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DURATION OF MITOSIS IN THE CORNEAL EPITHELIUM AND SPLEEN CELLS OF MICE WITH LEUKEMIA La

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The diurnal rhythm of mitosis and its duration were investigated in the corneal epithelium and spleen cells of mice with leukemia La. Correlation was found between changes in the mitotic index and duration of mitosis during the 24-h period. It is suggested that the more rapid course of mitosis in tissues with intensive cell proliferation and its slower course in tissues with low proliferative ability are reflections of a general rule.

KEY WORDS: *leukemia La; diurnal rhythm of mitosis; corneal epithelium; spleen.*

Investigations have shown differences in the duration of mitosis at different times of the 24-h period in normal animal tissues and in tumors [1, 5, 7, 8, 10]. At times of day when the mitotic index reaches a maximum, the duration of mitosis has been found to be shortest and, conversely, a fall in the mitotic index is accompanied by a rise in the duration of mitosis. Definite correlation thus exists between the number of cells starting mitosis and the duration of mitosis. The relationship between mitotic activity and the duration of mitosis in tissues with different levels of cell proliferation is an interesting topic for study.

This paper describes the results of an investigation of the duration of mitosis in two different tissues from the same mice: in the corneal epithelium, in which the mitotic cycle lasts 3-4 days [9] and in spleen cells in leukemia La, with a mitotic cycle of 12 h [3].

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EXPERIMENTAL METHOD

Experiments were carried out in July on male C57BL mice weighing 15-20 g. Acute leukemia La was transplanted by intraperitoneal injection of two million leukemic spleen cells. The mice usually died on the seventh to eighth day after this injection. Mitoses were counted in each case in 8000-9000 nuclei in areas of the spleen solidly packed with leukemic cells. In the corneal epithelium of both eyes 18,000-20,000 cells were examined. The mitotic index (MI) and index of colcemid-blocked mitoses (MI_{col}) were expressed in promille. The scheme of the experiments was as follows. The animals of group 1 (control) were killed in groups of 3 or 4 at a time every 2 h, starting from noon on the 6th day of development of leukemia. The animals of group 2 (experimental) received an injection of colcemid in a dose of 5 mg/kg body weight 4 h before sacrifice. These mice were killed at 4 and 10 p.m., midnight, 4 and 8 a.m., and noon, 6 at each time. No late phases of mitosis were found. The duration of mitosis was calculated by the equation:

$$tm = \frac{MI \cdot t}{MI_{col}},$$

where tm is the duration of mitosis; MI is the mean MI of the control animals sacrificed at three consecutive times; MI_{col} is the mean index of colcemid-blocked mitoses; t is the duration of action of colcemid (in h).

Statistical analysis was carried out by the Fisher–Student method.

EXPERIMENTAL RESULTS

It will be clear from Table 1 that the mitotic index and index of c mitoses changed considerably in the course of the 24-h period. The highest value of MI_{col} was found between 4 and 8 a.m. and the lowest between 8 p.m. and midnight ($P = 0.001$). Over a period of 8 h (from 4 a.m. to noon) 58.4% of the cells dividing during the 24-h period started mitosis. Consequently, cell division in the corneal epithelium is considerably synchronized in time. It also follows from these observations that the rhythms of entry of the cells into mitosis and the rate of mitosis are in harmony with each other: Duration of mitosis was shorter at a time of high mitotic index and longer when the mitotic index was low. The limits of variation during the 24-h period — from 1.2 to 4.3 h ($P = 0.04$) — are very close to those discovered in the corneal epithelium of healthy animals [5].

The rhythm of the index of mitosis was ill defined in the leukemic spleen cells although MI was higher at 8 p.m. than at 4 a.m. ($P = 0.03$). In a previous investigation [2] a definite rhythm of division of leukemic cells was found with a maximum at 7 p.m. and a minimum at 4 a.m. Changes in the index of c mitoses during the 24-h period were more marked. In the period from 4 to 8 p.m. accumulation of metaphases was more considerable than between 8 a.m. and noon ($P = 0.027$). The duration of mitosis between 4 and 8 p.m. was minimal (23 min). Conversely, the duration of mitosis increased to 33 min (by 43%) in the period from 8 a.m. to noon ($P = 0.009$ for this period), when the accumulation of mitoses was minimal. In a tissue

TABLE 1. Diurnal Changes in Number of Mitoses, Number of Blocked Metaphases, and Duration of Mitosis in Corneal Epithelium and Spleen Cells of Leukemic Mice

Time of day	Corneal epithelium			Leukemic spleen cells		
	MI, ‰	MI_{col}, ‰	tm, h	MI, ‰	MI_{col}, ‰	tm, h
noon-4 p.m.	12,8	36,2	1,4	15,9	119,0	0,53
4-8 p. m.	8,9	26,5	1,3	16,2	162,0	0,40
8 p.m.-midnight	6,5	6,1	4,3	17,1	140,0	0,48
midnight-4 a.m.	15,6	15,6	4,0	15,3	120,0	0,51
4-8 a.m.	18,9	62,8	1,2	16,1	128,0	0,50
8 a.m.-noon	19,8	56,0	1,4	15,7	111,0	0,56
Mean for the 24-h period	13,3		2,3	15,8		0,49
Total		203,2			780,0	

with a short mitotic cycle, with a proliferative pool of close to 1.0, and with a mean diurnal duration of mitosis of about 30 min, it is difficult to expect any sharp fluctuations in tm during the 24-h period, but the presence of small fluctuations in this index at different times of the 24-h period was found previously in lymphocytes of the thymus [6]. These fluctuations in tm also took place in a definite relationship with changes in the mitotic index in the cortical zone of the thymus. It can accordingly be concluded that the results exhibit definite reproducibility and indicate correlation between the number of cells starting mitosis per unit time and the duration of mitosis at different times of the 24-h period in tissues with a high level of proliferation, such as the thymus and the leukemic spleen.

When the mean diurnal values of MI (in leukemic spleen cells 15.8 °/₀₀, in corneal epithelial cells 13.3 °/₀₀), are compared the impression may be created that the intensity of cell multiplication was almost the same in the two tissues; however, the total number of cells starting mitosis during the 24-h period was 78.0% in the leukemic spleen and only 20.3% in the corneal epithelium, i.e., in the ratio of 3.8:1. Consequently, the mitotic index incompletely reflects the proliferative activity of the tissues. For a more correct assessment of mitotic activity, it is essential to know the rate of mitosis [4]. The experiments showed that the mean diurnal duration of mitosis in the corneal epithelium is 2.3 h, whereas in the leukemic spleen cells it was 0.5 h, i.e., in a ratio of 4.7:1. The shorter course of mitosis in the leukemic spleen than in the cornea evidently reflects a general rule: a more rapid course of mitotic division of the cells in tissues with a short mitotic cycle and a high proliferative pool and a slow passage through mitosis in tissues with a lower intensity of cell proliferation.

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