SEASONAL AND CIRCADIAN RHYTHMS OF BLOOD BIOCHEMICAL PARAMETERS IN MICE

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In publications devoted to the study of seasonal rhythms [1, 2, 4-6] there is exceedingly little information about blood biochemistry. Moreover, such investigations are frequently undertaken without regard to circadian rhythms, and this may distort the general picture of variability of values obtained in different seasons of the year. The study of seasonal rhythms of parameters of physiological processes in the course of the 24-h period can not only give a truer picture of the presence or absence of a seasonal rhythm, but can also be used to determine correlation between circadian and seasonal biorhythms, and in turn, this provides the prospects for a deeper understanding of the principles governing the temporal organization of biological systems.

EXPERIMENTAL METHOD

Four series of experiments were carried out on 320 mature noninbred male mice weighing 19-21 g: 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 were kept with natural alternation of daylight and darkness, and with free access to food and water. 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, after which the following parameters were determined in the plasma by means of an SMA 12/60 automatic analyzer (Technicon, Ireland): aspartate- and alanine-aminotransferase activity (AST and ALT, respectively), concentrations of protein, bilirubin, glucose, cholesterol, creatinine, and urea nitrogen, and also Cl^-, K^+, and Na^+ levels in the blood plasma. The numerical results were subjected to statistical analysis by the Fisher–Student test.

EXPERIMENTAL RESULTS

The most marked seasonal changes were observed in AST and ALT activity and in the creatinine and urea nitrogen concentrations (Figs. 1 and 2). Maximal AST activity was observed in the spring: the mean activity in the 24-h period was 299.0 mU/ml, 3.7 times higher than the minimal level, observed in the fall ($p < 0.05$). ALT activity followed a rather different pattern: its highest value was observed in the winter, when the mean level for the 24-h period was 123.5 mU/ml, 2.9 times higher than the corresponding value in the fall ($p < 0.05$). The curve reflecting the seasonal rhythm of AST activity is monomodal, that of ALT bimodal in character. The inverted character of the circadian fluctuations of activity of these enzymes in the winter and spring will also be noted (Fig. 1); it was during these seasons that their circadian differences had their greatest amplitude. The maximal creatinine concentration, which was observed in the summer (mean value for the 24-h period 0.95 mg%) was 2.5 times higher than the minimal value, observed in the winter ($p < 0.05$). The curve of the seasonal rhythm in this case was monomodal in character. A similar pattern also was observed with urea nitrogen. Its maximal level, observed in the summer (33.7 mg%) was almost 2.5 times higher than the minimum, reached in the fall ($p < 0.05$). The seasonal rhythm of the urea nitrogen concentration, like that of creatinine, can be regarded as monomodal in character, with its highest level in the spring and summer. Circadian fluctuations of these parameters, incidentally, were most marked in the summer, and definite synchronization of the character of their circadian curves was observed in that same period (Fig. 2).

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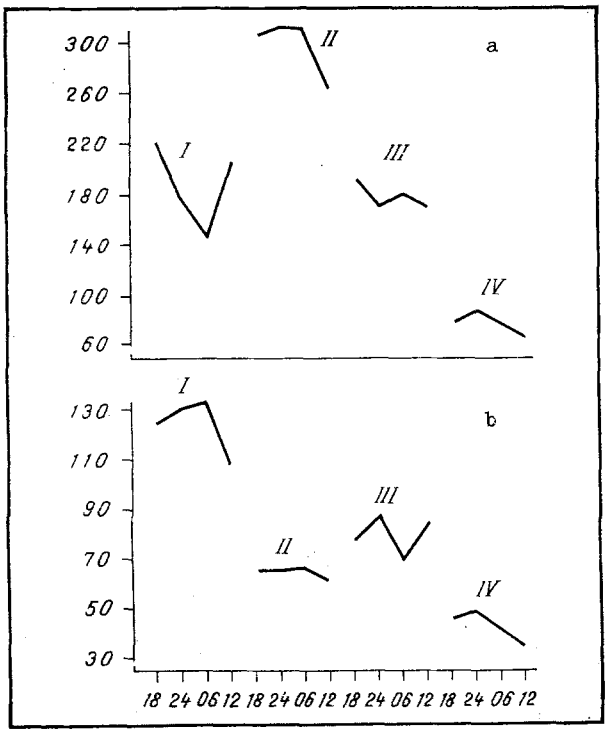

Fig. 1

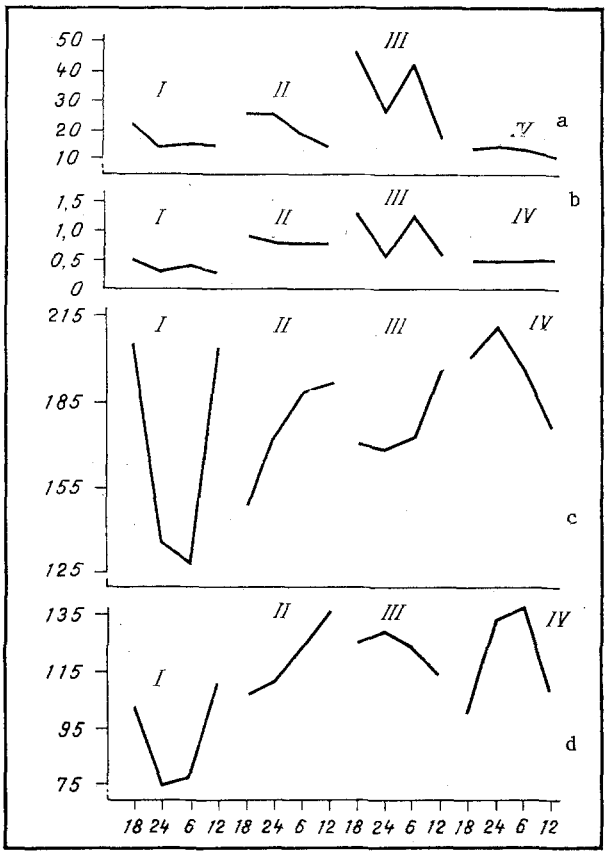

Fig. 2

Fig. 1. Seasonal and circadian changes (in mU/ml) of AST (a) and ALT (b) concentrations. Here and in Figs. 2 and 3: abscissa, clock time (in h); I) winter, II) spring, III) summer, IV) fall.

Fig. 2. Seasonal and circadian changes in concentration (in mg%) of urea nitrogen (a), creatinine (b), glucose (c), and cholesterol (d).

Among the other parameters studied, significant seasonal differences were found for bilirubin: its level in spring was higher (0.4 mg%) than in the other seasons of the year (Fig. 3). A similar picture also was observed in the case of total protein, the concentration of which was slightly but significantly ($p < 0.05$) higher in the spring than in the summer, fall, and winter. Cholesterol concentration (Fig. 2) was significantly ($p < 0.05$) lower in the winter than in the summer and fall. The distinct scatter of the circadian fluctuations of the cholesterol level will be noted, greatest in the winter. In addition, the character of the circadian rhythm curves differed greatly in the different seasons. The course of the cholesterol concentration at different times of the 24-h period in the winter can be considered to be inverted relative to that in the fall and summer. No seasonal changes were found in the glucose concentration (Fig. 2). However, in this case also, considerable circadian variations were found, and these also were most marked in the winter. Comparison of the circadian curves of glucose and cholesterol concentration in the different seasons of the year shows that these circadian fluctuations were synchronized in character in the winter, spring, and fall, but in the summer some degree of inversion was noted.

The Cl^-, K^+, and Na^+ concentrations were virtually unchanged in the different seasons of the year (Fig. 3). However, whereas in the spring, summer, and fall circadian fluctuations were not significant, in the winter, at different times of the 24-h period they were more marked and the character of the curves was similar to those for glucose and cholesterol. Analysis of the circadian rhythms of the various parameters in different seasons of the year indicates that maximal synchronization of the circadian rhythm occurs in the winter: for AST, glucose, cholesterol, protein, Cl^-, K^+, and Na^+, and also to some degree for urea nitrogen, the character of circadian quantitative distribution is similar. In the fall synchronization of the circadian parameters also is observed, but the character of the curves is inverted in form (AST, ALT, glucose, cholesterol, and urea nitrogen; fluctuations in the bilirubin, Cl^-, and Na^+ levels, although not significant, do not contradict this conclusion).

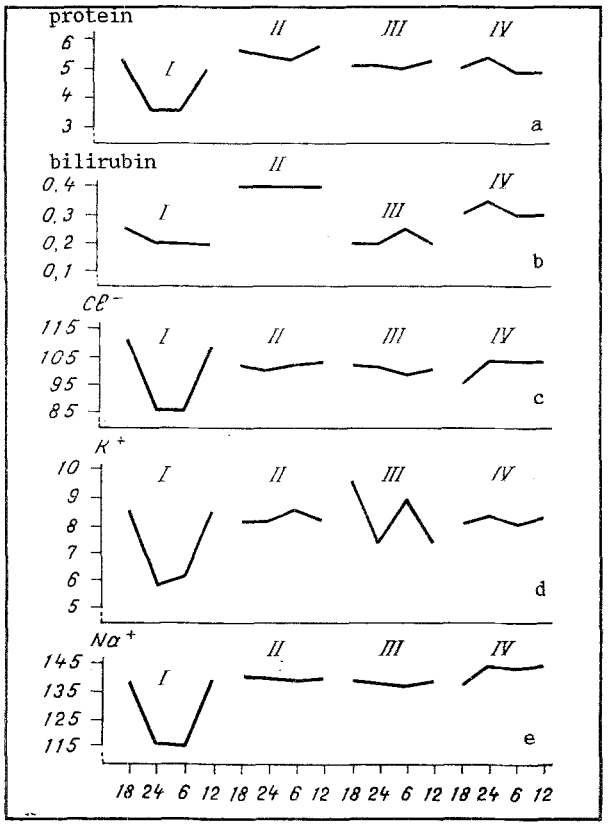

Fig. 3. Seasonal and circadian changes in protein (in g%, a), bilirubin (in mg%, b), Cl^-, K^+, and Na^+ (in meq/liter) concentrations (c, d, and e, respectively).

The curve reflecting these rhythms is monomodal in type: in the winter the concentrations are highest in the afternoon and evening, lowest at night and in the early morning; in the fall the opposite is the case. In the summer circadian synchronization is less noticeable (for AST, creatinine, and urea nitrogen, for K^+, and to some extent also for bilirubin); the circadian rhythm curve is bimodal. The character of the rhythm for ALT is inverted. Finally, in the spring, virtually no circadian synchronization can be detected, except for some degree of matching for AST and ALT.

The fact that circadian synchronization of these parameters was most marked in the winter and also in the fall can be attributed to the relative length of daylight and darkness during the 24-h period. Evidently in the case of nocturnal animals kept under natural conditions of alternation of daylight and darkness, synchronization of the biological rhythms during the 24-h period will be most marked when the duration of the dark period increases. Similar results were obtained previously in a study of mitotic activity in the corneal epithelium of rats; in this case synchronization of the circadian rhythm of mitosis was most marked in December, less so in February and May [3]. It is difficult as yet to explain the opposite phases of the rhythms of the biochemical parameters in the winter and fall, whereas the virtual absence of circadian synchronization in the spring can evidently be attributed to readjustments taking place in the animals, which probably lead also to a disturbance of the temporal organization of biological rhythms.

To conclude, the most important rhythms, both seasonal and circadian, affect parameters of the function of those organs, such as the liver (ASR, ALT) and kidneys (creatinine, urea nitrogen) which participate the most in maintaining resistance of the animal to various factors, by comparison with parameters whose stability is evidence of maintenance of homeostasis at the cellular, tissue, and whole-body levels. The results also point to the need for a study of seasonal fluctuations of physiological parameters during the 24-h period, for only by adopting that approach will it be possible to obtain a true picture of the presence or absence of biological rhythms in different seasons of the year.

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MATHEMATICAL MODEL OF PROTEIN LOSS ASSOCIATED WITH PROLONGED BLOOD LOSS

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For various reasons direct measurements of protein lost by the body as a result of effusion of blood or hemorrhage are not usually undertaken because it is assumed that the protein loss corresponds to the volume of lost blood. Data in the literature [6] and our own investigations [3] show that this hypothesis is more or less valid only at the beginning of blood loss. In the course of a pathological process, the protein loss is appreciably affected by changes in the hematocrit index, the concentrations of total protein and its fractions, and other factors also.

The aim of this investigation was a quantitative description of protein loss during repeated experimental effusion of blood and to predict changes in this parameter depending on the initial state of the animal and the experimental conditions.

EXPERIMENTAL METHOD

Data obtained in two series of experiments on 50 mongrel dogs were used to construct the mathematical model [3]. The experimental part of the work was described in detail previously [3, 4]. A model of prolonged blood loss [2] was created by repeated (every 15 min) bleeding from the femoral artery until the arterial blood pressure (BP) was 5.3 kP (40 mm Hg). If BP did not rise during the 15-min period, the interval between two consecutive bleedings was increased to 30, 45, or 60 min. In series I (n = 28) blood samples were taken from the animals before the experiment (under anesthesia), 1 h after the beginning of bleeding, and at the end of the phase of compensation of vascular tone, namely after spontaneous lowering of BP to 4.0-3.3 kP (30-25 mm Hg). In series II (n = 22) samples were taken before the experiment and from every portion of removed blood. The volume of removed blood was measured at each bleeding: the hematocrit index was determined at the beginning and end of the experiment and the total plasma protein concentration was measured spectrophotometrically. The protein loss during bleeding (Y, g/kg) was calculated from the results of the measurements:

$$Y = \boldsymbol{C} \cdot \boldsymbol{V} (1 - 0.96\mathrm{H}),$$

where **C** denotes the total plasma protein concentration (in g/liter), **V** the volume of blood removed (in liters/kg body weight); and H the hematocrit index (in liters/liter).

For all the portions of blood except the first to be removed, the hematocrit index was considered to be the same as that at the end of the experiment. The total protein loss after m bleedings was taken to be

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