SEASONAL AND CIRCADIAN FLUCTUATIONS OF BLOOD BIOCHEMICAL PARAMETERS IN MICE EXPOSED TO NATURAL AND CONSTANT LIGHT

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A previous investigation [2] showed unequal variability of changes in various biochemical parameters of experimental mice both in the course of the 24-h period and in different seasons of the year; the degree of these circadian and seasonal fluctuations largely depended, moreover, on whether these parameters studied related to the activity of organs actively involved in maintaining the resistance of the animal, or whether their main function was to maintain functional homeostasis at different time intervals during the animal's life.

It is accordingly very interesting to compare circadian changes in biochemical parameters in different seasons of the year, during natural alternation of daylight and darkness and also under conditions of desynchronization caused by keeping experimental animals in constant light, a procedure which ought to reveal the role of geoclimatic factors in the formation of a circannual rhythm during desynchronization, and also the effect of constant light and the formation of a circadian rhythm in different seasons of the year.

EXPERIMENTAL METHOD

Eight series of experiments were carried out under synchronized conditions, on 660 mature noninbred male mice weighing 19-21 g: 320 animals in four series, in which the animals were kept under natural conditions of daylight and darkness (LD) and 340 mice in four series in which the animals were exposed to continuous light (LL). The intensity of illumination was about 60 lux. The experiments were carried out as follows: in winter on January 24 and 25, in spring on April 26 and 27, in summer on July 3 and 4, and in the fall on October 5 and 6. The mice in all experiments were allowed free access to food and drink. The animals were killed by rapid decapitation at 6 p.m., midnight, 6 a.m., and noon, 20 mice at each time. Blood was centrifuged and the plasma separated, and the following parameters of the plasma were determined on an SMA 12/60 automatic analyzer ("Technicon," Ireland): activity of aspartate and alanine aminotransferase (AST and ALT respectively), concentrations of protein, bilirubin, glucose, cholesterol, creatinine, and nonprotein nitrogen, and also plasma levels of Cl^-, K^+, and Na^+. The results were subjected to statistical analysis by Fisher's and Student's tests, and also by cosinor analysis [5]; acrophases (∅) were given only if statistically significant.

EXPERIMENTAL RESULTS

It will be clear from Figs. 1-3 that the most marked seasonal fluctuations were observed with transaminases both under natural conditions and during constant light. Maximal AST activity in both LD and LL was observed in spring, minimal in the fall ($p < 0.01$). Consequently, both in LD and LL, AST has a distinct unimodal seasonal

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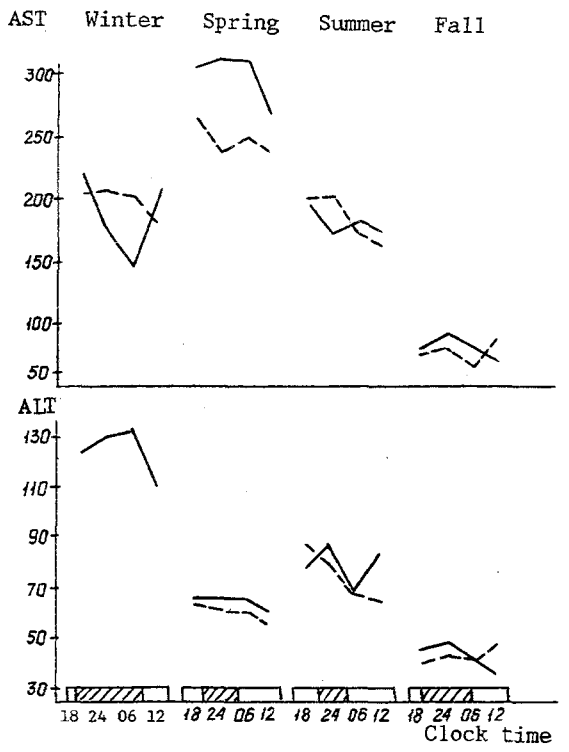

Fig. 1. Circadian and seasonal changes in content (in mU/ml) of AST and ALT under natural conditions (continuous line) and constant light (broken line).

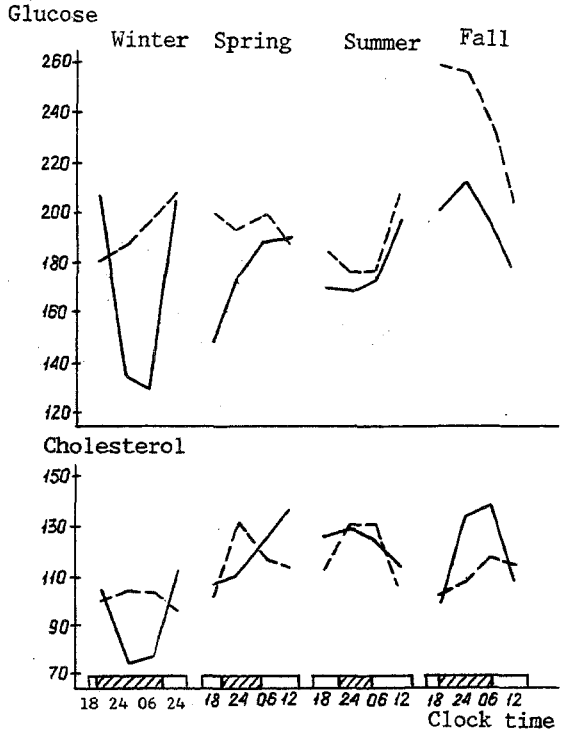

Fig. 2. Circadian and seasonal changes in content (in mg%) of glucose and cholesterol and at natural conditions (continuous line) and during constant light (broken line).

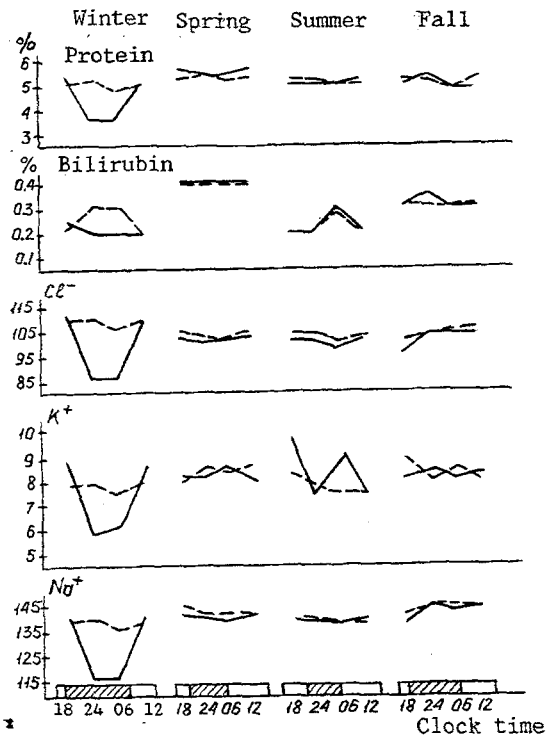

Fig. 3. Circadian and seasonal changes in content of protein (in g%), bilirubin (in mg%), and Cl^-, K^+, and Na^+ (in meq/liter) under natural conditions (continues lines) and during constant light (broken lines).

rhythm with peak activity in spring. Cosinor analysis of circadian fluctuations of AST under natural conditions revealed two significant acrophases of activity: in winter ∅ at 4.32 p.m. and in the fall ∅ at midnight. Considering the times of sunset (4.47 p.m.) and sunrise (8.36 a.m.) in the winter, it can be stated that in winter acrophase occurs during twilight, i.e., before the sun has fully set. In the fall, however, the acrophase was observed in the middle of the dark period of the 24 hours (sunset at 5.56 p.m., sunrise at 6.24 a.m.), i.e., a shift of the acrophase was observed into the night period. Particular attention must be paid to the smoothing of amplitude (A) of the fluctuations in AST activity in LL compared with LD in the winter period. The range of circadian fluctuations in LD (A = 40.2) was more than two times wider than the range of fluctuations in LL (A = 12.8), where no acrophase could be detected. The peak level of ALT activity under natural conditions was observed in winter, the minimal level, both in LD and in LL, in the fall. Cosinor analysis of fluctuations of ALT in the course of the 24-hour period in different seasons of the year revealed a statistically significant acrophase of activity only in the fall, when it was found in the middle of the dark section of the 24-h period (∅ = 11.08 p.m.), close to the AST acrophase. The bimodal character of the circadian curves of activity of both AST and ALT in the summer period, in animals kept under natural conditions, also will be noted.

The peak level of activity of creatinine and nonprotein nitrogen under natural conditions was observed in summer, the minimal level in the fall and winter. A similar pattern of seasonal fluctuations of the above-mentioned parameters also was noted in LL. Cosinor analysis revealed an acrophase of the circadian rhythm of nonprotein nitrogen in LD in the fall at 11.56 p.m.; it occurred in the middle of the dark section of the 24-h period, just as with the transaminases. The bimodal character of the circadian fluctuations of nonprotein nitrogen and creatinine in the summer period, when the daylight part of the 24-h period was considerably lengthened, must be emphasized once again.

No statistically significant seasonal differences in the glucose level were observed during alternation of light and darkness. The most important fact that is in all seasons of the year the glucose level was higher in LL than in LD, and the rise of the mean daily glucose level during constant light suggests that light has a significant influence

on carbohydrate metabolism, leading to an increase in the plasma glucose concentration. Cosinor analysis revealed two statistically significant acrophases of the circadian rhythm of the glucose concentration in the mice during natural alternation of daylight and darkness: in winter ∅ = 3.12 p.m., and in the fall, ∅ = 11.28 p.m. Consequently, during alternation of the seasons a shift of the acrophase in the period was observed, from twilight in winter to the dark period of night in the fall. Under LL conditions no significant circadian acrophase could be found in any of the seasons studied. Meanwhile, attention must be drawn to the considerable (by 3.8 times) fall of the amplitude of circadian fluctuations and glucose in the winter period in animals kept in LL (A = 13.5) compared with those kept in LD (A = 51.5). During investigation of the cholesterol concentration in animals in LD a statistically significant decrease was observed in its winter level compared with the other seasons of the year. The circadian rhythm of cholesterol in animals kept under natural conditions was discovered in the winter and fall. The acrophase (∅ = 2.16 p.m.) of the circadian rhythm of cholesterol in winter occurred in the second part of the daylight period, but in the fall (∅ = 4.40 p.m.) it occurred in the second half of the dark period; meanwhile, in animals kept under constant light conditions, smoothing of the amplitude of the circadian fluctuations of cholesterol in these seasons was observed. In winter the amplitude in LL was almost five times less than the amplitude in LD, and in the fall, the reduction was almost threefold. Determination of the total protein level in animals in LD in different seasons of the year revealed a small but statistically significant increase in the spring period. Cosinor analysis revealed a significant acrophase of the circadian rhythm of protein in winter in LD (∅ = 3.27 p.m.), i.e., the acrophase in this case also occurred in the twilight period. Under LL conditions, in this period of the year smoothing of the amplitude of fluctuations in the protein level took place, and no circadian rhythm could be seen. There were significant differences in the bilirubin level in different seasons of the year. The highest level under natural conditions was found in the spring. Under continuous light conditions the seasonal rhythm was similar in principle to that in LD.

The study of blood electrolyte levels (chloride, potassium, and sodium) in animals kept under natural conditions revealed no statistically significant seasonal differences, and a similar pattern also was observed in the animals kept in LL. The most important general rule, from our point of view, was that found in winter on comparing the circadian rhythm in animals kept in LD and LL. The peak of the electrolyte concentrations with significant acrophases was observed as follows: for chloride ∅ = 3.16 p.m., for potassium ∅ = 2.48 p.m., and for sodium ∅ = 3.04 p.m. Consequently, the highest level of the electrolytes in winter occurred in the second half of the daylight period. Under constant light conditions, in the same period of the year, a considerable (by 10-17 times) fall of the amplitude of the circadian fluctuations of electrolytes was observed, and as a result, the circadian rhythm for chloride, potassium, and sodium disappeared.

Analysis of the results shows that most biochemical parameters have a seasonal rhythm. Moreover, comparison of the mean daily values shows that the character of the changes in different seasons, for each parameter taken separately for animals kept under natural conditions, was very similar to the character of the seasonal fluctuations of the same parameter in animals kept in continuous light, suggesting that the influence of external environmental geoclimatic factors on circadian rhythm formation is stronger than the artificial desynchronizing effect of constant light. The most marked circadian rhythm was observed in the fall and, in particular, in winter, a fact which we associate with the longest duration of darkness in the course of the 24-h period in these seasons. The presence of well defined circadian synchronization in the winter season is also confirmed by the fact that the scatter of acrophases in that period for the 7 parameters studied is minimal and occurs in the twilight period: from 2.16 to 4.32 p.m. In the fall the acrophases shifted into the dark portion of the 24-h period, and in this case also a high degree of circadian synchronization was observed, and was discovered for 5 of the parameters studied, whose acrophases occur within a limited period of time, namely from 11.08 p.m. to 4.40 a.m. [3].

The fact that the abundance of circadian synchronization was greatest in the winter and fall suggests that these seasons are optimal for the activity of nocturnal animals. This evidently explains why the greatest desynchronizing effect of continuous light is manifested in winter and the fall, and in most cases leads to disappearance of the circadian rhythm. This is confirmed by the fact that in the summer months, when the period of darkness is shortened to 6.33 h, i.e., is reduced by 2-3 times, and the period of daylight becomes long, namely 17.27 h, we found no acrophases of a circadian rhythm, and the character of the circadian fluctuations itself was bimodal in pattern, as was observed previously in study of rectal temperature in mice kept under constant light conditions [3], and in man when working round the clock [1, 4]. We defined this phenomenon as "a state of physiological strain."

Finally, the study of circadian rhythms of biochemical parameters in different seasons of the year can help to elucidate the mechanisms responsible for chronoresistance of the body to various toxic agents and during different periods of physiological activity [6, 7].

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CONTRIBUTION OF GENETIC CONSTITUTION TO FORMATION OF CIRCADIAN RHYTHMS OF SOME HEMODYNAMIC PARAMETERS

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Studies in twins are of great value to the elucidation of the relative role of environment and heredity in the formation of biorhythms. The main task of analysis of quantitative traits in twins is the ability to evaluate the relative role of heredity and environment in the realization of the phenomenon chosen for study. Circadian changes in a number of parameters are known to be more similar in monozygotic than dizygotic twins [1]. This suggests the existence of a sufficiently important genetic component in the formation of circadian rhythms of certain parameters, and in turn, this raises the question of mathematical methods for evaluating this importance.

The first studies using analysis of twins in biorhythmology [1-3] were based on separate analysis of observations at each time point. As a result of this method, the number of coefficients of genetic and environmental determination is proportional to the number of time cuts, and this makes evaluation of the degree of genetic determination of the rhythm of the test parameter difficult. This method also is more suitable for solving the problem of the rhythm of genetic (or environmental) determination than the problem of genetic determination of the rhythm, which is by no means the same thing. The main idea of our suggested generalization of unidimensional procedures in work with twins to the multidimensional case (which is what distinguishes analysis of chronobiological data from analysis of ordinary data) is that the initial time series is replaced by an acrophase-amplitude point on a plane, after which the resulting two-dimensional distribution is analyzed.

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