

## **State of Nucleolar Apparatus and Ribosome Genes Activity of Peripheral Blood Lymphocytes in Rats Effected by 2,4 - DA.**

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### 1. INTRODUCTION.

High multifunctional biological activity of dioxines accumulating in environmental abiotic components as well as in the body of man and animals and exercising hormone-like effect necessitates the assessment of ecotoxicologic and medical consequences of this toxic substance effect. The increase of people's exposure to dioxines in the production area, accidents at the chemical enterprises, consuming dioxin contaminated food and dwelling in polluted districts makes the problem more urgent. The problem is complicated by specific toxic effect of dioxins on cells, tissue, age and sex of the population, dependence of their toxicokinetics and pathogenesis of intoxication on exposure, genetic and environmental peculiarities. All this requires new, unconventional approach to methods of study, effect and mode of influence of these substances on man's health [1].

In the present study we endeavoured to estimate the peculiarities of nucleolar genesis in the peripheral blood lymphocytes and their metabolism depending on the dose and time of exposure to 2,4 - D (2,4-dichlorophenoxivinegar acid) in experimental animals in a subacute trial.

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## 2. MATERIALS and METHODS.

The experiment was carried out on mature white rats (n=48), which were divided into six groups according to the intragastric administration of 2,4 - D amino salt dose: 1gr. - LD50; 2gr. - 1/2LD50; 3gr. - 1/4LD50; 4gr. - 1/20LD50; 5gr. - 1/200LD50 and 6gr. - control animals. The exposure time was as follows: 1 hour, 24 hours, 7 days, 14 days, 21 days and 28 days for each group of animals

The assessment of cytochemical reaction of the nucleolus structure with silver nitrate, as well as the reaction itself, was performed in accordance with V.M. Pogo-relov's modification [2].

This method consists in silver impregnation of interphasal cells stained with methylene blue. The following values were estimated: nucleolar coefficient (NC), Ag - negative cells ratio (%), K1 - the number of Ag - positive NOR (nucleoly - organizing region) in the cell, K2 - the average size of Ag - positive NOR 50 - 100 cells were studied in every case. Statistic data were calculated using STAT1-STAT2 programme.

## 3. RESULTS.

The decrease in Ag - positive NOR's value was revealed within the first hours of exposure in the first three experimental groups. The value decrease was detected on the 14th experimental day following 1/20 LD50 and 1/200LD50 administration. Thus, a more prolonged exposure time to 2,4 - DA is required to achieve the agent concentration level in animals capable of causing this shift. The decrease in Ag - positive NOR count proves that following 2,4 - DA exposure the decrease in transcriptionally active NOR content is observed in the lymphocytes nucleoli. Consequently, 2,4 - DA affects the transcription and processing of pre - ribosome RNA which results in r - RNA synthesis inhibition and finally cell metabolism inhibition.

The increase in NC occurs in an hour following the administration of large doses of 2,4 - DA (LD50; 1/2LD50 and 1/4LD 50). Thus it implies that 3 - 4 nucleoli - containing lymphocytes appear in the peripheral blood in rats. The phenomenon is considered abnormal as normally the peripheral blood lymphocytes contain 1 or, rarely, 2 nucleoli. The similar phenomenon is observed only on the 14th experimental day following the administration of low doses (1/20LD50 and 1/200LD50). Afterwards, the average number of cell nucleoli becomes normal and even decreases as compared to the control values on the 21 - 28 day irrespective of the dose. That is 3-4 nucleoli containing lymphocytes are observed in the peripheral blood.

Similar initial multinucleolar lymphocytes are in accordance with the previous index as regards the r - RNA synthesis inhibition when ring-like nucleolar lymphocytes transform into a point or micronucleolar ones. When observed under the electronic microscope they are characterized by consolidation (degranulation) and granular component isolation from the fibrillar centres. Burch and Smetana (1970) defined this phenomenon as "nucleolar segregation", which occurs in case of cellular degeneration.

Moreover, there is an obvious increase in the average size of Ag - positive NOR in the first two groups (LD50 and 1/2LD50) within the first hours of exposure. Then these values return to normal. Such argentic nucleoli (S Ag) are associated with the decrease in the number of Ag - stained NOR and nucleolar segregation. 2,4 - DA directly or indirectly inhibiting the transcription of t - RNA break off merging of prenucleolar bodies into a mature nucleus. It results in the increase of interphasal "nucleoli" number which being stained reveals tense r - RNA - related (C23 protein) Ag - granules. But they are not associated with a newly synthesized r - RNA molecule (B23 protein), Ag - granules [4,5]. In this case such an increase in silver granules proves its inhibition rather than the increase in r - RNA - synthesis.

Though Ag - negative cells value failed to reveal obvious changes the tendency discovered could be explained in the following way. This drop of value following the administration of LD50 and 1/2LD50 within an hour and 24 hours with its subsequent increase suggests that the initial increase in lymphocytes with Ag - positive nucleoli - forming regions of chromosomes in nucleoli is attributed to the compensative reverse lymphocytes activity and the presence of additional ribosome genes copied [3]. However, the functional activity of "intact" cells becomes finally exhausted and their gradual irreversible destruction occurs, the fact being confirmed by relative and absolute lymphocytes count values. In the first three groups ( LD50, 1/2 LD50 and 1/4 LD50 ) the values remained stable within 24 hours, later within the following 21 days relative and absolute lymphocytosis is observed, the values becoming normal on the 28 day. These values tend only to increase following the administration of 1/20LD50 and 1/200LD50 doses on the 28 day. The fact suggests that a considerable number of reserve lymphocytes are released into the peripheral blood in response to the destructive effect of the agent. In this case the increase in cells number compensates their qualitative (functional) incompetence.

Consequently, nucleogenesis values dynamics in response to 2,4 - DA effect testifies to the fact that the substance in question causes deep

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structural, functional and regulative transformations in the cells (lymphocytes).

## 4. REFERENCES.

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