DIURNAL PERIODICITY OF MITOTIC DIVISION IN SMALL INTESTINE CRYPTS OF ALBINO RATS AND MICE

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Mitotic activity of crypt cells of the small intestine decreases as its cells differentiate. In different parts of the crypt (lower, middle, and upper thirds in the direction from fundus to neck) the dynamics of the diurnal rhythm of mitosis varies. It is concluded that the diurnal rhythm of cell division and the intensity of cell reproduction are dependent on differentiation processes.

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The small intestine was one of the first objects in which the diurnal rhythm of mitosis was studied in animal tissues [17, 18]. Most investigators describe an increase in mitotic activity in the crypt cells of the small intestine of adult rats in the morning and in the first half of the afternoon [2, 4-6, 8, 12]. However, according to some investigators, two peaks in the number of mitoses occur in the crypt during the 24-h period [3, 7, 11]. In some investigations of crypts of the mouse small intestine the curve of the diurnal rhythm of mitosis is unimodal [2], whereas in other investigations it is bimodal [1, 11]. Pilgrim and coworkers [13] in general failed to find any diurnal rhythm of mitotic activity in the mouse small intestine.

Taken as a whole, the crypt cells consist of populations of cells of different degrees of differentiation, as reflected in the unequal ability of cells of different parts of the crypt to begin mitosis and enter the phase of DNA synthesis [9, 10, 14-16].

The object of the present investigation was to study mitotic activity throughout the 24-h period in different parts of the crypt of the small intestine in order to determine the effect of cell differentiation on the diurnal rhythm of mitosis.

EXPERIMENTAL METHOD

Experiments were carried out on 48 albino rats with a mean weight of 31 g (series I), 40 male albino rats with a mean weight of 43 g (series II), and 40 noninbred albino mice with a mean weight of 25 g (series III). The animals received a natural diet in the animal house and were kept under conditions of natural illumination. The experiments of series I were carried out in February, of series II in April, and of series III in January. The animals of series I and II were investigated during the course of the 24-h period at 10 A. M., 1, 4, 7, and 10 P. M., and 1, 4, and 7 A. M., while the animals of series III were investigated at 11 A. M., 2, 5, 8, and 11 P. M., and 2, 5, and 8 A. M. At each time 5 or 6 animals were sacrificed. The overall mitotic index (OMI) of the epithelium of the small intestine was calculated per 4000-5000 cells in 50 longitudinally divided crypts. In addition, mitotic indices were determined in the lower third of the crypt (MI_1), and its middle (MI_2) and upper thirds (MI_3) in the direction from the fundus to the neck of the crypt (the wall of the crypt was divided into three equal parts). The mitotic indices were expressed in promille.

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EXPERIMENTAL RESULTS

Series I. The values of OMI reached a maximum at 1 and 10 A. M. (29.5±5.2 and 29.7±4.1 ‰), and a minimum at 7 and 10 P. M. (22.6±5.4 and 22.9±2.9‰; P ≈ 0.026). The number of mitoses in the lower third of the crypt reached a minimum at 7 P. M. (P = 0.049), and in the middle third at 10 P. M. and 7 A. M. (P < 0.0001-0.049). MI_3 was greater between 1 and 10 A. M. than between 1 and 10 P. M. (P < 0.009-0.050). The mean values of OMI, MI_1, MI_2, and MI_3 for the 24-h period were 26.0±5.0, 34.3±7.4, 32.0±6.9, and 11.8±5.1‰ respectively. It is clear from these results that mitotic activity was sharply reduced (on the average by two-thirds) in the upper third of the crypt compared with its other divisions (P < 0.0001). Hence, whereas the curves of the diurnal rhythm of mitosis in the lower and upper thirds of the crypt are bimodal in character (this applies to the dynamics of the diurnal changes in OMI also), the curve for cells of the upper third of the crypt is not bimodal, and reflects an increase in mitotic activity once a day over a fairly long period of time (about 9 h). In the upper third of the crypt, the deviations in the mitotic index in the course of the 24-h period from the mean for that period were more marked (±43%) than in the lower and middle thirds (from −18 to +13%).

Series II. OMI in the epithelium of the crypt reached a maximum at 7 P. M. (34.2±7.0‰) and a minimum at 10 A. M. (25.5±4.9‰; P = 0.050). A similar dynamics in the course of the 24-h period was observed in the changes in MI_1 and MI_2 (P < 0.036-0.049; Fig. 1B). MI_3 increased between 4 and 10 P. M. and decreased at 1 and 7 A. M. and 1 P. M. (P < 0.0001-0.048). The mean values of OMI, MI_1, MI_2, and MI_3 for the 24-h period were 29.5±5.7, 41.6±10.1, 33.1±6.6, and 14.2±6.9‰, respectively. Consequently, in the animals of this series the mitotic activity in the middle third of the crypt was 1.3 times weaker than in the lower third (P = 0.001), and the number of mitoses in the upper third of the crypt was 2.5-3 times less than in the other parts of the crypt (P < 0.0001). The curves of the diurnal rhythm of mitosis in different parts of the crypt were unimodal, although it must be noted that an increase in mitotic activity in the upper third of the crypt was observed during a longer period of time (6 h) than in the lower and middle thirds. The greatest deviations of the mitotic indices in the course of the 24-h period from the mean values for that period in the lower and middle thirds of the crypt were comparatively small (from +15 to +10%), but in the upper third they were on a larger scale (from −43 to +35%).

Series III. The OMI reached a maximum at 2 P. M. (51.8±9.9‰) and was much lower at 5 P. M. (39.8±5.5‰; P = 0.050). As Fig. 1C shows, the values of MI_1 reached a maximum at 2 and 11 P. M. compared with other times of day (P = 0.050). In the middle third of the crypt the number of mitoses showed a tendency to increase at 2 P. M., but these changes in MI_2 were not significant. MI_3 was highest in the period from 8 A. M. to 8 P. M. (P = 0.050). The mean values of OMI, MI_1, MI_2, and MI_3 for the 24-h period were 44.2±6.7, 52.5±8.7, 64.0±10.6, and 16.1±6.0‰, respectively. Hence, according to the mean values for the 24-h period, MI_2 was 1.2 times higher than MI_1 and MI_3 was 3.3-4 times less than MI_1 and MI_2 (P < 0.0001). The diurnal rhythm of mitosis in the lower third of the crypt was expressed graphically by a

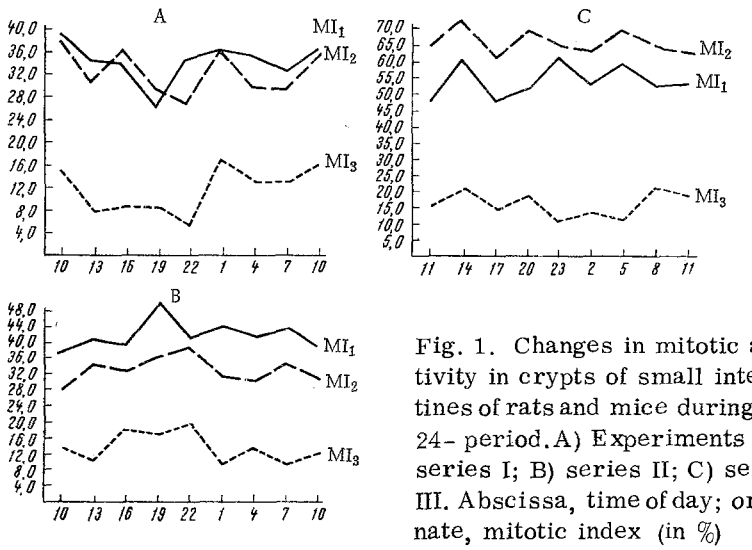

Fig. 1. Changes in mitotic activity in crypts of small intestines of rats and mice during the 24- period. A) Experiments of series I; B) series II; C) series III. Abscissa, time of day; ordinate, mitotic index (in ‰)

bimodal curve, in the middle third it could not be confidentially demonstrated, and in the upper third only one peak of mitotic activity was present during the 24-h period, occupying a comparatively long time (12 h). In the lower and middle thirds of the crypt, the greatest deviations of the mitotic indices during the 24-h period from the mean values for that period were from −10 to +18%, compared with from −17 to +32% in the upper third.

The results of this investigation show that mitotic activity varies in cells of the crypts of the small intestine of rats and mice at different stages of differentiation. The most highly differentiated crypt cells (in its upper third) are characterized by considerable weakening of mitotic activity. The dynamics of the diurnal rhythm of mitosis also varies in different parts of the crypt. Although two peaks in the number of mitoses occur in the lower third of the crypt, only one peak of mitotic activity can be observed in the upper third (experiments of series I and III). In the animals of series III, no diurnal rhythm of mitosis was present in the middle third of the crypt, although in its other divisions the rhythm of cell division was clearly marked.

Cells of the upper third of the crypt, as the results of all series of experiments showed, differ in the more constant character of the diurnal rhythm of mitosis (increase in mitotic activity once in the 24-h period, occupying a long period of time).

Differentiation of the cells thus affects not only the level of their reproduction in different parts of the crypt, but also affects the diurnal rhythm of mitosis. It can be postulated that changes in intracellular metabolism arising during development of the cells are one of the factors determining the dynamics of the diurnal periodicity of mitosis.

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