DIURNAL CHANGES IN THE DURATION OF MITOSIS IN SOME TISSUES OF YOUNG ALBINO RATS

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The duration of mitosis in the acinar cells of the pancreas varied from 1.7 to 2.5 h, in the hepatocytes from 1.3 to 5.2 h, and in the epidermis from 1.5 to 2.9 h. Diurnal changes in the mitotic index in the tissues investigated were due to both changes in the duration of mitosis and differences in the rate at which the cells commenced to divide. The renewal time of the tissues studied, although differing sharply in adult animals, was found to be about equal in rats aged 7 days (12.3 days in the pancreas and 20 days in the liver and epidermis).

Key words: duration of mitosis; 7-day-old rats; colcemid.

Changes in the duration of mitosis during the 24-hour period have been described in adult animals. However, different interpretations have been put on these findings. According to some workers [5, 6] changes in the duration of mitosis determine the character of the diurnal rhythm in the number of cell divisions, whereas according to others [2-4, 7], the diurnal rhythm of mitosis depends on the rate at which the cells start to divide.

There is no information on diurnal changes in the duration of mitosis in the tissues of young animals. Yet such data are necessary before the levels of motitic activity can be determined in different tissues during ontogenetic development of animals. The investigation described below was carried out for this purpose.

TABLE 1. Diurnal Changes in MI, MIC, and t_m in Pancreas and Liver of 7-Day-Old Rats (dose of colcemid 1.5 mg/kg)

Time (24-h clock)	Pancreas					Liver				
	Control			Experiment		Control			Experiment	
	MI (in ‰)	Period of 24 h	Mean MI (in %)	MI (in ‰)	t_m (in h)	MI (in ‰)	Period of 24 h	Mean MI (in %)	MIC (in ‰)	t_m (in h)
10	6,0					3,6				
12	4,7	10—14	5,5	11,0	2,0	3,3	10—14	3,7	11,3	1,3
14	5,9					4,3				
16	5,1	14—18	4,9	11,2	1,7	3,7	14—18	4,4	5,0	3,5
18	3,8					5,7				
20	6,1	18—22	5,8	12,5	1,8	2,2	18—22	3,9	10,8	1,4
22	7,7					4,6				
0	4,6	22—2	7,7	14,7	2,1	4,1	22—2	5,5	4,2	5,2
2	10,8					8,3				
4	7,6	2—9	9,1	15,5	2,5	6,0	2—6	6,9	10,7	2,5
6	8,9					6,4				
8	7,5	6—10	7,5	16,1	1,8	5,6	6—10	5,2	8,1	2,5
Mean diurnal value			6,7	13,5	1,9			4,9	8,4	2,7

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EXPERIMENTAL METHOD

Three series of experiments were carried out on 200 noninbred albino rats aged 7 days. The object of series I was to determine the effective dose of colcemid. Six groups of rats, with ten rats in each group, were used. Colcemid was injected intraperitoneally at 10 a.m. in doses of 0.5, 1, 1.5, 2.5, and 3 mg/kg, respectively.

In series II and III diurnal changes in the duration of mitosis were studied in the acinar cells of the pancreas, the parenchymatous cells of the liver, and the epidermis of the skin of the trunk after the administration of different doses of colcemid: 1.5 mg in series II and 0.5 mg/kg in series III.

The animals were killed 4 h after the injection of colcemid. Control animals were killed every 2 h during the 24-hour period, 3 rats at each time. The material was fixed in Carnoy's fluid. Microscopic preparations were stained with Carazzi's hematoxylin. The phases of mitosis were counted in the pancreas for an average of 9000 cells, in the liver for 10,000-15,000 cells, and in the epidermis for 3000-4000 cells of the stratum basale of each animal.

The mitotic index (MI) and index of blocked metaphases (MIC) were calculated per 1000 cells. The duration of mitosis was determined by the equation

$$t_m = \frac{MI \cdot t}{MIC},$$

where t is the duration of action of colcemid.

The results were subjected to statistical analysis.

EXPERIMENTAL RESULTS

In series I, doses of colcemid of 2, 2.5, and 3 mg/kg proved lethal for the 7-day-old rats. The animals died during the first 2 h after the injection. Rats of the remaining 3 groups remained alive for 4 h after injection of the substance although their condition (except the group receiving 0.5 mg/kg) was depressed and some of the rats had died 5 h after the beginning of the experiment.

Investigation of the preparation showed that doses of colcemid of 1.5, 1, and 0.5 mg/kg reliably arrested mitosis at the metaphase stage.

The results of the experiments of series II are given in Table 1. Clearly a well-marked diurnal rhythm of mitosis was present in the cells of the pancreas and the hepatocytes of the control rats. MI in the liver was significantly altered from 18 to 20 h (P = 0.00) and from 20 to 2 h (P = 0.004), and in the pancreas from 18 to 22 h (P = 0.00) and from 18 to 2 h (P = 0.01).

TABLE 2. Diurnal Changes in MI, MIC, and t_m in Epidermis of 7-Day-Old Rats (dose of colcemid 0.5 mg/kg)

Time (24-h clock)	Control			Experiment	
	MI (in ‰)	Period of 24 h	Mean MI (in %)	MIC (in ‰)	t_m (in h)
10	3,8				
12	5,3	10—14	4,5	8,8	2,0
14	4,0				
16	3,9	14—18	3,8	7,4	2,1
18	3,6				
20	3,6	18—22	4,5	11,3	1,6
22	6,2				
0	3,6	22—2	4,6	6,3	2,9
2	4,0				
4	4,8	2—6	4,7	6,8	2,0
6	5,3				
8	2,8	8—10	3,9	9,5	1,5
10	2,7				
Mean diurnal value			4,3	8,4	2

The largest number of dividing cells in these organs was found at night and in the early morning (02.00-08.00 h). These results agree with those obtained by the writers previously [1], although the maximal values of MI were found at 02.00 h, compared with at 22.00 h in the previous investigation, and the mean diurnal value of MI was lower (4.9% in the liver, 6.7% in the pancreas, compared with 6.9 and 9.8, respectively).

The accumulation of blocked mitoses in the pancreas and liver followed a different course. MIC in the cells of the pancreas fell significantly from 06.00-10.00 to 14.00-18.00 h (P = 0.01) to correspond to changes in the number of mitoses in the control animals. High values of MIC were found in the liver for a considerable part of the 24 hours. Only in the period from 14.00 to 18.00 and from 22.00 to 02.00 h was a significant decrease in the number of colcemid metaphases observed (P = 0.01 and P = 0.001, respectively). The accumulation of blocked mitoses thus remained at almost the same level both when MI was at its highest (02.00-10.00 h) and when it was minimal (10.00-14.00 h).

The duration of mitosis in the cells of the pancreas changed only very slightly during the 24 hours. The longest duration of mi-

tosis (2.5 h) corresponded to the time of the maximal MI. At other times of the 24-h period the duration of mitosis remained approximately the same (1.7-2.1 h). The change in the duration of mitosis in the periods of 02.00-06.00 and 14.00-18.00 h was close to significant ($P = 0.03$).

The duration of mitosis in the liver changed during the 24-h period from 1.3 to 5.2 h. The course of mitosis was particularly slow in the period from 22.00 to 02.00 h, when the number of dividing cells started to increase and the accumulation of colcemid mitoses was minimal. Differences in the duration of mitosis in the periods 18.00-22.00 and 22.00-02.00 h were significant ($P = 0.000$).

It can be concluded from the results that the diurnal changes in MI in the liver are mainly determined by changes in the duration of mitosis, for the longest duration of mitosis coincided in time with the rise in MI. However, since at some periods of the 24 hours there was a significant decrease in the number of cells starting to divide, the possibility cannot be ruled out that this factor may influence the character of the diurnal rhythm of mitosis.

The longest duration of mitosis in cells of the pancreas also was observed at the time of the maximum of MI (02.00-06.00 h). Consequently, in this tissue also the duration of mitosis at this period was due to an increase in the number of cell divisions observed. At other times of the 24-h period the duration of mitosis remained almost unchanged, whereas MI fell significantly. This suggests that diurnal changes in MI in the acinar cells of the pancreas are determined not only by fluctuations in the duration of mitosis, but also by differences in the number of cells starting to divide at different times of the 24-h period.

The results of the experiments of series III are given in Table 2.

No significant changes in MI in the epidermis of the control animals were found during the 24 hours. This can be explained by an inadequate number of control animals. However, the character of the change in MI in this experiment was similar to the changes observed by the writers previously [1]. An increase in the number of cell divisions was observed at 22.00 h.

MIC fell significantly only within the periods 18.00-22.00 and 22.00-02.00 h and 22.00-02.00 and 06.00-10.00 h ($P = 0.009$ and 0.02, respectively).

The results obtained by the study of the epidermis showed that at some times of the 24-h period both the rate at which the cells started to divide and the duration of the process of division itself changed.

Since the changes in MI in this experiment during the 24-h period were not significant, it is impossible to draw any definite conclusion regarding the dependence of this parameter on the factors studied.

Knowing the mean diurnal value of MIC, the time of renewal of the cells in the tissues studied can be determined. It was 12.3 days for the cells of the pancreas and 20 days for the parenchymatous cells of the liver and for the epidermis.

The renewal time of these tissues, which differs sharply in adult animals, was thus approximately the same in rats aged 7 days.

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