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Circadian Rhythms Of Some Metabolites Of Rats At The Age Of 18 Months In Conditions Of Constant Illumination.

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ABSTRACT

The aim of the study was to investigate the circadian rhythmicity of a number of metabolites under a fixed light regime and also the nature of their alteration under constant illumination in aged rats. The study was carried out on two groups of 20 Wistar rats at the age of 18 months in each group. During the whole experiment the rats of the first group were housed under a fixed illumination, L:D 12:12 (±180 lux, respectively; 8:00 AM lights on) (unless mentioned otherwise) for three weeks. The rats of second group were studied at the light regime, representing constant light (LL, ±180 lux) for the same time. In the blood plasma samples, collected at 9.00 h, 15.00 h, 21.00 h and 3.00 h, the levels of alanine aminotransferase, aspartate aminotransferase, total bilirubin, cholesterol, triglycerides, total protein, albumin and uric acid were determined. 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 results of the study show that a decrease in the functional activity of the pineal gland under the influence of constant illumination leads to various disturbances in the circadian rhythmicity of the studied metabolites. These disorders are manifested in desynchronosis, changes in parameters characterizing the circadian rhythm (a shift of the acrophase, changes in the amplitude) or in the disappearance of a reliable circadian rhythm as such.

Keywords: circadian rhythm, desynchronosis, light regime.

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INTRODUCTION

Circadian (24 hour) clocks are fundamentally important for coordinated physiological functions in organisms as diverse as bacteria and humans.

The circadian system of mammals includes three key components: endogenous "clock" that generates a circadian rhythm; afferent pathway, which determines the circadian rhythm in accordance with the astrophysical day; efferent pathway that distributes signals from the central generator to peripheral organs.

All the biological rhythms of the organism are strictly subordinate to the main pacemaker located in the suprachiasmatic nuclei of the hypothalamus, where through a retinohypothalamic path information on illumination from a retina of eyes arrives(1,2). The molecular mechanism of SCN is formed by "clock-genes" (*Per1, Per2, Per3, Cry-1, Cry-2, Clock, Bmal1/Mop3, Tim,* etc.). It is shown that light directly affects the work of those of them that provide a circadian rhythm. These genes regulate the activity of the genes of the key cell-division cycle and the genes of apoptosis (3,4).

A hormone mediator that brings the regulatory signals to organs and tissues is melatonin. In this case, the nature of the response is regulated not only by the level of the hormone in the blood, but also by the duration of its night secretion. In addition, melatonin provides adaptation of endogenous biorhythms to constantly changing environmental conditions, regulates reproductive functions, participates in antioxidant and antitumor protection of the body. The regulatory role of melatonin is universal for all mammalian organi7sms, as evidenced by the presence of this hormone and the clear rhythm of its production (5-7).

Most of the melatonin is synthesized in the pineal gland from tryptophan through an intermediate synthesis of serotonin, which is formed from the amino acid tryptophan that comes with food. The activity of enzymes involved in the conversion of serotonin to melatonin is suppressed by illumination, the biosynthesis of the hormone occurs at night (8,9).

Extrapineal melatonin, produced by enterochromaffin cells of the intestine, is not susceptible to circadian fluctuations (10-12).

Human circadian rhythms are synchronized to light/dark cycles. Over time, organisms adapts to diurnal variations in their physiology and metabolism. These rhythms are regulated by molecular circadian clocks. However, in modern time formation of human's life regimes have shifted away from the naturally occurring solar light cycle to artificial, irregular light schedules produced by electrical lighting. Long illumination at nighttime promotes oppression of melatonin-forming function of the pineal gland and entails acceleration of aging processes of this organ (13,14). Violations of the light status in general, and especially the presence in conditions of continuous illumination, lead to significant violations; the modifying effect of continuous illumination on the physiological processes of an organism is described by many reseachers. The impact of continuous lighting on an organism of rats at a young age leads to disruption of homeostasis, an increase in age pathology and the risk of developing a metabolic syndrome, contributes to a decrease in life expectancy and development of oncogenesis (15-17). Constant exposure to light also induces alterations in melatonin levels and circadian rhythms in rats, decrease of food intake, visceral adiposity. Exposure of adult female rats to continuous light leads to the gradual development of chronic anovulation. It was established that changes in the light-dark cycle *in vivo* entrain the phase of islet clock transcriptional oscillations, whereas prolonged exposure (10 weeks) to LL disrupts islet circadian clock function through impairment in the amplitude, phase, and interislet synchrony of clock transcriptional oscillations. It is also reported that exposure to LL light regime leads to decrease in glucose-stimulated insulin secretion due to a decrease in insulin secretory pulse mass (18).

The studies indicates that continuous light as such does not affect the endocrine function of testis but abolishes suppressive effects of melatonin on the steroidogenic activity of the testis in rat. Role of light in the mediation of acute effects of a single afternoon melatonin injection on steroidogenic activity of testis in the rat. Both continuous illumination and mild deprivation led to an increase in the number (density) of c-Fos immunopositive units in medial parvicellular subnucleus of the hypothalamic paraventricular nucleus (mpcPVN). The geometrical dimensions of nuclei of the neurons of this structure demonstrated a dependence on both illumination conditions and periodicity, also discovered earlier in the SChN neuron (19, 20).

There is evidence that a number of circadian rhythms are smoothed or distorted during aging, both under normal conditions and under the influence of stressors (21-23). This kind of age-related changes can be due to ontogenetic disorders in the functioning of the epiphysis (24,25).

Based on the above, we found it relevant to study the content of some metabolites in the blood of rats at the age of 18 months and the nature of their circadian rhythmicity at a fixed light regime (L:D 12:12) and constant illumination (LL).

MATERIALS AND METHODS

Animals

The study was carried out on 40 Wistar rats at the age of 18 months**.** Animals of body weight in diapasone of 200–250 g were used. 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).

Treatment design

For the first group of animals, 20 male Wistar rats were used. During the whole experiment, the rats were housed under a fixed illumination, L:D 12:12 (±180 lux, respectively; 8:00 AM lights on) (unless mentioned otherwise) in a temperature-controlled environment with ad libitum access to tap water and food (rat chow). The 20 rats of second group were studied under the same experimental conditions except for 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 three 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 in according to the compliance with EC Directive 86/609/EEC and with the Russian law regulating experiments on animals.

Biochemical studies

In the blood plasma, using the StatFax-3300 (USA) analyzer and corresponding «Spinreact» kits (Spain), the levels of alanine aminotransferase (ALT), aspartate aminotransferase (AST), total bilirubin, cholesterol, triglycerides, total protein, glucose, albumin and uric acid were determined.

Statistical Analysis

The obtained data, analyzed using Graph Pad Prism6.0, were expressed as Mean±SD. The statistical difference 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-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

According to the results of cosinor-analysis, the character of circadian rhythm of ALT is unreliable, both in the initial light regime and under conditions of constant illumination. The AST rhythm with constant illumination is characterized by an amplitude increase up to 44.8 U/l against 37.43 U/l at a constant light mode, and the acrophase of the rhythm shifts from 2.16 h to 22.26 h.

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At analysis of chronograms of the ALT and AST content we found, that under the constant illumination regime the AST circadian rhythm varies little. As at a fixed light regime, the maximum content of the enzyme is observed at 21.00 h, and the minimum at 9.00 h.

The circadian dynamics of ALT under conditions of constant illumination changes more significantly. Thus, if at the fixed light mode, the maximum of content of substance is noted at 3.00 h, and minimum – at 15.00 h, at constant illumination the chronogram becomes smoother, with a maximum at 21.00 h and a minimum at 3.00 h (Fig.1).

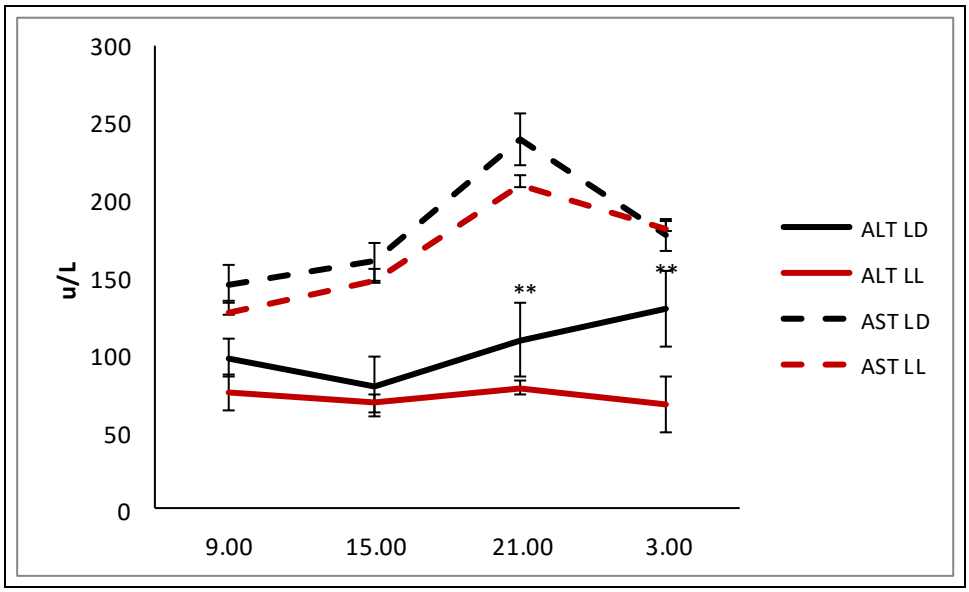

Figure 1: Dynamics of AST and ALT levels in rat plasma under LD and LL light modes.

Cosinor-analysis of the diurnal dynamics of cholesterol did not reveal a reliable circadian rhythm of this substance both at the L:D mode and under constant illumination. However, at a fixed light mode the minimum values of cholesterol are detected at 9.00 h, and the maximum at 3.00 h, but under constant light conditions the minimum occurred for 15.00 h, and the highest values are noted at 3.00 h.

Cosinor-analysis of the rhythm of bilirubin for a fixed light regime is reliable, characterized by an acrophase at 4.43 and an amplitude of 31.19 umol/L. Switching to the constant lighting mode leads to disorder of the rhythm. When analyzing the circadian rhythm of bilirubin, the maximum concentration is at 15.00 h, later it decreases to a minimum at 3.00 h. At constant light the bilirubin rhythm is significantly smoothed, with a maximum at 3.00 h and a minimum at 9.00 h (Fig.2).

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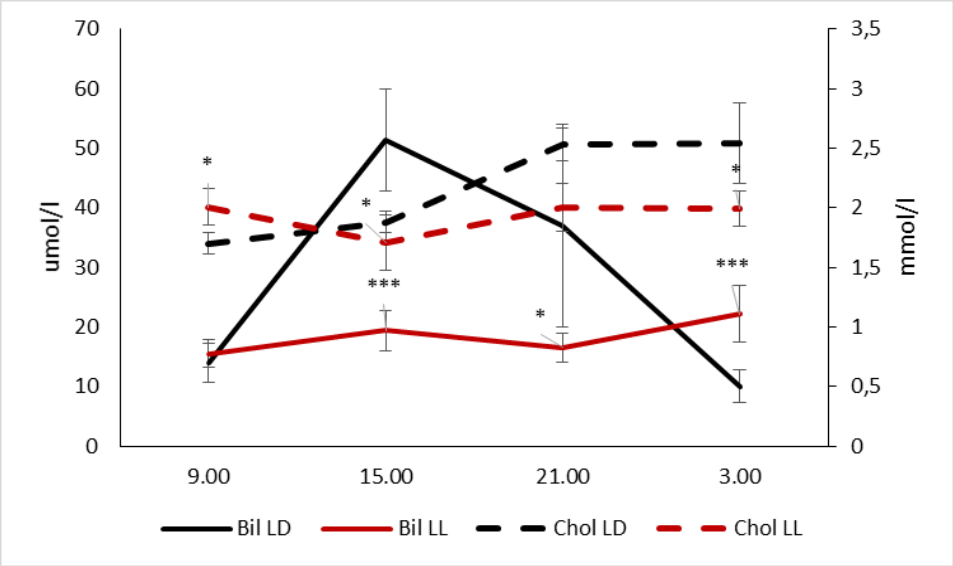

Figure 2: Dynamics of cholesterol (Chol) and bilirubin (Bil) levels in rat plasma under LD and LL light modes.

Analysis of diurnal dynamics of the total protein content in the blood of rats indicates the presence of a pronounced circadian rhythm at a fixed light regime with an acrophase at 20.54, and an amplitude of 7.52 g/l. The change in the light regime leads to the disappearance of a reliable circadian rhythm. When analyzing the chronogram, it is seen that a graph illustrating the daily dynamics of the total protein content in rat plasma under constant illumination is "mirror" with respect to that in a fixed light regime, with a maximum at 9.00 h and a minimum at 3.00 h (Fig.3).

The circadian rhythm of blood plasma albumin at a fixed light regime is characterized by an acrophase, occurring at 11.17 and an amplitude equal to 5.75 g/l. The transition to the constant lighting regime leads to a break in the circadian rhythm.

At that, the diurnal dynamics of albumin, as well as the total protein, after changing the mode of illumination acquires a "mirror" character in comparison with the same dynamics under a fixed light regime with a maximum at 9.00 h and a minimum at 3.00 h.

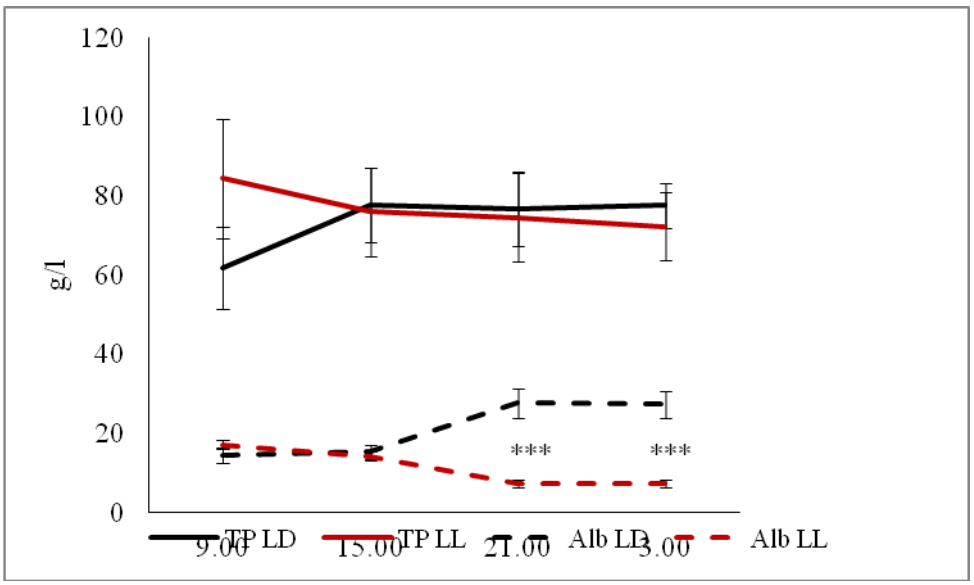

Figure 3: Dynamics of albumin (Alb) and total protein (TP) in rat plasma under LD and LL light modes

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The dynamics of the glucose level shows a pronounced circadian rhythm in both at the fixed light regime and after its change. A ta fixed light mode the rhythm is characterized by an acrophase, occuring at 16.10 h and a rhythm amplitude equal to 0.49 mmol/l. At constant illumination the acrophase shifts by 19.15 h, and the amplitude of the rhythm increases to 1.15 mmol/l. In both lighting modes, the maximum is fixed at 15.00 h, and the minimum at 9.00 h.

Just like the blood glucose level, the triglyceride content in the blood retains circadian rhythmicity in both modes of illumination. At a fixed light regime, the acrophase of the rhythm of triglycerides was noted at 4.43, the amplitude was 0.65 mmol/l. After changing the lighting mode, the rhythm's acrophase shifts by 22.24 h, while the rhythm amplitude increases to 2.59 mmol/l. In both modes of lighting, the maximum is at 21.00 h, and the minimum is at 15.00 h (Fig. 4).

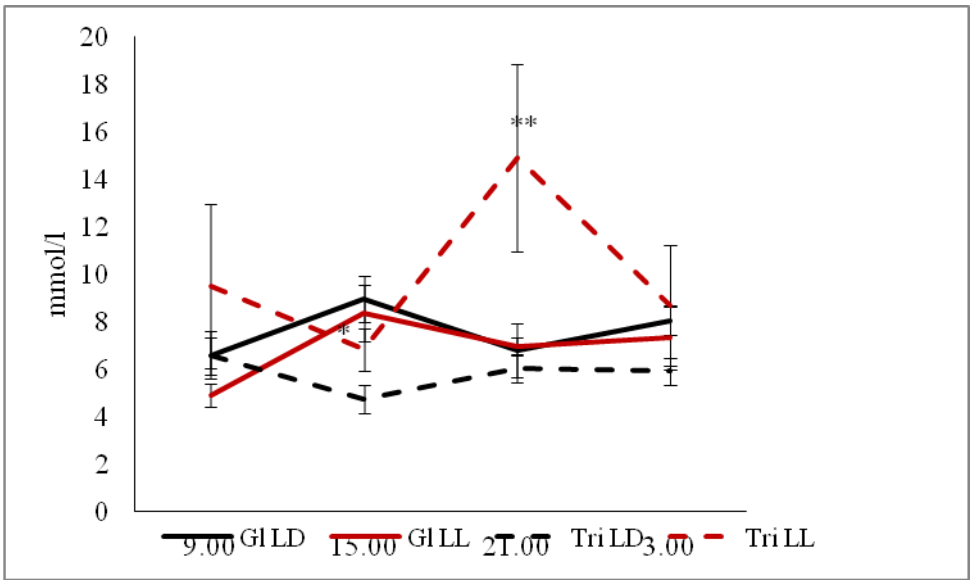

Figure 4: Dynamics of triglyceride (Trigl) and glucose (Gl) levels in rat plasma under LD and LL light modes.

The uric acid content also shows circadian rhythmicity with an acrophase at 18.51 h and an amplitude of 96.94 umol/L at a fixed light regime, and under LL mode the acrophase shifts by 22.59 h, the amplitude increases to 131.41 umol/L. At the same time, the maximum content of the substance is observed at 21.00 h, and the minimum at 3.00 h at fixed light and at 15.00 h with constant illumination (Fig. 5).

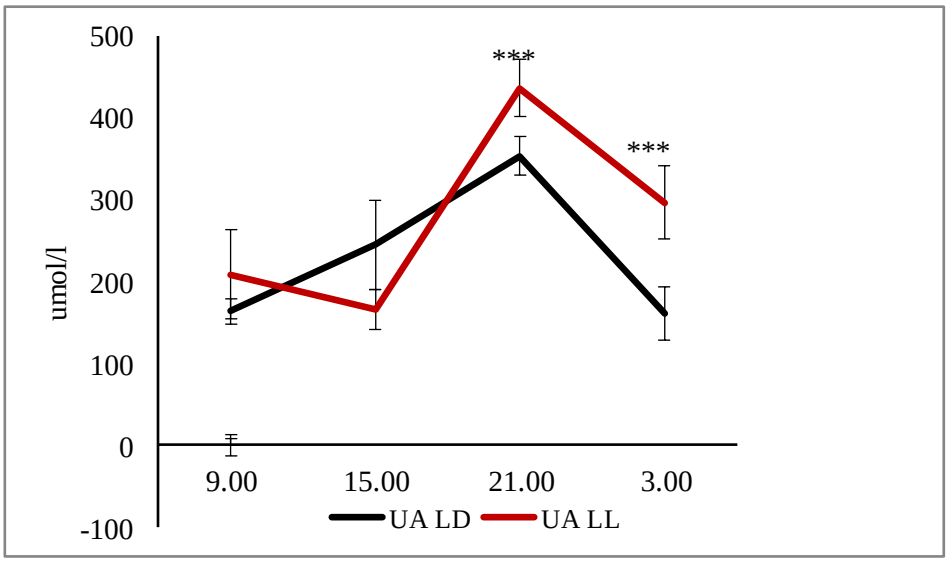

Figure 5: Dynamics of uric acid (UA) in rat plasma under LD and LL light modes.

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DISCUSSION AND CONCLUSION

Studied biochemical parameters, except of ALT showed marked circadian fluctuations over a 24-h period related to light–dark cycles in Wistar rats (26,27).

An absence of a reliable circadian rhythm of ALT can be one of age features of functioning of an organism of mammals. It is well known that circadian rhythms are rather plastic and can change considerably during an ontogenesis (28-31). Besides, it is shown that constant illumination from a one-month age induces a spontaneous carcinogenesis at rats and reduces average and maximal life expectancy. On the contrary, impact of constant illumination since a 14-month age exerts the modulating impact on age pathology on laboratory animals: reduces quantity of benign and malignant neoplasms, increases average and maximal life expectancy in comparison with males in the conditions of the standard light. At that, the data on diurnal dynamics of ALT and AST obtained by us on the whole coincide with the data of a number of authors, indicating an increase in the level of these enzymes at night (32). The level of cholesterol in rats under L:D conditions rises at night, which is consistent with the results of previous studies (33,34).

In general, the fact that the maxima of the parameters of rats under study at a fixed light regime comes on the dark phase of the day, may be related to their nocturnal rhythmicity, where their activity and feeding occur in night. Significant fluctuations in the level of glucose during the day can be associated with the food intake, digestion, and accumulation of glucose in the blood. Nevertheless, the maximum level of glucose at a fixed light regime is observed in the daytime, which is due to the fact that, in contrast to melatonin, the minimum insulin level in rats is observed in the daytime, since the main function of insulin - the control of metabolism in a state after eating - should not be realized at the light (35).

Constant lighting during the day leads to a significant disruption of the circadian rhythm of all the studied parameters and to desynchronosis.

Thus, in the case of bilirubin, total protein and albumin, according to the results of cosinor-analysis there is no reliable circadian rhythmicity at all, although the daily dynamics of these parameters varies significantly.

In the case of other examined parameters, the cosinor-analysis confirms the presence of a reliable circadian rhythm, but the acrophases of the rhythms move relative to the acrophase at a fixed light regime, and the amplitude of the rhythms increases significantly.

Such changes may be associated with impaired functioning of the pineal gland as an oscillator at constant illumination. Thus, it has been shown that in animals with a surgically removed pineal gland the secretion of insulin and glucose homeostasis changes. Pinealectomy in rats leads to insulin resistance of the liver, activation of gluconeogenesis (36) and an increase in the level of glycemia at night (37). An increase in glucose-stimulated insulin secretion and a violation of the amplitude of its rhythms was detected in cultured β-cells of rats subjected to pinealectomy (38). Removal of the pineal gland in rats with a model of type 2 diabetes mellitus (OLETF line) leads to hyperinsulinemia and accumulation of triglycerides in the liver (39).

At the same time, it can be assumed that the circadian rhythm of the total protein, albumin and bilirubin largely depends on the activity of the central photoperiodic system, and therefore the regime of constant illumination leads to a breakdown in the circadian rhythms of these substances.

The circadian rhythm of metabolites, for which a reliable daily rhythm was found in a constant light, may be subordinated to the modulating effect of secondary oscillators.

As is known, three ways of synchronization of individual physiological rhythms are distinguished: 1) extrasynchronization is caused by interactions of the circadian system with external time sensors; 2) intersynchronization is determined by the interactions between the oscillations of different physiological functions; 3) intrasynchronization is a process of internal temporary self-organization, features of the endogenous rhythmicity of individual biological functions.

The circadian system is plastic, its internal connections are highly dynamic (40-42). At changes of the state of neuroendocrine mediators, and primarily the pineal gland, the internal desynchronism of the physiological functions is expected, since the secondary oscillators begin to function at their own frequency.

In addition, the circadian rhythms of individual physiological functions differ in their degree of conjugation to the central pacemaker (43- 47), presence of their own diurnal periods, ability to intersynchronization (48- 51).

Also, a decrease or increase in the level of the mesor is often accompanied by the appearance of ultradian rhythms (52,53). The deviation of diurnal curves of physiological functions from endogenously specified programs is accompanied by shifts of acrophases, internal phase desynchronization of physiological rhythms and, consequently, the appearance of ultradian components of the rhythm.

The results of the study show that a decrease in the functional activity of the pineal gland under the influence of constant illumination leads to various disturbances in the circadian rhythmicity of the studied metabolites. These disorders are manifested in desynchronosis, changes in parameters characterizing the circadian rhythm (a shift of the acrophase, changes in the amplitude) or in the disappearance of a reliable circadian rhythm as such.

At present, a fairly large number of people are exposed to light pollution (night lighting), conditioned by the profession or lifestyle. This phenomenon is accompanied by a number of serious disorders of behavior and health, including cardiovascular diseases and cancer. The probable delayed nature of health problems in this way of life confronts researchers with not only interesting, but also practically meaningful tasks.

The physiological responses of the mammalian organism to constant illumination that we discovered help us to better understand the role of the light regime in maintaining and regulating homeostasis and take into account its role in various aspects of practical activity.

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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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