DIURNAL CHANGES IN THE DURATION OF MITOSIS IN RATS

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Diurnal changes in the duration of mitosis were studied by the colchicine method in rats aged 45 days. In adult animals the mean diurnal duration of mitosis in the epithelium of the pancreas and liver and in the epidermis was reduced by almost half compared with that at the age of 7 days. Diurnal changes in the mitotic indices for the tissues studied may be due to changes in the rates in which the cells start to divide and also in the duration of mitosis.

KEY WORDS: liver; colcemid; duration of mitosis; diurnal changes in mitotic indices.

The study of diurnal changes in the duration of mitosis, together with investigation of the diurnal rhythm of mitosis, is essential for a more accurate estimation of mitotic activity of the tissues and the rates of their cell renewal. This information is also important in order to discover whether diurnal changes in the number of mitoses are determined entirely by the rate at which the cells commence mitosis or whether a role is played also by change in the duration of mitotic cell division at different times of the 24-h period [3-8].

In the investigation described below diurnal changes in the duration of mitosis were studied in the exocrine epithelium of the pancreas, the epithelium of the esophagus, the liver, and epidermis of rats.

EXPERIMENTAL METHOD

Experiments were carried out on 72 rats aged 45 days. Animals of the control group were killed at intervals of 2 h in the course of the 24-h period, three rats at each time. The experimental animals received an intraperitoneal injection of colcemid 4 h before sacrifice in a dose of 3.5 mg/kg and they were killed at intervals of 4 h, six rats at each time. The mitotic index (MI) and the index of blocked mitoses (MIC) were determined per thousand cells on the basis of examination of 10,000-15,000 hepatocytes and esophageal epithelial cells, 3000-4000 cells of the stratum basale of the epidermis, and 15,000-18,000 cells of the pancreatic acini. The duration of mitosis (t_m) was determimed by the equation

$$t_m = \frac{\mathrm{MI} \cdot t}{\mathrm{MIC}}.$$

EXPERIMENTAL RESULTS

The results are given in Tables 1 and 2.

Pancreas. In the epithelium of the control animals a diurnal rhythm of mitoses was found with a maximum at 10 a.m. ($P_{2-10} = 0.001$). The decrease in MI in the period from 6 to 8 a.m. was not significant ($P = 0.14$). The minimal number of mitoses was observed at 8 p.m. ($P_{16-20} = 0.05$).

Changes in MIC were similar to the changes in MI. The largest number of MICs also was observed between 6 and 10 a.m. and the smallest between 10 p.m. and 2 a.m. ($P = 0.008$).

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TABLE 1. Diurnal Changes in MI, MIC, and t_m in the Pancreas and Liver of Rats Aged 45 Days

Time of day	Pancreas					Liver				
	control	treatment with colcemid				control	treatment with colcemid			
	MI (in ‰)	period of day (hours)	mean MI (in ‰)	MIC (in ‰)	t_m (in h)	MI (in ‰)	period of day (hours)	mean MI (in ‰)	MIC (in ‰)	t_m (in h)
12 noon	0,6	10—14	1,2	4,3	1,1	0,2	10—14	0,4	1,5	1,2
2 p.m.	1,8					0,2				
4 p.m.	1,3	14—18	1,3	5,3	1,0	0,1	14—18	0,2	1,3	0,6
6 p.m.	0,9					0,3				
8 p.m.	0,5	18—22	0,9	5,4	0,7	0,2	18—22	0,2	0,7	1,1
10 p.m.	1,3					0,0				
Midnight	1,2	22—2	1,2	3,7	1,3	0,4	22—2	0,3	0,5	2,4
2 a.m.	1,0					0,6				
4 a.m.	1,4	2—6	1,5	5,3	1,1	0,4	2—6	0,4	0,8	2,2
6 a.m.	2,0					0,3				
8 a.m.	0,9	6—10	2,0	14,9	0,5	1,5	6—10	0,9	2,2	1,6
10 a.m.	3,2					0,9				
Mean values			1,4	6,5	0,9			0,4	1,2	1,5

TABLE 2. Diurnal Changes in MI, MIC, and t_m in the Esophagus and Epidermis of Rats Aged 45 Days

Time of day	Esophagus					Epidermis				
	control	treatment with colcemid				control	treatment with colcemid			
	MI (in ‰)	period of day (hours)	mean MI (in ‰)	MIC (in ‰)	t_m (in h)	MI (in ‰)	period of day (hours)	mean MI (in ‰)	MIC (in ‰)	t_m (in h)
12 noon	2,9	10—14	4,7	22,3	0,8	2,9	10—14	3,2	13,5	0,9
2 p.m.	2,6					1,9				
4 p.m.	2,5	14—18	2,8	13,4	0,8	1,9	14—18	1,6	11,7	0,6
6 p.m.	3,4					1,1				
8 p.m.	3,4	18—22	2,6	6,8	1,5	1,5	18—22	1,1	5,9	0,7
10 p.m.	1,0					0,7				
Midnight	5,5	22—2	4,1	55,0	0,3	5,2	22—2	4,9	11,2	1,7
2 a.m.	6,0					8,9				
4 a.m.	10,1	2—6	8,6	45,6	0,7	8,7	2—6	9,2	15,8	2,3
6 a.m.	9,7					10,2				
8 a.m.	7,8	6—10	8,7	53,8	0,6	7,0	6—10	6,7	18,9	1,4
10 a.m.	8,7					4,0				
Mean values			5,2	36,1	0,8			4,5	12,8	1,2

The longest duration of mitosis (1.3 h) was observed in the period from 10 p.m. to 2 a.m. and the shortest (0.5 h) from 6 to 10 a.m. ($P = 0.001$).

Comparison of the data for MIC and t_m with changes in MI leads to the conclusion that the high values of MI in the period from 6 to 10 a.m. were due mainly to the rate at which the cells began to divide, for MIC at this time reached its maximum and t_m was minimal. However, in the period from 10 p.m. to 2 a.m. the rate at which the cells began to divide was the lowest and t_m was maximal. At this time of the 24-h period the low value of MI was evidently largely due to the long duration of mitosis.

The Liver. A diurnal rhythm of the number of mitoses was found in the control animals with a maximum at 8-10 a.m. and a minimum during the afternoon and evening (12 noon to 10 p.m.).

Differences in the values of MI were significant if MI for the period noon to 10 p.m., when the number of cell divisions was small, was compared with the other periods of the investigation (midnight to 10 a.m.), during which mitoses were more numerous ($P = 0.009$).

The value of MIC was maximal between 6 and 10 a.m., after which it fell and reached a minimum between 10 p.m. and 2 a.m.

The value of t_m varied from 0.6 to 2.4 h but changed significantly only during the period from 2-6 p.m. to 10 p.m.-2 a.m. ($P = 0.01$). The highest value of t_m occurred between 10 p.m. and 2 a.m., when MI

in the control group started to increase and the rate at which the cells began to divide, as judged by the rate of accumulation of blocked mitoses, was at its lowest level. Conjecturally t_m in this part of the 24-h period affected the increase in MI. However, in the period between 10 p.m.-2 a.m. and 6-10 a.m. MI increased threefold; the rate at which the cells began mitosis rose significantly ($P = 0.008$), but t_m remained almost unchanged. The decrease in t_m in the period from 2-6 to 6-10 a.m. was not significant ($P = 0.3$). Consequently, within this time interval the changes in MI were mainly due to the rate at which the cells began to divide.

Comparison of the results of this experiment with those of experiments on 7-day-old rats [2] showed that the mean diurnal values of these parameters decreased by half or more with age. The time for the number of cells to double, calculated from the total number of mitoses blocked during the 24 h, increased with the age of the animals: in the pancreas from 12.3 to 25.6 days and in the liver from 20 to 143 days.

The Esophagus. In the control group of animals a distinct diurnal rhythm of the number of mitoses was found with a maximum at 4-10 a.m. and a minimum at 10 p.m. The increase in MI in the period from 10 p.m. to 4 a.m. was significant ($P = 0.02$).

The highest value of MIC was observed at night and in the morning, the lowest between 6 and 10 p.m. The changes in MIC from 6-10 p.m. to 10 p.m.-2 a.m. were significant ($P = 0.002$). Considering that the time of mitosis did not change significantly over the greater part of the 24-h period (from midnight to 4 p.m.) it can be concluded that during this period of time the changes in MI in the esophagus were independent of t_m. However, since t_m was much higher between 6 and 10 p.m. than at all other times of day (P_{18-22} and $P_{22-2} = 0.001$), and since the rate of increase in the number of mitoses was lowest, it can be postulated that the changes in MI at this time also were determined by the value of t_m.

The time taken for the number of cells in the esophagus to double in rats aged 45 days was 5 days.

The Epidermis. MI, the rate at which the cells started to divide (MIC), and t_m showed similar changes in the epidermis. The highest values of the parameters studied were observed at night and in the morning (midnight to 8 a.m.). In the afternoon and evening all three parameters studied fell significantly. Minimal values of MI, MIC, and t_m were observed between 6 and 10 p.m.

Since t_m changed significantly only during one period (from 2-6 p.m. to 2-6 a.m.) but the changes in MIC were significant over a long interval of time, it seems that the diurnal fluctuations in MI were due more to changes in the rate at which the cells started mitosis than to changes in t_m.

It can be concluded from a comparison of the results of these experiments with those obtained previously on rats aged 7 days [2] that the mean diurnal values of MI in the epidermis do not change with age of the animals; MIC rises from 8.4 to 12.8 ‰, t_m falls from 2 to 1.2 h. The time for the number of cells in the epidermis to double is reduced from 20 to 13 days.

The absence of age changes in MI in the epidermis is evidently due to two opposite processes: on the one hand, an increase in the rates at which the cells begin mitosis, as judged from the mean diurnal increase in MIC, and on the other hand, a decrease in t_m.

In all the tissues studied diurnal changes in t_m were thus found. At the same time it was established that t_m in the epidermis, liver, and pancreas fell by about half with increasing age of the animals. Age changes in the diurnal ryhthm of the number of mitoses can be due both to changes in the rate of mitosis and in the rate at which the cells start to divide.

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