EFFECT OF INVERSION OF DAYLIGHT AND DARKNESS ON THE GENERAL STRUCTURE OF BIOLOGICAL RHYTHMS OF MITOTIC ACTIVITY IN THYMOCYTES

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In research into biological rhythms of mitotic activity of thymocytes, only its seasonal and circadian variations have been investigated [6, 8, 9, 10]. No attempt has been made to study rhythms of changes in the mitotic index (MI) of thymocytes with other, including ultradian, rhythms. Yet at the present time attention is being increasingly drawn to the study of biological rhythms to a particular function, which differ in the length of their period and which, together, form a general structure of temporal variations of that function. Considering the important role for biological rhythms of a time transducer such as the photoperiod, it is interesting to determine its effect on that structure. This is particularly so because of data in the literature indicating absence of changes in circadian fluctuations of thymocyte MI after reversal of daylight and darkness [2]. According to Filippovich and Kuzin, reorganization of the circadian rhythms of MI after daylight/darkness inversion (photoinversion) takes place earlier in rapidly renewing tissues (after 2-3 weeks in the esophageal epithelium) than in slowly renewing tissues (after 3-4 weeks in the hepatic parenchyma). Thymocytes are known to possess an extremely high rate of proliferation [6].

The aim of this investigation was to study the general structure of rhythms of thymocyte division and its changes 5 days after photoinversion.

EXPERIMENTAL METHOD

Experiments were carried out on 420 noninbred male mice weighing 18-19 g, divided into two series: I) control, II) after photoinversion. Initially all the animals were kept for 7 days under standard conditions of daylight (L) and darkness (D): L:D = 12:12 (daylight from 2 a.m. until 6 p.m.). The mice of series I were then killed for investigation, whereas the mice of series II were kept for a further 5 days under photoinversion conditions (D:L = 12:12; daylight from 6 p.m. until 6 a.m.); films of the thymus were obtained every 2 h during the 24-h period, and every 20 min from 10 a.m. to 4 p.m. and from 10 p.m. to 4 a.m. Five animals were used at each experimental point. The films of the thymus were fixed twice in ethanol, hydrolyzed for 6 min in 1 N HCl at 56°C, washed with water, and stained with methylene blue. MI was determined in promille, 10,000 cells being counted in films from each animal. The numerical results were analyzed on an M-4030 computer by programs for identifying concealed rhythms in biological processes. The material for study was regarded as the sum of a series of harmonics and of random noise. To discover the periods of the oscillations (in h) composing the possible spectrum of biological rhythms, classical assessments of spectral analysis [4] were used. Periodic components of the model were collected to correspond to peaks of spectral density. The best combination of periods of components was selected in the hyper-region covering near-peak frequencies. The optimal model of the process was chosen by regression analysis [7]. Amplitudes of oscillations (in $^0/_{00}$), found by regression analysis, were used in order to compare their power and to detect the predominant frequency of oscillations. The pool of dividing thymocytes in the course of 24 h, the pool of dividing cells in active phases of circadian rhythmic changes in MI, and the ratio between the latter

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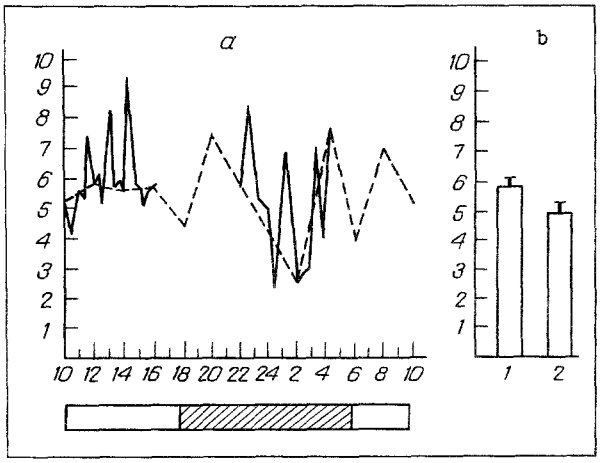

Fig. 1. Changes in MI of thymocytes and its mean value in control animals during light and darkness. Abscissa: a) clock time (in h); b: 1) daylight, 2) darkness; ordinate, MI (in $^0/_{00}$). Here and in Fig. 2a: continuous line, MI every 20 min; broken line, every 2 h; 3) mean values of MI in darkness and in light (for measurements made every 20 min).

and the 24-hourly pool, were determined by a graphic-parametric method [5]. The numerical results were subjected to statistical analysis by the Fisher–Student test, with a $p \leq 0.05$ level of significance of differences.

EXPERIMENTAL RESULTS

The time course of MI of the thymocytes of the control animals and on the 5th day after photoinversion is shown in Figs. 1 and 2. Clearly MI of the thymocytes in the control varied throughout the 24-h period. Its determination every 2 h showed that from 10 a.m. to 6 p.m. MI did not change significantly. Toward 8 p.m. an increase in MI was observed ($p = 0.0005$), after which until 2 a.m. it fell to its lowest value for the 24-h period ($p = 0.0005$). MI increased at 4 a.m. ($p = 0.005$), and this was followed by a decrease at 6 a.m. ($p = 0.005$). MI then increased again ($p = 0.0005$). Thus in the course of 2-hourly determinations of MI during the 24-h period complex temporal changes in MI were found, and in particular, during the period of testing it increased threefold. The 1st and 2nd times that this happened was at the beginning and end of the period of darkness, and the 3rd time (4 h after the 2nd time) it was at the beginning of the period of daylight. The increase in MI took place after unequal time intervals, and as a result, changes in this index of the thymocytes during the 24-h period were irregular and were not like a circadian rhythm. The greatest difference between extreme values of MI during the 24-h period (2 and 4 a.m.) was 5.3 $^0/_{00}$, and the average daily value of MI was 5.5 $^0/_{00}$. The 24-hourly pool of dividing thymocytes (t_m was taken to be 30 min [3]) was 396.0 $^0/_{00}$, and the number of cells in active phases of circadian rhythmic changes in MI was 77%.

Spectral analysis of the results of determination of MI of thymocytes at 2-hourly intervals showed the presence of ultradian oscillations with a period of 7.0 h. The amplitude of these oscillations was 1.6%.

The study of the time course of MI, by determining it every 20 min, showed that during both light and dark periods of the day high-frequency oscillations of MI of the thymocytes were present. During daylight the period of these oscillations was 2.4 and 1.6 h, and their amplitude was the same, namely 0.7%. During darkness the oscillations had periods of 2.2 and 1.2 h, and their amplitudes were 1.6 and 0.9%, respectively. The oscillations found can evidently be classed as circumhoralian biological rhythms [1], one feature of which is variation of the period within quite wide limits. However, it follows from the results that during darkness oscillations with a period of 2.2 h were predominant, whereas oscillations of that kind (2.4 h) were less abundant in daylight. On that basis, and also on the basis of visual analysis of Fig. 1a, it can be concluded that the period of circumhoralian oscillations of MI of thymocytes in the dark interval in the control animals was on average longer than in daylight. Incidentally, the mean value of MI in these oscillations was significantly lower at night than during the day (4.9 ± 0.30 $^0/_{00}$ and 5.9 ± 0.13 $^0/_{00}$, $p = 0.01$, respectively). Thus shortening of the period of the circumhoralian oscillations of MI was combined with an increase in its values in measurements throughout the 24-h period.

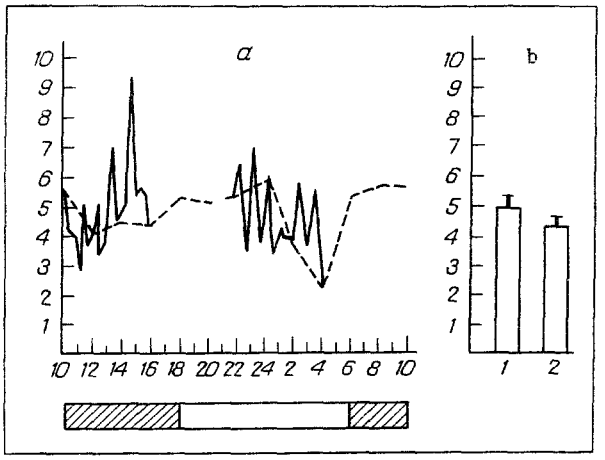

Fig. 2. Changes in MI of thymocytes and its mean values during light and darkness, in mice after 5 days of photoinversion. Abscissa: a) clock time (in h); b: 1) darkness, 2) daylight; ordinate, MI (in $^0/_{00}$).

It will be clear from Fig. 2 that the curve of changes in MI of the thymocytes on the 5th day after photoinversion, measured every 2 h, has two maxima during the 24-h period: one in the middle of daylight (midnight), the second at the beginning of darkness (8 a.m.). Minimal values of MI of the platelets were observed at the end of daylight (4 a.m.) and in the middle of darkness (noon). The amplitude of the circadian changes in MI was 4.4 $^0/_{00}$, the mean daily value of MI was 4.9 $^0/_{00}$, and the 24-hourly pool of dividing cells 362.6 $^0/_{00}$, whereas the number of dividing cells in the active phases was 62 $^0/_{00}$. Thus on the 4th day after photoinversion, definite changes took place in the character of circadian oscillations of MI of the thymocytes compared with the control, namely that only two maxima were noted during the 24-h period. The results were confirmed by spectral analysis of the MI curve with observations at 2-hourly intervals. It showed that an oscillation with a period of 13.2 h became dominant. Its amplitude was 1.1 $^0/_{00}$. Besides, oscillations of frequency similar (period 7.7 h) to those present in the control, but with only half their amplitude (0.8 $^0/_{00}$), were found by spectral analysis. The study of the MI curve of the thymocytes, plotted from the results of tests of the experimental material every 20 min, showed that on the 5th day after photoinversion, high-frequency rhythms were preserved in the course of thymocyte reproduction. The decrease in the period of these oscillations as a whole, compared with the control, must be noted. For instance, during darkness oscillations with a period of 1.2 h (amplitude 1.2 $^0/_{00}$) were predominant, but in this case oscillations with a period of 1.6 h (amplitude 0.9 $^0/_{00}$) were present. During daylight, only oscillations with a period of 1.0 h could be found (amplitude 1.1 $^0/_{00}$). This was the highest frequency of oscillations observed during the study of MI of thymocytes in the control and experimental animals. Thus after photoinversion definite changes were observed in the spectrum of high-frequency oscillations of thymocyte MI compared with the control. As in the control, however, the frequency of these oscillations was higher during daylight than in darkness. The mean value of MI in the above-mentioned oscillations, however, did not differ significantly in the periods of daylight and darkness (5.0 ± 0.16 $^0/_{00}$ and 4.7 ± 0.11 $^0/_{00}$, respectively). Consequently, after photoinversion, correlation of changes in the period of high-frequency oscillations was found only with the phases of light and darkness during the circadian rhythm of illumination, but not with circadian fluctuations in thymocyte proliferation.

The results are evidence that rhythms of thymocyte proliferation are characterized by a definite spectrum of these oscillations, which include ultradian and circumhoralian rhythms. Circadian rhythms of cell division were not found in the thymus of intact mice. Changes began to appear 5 days after photoinversion in both ultradian and circumhoralian oscillations of MI. They mainly took the form that in both cases oscillations with periods not present in the control mice appeared. This indicates that after photoinversion there is a restructuring of the biological rhythms of mitotic activity of the thymocytes, which is still not complete 5 days after the event.

The investigation showed that the length of the period of circumhoralian oscillations of thymocyte MI in intact animals is longer at night than during the day, and its increase depends directly on mitotic activity of the thymocytes during the 24-h period. In animals after photoinversion, a connection still remains between changes in the period of circumhoralian oscillations and the level of illumination, whereas connection with the phases of the circadian rhythm of MI disappears. Fuller details of this relationship must await further investigations.

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ACTION OF REMANTADINE ON FUSION OF THE LIPID ENVELOPE OF INFLUENZA A VIRUS WITH PLASMA AND INTERNAL MEMBRANES IN LYMPHOBLASTOID CELLS

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For transcription of the virus genome to begin in cells infected by enveloped viruses, the nucleocapsid of the virus must be released from external proteins and the lipid envelope. For this purpose, the influenza virus utilizes the path of receptor-mediated endocytosis: it is bound by receptors on the plasma membrane, and penetrates through covered pits and vesicles into the endosomes, where at low pH fusion of the lipid envelopes of the virus and endosomes takes place, leading to release of the nucleocapsid [4, 11]. Fusion of the lipid envelope of the virus with cell membranes was studied until very recently by inducing this process artificially by lowering the pH, and thus observing only fusion of the lipid envelope of the virus and the plasma membranes of the cells [8, 14]. By the fluorescence quenching method [12] the fusion of the lipid envelope of the virus both with plasma membranes of cells and with membranes of endosomes can be evaluated quantitatively. In this method a fluorescent probe with hydrocarbon chain, determining incorporation of the probe molecule into the lipid envelope of the virus, is used. If the virus membrane fuses with the cell membranes, redistribution of the probe on the membrane takes place, its concentration is reduced, quenching is reduced, and accordingly fluorescence increases, and an effect of dequenching of fluorescence (DQF) is observed.

Remantadine is widely used nowadays in the chemotherapy of influenza, but its mechanism of action has not yet been explained [1]. According to one group of investigators remantadine is a substance which, like NH_4Cl and chloroquine, inhibits fusion of the lipid envelope of the virus and endosomes. On the other hand, there is evidence [6, 7] that remantadine blocks the stage of RNP release from the nucleocapsid, interfering with the beginning of transcription. The aim of the present investigation

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