DIURNAL VARIATIONS IN THE DURATION OF MITOSIS IN THE ESOPHAGEAL EPITHELIUM OF MICE

L. D. Liozner and I. V. Markelova

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Experiments on C57BL/6J mice showed that the mitotic index in the esophageal epithelium differs considerably in the morning and evening. The use of the colchamine (colcemid) method showed that the duration of mitosis in the morning and evening is about equal, namely 34-49 min (the results varied depending on the dose of colchamine and the duration of its action). Diurnal differences in the mitotic index of the esophageal epithelium thus depend on differences in mitotic activity (the number of mitoses in unit time). The need for observing several demands when the colchicine method is used, in order to obtain reliable results, is emphasized.

KEY WORDS: duration of mitosis; esophageal epithelium; colchamine; diurnal rhythm of mitosis.

Diurnal variations in the duration of mitosis and furthermore, the importance of these variations for the diurnal rhythm of mitosis, have been inadequately studied. The statement of some workers that the diurnal rhythm of mitosis is due to corresponding changes in the duration of mitosis [7-9] was not confirmed by later research [6, 10]. Meanwhile papers continue to appear in which it is concluded from the investigations described in them that sharp fluctuations take place in the duration of mitosis during the 24-hour period [3, 4]. These investigations were carried out with the use of colchicine or its derivatives.

With this in mind, and in order to test the hypothesis that the diurnal rhythm of mitosis depends on its duration, experiments were carried out on the esophageal epithelium, a tissue in which differences in the percentage of mitosis in the morning and evening considerably exceed the possible individual variation of this index.

EXPERIMENTAL METHOD

Female C57BL/6J mice weighing 15 g were used. The animals were divided into two groups (60 in each group). The experiments on the mice of group 1 were carried out in the morning and on the mice of group 2 in the evening. The 20 mice of group 1 received an intraperitoneal injection of 5 mg/kg colchamine (a preparation analogous to colcemid) in Hanks' solution in a dose of 0.2 ml at 4 A.M. 20 mice received an injection of 0.1 ml of the same solution (a dose of 2.5 mg/kg). Twenty mice remained intact. The experimental mice were killed 1 and 3 h after the injection, 10 at each time. Intact mice were killed 30 min before the first sacrifice of the experimental mice (control 1) and 30 min after the second sacrifice (control 2).

The evening (second) group consisted of analogous subgroups. Colchamine was injected at 4:00 p.m. The experimental mice were killed at 5 and 7 P.M. and the intact mice at 4:30 and 7:30 p.m. Mitoses were counted in sections in the basal layer of the esophageal epithelium, 2000 cells being examined from each mouse. The duration of mitosis was determined by the equation:

$$t_m = \frac{MI \cdot A}{MI_c},$$

Institute of Human Morphology, Academy of Medical Sciences of the USSR. Institue of Medical Genetics, Academy of Medical Sciences of the USSR, Moscow. (Presented by Academician of the Academy of Medical Sciences of the USSR A. P. Avtsyn.) Translated from Byulleten' Éksperimental'noi Biologii i Meditsiny, Vol. 79, No. 6, pp. 89-92, June, 1975. Original article submitted July 12, 1974.

TABLE 1. Number of Mitoses in Mice of Morning and Evening Groups

Group of animals	Subgroup	Dose of colchamine (in mg/kg)	Time of sacrifice	MI (in %)	Number of c-mitoses per 1000 cells
Morning	Control 1	—	4 h 30 min	13,7±1,6	—
	Experiment 1	5,0	5 h min	24,6	22,8±5,0
	Experiment 2	2,5	5 h min	25,2	22,5±4,2
	Experiment 3	5,0	7 h min	74,6	69,2±8,6
	Experiment 4	2,5	7 h min	72,6	55,6±6,3
	Control 2	—	7 h 30 "	23,9±3,9	—
	» mean	—	4 h 30 "	18,8±4,1	—
			7 h 30 "		
Evening	Control 1	—	16 h 30 "	3,3±1,0	—
	Experiment 1	5,0	17 h "	2,4	2,3±0,5
	Experiment 2	2,5	17 h "	3,3	3,0±0,8
	Experiment 3	5,0	19 h "	11,1	10,1±2,3
	Experiment 4	2,5	19 h "	15,0	14,5±5,3
	Control 2	—	19 h 30 "	2,1±0,4	—
	» mean	—	16 h 30 "	2,7±1,1	—
			19 h 30 "		

TABLE 2. Mitotic Activity (in $^0/_{00}$/h) in Morning and Evening

Group of animals	Dose of colchamine (in mg/kg)	Period of action of colchamine after its injection		
		first hour	second-third hour	first-third hour
Morning	5,0	22,8±5,0	23,2±4,8	23,1±2,9
	2,5	22,5±4,2	23,0±3,8	22,8±2,1
Evening	5,0	2,3±0,5	3,9±1,2	3,4±0,8
	2,5	3,0±0,8	5,8±2,7	4,8±1,8

TABLE 3. Duration of Mitosis (in min) in Morning and Evening

Group of animals	Dose of colchamine (in mg/kg)	Period of action of colchamine after its injection		
		first hour	second-third hour	first-third hour
Morning	5,0	36,0±8,9	48,6±11,8	49,2±8,2
	2,5	36,6±8,3	49,0±9,7	49,0±7,0
Evening	5,0	85,8±34,2	42,0±15,4	48,0±12,4
	2,6	66,0±28,2	28,2±14,3	33,5±14,3

where t_m is the duration of mitosis (in h), A the time of action of the colchamine (in h), MI the mean mitotic index in the control (in %), and MI_c the number of metaphases blocked by colchamine (c-mitoses) at the end of the experiment.

If the duration of mitosis was calculated for a definite interval of the total duration of the experiment, corresponding changes were introduced into the equation, as follows:

$$t_m = \frac{MI\ (A_2 - A_1)}{MI_{cx_2} - MI_{cx_1}},$$

where A_1 and A_2 are the beginning and end of the time interval analyzed respectively and MI_{cx_1} and MI_{cx_2} are the number of blocked metaphases at the beginning and end of that interval respectively. The standard error was determined for the mean number of metaphases which accumulated and the mean mitotic index. Knowing these errors, the error of the duration of mitosis and of mitotic activity (the number of mitoses per hour) was calculated by the method of Worthing and Geffner [2, 5].

EXPERIMENTAL RESULTS

As Table 1 shows, MI for the intact mice of the morning group (1) was considerably higher than in the intact mice of the evening (2) group (18.8 and 2.7 % respectively).

A dose of colchamine of 5 mg/kg completely arrested mitosis in the mice of both groups, but a dose of 2.5 mg/kg did so only in the mice of the evening group. Late phases – anaphases, telophases, a stage of reconstruction of the nuclei (Fig. 1) – were found in five mice of the morning group. In neither group were gross disturbances of the rate at which the cells proceeded into mitosis observed (Fig. 1).

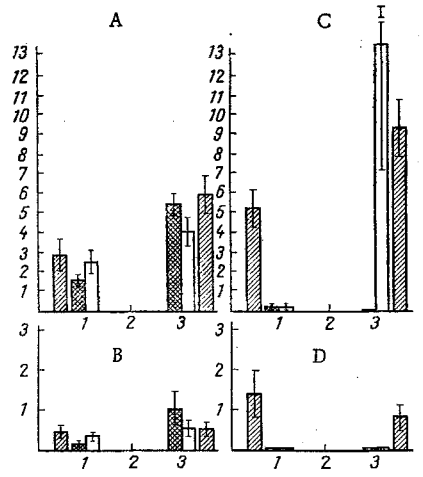

Fig. 1. Number of prophases and late phases of mitosis (anaphases, telophases, and stage of reconstruction of nucleus) in experimental and control groups (M ± m): A, B) prophases in morning and evening groups of experiment respectively; C, D) late phases of mitosis in morning and evening groups of experiment respectively. Obliquely shaded columns show control; cross-hatched columns colchamine 5 mg/kg; unshaded columns colchamine 2.5 mg/kg. Abscissa, time after injection of colchamine (in h); ordinate, number of different phases of mitosis (in %).

Mitotic activity, like MI, was significantly higher in the morning than in the evening (23.2-23 compared with 3.9-5.8 %). The accumulation of mitoses under the influence of colchamine took place more or less uniformly, and for that reason the values of mitotic activity at the different times of the experiment for both the morning and the evening groups were similar (Table 2).

Although in some cases, especially if calculations were made on the basis of the accumulation of mitoses in the first hour of the experiment, considerable differences were found in the duration of mitosis in the morning and evening; however, they were not statistically significant (Table 3). Most of the calculations showed that the duration of mitosis was 42-49 min. Closely similar values were obtained in rats by Bardik [1]. In experiments in which colchamine was used in a dose of 5 mg/kg the duration of mitoses (allowing for the action of colchamine for 3 h) was the same in the morning and evening. When colchamine was given in a dose of 2.5 mg/kg (also allowing for its action for 3 h) the duration of mitosis was shorter in the evening, but it must be remembered that this dose in the morning did not completely block metaphases. It must be emphasized that not absolutely identical results were obtained with different doses of colchamine. Although the differences in a given case were not statistically significant, this fact must nevertheless be taken into account when colchamine is used to determine the duration of mitosis.

The results thus showed, first, that real differences exist in the esophageal epithelium between the value of mitotic activity in the morning and evening ($P = 0.0001$) and, second, that these differences are independent of the duration of mitosis, which was about the same in the morning and evening.

It is, of course, impossible on the basis of these results to rule out the existence of diurnal differences in the duration of mitosis in different organs. However, they show the need for caution when drawing conclusions regarding diurnal differences in the duration of mitosis. Careful steps must be taken to ensure that the colchamine is "working" properly when it is used for the determination of the duration of mitosis. If the colchicine method is to be used properly, certain conditions must by obeyed: uniform accumulation of c-mitoses at consecutive times of action of the preparation, absence of late (following metaphase) phases of mitosis, and absence of effect on the entry of the cells into mitosis (maintenance of the previous percentage of prophases). The use of statistical analysis for calculating not only the differences in MI, but the duration of mitosis itself, is also obligatory.

In order to draw valid conclusions on the dependence of the diurnal rhythm of mitosis on its duration, the material must be large, impeccable, and well reproducible. In addition, it is desirable to confirm conclusions drawn by workers using the colchicine method by means of any other method, such as inhibition of mitosis by x-ray irradiation.

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