EPR Study of Free Radicals Produced by Pesticides in the Blood of Mammals

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ABSTRACT

This article reveals the impact of pesticides on the free radicals in mammalian blood and on the paramagnetic centers. It has been discovered first time that pesticides ability to increase the quantity of free radicals, the influence of pesticides together with nitrates on the change of the paramagnetic centers of blood.

Keywords: pesticides, nitrate, blood, hemoglobin, nitrogen oxide, free radicals, paramagnetic centers, Electron paramagnetic resonance (EPR) spectrometer.

Introduction. Pesticides and free radicals are closely related to each other; therefore, pesticides have a toxic, allergic and mutagenic effect on the body of mammals. In a number of studies, it was noted that in those areas where pesticides and nitrates are intensively used, the frequency of diseases is much higher compared to other areas [1-3].

Pesticides can lead to a decrease in the number of erythrocytes and leukocytes, reduce the content of hemoglobin and total protein in blood plasma, and increase the activity of transamination in blood serum [4,5]. However, the mechanism of the effect of pesticides on blood transport proteins, enzymes, and hemoglobin still remains unexplored. The mechanisms of formation of a number of radicals and paramagnetic centers that can take part in the stimulation of lipid peroxidation, which are part of the membranes of blood cells, also remain unclear.

Pesticides are often used together with mineral fertilizers. Due to the widespread use of fertilizers, there is a risk of excessive accumulation of nitrates and nitrites in soil, ground water and plant foods. Nitrates are far from indifferent to the body of animals and humans. Entering the body of mammals, these substances under the action of the microflora of the oral cavity and the gastrointestinal tract can turn into nitrites [6,7]. There is information in the literature about the toxic effect of nitrites on the human body, animals and modeling of the EPR spectra of paramagnetic blood centers on a computer [8-11]. The main toxic effect of nitrites is their methemoglobin-producing activity [12]. However, along with the formation of methemoglobin in the blood, nitrites can be reduced to nitric oxide [13]. Nitric oxide, interacting with hemoglobin, forms $R-$ и $T-$ conformers of $Nb-NO$ complexes [14-15]. Entering the body of humans and animals, pesticides and nitro compounds can lead to severe tactic effects, the mechanism of which remains largely unexplored.

Materials and research methods.

It is convenient to consider blood in the form of two components of plasma and formed elements of erythrocytes, white blood cells. In blood plasma, the main paramagnetic centers are ceruloplasmin containing copper,

transferrin containing iron, semiquinone forms of blood coenzymes and ascorbic acid. When studying the effect of pesticides on the paramagnetic properties of mammalian blood, human donor blood and Wistar rat blood and hemoglobin were used as the object of study.

The EPR spectra were recorded using a 3-cm radio spectrometer "Rubin" with high-frequency modulation of the magnetic field (100 kHz) and a magnetic field sweep controlled by a computer.

The following pesticides were used in the experiments: diquat, prometrin, hydrel, kotaran, atrazine, zencor, devrinol, tenoran.

The effect of pesticides was studied on 40 Wistar rats weighing 190-200 grams, they were divided into four groups. The first, second and third groups of animals were injected intraperitoneally and intravenously at different doses of pesticides. The fourth group served as control. Animals were killed by decapitation after administration of the above substances after 40 min. Blood was frozen in 0.6 ml Teflon cartridges and frozen in liquid nitrogen. The amplitude values of the EPR signals were related to the weight of the wet sample and then averaged for the samples of one group.

Hemoglobin was obtained according to the method of Antonini and Brunori (Antonini Branori, 1971) [16].

In experiments in vitro, blood and hemoglobin were incubated with the above pesticides (at different concentrations).

Results and discussion. Free radicals, by definition, are particles that have unpaired electrons. They can be positively charged, negatively charged and neutral, and all three types of radicals play an important role. Radicals have different reactivity, depending on the temperature and concentration of the surrounding molecules. At very low temperatures, even very active radicals can be immobilized in highly viscous glasses or crystals and can live for a very long time; reactive radicals are often "trapped" at low temperatures for study by electron paramagnetic resonance (EPR).

We studied the effect of intraperitoneal injection of pesticides: diquat, prometrin, hydrel, kotaran, atrazine, zenkor, devrinol and tenoran on changes in the content of free radicals, as well as paramagnetic centers of ceruloplasmin and transferrin in the blood of rats.

It has been shown that the pesticides atrazine, zencor, devrinol, tenoran do not significantly affect the content and composition of paramagnetic blood centers. At the same time, intraperitoneal administration of diquat, prometrin, hydrel and cotoran in doses of LD_{50} resulted in a 2-6-fold increase in the content of free radicals in the blood in 30-60 minutes. The greatest change in the content of free radicals was observed with intraperitoneal administration of prometrin (3-5 times) and diquat (4-6 times). A smaller change in the content of free radicals was noted with the intraperitoneal administration of Hydrel (2-4 times) and Kotoran (2-3 times).

In order to understand the nature of the effect of pesticides on paramagnetic blood centers, experiments were undertaken to study the effect of this substance on paramagnetic Nb-NO complexes. The EPR spectra of Nb-NO complexes are well studied [11-15].

It is also known that conformational changes in Nb-NO complexes under the influence of a number of substances (2,3-diphosphoglycerote, ATP, sodium dodecyl sulfate) lead to a change in the shape of the EPR spectra.

Therefore, it could be expected that the complexes could be an experimental model for studying the possible effect of pesticides on the protein frequency of the blood pigment. In addition, as mentioned earlier, pesticides can enter the body of humans and animals against the background of nitrate-nitrite intoxication. Therefore, the course of this form of intoxication may have its own characteristics.

Literature data on the effect of pesticides on paramagnetic blood centers under conditions of nitrate-nitrite intoxication are unknown to us. In this regard, Nb-NO complexes formed during nitrate-nitrite intoxication could be not only an experimental model for studying the effect of pesticides on proteins, but also be of independent importance for studying the

binary action of pesticides and nitro compounds.

Bring it out. For the first time, the effect of pesticides on the EPR spectra of animal blood was shown, and it was also found that the EPR spectra of blood significantly depend on the presence of nitrites in the environment. Thus, strengthening of the combined action of pesticides and nitrites was found, which makes it possible to organize a service for monitoring the action of harmful environmental factors.

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