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THE EFFECT OF CONSTANT LIGHTING ON THE ORGANIZATION OF CIRCADIAN RHYTHMS OF HEMATOLOGICAL PARAMETERS IN WISTAR RATS AT AGE OF 6 MONTHS

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ABSTRACT: The effect of constant lighting on the level of some hematological parameters and their daily dynamics was studied in male Wistar rats at the age of 6 months. It was found that staying in constant light for 3 weeks leads to a rise in hematocrit level, mainly due to an increase in the number of red blood cells, while a decrease in the oxygen-transporting properties of these cells is noted. An analysis of the diurnal dynamics of the studied parameters, it was found that the keeping of animals under constant illumination destroys the circadian rhythms of HGB, MCV, RDWc, PCT, MPV, PDWc, and MCHC. The destruction of the circadian rhythms found in control is most likely due to both a decrease in the level of melatonin in constant light and a violation of its own circadian rhythm in the absence of a pacemaker, which role is played by the alternation of light and darkness.

INTRODUCTION: The rhythmicity of functioning is the fundamental property of all living systems, which plays one of the main roles in ensuring normal vital functions. On the basis of biological rhythms, periodic programs are built that provide the necessary order of the course of bioprocesses, the optimal level of functioning of the organism at any given moment of time. It is natural that the blood system, which response to all changes in the external and internal environment, has biorhythms of the processes of proliferation, differentiation, migration, metabolism, and apoptosis of its cellular elements.

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A quantity of circulating blood cells and their functions, particularly, the phagocytosis, activity of natural killer cells, kind of reaction on mitogens, and also formation of blood cells in the bone marrow and its reaction on toxical (for example, chemotherapeutic) agents show a presence of biorhythms at several frequencies, of which circadian rhythms are most intensively studied.

Some of these rhythms show sufficiently large amplitudes to be clinically important, especially if it is necessary to evaluate successive samples of the same patients. Violation of the rhythm characterizes hematological and immunity-related diseases, such as for example, HIV infection. Circadian rhythms of platelet aggregation and adhesion enhance the blood coagulation properties in the morning, which is believed to lead to a peak incidence of myocardial infarction, cerebral infarction, and sudden cardiac death [1]. For example, a number of researchers show that the

count of circulating leukocytes (WBCs) involved in immune defense is prone to rhythm [2, 3]. Periodic changes in the number of white blood cells circulating in the peripheral blood can be caused by several factors. These include the distribution of circulating and marginal cell components of tissues and organs, the influx from storage sites, cell proliferation, the release of de novo cells into the blood circulation, and the destruction and removal of cells. The main mechanisms of circadian changes in circulating blood cells have not been fully elucidated [4, 5, 6]. The temporal organization of the blood system, as well as other mammalian systems, is endogenous and genetically determined, but, nevertheless, it is modulated by periodic environmental factors – synchronizers, or "time cues". It is known that the light regime is one of the most powerful synchronizers of daily biological rhythms in mammals. Violation of the lighting regime can cause a state of desynchronosis, which is a powerful stressful factor that can lead to the development of a particular pathology, especially if there is a predisposition to it or the adaptive capabilities of the organism are weakened.

According to the hypothesis of circadian destruction, exposure to light at night disrupts the endogenous circadian rhythm, suppresses night time secretion of melatonin by the pineal gland, which leads to a decrease in its concentration in the blood [7]. This fact actualizes the significant contemporary problem of light pollution, in other words, the fact that an increasing number of people, due to profession or other social reasons, are exposed to lighting in the dark. Excessively continuous lighting has become an essential part of modern lifestyles, which is accompanied by many serious behavioural and health disorders, including cardiovascular disease and cancer [8, 9, 10].

In particular, such a common phenomenon as a violation of the CR during shift work leads to an increased risk of cardiovascular disease, metabolic syndrome, and type II diabetes mellitus [11]. In addition, animal studies have shown that even replacing of complete darkness with dim lighting at night causes metabolic disorders and obesity [12, 13]. Based on the foregoing, it looks actual to study the values of some hematological parameters and their daily dynamics in Wistar rats under constant lighting.

MATERIALS AND METHODS:

Animals: The research was conducted at 6-months-oldmaleWistarrats. Animals were taken from the Stolbovaya nursery (the "Stolbovaya" affiliate of the Federal State Budgetary Institution of Science "Scientific Center for Biomedical Technologies of the Federal Medical and Biological Agency). The study used 80 animals, divided into 2 equal groups. Animals were kept in cages (5 rats per cage), at standard conditions (temperature 16–18 C) and fed with granular feed with *ad libitum* access to tap water.

Treatment Design: Animals of the first group were kept under a fixed illumination, L:D 14:10 (±180 lux, respectively; 8:00 AM lights on) (unless mentioned otherwise) during the whole experiment. Animals of the second group were studied under the light regime, representing constant light (LL ±180 lux). Both the first and second groups of animals were kept at the specified light regime for 3 weeks. After three weeks, euthanasia of the animals in the carbon chamber was performed at 9.00 h, 15.00 h, 21.00 h, and 3.00 h, blood was collected for biochemical studies. All animal experiments were performed according to EC Directive 86/609/EEC compliance and with the Russian law regulating experiments on animals.

Blood Sampling and Analyses: Peripheral blood samples were collected from the tail vein in EDTA tubes. In blood samples the selected hematological parameters (WBC – total white blood cell count, LYM – lymphocytes count, MID – medium size cells count, GRA – granulocytes count, LYM% – lymphocyte percentage, MID% – medium size cells percentage, GRA% – granulocytes percentage, RBC – red blood cell count, HGB – hemoglobin, HCT – hematocrit, MCV – mean corpuscular volume, MCH – mean corpuscular hemoglobin, MCHC – mean corpuscular hemoglobin concentration, RDWc– red cell distribution width, PLT – platelet count, PCT – platelet percentage, MPV – mean platelet volume, PDWc– platelet distribution width) were measured using hematological analyzer Abacus junior VET (Diatron®, Austria).

Statistical Analysis: The obtained data, analyzed using Graph Pad Prism 6.0, were expressed as Mean ± SD. The statistical difference was determined using Student t-tests. A p-value of <

0.05 was considered statistically significant. For the analysis of characteristics of circadian rhythm of the studied substances, the cosinor-analysis carried out by means of the Cosinor Ellipse 2006-1.1 program was used. The presence of a reliable circadian rhythm and also its acrophase and amplitude were determined. Acrophase is the measure of peak time of the total rhythmic variability in a 24-h period. Amplitude corresponds to half of the total rhythmic variability in a cycle. The acrophase is expressed in hours; amplitude values are expressed with the same units as the documented variables.

RESULTS: Resulting from the research, it is revealed that keeping animals in conditions of constant lighting leads to a change in values of some haematological parameters. In particular, constant lighting influence leads to an increase of hematocrit (HCT) from 51.13 ± 1.93% in control group to 53.36 ± 1.95% in the experimental group.

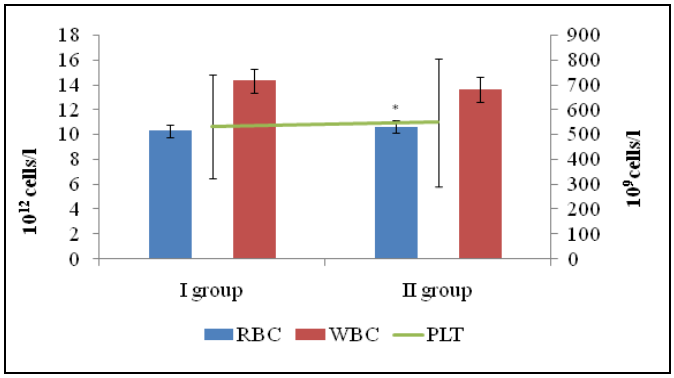


FIG. 1: INFLUENCE OF CONSTANT LIGHT ON TOTAL LEUCOCYTES, ERYTHROCYTES AND THROMBOCYTES COUNTS

The analysis of total white blood cell count (WBC), red blood cell count (RBC) and platelet count (PLT) at blood of rats revealed that staying of rats in conditions of constant lighting results in increase of red blood cells count from 10.31 ± 0.48 × 10^{12} cells/Lat control group to 10.63 ± 0.51 × 10^{12} cells/Lat experimental group. At the same time, the parameters of leucocytes and thrombocytes count stay unaltered, 14.33 ± 4.80 × 10^9 cells/L and 534.80 ± 209.00 × 10^9 cells/Lat fixed light regime, 13.62 ± 5.17 × 10^9 cells/L and 551.00 ± 256.60 × 10^9 cells/L in conditions of constant light **Fig.1**. The study of hemoglobin content (HGB) showed the absence of significant differences of this parameter between rats of the control and experimental groups. When the rats were kept under constant illumination, there was a tendency to an increase in the hemoglobin content in the whole blood of rats from 166.40 ± 6.28 g/l to 168.20 ± 9.03 g/l. At the same time, we noted the reliable decrease in mean corpuscular hemoglobin concentration (MCHC) from 325.50 ± 12.09 g/l to 315.00 ± 9.28 g/lat studied groups. When analyzing the effect of constant illumination on the average hemoglobin content in an individual red blood cell (MCH), the decrease of this parameter from 16.15 ± 0.73 pg in control to 15.83 ± 0.79 pg in the experiment is shown **Fig. 2**. The mean volume of erythrocytes (MCV) increases from 49.63±1.82 mkm to 50.3 ± 2.03 mkm.

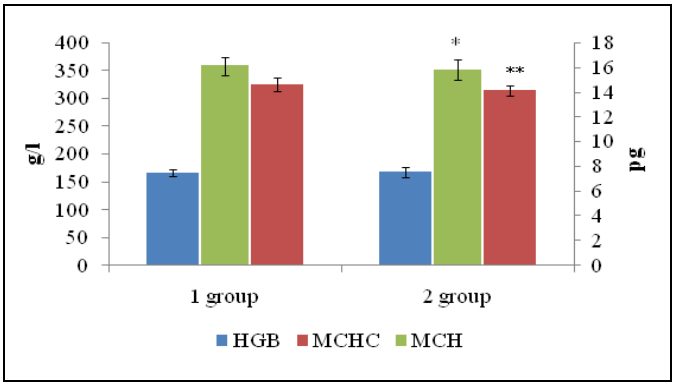


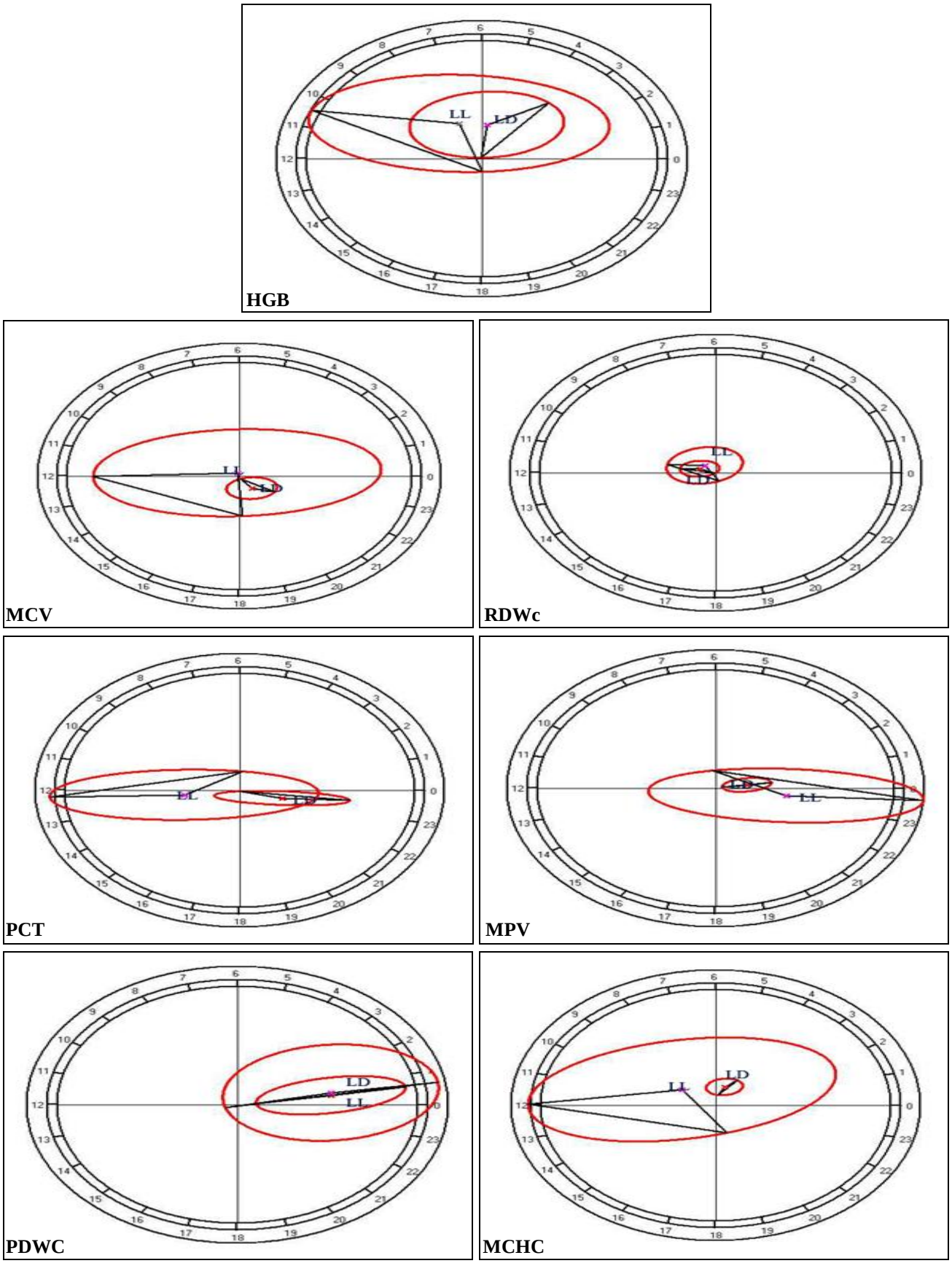
FIG. 2: THE EFFECT OF CONSTANT ILLUMINATION ON THE HEMOGLOBIN CONTENT IN RAT BLOOD AND THE AVERAGE CONCENTRATION OF THE HEMOGLOBIN IN THE ERYTHROCYTE MASS

Studying the diurnal dynamics of the considered parameters at a fixed light regime according to the results of cosinor analysis, we found reliable circadian rhythms for a number of them **Table 1**.

TABLE 1: AMPLITUDE-PHASE CHARACTERISTICS OF RELIABLE CIRCADIAN RHYTHMS OF SOME HEMATOLOGICAL PARAMETRS OF RATS UNDER A FIXED LIGHT REGIME (BASED ON RESULTS OF COSINOR ANALYSIS)

Parameter	HGB,	MCV,	RDWcv,	PCT,	MPV,	PDWc,	MCHC,	P-LCC,	PLC-R,
Amplitude of rhythm	3.71	1.12	0.41	0.127	0.34	1.25	5.99	25.23	1.94
Acrophase of rhythm, h	1.10	-3.30	10.20	-0.50	0.42	0.04	4.46	0.0.24	0.31

In this case, the presence of animals in constant light leads to the destruction of some of them. This applies to circadian rhythms of HGB, MCV, RDWc, PCT, MPV, PDWc, and MCHC **Fig. 3**.



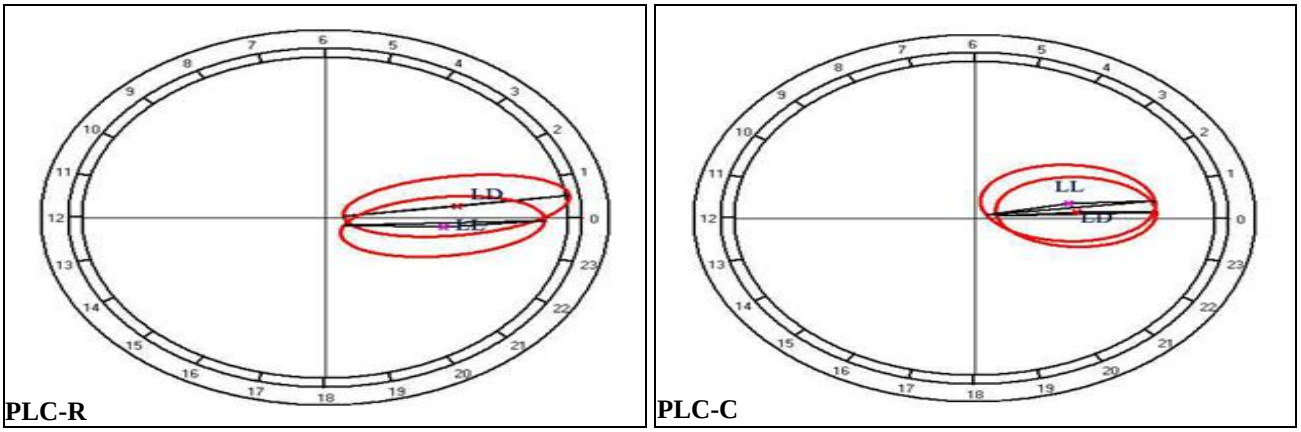


FIG. 3: THE EFFECT OF CONSTANT LIGHTING ON THE CIRCADIAN RHYTHMS OF SOME HEMATOLOGICAL PARAMETERS ACCORDING TO THE RESULTS OF COSINOR ANALYSIS

In the case of PLC-C and PLC-R both the phase and amplitude characteristics of rhythm are practically not changed, and at an analysis of diurnal rhythmicity of PDWc the amplitude of rhythm sufficiently increases, but its acrophase stays unaltered.

DISCUSSION AND CONCLUSION: The conducted results testify that staying of animals in conditions of constant lighting for three weeks leads to raise in level of hematocrit, predominantly due to an increase of the quantity of erythrocytes. At the same time, the decrease in the level of mean corpuscular hemoglobin on the background of mean erythrocyte volume growth is noted.

Such effects, in our opinion, confirmed by a number of sources, are associated with a prolonged and persistent decrease in the concentration of melatonin in conditions of constant light. It is well known that the pineal gland carries out the correction of activity of the central nervous system and immune status, mainly through the secretion of melatonin. In addition, direct hormonal regulation of blood cell activity through a membrane and nuclear specific melatonin binding points cannot be ruled out [14, 15]. It was shown that melatonin not only has a stimulating effect on erythropoiesis but also optimizes morpho-functional characteristics of blood cells [16, 17].

The protective effect of melatonin on red blood cells is apparently mediated by a complex of cellular and systemic mechanisms. The first concerns to its ability to restrain the processes of oxidative stress and cause an increase in the antioxidant status of the organism as a whole, to provide protection for mitochondrial membranes, and ensure anti-inflammatory and immuno-modulatory effects. Of the systemic mechanisms of influence on the blood system, rhythm-organizing and anti-stress capabilities of the hormone are of the greatest importance [18, 19].

The violation of namely the rhythm-organizing function of melatonin is manifested when considering the effect of constant lighting on the circadian rhythm of the studied hematological parameters. The destruction of the circadian rhythms found in control is most likely due to both a decrease in the level of melatonin in constant light and a violation of its own circadian rhythm in the absence of a pacemaker, which role is played by the alternation of light and darkness.

Based on the results of the study, we can conclude that the impact of constant lighting entails the development of hypohemoglobinemia. As a result, there is a compensatory reaction to a decrease in the oxygen transport function of blood, which manifests itself in an increase of mean volume of erythrocytes (MCV) and total red blood cell count.

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CONFLICTS OF INTEREST: The authors declare that there is no conflict of interest regarding the publication of this paper.

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