DURATION OF MITOSIS AND DIURNAL RHYTHM OF MITOTIC ACTIVITY

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A study of the duration of mitosis, using colchicine, and of mitotic activity in the bone marrow, the crypts of the small intestine, and the thyroid gland of rats during the 24-h period showed that the daily increase in mitotic activity is associated with the starting of mitosis by a larger number of cells and not with an increase in the duration of mitosis. The most highly differentiated cells in the crypts of the small intestine and in the thyroid gland are characterized by diminished ability to take part in mitosis.

Experiments have shown that the duration of mitosis in animal tissues varies in the course of the 24-h period. Kreyberg and co-workers [4], in experiments on hairless mice, showed that changes in the duration of mitosis in the epidermis correlate with changes in mitotic activity at different times of day or night. Gyergyay-Malatinszky and Gyergyay [3] observed an increase in the duration of mitosis in the epithelium of the small intestine and tongue of mice during an increase in mitotic activity. This contradicts the existing views regarding changes in the diurnal rhythm of mitotic activity as the result of differences in the number of cells starting on mitosis in the course of the 24-h period.

The relationship between changes in the duration and number of mitoses during the 24-h period in the bone marrow, small intestine, and thyroid gland was investigated.

EXPERIMENTAL METHOD

Experiments were carried out on 56 male albino rats (mean weight 47 g). Control animals (group 1) were sacrificed at 10 A. M., 1, 4, 7, and 10 P.M., and 1, 4, and 7 A.M. At each time of the experiment, 5 animals were sacrificed. The animals of group 2 received an intraperitoneal injection of colchicine (1 μg/g) 6 h before sacrifice, and they were sacrificed at 10 A.M., 4 and 10 P.M., and 4 A.M. Mitotic activity in the tissues was judged from the results of determination of the mitotic index (MI) in pro mille. MI of bone marrow cells was calculated for 5000-7000 cells in aceto-orcein impression films. The overall mitotic index (OMI) of epithelium of the small intestine was calculated for 4000-5000 cells in 50 longitudinally cut crypts. MI was also determined in the lower, middle, and upper thirds of the crypt (MI_1, MI_2, and MI_3, respectively, in the direction from the bottom to the neck of the crypt). MI of the thyroid epithelium was calculated for 40,000-60,000 cells in each case. The duration of mitosis was calculated by the formula $t_m = MI \cdot A/MI_{colch.}$, where A represents the time of action of colchicine.

EXPERIMENTAL RESULTS

A diurnal rhythm of mitosis exists in the bone marrow cells of young rats, with a maximum between 4 and 10 A.M. and a minimum between 4 and 10 P.M. ($P = 0.002$) (Fig. 1). The greatest number of C-mitoses also was observed between 4 and 10 A.M., and the smallest number between 10 P.M. and 4 A. M.

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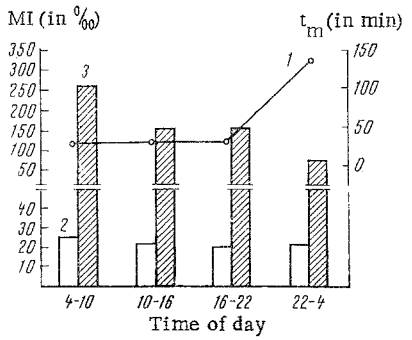

Fig. 1. Changes in duration of mitosis (1), in MI (2), and in $MI_{colch.}$ (3) during the 24-h period in bone marrow cells.

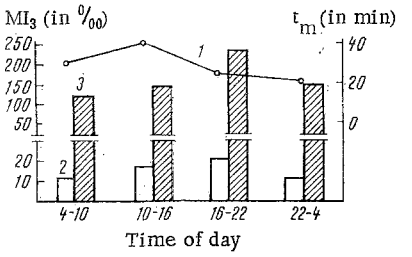

Fig. 2. Changes in duration of mitosis (1), MI (2), and $MI_{colch.}$ (3) during the 24-h period in cells of the upper third of a crypt of the small intestine.

($P < 0.0001$). The duration of mitosis varied during the 24-h period, being shorter between 4 and 10 A.M. (29 min), i.e., at the period of maximal mitotic activity. The longest duration of mitosis was found between 10 P.M. and 4 A.M. (141 min), shortly before the increase in mitotic activity. The increase in the duration of mitosis, in the absence of an increase in MI, evidently indicated delay in the starting of mitosis at this time by a certain proportion of the cells.

The mean values of MI, $MI_{colch.}$, and t_m for the 24-h period were 20.6 ±4.1 and 166.2 ±92.0 ‰, and 45 min, respectively.

In the lower and middle thirds of the crypt of the small intestine, the highest values of MI and $MI_{colch.}$ were found between 4 and 10 P.M. ($P < 0.050$). However, the duration of mitosis in these parts of the crypt changed very little during the 24-h period (from 46 to 54 and from 42 to 52 min, respectively). The mean values of MI_1, $MI_{1colch.}$, and t_m for the 24-h period were 41.6 ±10.1 and 311.6 ± 93.0 ‰, and 48 min, and for MI_2, $MI_{2colch.}$, and t_m 33.1 ±6.6 and 255.8 ± 56.0 ‰, and 47 min, respectively.

In the upper part of the crypt the diurnal rhythm of mitosis was more marked. Here many more normal and C-mitoses were observed between 4 and 10 P.M. than at other times of day or night ($P < 0.040$) (Fig. 2). The duration of mitosis also varied during the 24-h period. At a time of increase of mitotic activity, the duration of mitosis was shorter (29 min), and immediately before this period, the duration of mitosis was greatest (40 min). The absence of an increase in MI at a time of decrease in the rate of mitosis in the upper third of the crypt, as in the bone marrow, evidently reflects delay in the starting of mitosis by a certain proportion of the crypt cells.

The mean values of MI_3, $MI_{3colch.}$, and t_m for the 24-h period were 14.2 ±6.9 and 167.8 ± 52.0 ‰, and 31 min, respectively.

In the upper third of the crypt of the small intestine, a considerable decrease in mitotic activity was thus observed compared with the lower and middle thirds ($P < 0.0001$). The mean number of normal and C-mitoses in the middle third of the crypt for the 24-h period was smaller than in the lower third ($P < 0.050$). These results show that during differentiation of the crypt cells, the duration of mitosis was reduced.

The largest number of normal and C-mitoses in the thyroid epithelium was observed between 4 and 10 A.M. (10.2 ±4.5 ‰, and 66.4 ±16.0 ‰, respectively), and the smallest number between 10 A.M. and 4 P.M. (2.5 ±1.5 ‰, $P = 0.034$ and 20.4 ±16.0 ‰, $P = 0.008$). The duration of mitosis showed only slight changes during the 24-h period (from 46 to 55 min). The mean values of the overall mitotic index (OMI), of $OMI_{colch.}$, and of t_m for the 24-h period were 4.8 ±4.2 ‰ and 35.6 ±23.0 ‰, and 49 min, respectively.

Besides the number of mitoses in the whole population of thyroid cells, MI also was calculated for individual classes of follicles. Size classes of follicles are present in the thyroid gland of young rats (class 1 – follicles containing 2-5 cells in their wall or section; class 2 – 6 to 15 cells; class 3 – 16 to 25 cells; class 4 – 26 to 35 cells; and class 5 – 36 to 45 cells). In the follicles of classes 1, 2 and 3, the highest values of MI and $MI_{colch.}$ were observed between 4 and 10 A.M., and the lowest values between 10 A.M. and 4 P.M. ($P < 0.039$). In the follicles of classes 4 and 5, besides an increase in mitotic activity and in the accumulation of C-mitoses in the period from 4 to 10 A.M., there was also a significant increase in MI and $MI_{colch.}$ between 4 and 10 P.M. ($P < 0.0001$-0.045). The duration of mitosis in the different classes of follicles varied only slightly during the 24-h period. According to the mean data for the 24-h period, the values of MI, $MI_{colch.}$, and t_m for the various classes of follicles were as follows: class 1 6.2 ±4.3 and 60.2 ±36.0 ‰, and 37 min; class 2 4.3 ±3.4 and 34.8 ±23.0 ‰, and 44 min; class 3 7.0 ±7.3 and 47.0 ±39.0 ‰, and 53 min; class 4 3.1 ± 3.1 and 20.2 ±20.0 ‰, and 47 min; and class 5 2.7 ±2.8 and 17.1 ±6.0 ‰, and 53 min.

The values for mitotic activity and accumulation of C-mitoses in the follicles of classes 4 and 5 are significantly lower than those for follicles of classes 1-3 ($P < 0.003$-0.047). Hence, cells in the larger follicles divide less frequently.

In all tissues the values of prophase index in the control and experimental rats were identical.

The mean values for the 24-h period thus obtained provide a basis for calculation of the duration of the mitotic cycle (T) of cells of the studied tissues, by means of the formula:

$$T = \frac{t_M \cdot N}{n},$$

where N is the total number of cells and n the number of dividing cells. The duration of the mitotic cycle for bone marrow cells was 35 h, for the cells of the lower third of the crypt 20 h, middle third 24 h, and upper third 38 h, and for cells of the thyroid gland 7.1 days ($P_C = 100\%$).

The results of this investigation show that the duration of mitosis may vary during the 24-h period. Changes in the duration of mitosis during the 24-h period have recently been established for the epithelium of the cornea and tongue and for the kidney cells of mice [1, 2]. In the present experiments no increase in the duration of mitosis was found at the time of day or night when mitotic activity of the tissues was increased. It can accordingly be concluded that the daily increase in the number of mitoses takes place because of the greater number of cells starting on mitosis.

The results of investigation of the small intestine and thyroid gland show that as the cells differentiate and develop, the mitotic activity in these organs is reduced.

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