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THE ULTRASTRUCTURAL CHANGES IN PERIPHERAL BLOOD LEUCOCYTES OF FISH EXPOSED TO THE PYRETHROID — CYPERMETHRIN

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Pyrethroids are the fourth class of insecticides. The insecticides utilize widely in agriculture. The pyrethroids are very toxic to water organisms, including fish. Cypermethrin is one of the synthetic pyrethroids The aim of the study was to get to know the pathological changes in leucocyte system of fish exposed to subtoxic concentration of cypermethrin, observed in electron microscope. The experiments were made on carp weighting 70±10 g, in aquaria conditions. The results showed that cypermethrin influence the internal mitochondrial membrane with her cristae of all kinds of leucocytes (disintegration process). The cytoplasm of the cells showed rarefacation, segregation and impoverishment. The cell nuclei were changed: the differences between euchromatin and heterochromatin were less expressed. Some of them took unusual, atypical shapes.

Key words*:* CARP (CYPRINUS CARPIO l.), CYPERMETHRIN, ULTRASTRUCTURAL CHANGES, LEUCOCYTES

Pyrethroids are important insecticides used largely because of their high activity and, coincidentally, low used doses. They are utilized widely in agriculture, forestry, horticulture, veterinary medicine and public health, and household. They are neurotoxins. The mechanism of action is modification of Na^+ channels activity, keeping them in the open state for an extremely long period, sometimes as long as several seconds [6]. It is known that pyretroids cause different cell toxic effects, too. Due to their lipophilic nature, pyrethroids are taken up by biological membranes and tissues. At the cellular level, pyrethroids act on a variety of biochemical and physiological target sites, between which the most important are cell membranes. The target sites in synaptosomal membranes and neuronal cells are ATPases [3, 4].The total ATP in neuronal cells cultures are changed, too.

Pyrethroids are very toxic to fish. Most pyrethroids are toxic to fish at extremely low concentrations, with LC_{50}s of generally less than 10 $\mu g \cdot dm^{-1}$. Cypermethrin, one of the most toxic active substances, was found to have LC_{50}s of 1,2, 0,9 and 0,5 $\mu g \cdot dm^{-1}$ in brown trout, carp and rainbow trout, respectively [8]. Sublethal and chronic studies of pyrethroids have demonstrated that fish are very susceptible to pyrethroids, especially early life stages. Efficient uptake of insecticides across the gill and into bloodstream can result in high toxicity to fish [1]. The lipophilicity helps the pyrethroids penetrate to fish organism very quickly. The aim of the study was to get to know the effects of cypermethrin on ultrastructure of fish peripheral blood leucocytes.

Materials and methods

Toxic substance:

Cypermethrin [isomer of 8 stereoisomers of ester of α-cyjano-3-phenoxybenzyl acid-cis-3-(2,2-dichlorovinyl)-2,2-dimethylcyclopropanecarboxylate $C_{22}H_{19}Cl_2NO_3$] — was obtained from Promochem Sp. Company, Poland and contained 99,7 % pure active substance. Cypermethin is one

of the type II of synthetic pyrethroid and contains an α-cyano-group. The tested substance was added to water only once, in sub-toxic concentration — 0,2 μg·dm^{-1}. The exposure time was 2 week.

Fish. The experiments were carried out on carp weighting 70±10 g, in aquaria conditions. The fish were keeping in river water with her parameters: BOD_5 — 2,2 mg O_2·dm^{-1}; dissolved oxygen — 10,5 mg O_2·dm^{-1}; pH — 7,7; total hardness — 4,5 mval·dm^{-1} (126 mg $CaCO_3$·dm^{-1}); temperature — 17±2 ˚C. The water was aeration during the whole time of the experiment. The carbon filters were used in aquaria. No fish death were observed during cypermethrin exposure. The fish were fed on the alternate days during the experiment. The fish, after the exposure period, were transferred to clean water for the next 4 weeks to find out if they can return to the homeostasis.

Biological material. Samples of peripheral blood were taken from 10 experimental and 5 control fish: after 3 and 14 days of exposure and in the end of convalescence time. Leucocytes were isolated from blood samples on gradient Lymphoprep^{TM} (Nycomed Pharma As Oslo, Norway). For electron microscopic study the cells were prepared in mixed solution: 2 % paraformaldehyde and 2,5 % glutaraldehyde buffered at pH 7,0 with 0,1 M cacodylate buffer for 2 h and then the cells were kept for at least 15–18 h in temperature 4 ˚C. After that they were washed and postfixed with 1 % osmium tetroxide in 0,1 M cacodylate buffer with pH 7,4 at 4 ˚C. Then the specimens were washed and contrast in 0,5 % aqueous solution of uranyl acetate for 2 h (room temperature). The specimens were dehydrated in graded alcohol series: 30, 50 and 70 % — 15 min at temp. 4 ˚C, 90, 95 and 100 % — 15 min at room temp. The next ethanol was replacing gradually with increasing concentration of propylene oxide up to 100 % of this substance. Propylene oxide was gradually replacing by Spurr Low Viscosity resin, finally with 100 % resin. The specimens were embedded in fresh resin and polymerized at 70 ˚C for 12 h. Thicker sections (60 nm) were cut and stained with 8 % uranyl acetate in 0,5 % acetic acid for 45 min and lead citrate for 10 min (Reynolds 1963), before examination with an electron microscope (Tesla BS-500).

Results and discussion

The results of the study show figures 1–5. Cypermethrin influences on fish leucocyte system during the exposure time and for long time after it (convalescence time). The most important pathological changes were observed in cell mitochondria. They were swollen, especially on the third day of exposure and the inner membranes with cristae were destroyed (Fig. 1).

They had been observed only in a few places of mitochondria. Many mitochondria were empty so they were electron light (Fig. 2). Process of lysis of the cell cytoplasm was observed, too, especially of peripheral cytoplasm. The cytoplasm was organelle-pure (Fig. 3). Some cells were completely destroyed. In the end of exposure the pathological changes were deeper. Some of the cells lost their round shape. The vacuolization of their cytoplasm and exocytosis was observed so the cells had very small organelle, were irregular. Differences between euchromatin and heterochromatin were indistinct (Fig. 4).

The convalescence time showed that organism of exposed fish had been coming back to the homeostasis (Fig. 5). Both pathologically changed cells and normal leucocytes could be observed. Vacuolization and exocytosis processes in cytoplasm were examined in pathological cells. Their nuclei had changed shapes and difference between euchromatin and heterochromatin were not clear.

Pathological changes in liver and skeletal muscle were studied [2, 7] during fish exposure to pyrethroid insecticide — neopybuthrin in concentration of 1/2 LC_{50} (2,1 mg·dm^{-1}) and LC_{50}. The mitochondria were swollen and degenerated and their cristae — difficult to be seen. The endoplasmic reticulum was showed fragmentation. The nuclei were poor in chromatin.

The toxicity of cypermethrin in neuronal cell cultures was studied by Kakko et al. (2004). The cytotoxic effects were studied by measuring total ATP and activity of mitochondrial enzymes. They observed dose-dependent decrease of total ATP in low concentration of cypermethrin, and

increase of the enzymes in low the pyrethroid concentration and decrease — in higher once. The reaction of total ATP to the pyrethroid exposure seems to be clear to explain in context of own results of the experiment. Evidence of cell stress was observed in mitochondrial functions as their reduction in mice given high doses of permethrin [5]. It seems that toxicological influence of cypermethrin, except neurotoxicity, concentrates in mitochondria, their ultrastructure and producing ATP in different types of cells.

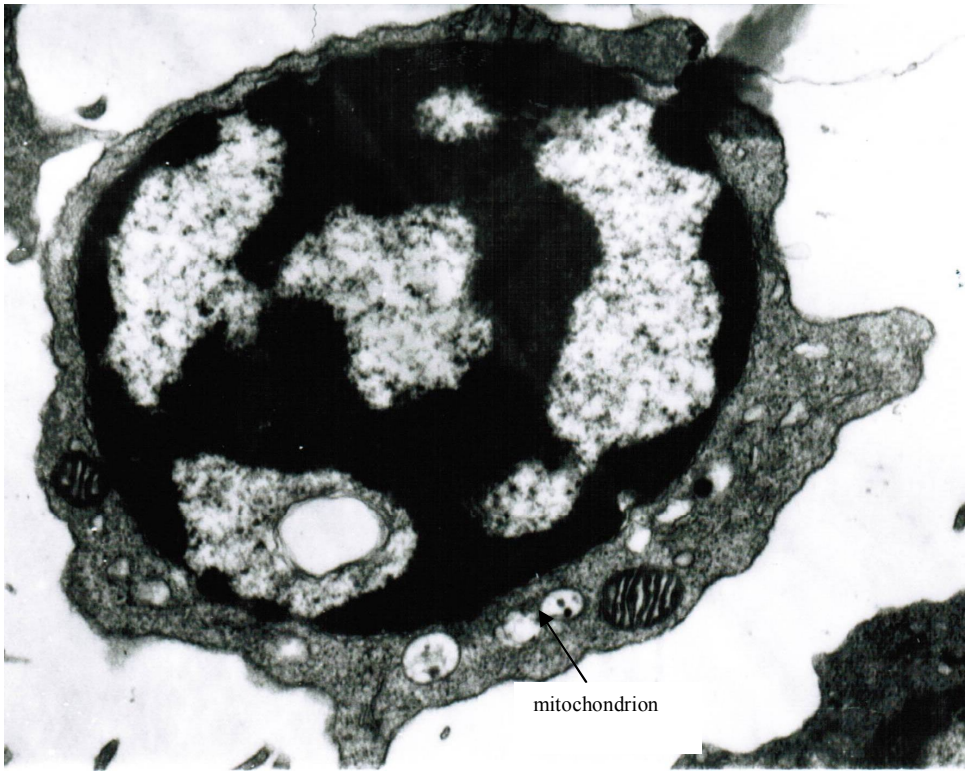

Fig. 1. Electron micrograph of a control lymphocyte of peripheral blood of carp showing normal mitochondrion and nucleus. Euchromatin and heterochromatin are clearly different. X 14000

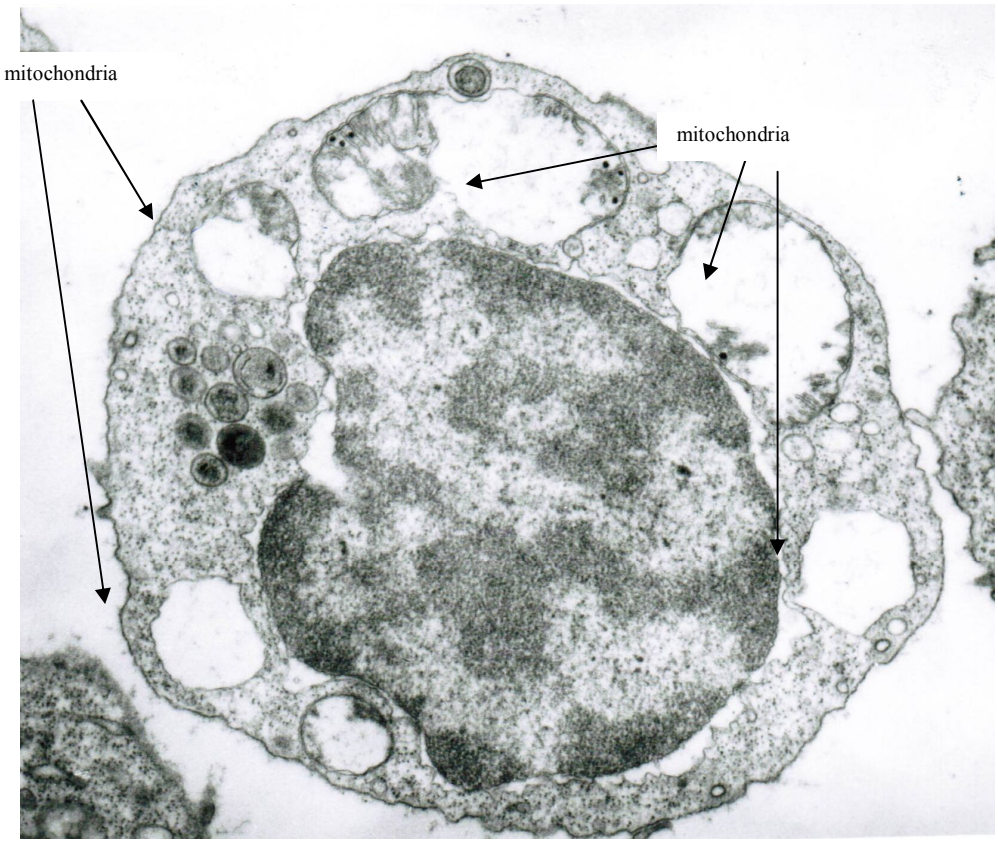

Fig. 2. Electron micrograph of lymphocyte of carp after 3 days of exposure to cypermethrin. Destroyed mitochondria with some cristae. The difference between euchromatin and heterochromatin is not clear. X 10000

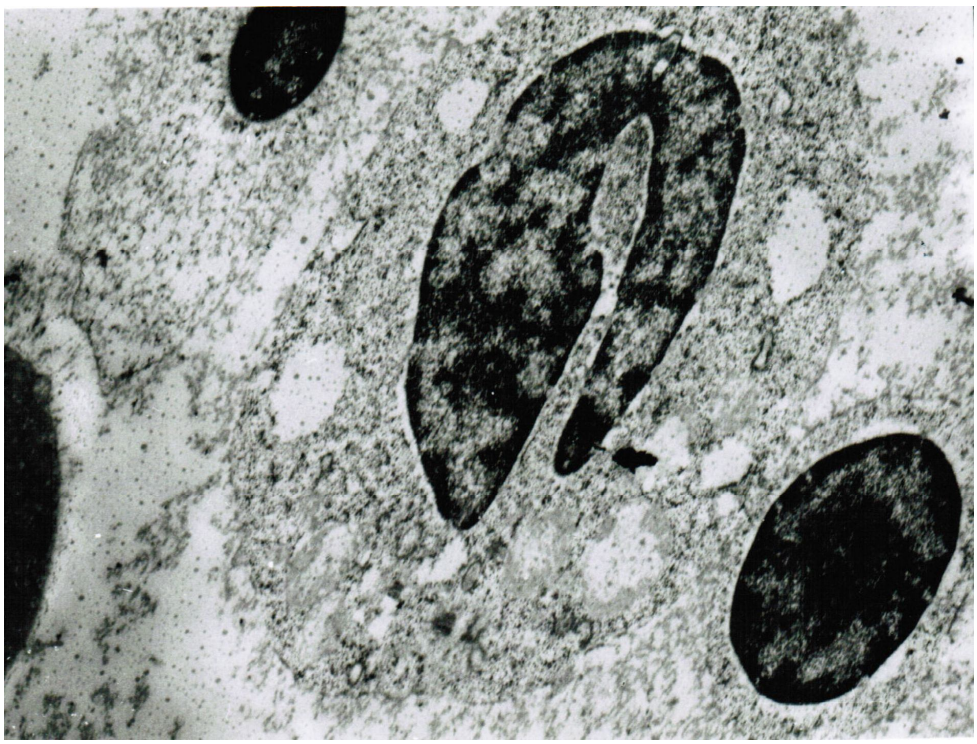

Fig. 3. Electron micrograph of leucocytes of carp after 3 days of exposure to cypermethrin. Completely destroyed cells. Lysis of cytoplasm. X 8000

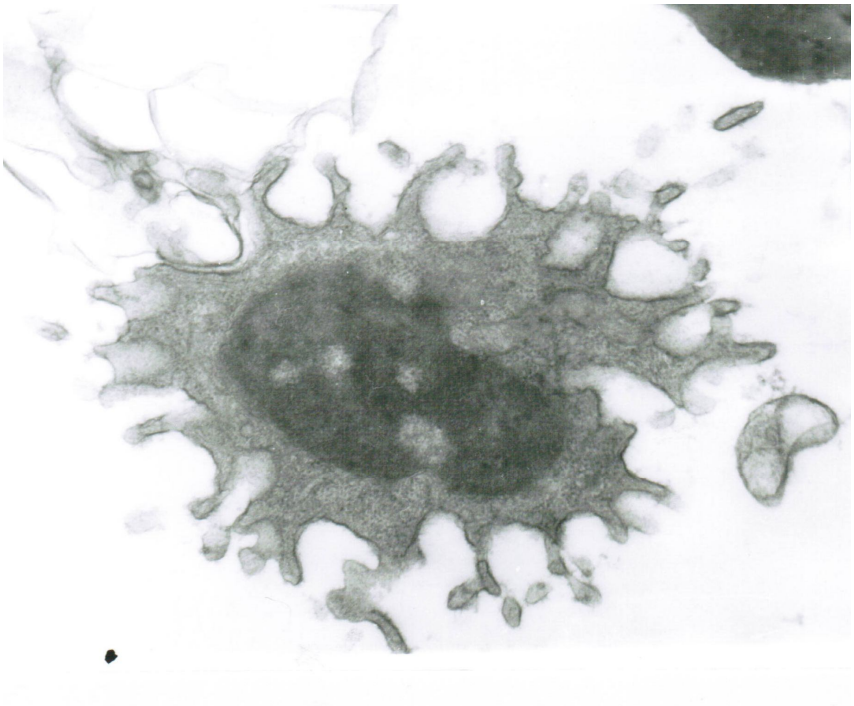

Fig. 4. Electron micrograph of lymphocyte of carp after 2 weeks of exposure to cypermethrin. Exocytosis of cytoplasm of the cell. X 18000

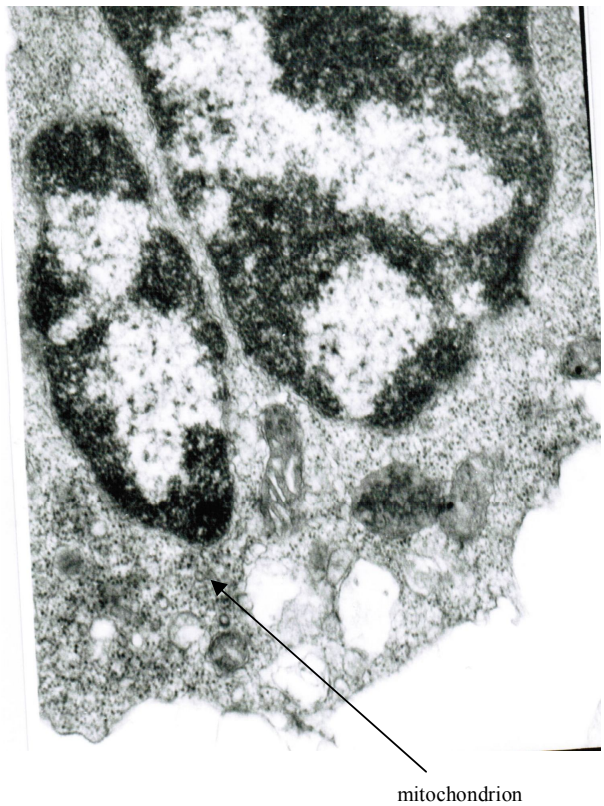

Fig. 5. Electron micrograph of lymphocyte of carp after 4 weeks of convalescence period. Normal cell structure. Correct structure of mitochondrion. X 18000

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УЛЬТРАСТРУКТУРНІ ЗМІНИ В ПЕРЕФЕРІЙНИХ ЛЕЙКОЦИТАХ КРОВІ РИБ ПІД ВПЛИВОМ ПІРЕТРОЇДУ-ЦИПЕРМЕТРИНУ

Р е з ю м е

Піретроїди належать до четвертого класу інсектицидів. Інсектициди широко використовують у сільському господарстві. Вони дуже токсичні для водних організмів, зокрема риб. Циперметрин є одним з піретроїхдів-синтетиків. Метою досліджень було встановити патологічні зміни у лейкоцитарній системі риб під впливом субтоксичних концентрації циперметрину. Досліди проводились на карпі вагою 70±10 г. Циперметрин впливає на внутрішню мембрану мітохондрій. Цитоплазма клітин зазнала сегрегації і зтоншання. Змінилось ядро клітини: відмінності між еухроматином і гетерохроматином були невиражені. Деякі з них мали незвичайну, нетипову форму.

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УЛЬТРАСТРУКТУРНЫЕ ИЗМЕНЕНИЯ В ПЕРЕФЕРИЙНИХ ЛЕЙКОЦИТАХ КРОВИ РЫБ ПОД ВОЗДЕЙСТВИЕМ ПИРЕТРОИДА-ЦИПЕРМЕТРИНА

А н н о т а ц и я

Пиретроиди принадлежат к четвертому классу инсектицидов. Инсектициды широко используют в сельском хозяйстве. Пиретроиди очень токсичные для водных организмов, включая рыб. Циперметрин является одним из пиретроихдив-синтетиков. Целью исследований было установить патологические изменения в лейкоцитарной системе рыб под воздействием субтоксичных концентрации циперметрина. Опыты проводились на карпе весом 70±10 г. Циперметрин влияет на внутреннюю мембрану митохондрий. Цитоплазма клеток испытала сегрегации и зтоншання. Изменилось ядро клетки: отличия между эухроматином и гетерохроматином были невыражены. Некоторые из них имели необычную, нетипичную форму.

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