neutral medium causes an increase in their harmful effect. This is possibly also connected with the fact that aluminum leads to a fall in the pH of water and its toxic action is thereby doubled. In an acid medium, the toxicity of aluminum rises all the more. Under the conditions of a low concentration of toxicant, adaptation mechanisms are retained to some degree, while at a high concentration of toxicant these mechanisms are suppressed and the resistance of the organism falls sharply.

EXPERIMENTAL

The material was collected from the Koncherzer reservoir in a single expedition to eliminate the influence of both seasonal and ecological variability. The experiment was set up by the scheme shown in Table 1. All six groups of hydrobionts were present in aquaria of the same volumes under otherwise identical conditions (illumination, temperature, etc.). In the experiments we used natural water from the Koncherzer reservoir. Acidification was carried out with 0.1 N sulfuric acid to pH 5.5. Solutions of aluminum salt ($AlCl_3$) were prepared in the initial and the acidified water. When aluminum chloride was dissolved in concentrations of 5.0 and 2.0 mg Al/liter, as a result of hydrolysis the pH fell to 6.0 (initial water) and 5.0 (acidified water).

For the preparation of one sample of lipid extract, a whole mollusk was taken. The fixation of the specimen and the isolation of lipids from it were carried out by the generally used Folch method [17]. The quantitative determination of the lipid fraction was carried out by spectrophotometric methods [18] after their separation on thin-layer plates.

The results were calculated as percentages and were treated statistically by the generally adopted methods [19]. To evaluate the differences between the experimental and control groups we used the Student criterion (the differences were considered significant at $t = 1.7$-3).

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