EXPERIMENTAL BIOLOGY

Effect of an Inverted Light Regime on the Biological Rhythms of the Mitotic Index of Mouse Esophageal Epithelium in Different Periods

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Translated from *Byulleten' Eksperimental'noi Biologii i Meditsiny,* Vol. 121, № 1, pp. 94-97, January, 1996
Original article submitted January 12, 1995

Changes of the mitotic index in the esophageal epithelium were studied every 20 min for 24 hours in control mice and in mice kept under conditions of an inverted light regime for 10 days. A monophasic circadian rhythm was revealed, which was virtually completely resynchronized to the new light regime after 10 days of photoinversion. Circahoralian fluctuations of the mitotic index with 1-2-hour periods were noted. In controls the period of these fluctuations was shorter in the active phase of the circadian rhythm than during the passive phase. After photoinversion this regularity was no longer observed. Hence, in contrast to the circadian rhythm, the hierarchical structure of the temporal organization of the mitotic index rhythm of the esophageal epithelium in different periods was not restructured during the 10 days of the new light/dark regime.

Key Words: *circadian rhythm; circahoralian rhythm; mitotic index; photoinversion; temporal organization*

The totality of biological rhythms of the organism's functions with different periods is one of the types of temporal organization of living systems [5]. However, the regularities of this type of temporal organization have not yet been studied in depth. Only a few reports have been devoted to studies of the mitotic activity of epithelial cells of the tongue [9,11] and thymocytes [8]. The classification of biorhythms by the length of the cycle of fluctuations [14] is universally acknowledged. The relationships between the circadian and so-called circahoralian rhythms are interesting from the viewpoint of the hierarchical structure of rhythmic fluctuations of different periods [1,2].

The aim of this study was to analyze the biological rhythms of the mitotic activity of mouse esophageal epithelial cells with different periods and the effect of an inverted light regime on their hierarchical structure.

MATERIALS AND METHODS

A total of 1300 outbred male albino mice weighing 18-19 g were used in the experiment. The animals were adapted to the control light regime (light:darkness= =12:12, light from 06:00 to 18:00 h) over 7 days. Then half of them were transferred to conditions of an inverted light regime (light:darkness=12:12, light from 18:00 to 06:00 h) and thus kept for 10 days. After this an esophagus was taken for investigation from control and photoinverted animals every 20 min for 24 hours. After standard histological treatment 5-μ slices of the organ were prepared and stained with Mayer's hematoxylin. The mitotic index (MI) was calculated as the

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0007-4888/96/0001-0086$15.00

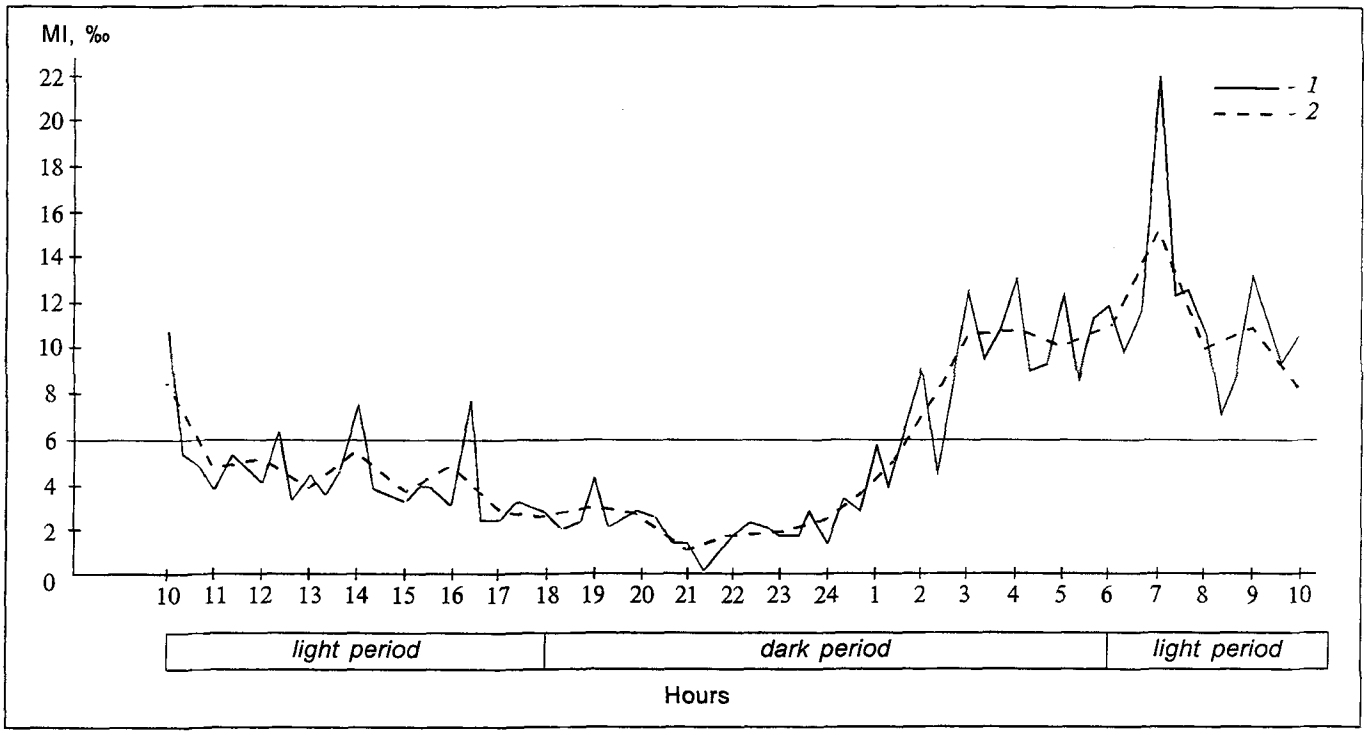

Fig. 1. Changes of MI in the esophageal epithelium of control mice. Here and in Fig. 2: *1*) MI every 20 min; *2*) mean MI after 1 h: horizontal line: mean diurnal MI.

fraction of dividing cells after examination of 10,000 cells of the basal layer of esophageal epithelium from every animal and expressed in promille. Based on the results, experimental curves were plotted representing MI changes in the control and experimental animals. Mathematical methods of studying the biorhythms were used in the research. An experimental curve was approximated to a sinusoid on segments of the observation interval, using computer software. The sinusoidal period was selected using the method of least squares. The period of the approximated sinusoid was regarded as the period of circahoralian fluctuation. Differences were considered reliable at $p<0.05$ (Student's t test). The mean hourly MI curves for 24-hour intervals for the control and experiment were processed by the graphic-parametric method of analyzing the circadian rhythms of proliferative activity [10]. The duration of mitosis was assumed to be 1 hour [7].

RESULTS

Figures 1 and 2 show that the time course of MI in the esophageal epithelium of control and photoinverted animals in the course of a 24-hour observation is characterized by a monophasic circadian rhythm. In controls the maximum MI at 07:00 h reliably differed from its minimum at 21:00 h ($p<0.002$). In the experimental animals the maximum MI was noted at 14:00 h and

TABLE 1. Graphic Parametric Analysis of Circadian Rhythms of Cell Mitosis in Esophageal Epithelial in the Control and after Photoinversion

Parameter	Control	Photoinversion
Mesor	6.1	5.1
Acrophase, h	7:00	14:00
AP, h	1:30-10:30	12:00-20:00
Duration of AP, h	9	8
Middle of AP, time of day	6:00	16:00
Absolute amplitude, ‰	14.3	10.0
Relative amplitude	14.0	8.1
MI synchronization coefficient in the rhythm, 1/h	1.4	0.9
Pool of dividing cells in 24 h, ‰	145.3	122.3
Pool of dividing cells in AP of rhythm, ‰	95.0	75.7
Pool of dividing cells in AP of rhythm/pool of dividing cells in 24 h, ‰	65.4	61.9

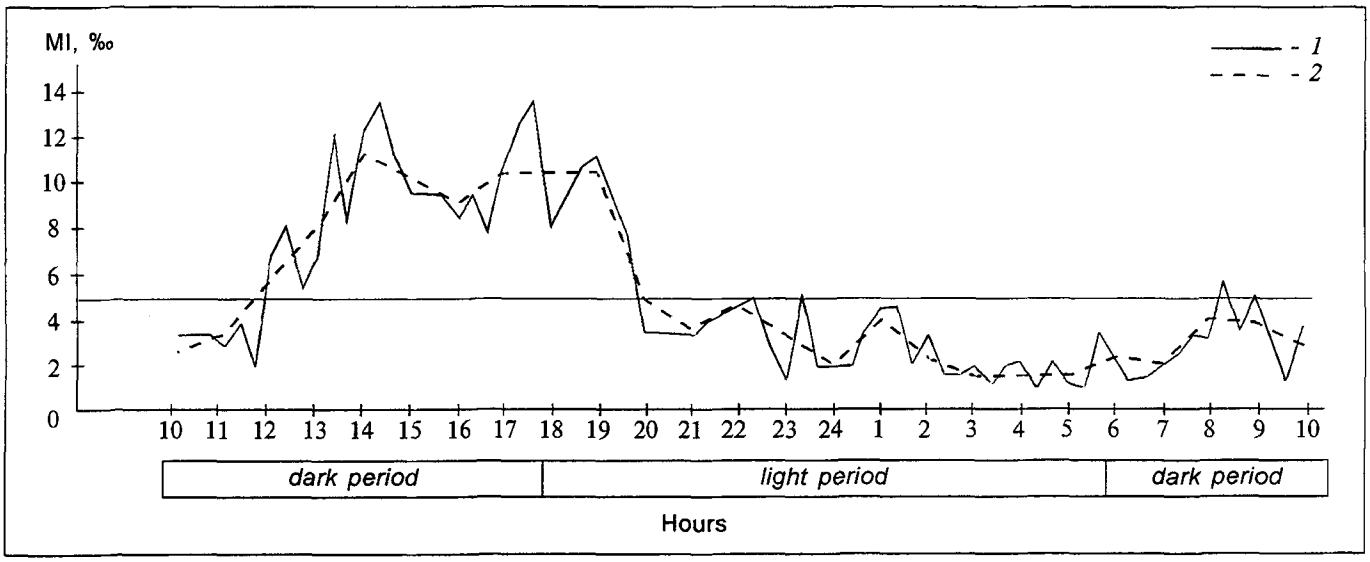

Fig. 2. Changes of MI in the esophageal epithelium of mice 10 days after photoinversion.

differed reliably from the minimum at 05:00 h ($p<0.02$). Table 1 shows that 10 days after inversion of the light regime the circadian rhythms of MI in mouse esophageal epithelium are virtually completely resynchronized with a phase shift to the right. The active phase (AP) of the daily rhythm in both experimental series falls in the second half of the dark period and at the beginning of the light period. The acrophase is shifted 7 hours and the middle of the AP shifted 10 hours. The mesor and duration of the AP are negligibly reduced. An appreciable decrease of the rhythm amplitude and synchronization coefficient is worthy of note. The diurnal pool of dividing cells is somewhat diminished in photoinverted animals in comparison with the control, but almost two-thirds of cells proliferating during 24 hours go through mitosis in the AP of the diurnal MI rhythm in both experimental groups. Our results agree with other scientists' reports about daily MI rhythms in the esophageal epithelium and the effects of light regime inversion on it [4,12,13].

TABLE 2. Periods of Circahoralian MI Rhythms in the Control and after Photoinversion

Control		Photoinversion	
segment, time interval	period, h	segment, time interval	period, h
24:00-1:40	1.0	24:00-1:40	1.5
2:00-3:40	1.0	2:00-4:20	1.0
4:00-6:20	1.0	4:40-5:40	1.0
6:40-8:40	1.5	6:00-8:00	1.5
8:00-10:20	2.0	8:40-9:40	2.0
12:00-14:00	2.0	12:00-13:40	1.0
14:40-16:00	2.0	17:00-18:40	1.5
18:20-20:40	1.0	19:00-20:40	2.0

High-frequency fluctuations of MI, which may be classed as circahoralian, were observed in both series of the experiment. The data in Table 2 and visual analysis of the curves indicate that 1-hour fluctuations predominate in the AP of the diurnal MI rhythm of control mice, whereas for the passive phase 2-hour fluctuations are more frequent. Hence, in the control a relationship is observed between the phases of the diurnal MI rhythms and the frequency of circahoralian fluctuations of this parameter. In the AP of the circadian rhythm the circahoralian fluctuations become somewhat more frequent in comparison with the passive phase. In photoinverted animals rhythms with 1-2-hour periods were observed over the entire 24 hours, but there was no clear-cut relationship between the phases of the diurnal MI rhythms and the frequency of the circahoralian fluctuations.

Hence, our study revealed biological rhythms of mitotic activity of mouse esophageal epithelium cells with different periods. A distinct monophasic circadian rhythm of mitosis with the AP in the second half of the dark period and beginning of the light period is observed in the esophageal epithelium. This indicates a synchronization of the diurnal fluctuations of MI with the 24-hour light/dark cycle. Besides the circadian rhythms, the proliferative system of esophageal epithelium is characterized by circahoralian MI rhythms, and there is a relationship between these two levels of hierarchical structure of cell-division biorhythms which is expressed as more frequent circahoralian fluctuations in the AP of the diurnal MI rhythm. Ten days after inversion of light regime we observed a virtually complete resynchronization of the diurnal MI rhythm with a new light/dark cycle, which occurred through a phase shift attended by negligible changes in other parameters of the rhythm.

Circahoralian fluctuations of MI were still observed in photoinverted animals, but they were no longer related to the diurnal rhythm phases characteristic of the controls. We may conclude that, although 10 days after photoinversion the daily rhythm of cell divisions is virtually completely restructured, the hierarchical structure of the MI rhythms of different periods, regarded as a systemic entity with certain relationships between the levels of its organization, has not yet completed its restructuring.

REFERENCES

1. V. Ya. Brodskii, *Tsitologiya,* **18,** № 4, 397-407 (1976).
2. V. Ya. Brodskii, V. I. Fateeva, S. I. Rapoport, *et al., Byull. Eksp. Biol. Med.,* **97,** № 5, 612-614 (1984).
3. F. I. Komarov, Yu. A. Romanov, and N. I. Moiseeva, in: *Chronobiology and Chronomedicine: A Handbook* [in Russian], Moscow (1989), pp. 5-17.
4. S. M. Kuzin and Yu. A. Romanov, *Byull. Eksp. Biol. Med.,* **91,** № 3, 365-367 (1981).
5. Yu. A. Romanov, *Topics in Chronobiology* [in Russian], Moscow (1989).
6. Yu. A. Romanov and I. K. Rakhmatullina, *Byull. Eksp. Biol. Med.,* **71,** № 5, 103-106 (1971).
7. Yu. A. Romanov and V. P. Rybakov, *Ibid.,* **72,** № 11, 93-97.
8. Yu. A. Romanov, V. V. Evstaf'ev, and S. S. Filippovich, *Ibid.,* **109,** № 5, 481-483 (1990).
9. Yu. A. Romanov, V. P. Rybakov, and V. V. Evstaf'ev, in: *The Epithelium and Connective Tissue under Normal, Experimental, and Pathological Conditions* [in Russian], Tyumen (1989), p. 201.
10. Yu. A. Romanov, S. S. Filippovich, S. M. Kuzin, *et al., Methods of Regeneration and Cell Division* [in Russian], Moscow (1979), pp. 44-53.
11. V. P. Rybakov, V. V. Evstaf'ev, and S. M. Kuzin, in: *Chronobiology and Chronomedicine* [in Russian], Astrakhan (1988), pp. 61-62.
12. V. P. Rybakov, Yu. A. Romanov, and A. V. Timofeev, *Tsitologiya,* **21,** № 4, 401-406 (1979).
13. S. S. Filippovich, in: *Reactivity and Plasticity of Epithelial and Connective Tissue under Normal, Experimental, and Pathological Conditions* [in Russian], Sverdlovsk (1974), pp. 208-209.
14. F. Halberg and G. S. Katinas, *Int. J. Chronobiol.,* **1,** № 1, 31-63 (1973).