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The Influence of Experimental Hypercalcemia on the Organization of Circadian Rhythms of Rectal Temperature, Some Metabolites and Red Blood Indices

Areshidze D.A., Kozlova M.A., Kuznetsova I.A.*, Makartseva L.A.

Moscow State Regional University, 10A, Radio st., Moscow, Russia

Abstract.

The results of the study indicate a very significant effect of experimental hypercalcemia on the organization of circadian rhythmicity of the studied metabolites, rectal temperature and red blood indices. The greatest influence of experimental hypercalcemia is manifested in the circadian rhythmicity of albumin, as the animals of the experimental group do not show a reliable rhythm of this substance according to the results of cosinor analysis. The circadian rhythm of blood glucose also undergoes significant changes, and the rhythm of creatinine is the least variable, as does the circadian rhythm of rectal temperature, although in both cases the acrophase of rhythms is shifting. The circadian rhythmicity of the studied blood parameters was also affected by the increased level of Ca in animals. The acrophases of the rhythms of most of the studied hematological parameters in the control group occur at night and early morning hours. In contrast to the control parameters, in the animals of the experimental group the acrophases shift for the light time of day and the beginning of the dark period. Experimental hypercalcemia, in addition to disturbing of circadian rhythmicity as such, leads to desynchronosis of the studied rhythms. Thus, a violation of the normal level of calcium, one of the important homeostatic parameters of an organism, entails significant changes in the circadian rhythms of various body parameters.

Key words: circadian rhythm, hypercalcemia, red blood, temperature, albumin.

INTRODUCTION

The regularities of biorhythmic processes in a organism may be used to improve the prevention, diagnosis and treatment of diseases, including the use of pharmacological drugs. Circadian oscillations of various physiological parameters can determine the effectiveness of drug therapy. Thus, studies of the regularities of the effect of medications administered to the body, on the parameters of its biorhythm can serve as a basis for improving of effective treatment courses and for recommendations on their intake time. Considering the fact that the majority of preclinical studies are carried out using laboratory animals, in particular rats, the characteristics of the circadian rhythms of their physiological functions should be taken into account for the correct interpretation of the obtained results. Most physiological processes in an organism of mammals follow daily oscillations. These rhythmic processes are governed by environmental cues (e.g., fluctuations in light intensity and temperature), an internal circadian timing system, and the interaction between this timekeeping system and environmental signals [1-3].

Circadian oscillations of physiological functions in the mammalian organism are a strictly hierarchical system, the realization of various processes in which is carried out in strict sequence [4-7].

Biological rhythms are one of the fundamental phenomena that serve as the basis of homeostasis. They are the basis of the protective-adaptive reactions of the organism, both in the momentary situations, and at long and powerful affecting of negative factors on an organism. Violation of the rhythm and synchronism of the functioning of systems entails a plume of hormonal-metabolic and morphological changes leading to the defeat of almost all functional systems.

Organization and maintenance of circadian rhythms is determined by a whole complex of exogenous and endogenous factors, the leading role among which occupies the light sensor of time, exact alternation of light and darkness. The formation of the structure of circadian rhythms, occurring in ontogeny in parallel with the maturation -of functional systems, is an indicator of the adaptive capabilities of the organism [8-12]. Consequently, the most sensitive indicator of the adaptive capabilities of an organism are biorhythms, in particular, circadian rhythms [13, 14].

The primary role in the coordination of circadian oscillations is performed by primary structures-pacemakers, which include the suprachiasmatic nuclei of the hypothalamus and the pineal gland, performing their functions as organizers of the daily rhythm of functions by interacting with both the endocrine system and secondary oscillators. Secondary pacemakers, although they control the oscillators of the next level, are hierarchically subordinated to the leading oscillators, and as a rule are connected with them by feedbacks [15-21].

In the event of disruption of the normal functioning of these units, or if the morphofunctional links between them are disturbed, the state of desynchronosis, as external and internal, may occur, and the adaptive capabilities of the mammalian organism may decrease [22-31].

One of the most important links in the system ensuring normal circadian rhythm are the parathyroid glands (PTG). In particular, their role in the regulation of the activity of the HPA axis is shown, and the involvement of the PTG in the regulation of circadian rhythm can be carried out either directly, through the production of parathyroid hormone, or indirectly, through a change in the level of calcium in an organism [32-36].

Despite the well-known important role of calcium in the functioning of the mammalian organism, the issues of the peculiarities of the organization of its circadian rhythms, of their reorganization in the norm and in pathologies remain relevant for study. So, it is well known that the circadian rhythm of calcium is clearly expressed, has a sufficiently high amplitude, in the blood plasma of mammals the rhythms of total and ionized calcium are in antiphase. In this case, the daily rhythm of the ion depends on many factors and can have high variability [37-46].

It is also important to study the effect of this ion on the organization of circadian rhythms of mammalian organs and systems, taking into account the principal role of calcium in their functioning. Given the fundamental role of Ca^{2+} signaling in biochemical integration and coordination, these intrinsic rhythms in mammals are ideally placed to impose circadian order across the many functions of a cell.

Studies of M.C. Harrisingh et al. [47] have shown that although circadian oscillation in dynamics of intracellular Ca^{2+} signals has been observed in both plant and animal cells, it has remained unknown whether Ca^{2+} signals play an *in vivo* role in cellular oscillation itself. To address this issue, it was modified the dynamics of intracellular Ca^{2+} signals in circadian pacemaker neurons *in vivo* by targeted expression of varying doses of a Ca^{2+} buffer protein in transgenic Drosophila melanogaster. Intracellular Ca^{2+} buffering in pacemaker neurons results in dose-dependent slowing of free-running behavioral rhythms, with average period >3 h longer than control at the highest dose. The rhythmic nuclear accumulation of a transcription factor known to be essential for cellular circadian oscillation is also slowed. It was also found in

this experiment that Ca^{2+} buffering interacts synergistically with genetic manipulations that interfere with either calmodulin or calmodulin-dependent protein kinase II function. The results of this experiment indicate a role for intracellular Ca^{2+} signaling in regulating of intrinsic cellular oscillation *in vivo*.

These and other data allow us to assume that a violation of calcium metabolism, such as hypercalcemia, can lead to disruption of the circadian rhythm of many functions of the mammalian organism.

The toxic effect of calcium is manifested only with prolonged admission and usually in persons with disturbed metabolism of this bioelement (for example, in hyperparathyroidism). Poisoning can occur at a regular intake of more than 2.5 grams of calcium per day. The main causes of calcium excess in an organism:

- Excess calcium intake with food, drugs or dietary supplements.
- Disturbance of calcium metabolism, including cases associated with disorders of regulation.
- Hypervitaminosis D.

According to a number of reports, the most common cause of hypercalcemia in the general population and among outpatients is hyperparathyroidism; other researchers report a relatively high frequency of hypercalcemia due to the use of thiazide diuretics, at thyroid diseases, the Burnett syndrome (milk-alkali), and long-term immobilization. Malignant neoplasms are more common among patients in the therapeutic hospital than in the general population, and, according to most reports, are the most common cause of hypercalcemia in this case.

Proceeding from the above, we considered it necessary to conduct a study of the effect of experimental hypercalcemia on the circadian rhythm of rectal temperature, albumin, creatinine and glucose blood content, levels of hematocrit and red blood parameters in Wistar rats at a fixed light regime.

MATERIALS AND METHODS

Animals.

The experiment was performed on male Wistar albino rats at the age of 6 months. In the experiment 60 rats divided into 2 groups were used.

Group I - intact animals (n = 30);

Group II - animals with experimental hypercalcemia (n = 30).

Modeling of hypercalcemia.

For modeling of hypercalcemia, rats were provided with ad libitum consumption of drinking water with the addition of calcium chloride at a concentration of 235 mg/dm^3, in terms of ionized calcium (daily consumption: 8.1-10.2 mg/kg of body weight) for two months. Water with the addition of calcium 235 of mg/dm^3 was prepared from a 10% solution of calcium chloride (AOA Dalkhimpharm, Russia) by dilution with tap water using the scheme: 6,526 cm^3 of a 10% solution per 1 dm^3 of water.

Study design.

During the whole experiment, the rats were housed under a fixed illumination, L:D 12:12 (±180 lux, respectively; 8:00 AM lights on) in a temperature-controlled environment with ad libitum access to tap water and food (rat chow).

Blood sampling for the study, as well as rectal temperature measurement, was carried out at 10, 14, 18, 22.2 and 6 hours.

Hematological analysis was performed using the hematological analyzer Abacus junior vet (Diatron, Austria). For measurement of rectal temperature, the BIO-TK8851 thermometer (Bioseb, USA) was used. In the blood plasma, using the StatFax-3300 analyzer and corresponding Spinreact kits, the levels of albumin, glucose and creatinine were determined.

All animal experiments were performed in according to the compliance with EC Directive 86/609/EEC and with the Russian law regulating experiments on animals.

Statistical Analysis.

The obtained data, analyzed using Graph Pad Prism 6., were expressed as Mean±SD and the statistical difference determined using paired Student t-test. A $P<0.05$ 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-hour 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 AND DISCUSSION

The results of the study testify to significant effect of experimental hypercalcemia on the structure of the circadian rhythms studied by us.

At analysis of the daily content of albumin in the blood plasma, we found that under conditions of a fixed light regime in animals of the control group the greatest content of this protein was noted in the morning hours with a maximum at 6 h, then there was a constant decrease to a minimum noted at 22 h. In animals with hypercalcemia, on the contrary, the maximum albumin content is noted at 22 h, with a gradual decrease to a minimum noted at 10 h (Fig. 1).

The results of cosinor analysis also indicate a significant difference in the organization of the circadian rhythm of albumin in the studied groups of animals. In control animals, the acrophase of rhythm is recorded at 7h58m, the amplitude of the rhythm is 0.52 g/l. In experimental animals, there is no reliable circadian rhythm, the amplitude of daily fluctuations in the level of albumin increases to 1.32 g/l (Fig. 3). In the study of the daily dynamics of creatinine, both in the control and in experimental animals, the maximum content of this substance is noted at 10 h, with a minimum at 2 h in the control group, and at 14 h in experimental animals. In addition, intact rats show a significant increase in creatinine level at 22 h, which is not observed in experimental rats (Fig. 2).

According to the results of cosinor analysis, the circadian rhythm of creatinine is found in both groups of animals. In intact animals, the acrophase of the rhythm is observed at 10h55m, the amplitude is 5.45 mmol/l, and in the rats of the experimental group the acrophase shifts at 9h11m with an increase in amplitude to 7.12 mmol/l (Fig. 3). In the daily dynamics of glucose in the blood plasma of the rats of the control group, the maximum at 2 h is noted, which is preceded by a minimum value of this metabolite, noted at 22 h. In animals of the experimental group, the maximum glucose content is observed at 18 h, and the minimum content at 10 h (Fig. 4). Cosinor analysis of the rhythm of glucose in animals of the control group showed an acrophase at 7h27m, and the amplitude of the rhythm was 0.36 mmol/l. In animals with hypercalcemia, the acrophase shifts to 18h00m, with the amplitude increasing to 0.6 mmol/l (Fig. 5).

Analysis of the daily rhythm of rectal temperature also revealed differences between the parameters of the control and experimental groups of animals. In the animals of the control group, the maximum temperature was noted at 14h00m, and the minimum temperature - at 2h00m. In the rats of the experimental group, the highest temperature was observed at 22 h, and the minimum occurred at 10 h (Fig. 6). The rhythm in both groups is characterized by close acrophases - 21h00m in the control and 22h30m in the experimental group, while the amplitude of the rhythm was 0.99°C in the control, and 0.55°C in the experimental animals (Fig. 5).

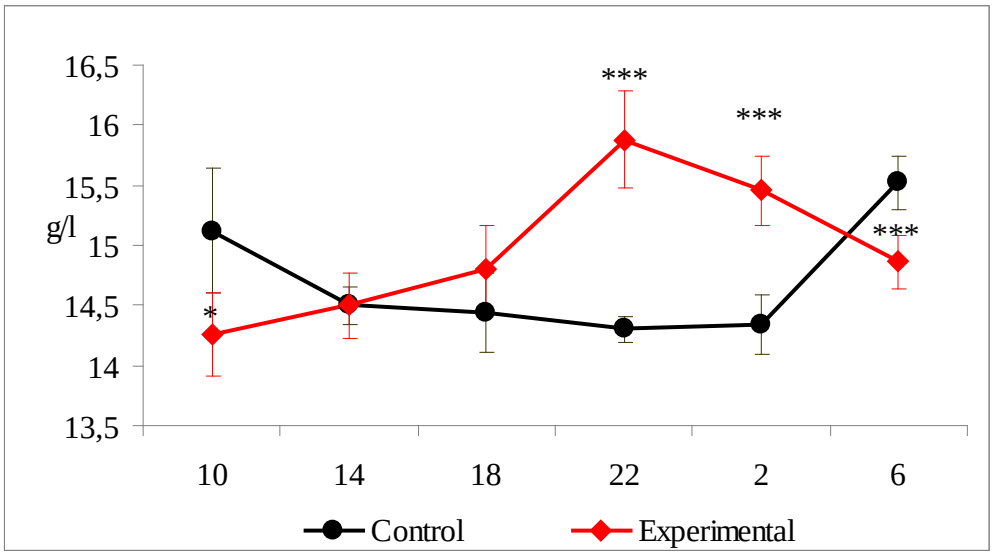

Fig. 1. Daily dynamics of albumin concentration in rat plasma.
Hereinafter marked values significantly different between groups (* – p≤0.05, ** –p≤0.005, *** – p≤0.0005).

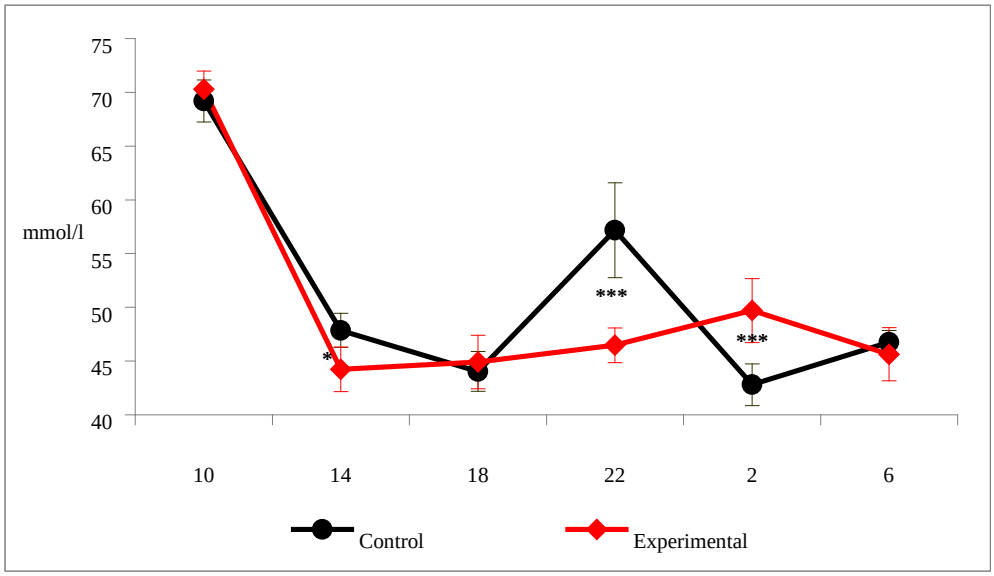

Fig. 2. Daily dynamics of creatinine concentration in rat plasma.

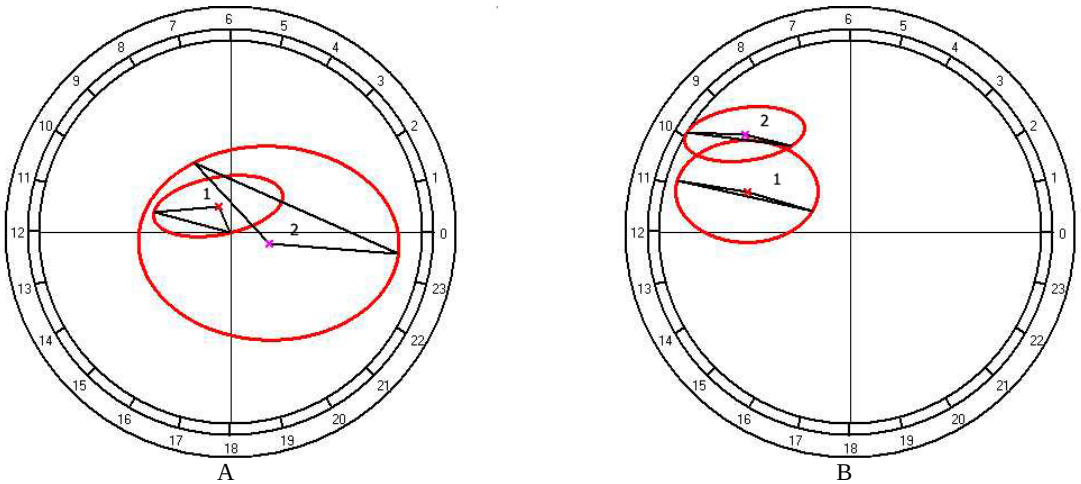

Fig. 3. Cosinor analysis of the circadian rhythm of albumin (A) and creatinine (B) in the rat plasma. Hereinafter 1 – control group, 2 – experimental group.

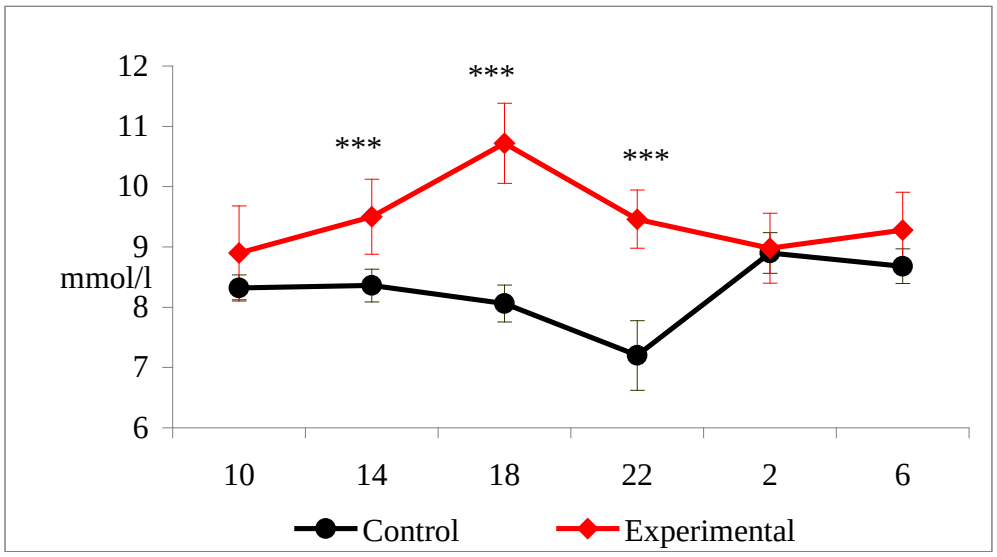

Fig. 4. Daily dynamics of glucose concentration in rat plasma.

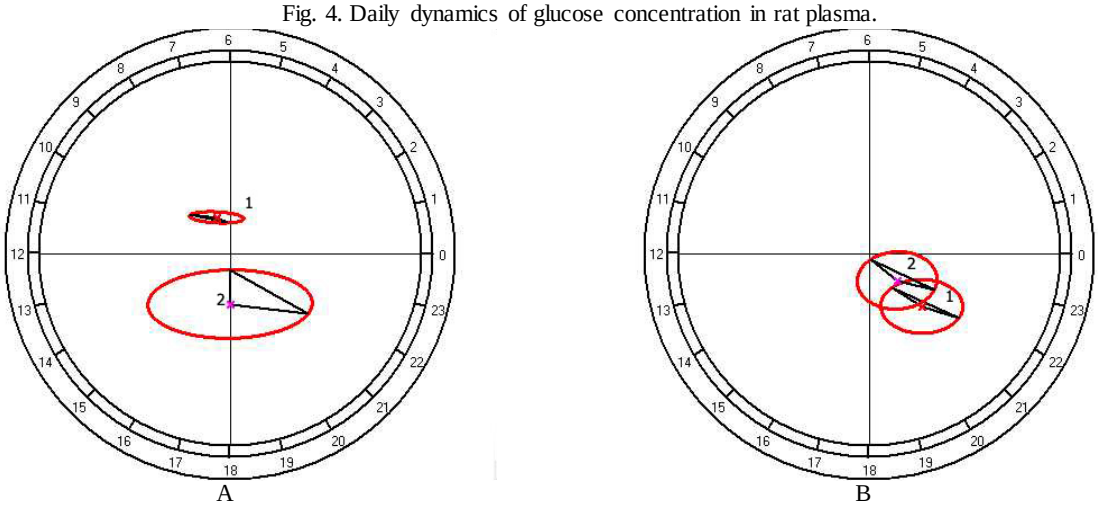

Fig. 5. Cosinor analysis of the circadian rhythm of glucose in the rat plasma (A) and rectal temperature (B).

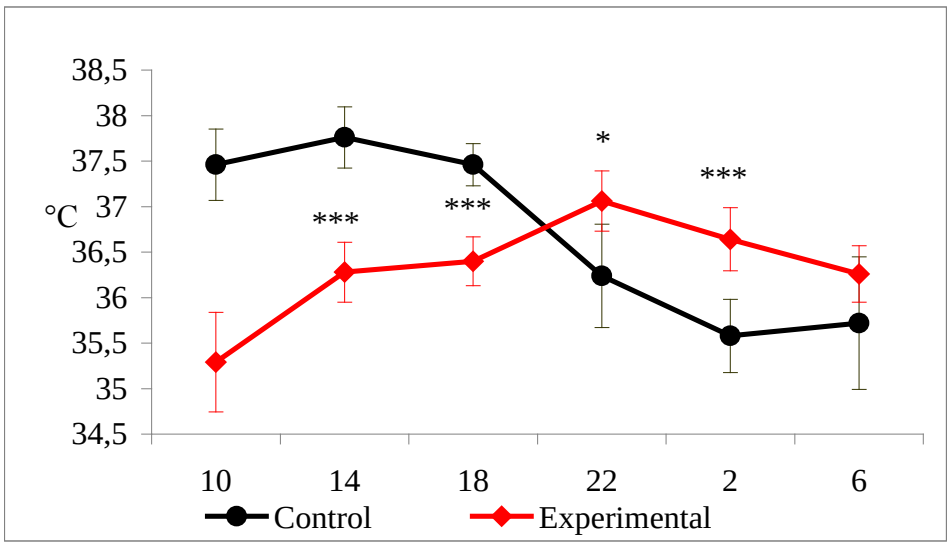

Fig. 6. Daily dynamics of rat rectal temperature.

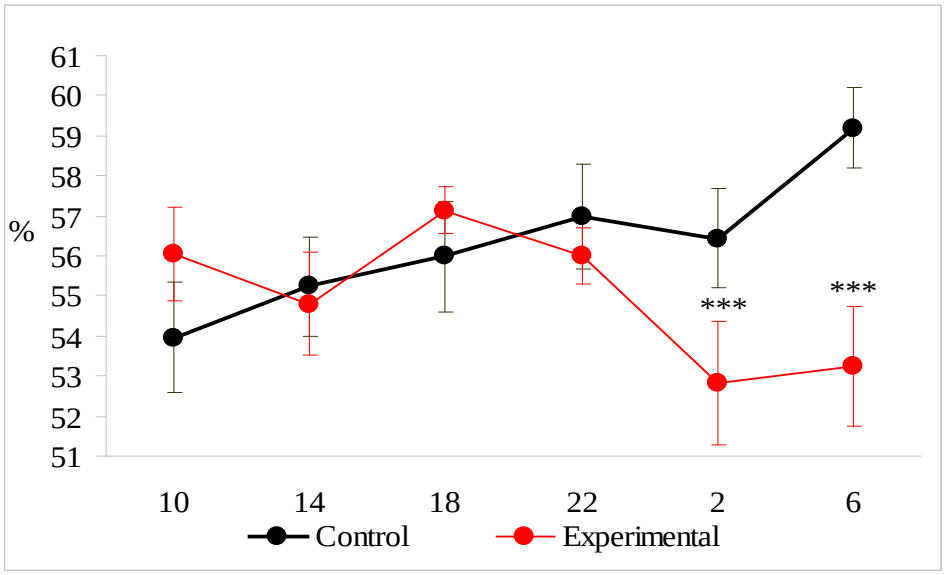

Fig. 7. Daily dynamics of hematocrit in the rat blood.

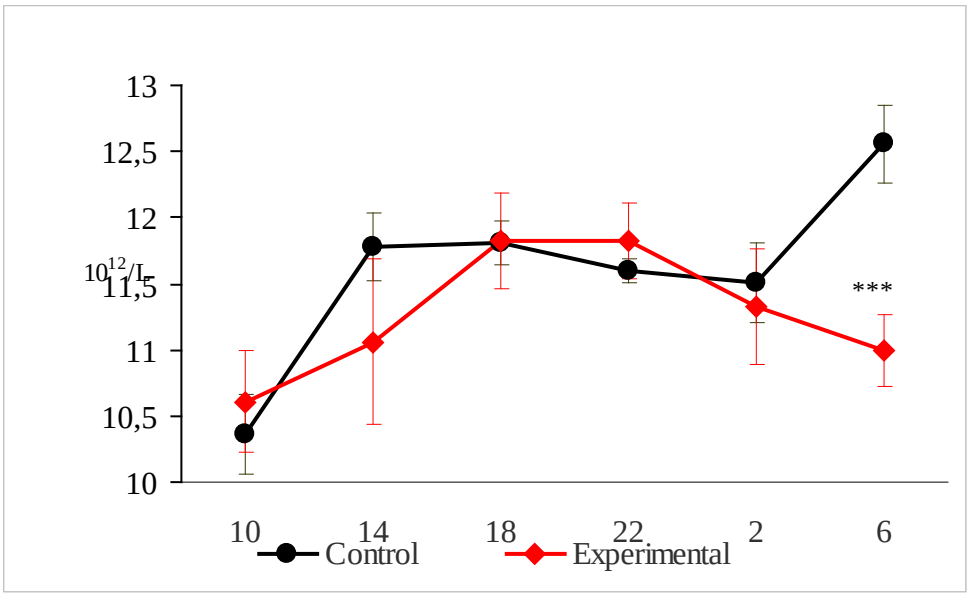

Fig. 8. Daily dynamics of red blood cell count.

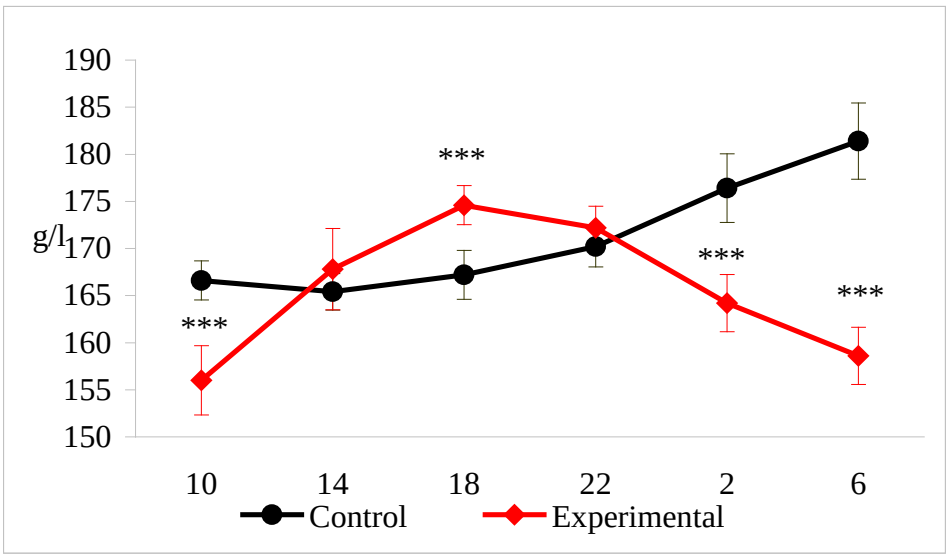

Fig. 9. Daily dynamics of hemoglobin content.

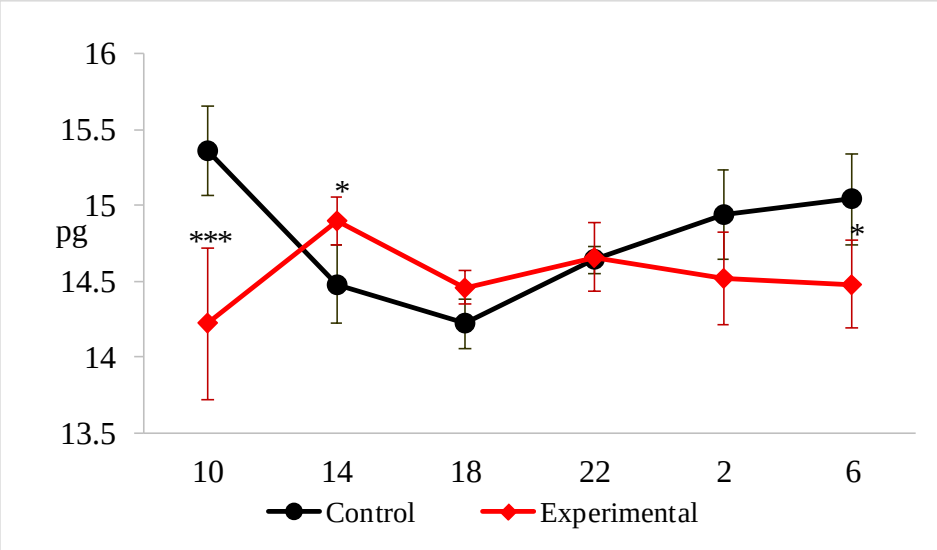

Fig. 10. Daily dynamics of MCH content.

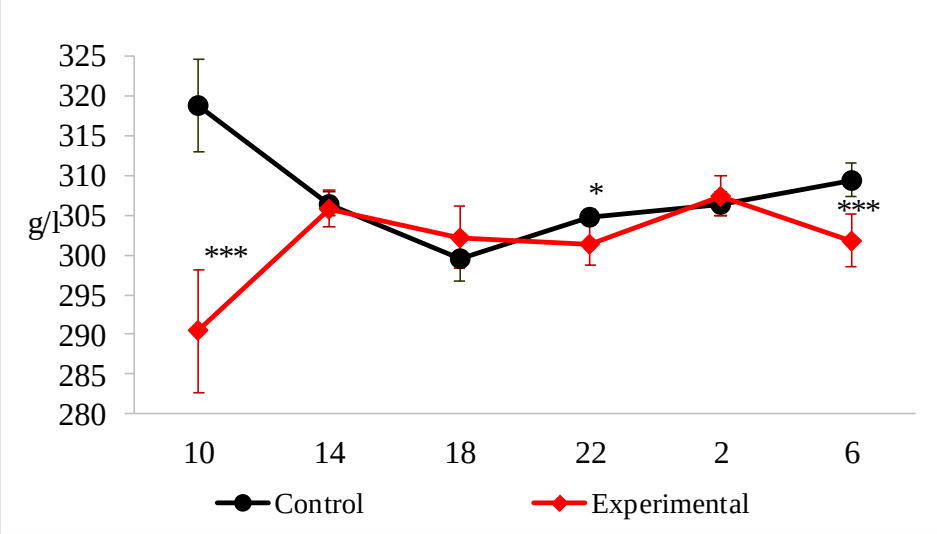

Fig. 11. Daily dynamics of MCHC content.

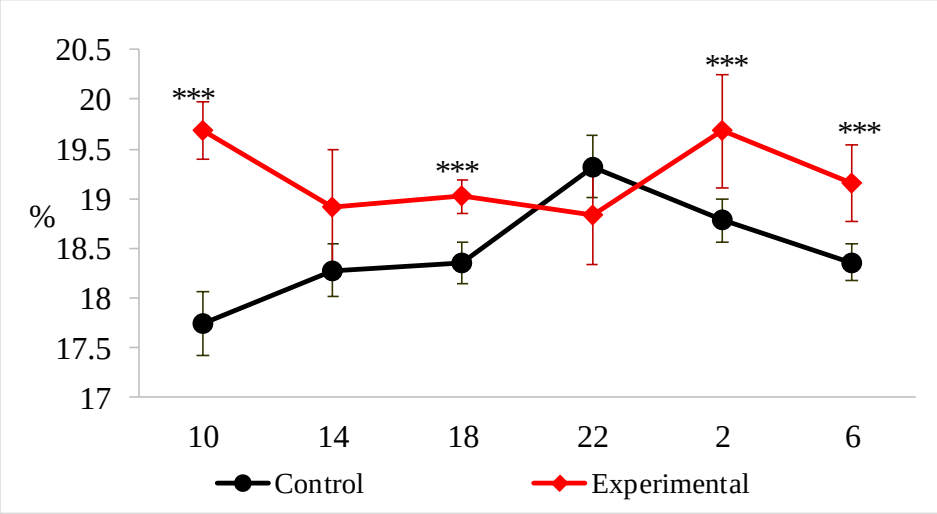

Fig. 12. Daily dynamics of RDW-SD.

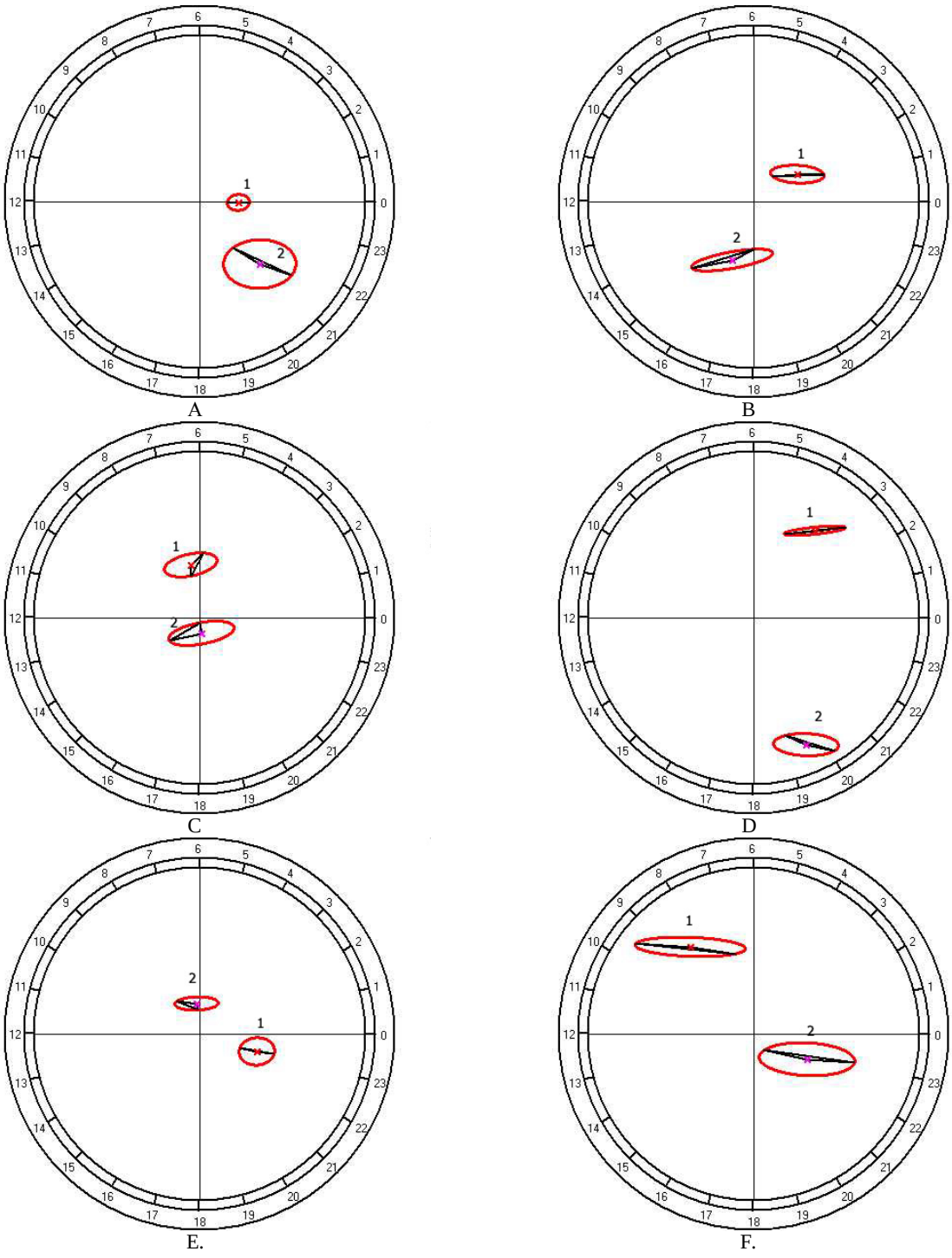

Fig. 13. Cosinor analysis of the circadian rhythms of red blood indices: red blood cell count (A); hematocrit (B); MCH (C); hemoglobin (D); RDW-SD (E); MCHC (F).

The results of hematological analysis indicate the presence of a marked circadian rhythm of some parameters. Thus, in rats of the control group during the day the maximum hematocrit is observed at 6 h, and its minimum is noted at 10 h. In rats of the experimental group, the maximum hematocrit was noted at 18 h, and the minimum – at 2 h (Fig. 7).

In this case, the rhythm acrophase in control rats is noted at 2h13m, and the rhythm is characterized by an amplitude of 0.75%, while in the rats of the experimental group the acrophase of the circadian rhythm is noted at 16h46m, and the amplitude is increased to 1.72% (Fig. 13). At analysis of the daily dynamics of red blood cell count it was found that the minimum content of these cells in rats of both groups was noted at 10 h, but in rats of the control group the maximum of erythrocytes was noted at 6 h, and in animals of the experimental group – at 18 h (Fig. 8).

The acrophase of the red blood cell count rhythm in the control group was noted at 00h02m, the amplitude of the rhythm was

0.28×10^{12}/L. In animals of the experimental group the rhythm acrophase is noted at 21 h, and the amplitude increases to 0.61×10^{12}/L (Fig. 13). The diurnal rhythm of blood hemoglobin content of control animals is characterized by a minimum at 14 h and a maximum at 6 h, while in experimental animals the minimum is noted at 10 h, and the maximum values are observed at 18 h (Fig. 9). The circadian rhythm of the hemoglobin content in the rats of the control group is characterized by acrophase, which is noted at 3h30m and with an amplitude of 2.21 g/l. In rats of the experimental group, at a practically unchanged amplitude, acrophase is noted at 19h38m (Fig. 13).

When considering the daily dynamics of mean corpuscular hemoglobin (MCH) it is established that in the control animals this parameter reaches a maximum at 10 h, and the minimum is observed at 18 h. In the rats of the experimental group the values are maximal at 14 h, and at 10 h a minimum is observed (Fig. 10). The acrophase of rhythm of MCH in control rats is on 6h06m, its amplitude is 0.48 pg. In the rats of the experimental group, the acrophase is shifted by 17h58m, and the amplitude of the rhythm decreases to 0.3 pg (Fig. 13).

The mean corpuscular hemoglobin concentration (MCHC) rhythm also shows a maximum at 10 h and a minimum at 18 h in the control rats, while the experimental rats have a maximum at 2 h and a minimum at 10 h (Fig. 11). According to the results of cosinor analysis in intact animals, the acrophase of the rhythm of the MCHC occurred at 8h39m, in the experimental group – at 22h30m. The amplitude of the rhythm in the control group was 3.54 g/l, and 3.82 g/l in the experiment (Fig. 13). Rhythm of RDW-SD of erythrocytes in rats with hypercalcemia was also different from the rhythm in control animals. Thus, in the control, the maximum values of this parameter are observed at 22 h, and then they decrease to a minimum, which is noted at 10 h. In experimental animals, the maximum is noted at 2 h, and the minimum values occurs at 22 h (Fig. 12). The acrophase of this rhythm in control animals occurred at 22h41m, the amplitude was 0.63%. In the experiment, the acrophase is noted at 6h15m, and the amplitude decreases to 0.31% (Fig. 13).

The results of the study indicate a very significant effect of experimental hypercalcemia on the organization of circadian rhythmicity of the studied parameters. In particular, the effect of experimental hypercalcemia on the circadian rhythmicity of albumin is the most pronounced, because the animals of the experimental group do not show a reliable rhythm of this substance according to the results of cosinor analysis. The circadian rhythm of blood glucose also undergoes significant changes, manifested in the displacement of the acrophase from the early morning hours to the evening hours and a significant increase in the amplitude of the rhythm. The rhythm of creatinine is the least variable, as does the circadian rhythm of rectal temperature, although in both cases the acrophase of rhythms is shifting.

The circadian rhythmicity of the studied blood parameters was also affected by the high level of Ca in animal organism. The acrophases of the rhythms of most of the studied hematological parameters in the control group fall at night and early morning hours: hematocrit at 2h13m; the number of erythrocytes - at 0h02m; hemoglobin - 3h30m; MCH - 6h06m and MCHC – at 8h39m. And only the acrophase of the rhythm of RDW-SD is noted at night, at 22h41m. In contrast to the control parameters, in the animals of the experimental group the acrophase of rhythm of RDW-SD is noted at 6h15m, and for all other parameters the acrophases shift to the light time of day and the beginning of the dark period: hematocrit – at 16h46m; the red blood cell count – at 21h00m; hemoglobin – 19h38m; MCH – 17h58m and MCHC – at 22h30m.

CONCLUSION

Considering the structure of circadian rhythms of the studied parameters, it is necessary to note the fact that experimental hypercalcemia, in addition to disturbing of circadian rhythmicity as such, leads to desynchronosis of the studied rhythms.

Thus, it can be argued that experimental hypercalcemia changes the nature of circadian rhythmicity of metabolites, rectal temperature, hematocrit and some parameters of red blood. Thereby, a violation of the normal level of calcium, one of the important homeostatic indicators of an organism, entails significant changes in the circadian rhythms of various body parameters.

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CONFLICT OF INTEREST

The authors declare that there is no conflict of interests regarding the publication of this paper.

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