# EXPERIMENTAL BIOLOGY

# Ultradian Biorhythms of Cell Proliferation

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We studied circadian and ultradian rhythms of mitotic index of epithelial cells of the esophagus, intestine, and dorsal surface of the tongue, and thymocytes from mice maintained at normal and inverted light regimens. It was shown that in normal animals, the period of ultradian oscillations was shorter during the active circadian phase compared to the passive phase. Photoinversion changed the correlation between ultradian oscillations of the mitotic index and its circadian phases, which was differently pronounced in various tissues. Thus, changes in daily illumination serve as the time indicator for circadian rhythms of cell proliferation, but not for ultradian oscillations of mitotic activity.

**Key Words:** *circadian rhythm; ultradian rhythm; mitotic index; photoinversion*

The concept of temporal organization of biological systems was recently intensively developed. An important component of this temporal organization is ultradian rhythms, which interact with other rhythms [2-4]. Various biorhythms of the same biological function provide a wide spectrum of perception, transmission, and reproduction of biological information of the same semantics and, when necessary, its differential selection [5]. Circadian rhythms of mitotic activity are studied in details. However, other biorhythms of this function are less studied. Here we studied ultradian rhythms of cell proliferation in various tissues.

## MATERIALS AND METHODS

Series I was carried out on 288 outbred albino male mice weighing about 30 g and maintained under standard conditions at 12:12-h light regimen (light from 8.00 to 20.00). The animals were sacrificed every 20 min during a day (3-5 mice per point). The epithelium of the tongue and intestine was isolated. The specimens were fixed in Carnoy fluid for 1.5-2.0 h, dehydrated in alcohol, embedded in paraffin, and cut into 4-7-μ sections. After deparaffinization, the sections were stained with Mayer's hematoxylin and post-stained with eosin. Mitotic index (MI) was determined after counting 5000-8000 epithelial cells in each preparation.

Series II was carried out on 1900 outbred albino male mice weighing 20 g. Some animals were kept under a standard light regimen (light:dark 12:12, light from 6.00 to 18.00 h), and others under an inverted regimen (light:dark 12:12, light from 18.00 to 6.00 h). Inversion was carried out after adaptation to normal regimen for one week.

The mice were sacrificed 3, 7, and 10 days after photoinversion. Thymocytes and esophageal epithelium were examined. Smears of the thymus were twice fixed in ethanol, hydrolized in 1 N HCl at 56°C for 6 min, washed in water, and stained with methylene blue. Esophagus specimens were subjected to routine histological treatment, cut into 6-μ sections, and stained with Mayer's hematoxylin. MI was calculated as the number of dividing cells per 10,000 cells from each animal expressed in $^{o}/_{oo}$.

The data were processed on a computer using programs revealing masked periodicity in biological processes [1]. Our program excluded temporal trend from the temporal row. Polynomial trend was found using

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step-by-step regression analysis. Polynomial degree did not exceed 2, because the polynomial of a higher degree can include the periodic component of the process.

The first part of the program is based on a stationary model of biorhythm. Biological process is viewed as the sum of harmonics and noise. The frequencies of biorhythm oscillations were found by spectrum analysis. The periodic components of the model were selected in accordance with spectral density peaks. The optimal combination of the periodic components was in the hyperarea close to the peak frequencies. The optimal model was selected using regression analysis. The oscillation amplitudes (in per mil) calculated by regression analysis were used for comparison and for identification of predominant oscillations.

## RESULTS

The study of the dynamics of mitotic activity of epithelial cells of the dorsal surface of the tongue revealed circadian oscillations including two active (AP, 2.40-8.20 and 12.00-15.00 h) and two passive phases (PP, 8.20-12.00 and 15.00-2.40 h). Ultradian oscillations with 2.6-, 1.6-, and 1.0-h periods were also found. One AP of mitotic activity was short (about 3 h), therefore ultradian oscillations within this phase cannot be determined. The frequency and amplitude of ultradian oscillations increased during AP of circadian rhythm and decreased during PP (Table 1).

In the intestinal epithelium, circadian rhythm of MI had 2 peaks with two AP (3.00-10.40 and 18.00-23.00 h) and two PP (10.40-18.00 and 23.00-3.00 h). However, no clear-cut dependency between ultradian rhythms and phases of circadian rhythm was revealed.

It should be noted that the spectrum of rhythms in cell populations widened from the lower to the upper third of the crypt. No significant ultradian oscillations were found in the lower and middle thirds of the crypt, but in the upper third a 1.5-h rhythm appeared.

MI of thymocytes from control mice was characterized by a biphasic rhythm with two AP (13.00-18.00 and 22.20-5.00 h) and two PP (5.00-13.00 and 18.00-22.20 h).

Table 2 presents changes in the period of ultradian oscillations of MI during AP and PP of the circadian rhythm. In control animals, the period of ultradian oscillations of MI was shorter during AP and longer during PP of the circadian rhythm.

Thus, spectral analysis of AP and PP of the circadian rhythm of MI of thymocytes from mice kept under normal photoregimen revealed a dependency of ultradian MI rhythm on its circadian phase.

The animals kept under inverted photoregimen for 10 days showed a biphasic rhythm of MI in thymocytes with two AP (16.20-23.00 and 1.20-10.00 h) and two PP (10.20-16.00 and 23.00-1.20 h). Table 2 showed that on day 10 of photoinversion circadian rhythm of MI in thymocyte population was not completely resynchronized with the new light regimen. No correlation between ultradian rhythm of MI and its circadian phase was observed in photoinverted animals.

The dynamics of mitotic activity in control esophageal epithelium was characterized by monophasic circadian rhythm with AP (1.20-10.20 h) and PP (10.20-1.20 h). During AP, 1-h oscillations of MI prevailed, while during PP 2-h rhythms were more pronounced (Table 2). Thus, the frequency of MI oscillations in esophageal epitheluim increased during AP. On day 10 of photoinversion the circadian rhythm of MI was completely resynchronized with the new photoregimen due to phase shift from 12.00 to 20.00 h in AP and from 20.00 to 12.00 h in PP. In photoinverted animals, ultradian oscillations of MI were preserved, but did

**TABLE 1.** Ultradian Rhythms of MI in the Epithelium of the Dorsal Surface of the Tongue and Small Intestine in Mice during Various Phases of Circadian Rhythm

| Group | Interval, h | Period, h | Amplitude, ‰ | Mean MI, ‰ |
|---|---|---|---|---|
| Tongue epithelium | | | | |
| AP | 2.40-8.20 | 1.0 | 3.0 | 17.3 |
| PP | 15.00-2.40 | 2.6 | 1.6 | 4.9 |
| | 8.20-12.00 | 1.6 | 1.7 | 6.5 |
| Small intestine | | | | |
| AP | 3.00-10.40 | 2.3 | 8.3 | 36.3 |
| | | 3.3 | 11.9 | |
| | 18.00-23.00 | 1.6 | 9.9 | 36.2 |
| | | 2.3 | 11.3 | |
| PP | 10.40-18.00 | 2.0 | 9.0 | 30.3 |
| | | 3.0 | 14.1 | |

**TABLE 2.** Periods of Ultradian Rhythms of MI in Thymocytes and Esophageal Epithelium during Various Phases of Circadian Rhythm

| Group | Interval, h | Period, h | Amplitude, ‰ | Mean MI, ‰ |
|---|---|---|---|---|
| **Thymocytes** | | | | |
| Control | | | | |
| AP | 13.00-18.00 | 1.1 | 0.3 | 1.2 |
| | 22.20-5.00 | 1.2 | 0.3 | 1.6 |
| PP | 5.00-13.00 | 2.6 | 0.4 | 1.0 |
| | 18.00-22.20 | 3.9 | 0.3 | 1.1 |
| Inversion | | | | |
| AP | 16.20-23.00 | 1.2 | 0.3 | 1.6 |
| | 1.20-10.00 | 3.6 | 0.3 | 1.5 |
| PP | 10.20-16.00 | 2.7 | 0.2 | 1.1 |
| | | 1.8 | 0.1 | |
| **Esophageal epithelium** | | | | |
| Control | | | | |
| AP | 1.20-10.20 | 1.0 | 2.5 | 8.7 |
| | | 1.6 | 0.7 | |
| PP | 10.20-1.20 | 1.6 | 0.4 | 3.5 |
| | | 2.0 | 2.3 | |
| Inversion | | | | |
| AP | 12.00-20.00 | 1.2 | 0.9 | 7.1 |
| | | 2.0 | 0.7 | |
| PP | 20.00-12.00 | 2.0 | 0.5 | 3.1 |

not correlate with the circadian phases (as control mice).

Thus, in normal animals, a correlation between circadian and ultradian oscillations of proliferative activity in various tissues was revealed. This correlation was manifested in decreased period of ultradian oscillations during AP and increased period during PP. Photoinverted animals showed different correlations between MI oscillations of different periods in various tissues. In these animals, circadian MI rhythms were rearranged in the epithelium and incompletely resynchronized in thymocytes. However, in both tissues photoinversion changed the correlation between ultradian oscillations and circadian phases of MI. Thus, changes in illumination can serve as the time indicator for circadian rhythms in cell proliferation, but not for ultradian MI oscillations.

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