CHANGES IN THE DIURNAL RHYTHM OF MITOSIS IN THE ESOPHAGEAL EPITHELIUM OF RATS DURING POSTNATAL DEVELOPMENT

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Changes in the diurnal rhythm of mitosis in the esophageal epithelium of rats of six age groups (from two to 90 days) were investigated. On the first days of postnatal development the diurnal mitotic rhythm was characterized by an increase in mitotic activity (MA) at 7-10 P.M. By the end of the first week of life the diurnal changes in the number of mitoses were slight and not significant. Starting from the age of 15 days the rhythm of mitosis became biphasic in character with a tendency for MA to increase in the morning, and later (at the age of three weeks) this led to the development of a maximum of the number of mitoses in the morning. By the age of 45 days the rhythm of mitosis was monophasic in character and similar to the rhythm in adult animals, which was clearly apparent in the rats at the age of three months. Administration of thyroxine (1 h before sacrifice) to rats aged 15 and 45 days led to a decrease in the MA level when investigated at many times during the 24-h period. The character of the diurnal rhythm of mitosis was unchanged. In no case was a significant increase in MA found after administration of the hormone.

The available facts confirming the appearance of diurnal rhythms of mitosis during individual development [1, 3] are insufficient to allow definite conclusions to be drawn regarding the general principle of modification and formation of rhythms during ontogeny. Meanwhile systematic research in this direction, besides shedding light on various special problems, would yield essential information for the establishment of the general principles governing the development of diurnal rhythms and would thereby help to solve the problem of their nature.

The object of this investigation was to study age changes in the diurnal rhythm of mitosis in the esophageal epithelium of rats. At the same time the possibility of modifying the level of mitotic activity (MA) and the diurnal rhythm of mitosis in animals of different ages by the action of thyroxine also was studied, for data have been published [5-8] to show that 1 h after administration of thyroxine to animals a significant increase in MA is observed in certain organs, including the esophageal epithelium, at certain times of the 24-h period. The authors cited consider that this effect is due to the transfer of cells from the reserve pool of the G_2-phase into mitosis (the "G_2-effect" of thyroid hormones).

EXPERIMENTAL METHOD

Rats of six age groups were used: 2, 7, 15, 22, 45, and 90 days. The animals were kept under natural conditions of lighting and with free access to food. The rats were sacrificed at three-hourly intervals during the 24-h period, four or five animals from each age group at a time. In the experiments to study the effect of thyroxine, the hormone was injected intraperitoneally in a dose of 10 μg/100 g body weight 1 h before sacrifice into animals aged 15 and 45 days (mean weight 25 and 80 g). Mitoses were counted in 9,000-10,000 cells for each animal in sections. The mitotic index (MI) was expressed per thousand cells. The results were subjected to statistical analysis.

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TABLE 1. Diurnal Changes in MI (in $^0/_{00}$) in Rats of Different Ages Receiving or Not Receiving Thyroxine

Time of day	MI (in $^0/_{00}$) for rats of different ages									
	1 day old		7-дневные		15 days old		22 days old		45 days old	
	norm		norm		norm	thyroxine	norm	thyroxine	norm	thyroxine
10	15,1		9,4		8,1	10,8	17,7	13,5	2,6	2,6
13	13,6		10,3		6,8	5,6	11,1	2,9	1,3	1,3
16	14,8		8,2		8,5	8,3	17,1	4,2	0,6	0,6
19	17,4		10,5		9,1	9,2	14,0	1,8	1,6	1,6
22	22,4		9,5		7,2	8,3	11,6	3,8	1,7	1,7
1	12,9		9,9		7,4	7,3	8,8	4,4	2,5	11,0
4	7,7		7,3		8,5	5,9	11,2	11,6	11,2	12,9
	11,1		9,3		9,8	8,6	14,5	7,3	7,7	5,9
Mean for 24 h	14,4		9,3		8,2	8,0	12,0	6,3	5,4	4,7
MI max	$P=0,006$		$P=0,045$		1,4	1,9	$P=0,021$	7,5	16,9	21,5
MI min	2,9		1,4		$P_{22-7}=0,005$	4,4				
	$P_{16-22}=0,003$				$P_{7-13}=0,03$	$P_{13-19}=0,007$	$P_{13-16}=0,037$	$P_{10-13}=0,002$	$P_{10-13}=0,001$	$P_{22-1}=0,005$
	$P_{22-1}=0,005$					$P_{19-4}=0,017$	$P_{16-19}=0,001$	$P_{10-19}=0,001$	$P_{13-19}=0,006$	$P_{16-4}=0,001$
	$P_{22-4}=0,001$					$P_{4-7}=0,03$	$P_{10-19}=0,027$		$P_{10-19}=0,000$	$P_{16-1}=0,000$
	$P_{4-10}=0,043$						$P_{19-22}=0,001$	$P_{19-1}=0,006$	$P_{19-4}=0,000$	$P_{16-7}=0,005$
							$P_{19-4}=0,001$	$P_{19-4}=0,009$	$P_{19-7}=0,000$	$P_{4-7}=0,037$
								$P_{19-7}=0,000$	$P_{7-10}=0,01$	$P_{4-10}=0,004$
								$P_{7-10}=0,04$		

EXPERIMENTAL RESULTS

The results are given in Table 1. On the second day after birth the diurnal rhythm of mitosis was bimodal in character (i.e., it had two maxima); the principal maximum of MA occurred at 10 P.M. ($P_{10\,P.M-4\,A.M.} = 0.001$) and a less marked increase in the number of mitoses occurred at 10 A.M. ($P_{10\,A.M.-4A.M.} = 0.043$); the minimal level of MA was observed at 4 A.M. However, the bimodal character of the rhythm was ill-defined for the decrease in the number of mitoses between 10 A.M. and 1 P.M. was not significant ($P = 0.133$).

On the seventh to eighth day after birth the altitude of the variations between the maximal and minimal levels of MA was considerably reduced (by about half) and the curve of the change in the number of mitoses assumed the shape of a broken line, with no significant change in MA during the 24-h period.

At the age of 15 days the rhythm of mitosis again became bimodal in character with maxima at 7 P.M. and 7 A.M. and minima at 1 and 10 P.M. Only the morning maximum of MA was statistically significant ($P = 0.005$). It is interesting to note that in the group of animals receiving thyroxine the mitotic rhythm also was bimodal in character, and the two maxima of MA (at 7 P.M. and 7 A.M.) were significant ($P \leq 0.03$).

At the age of 22 days, simultaneously with the marked increase in altitude (by about three times) of the daily fluctuations in MA the curve of the mitotic rhythm again assumed the shape of a broken line. The number of cell divisions reached a maximum in the morning and afternoon (7-10 A.M. and 4 P.M.) and a minimum at 7 P.M. An increase in MA also was observed at 10 P.M. ($P = 0.001$).

In the rats aged 45 days the diurnal rhythm of mitosis was again unimodal in character with a maximum in the morning and a minimum in the afternoon and evening ($P \leq 0.009$). The altitude of the diurnal fluctuations in MA was increased chiefly because of a marked decrease in the number of mitoses in the period of minimal MA (7 P.M.). The same character of the diurnal rhythm of mitosis was found in animals receiving thyroxine, but in this case the decrease in MA from 4 to 7 A.M. was significant ($P = 0.03$).

In the rats aged 90 days the diurnal rhythm of mitosis was definitely unimodal in character, with a period of increased MA from 1 to 7 A.M., reaching a maximum at 4 A.M., and a period of low MA from 1 to 10 P.M., with a minimum at 4 P.M. The differences were significant ($P \leq 0.005$). The altitude of the diurnal fluctuations of MA was increased almost threefold compared with the age of 45 days.

The diurnal rhythm of mitosis in the esophageal epithelium during the first days after birth was thus characterized by an increase in the number of mitoses at 7-10 P.M. The small increase in MA at 10 A.M. evidently reflects the synchronizing influence of the mother.

By the end of the first week of life the degree of synchronization of the cell divisions was considerably reduced, with

the appearance of very slight variations, not statistically significant, in MA during the 24-h period. At the age of 15 days, while a slight degree of synchronization of cell division persisted, the frequency of the variations in MA was reduced and the mitotic rhythm began to show a tendency toward an increase in MA in the morning. The age of three weeks is evidently a critical period in the course of establishment of the diurnal rhythm of mitosis, for it is evidently at that age that the mechanisms of synchronization of cell division responsible for rhythmic changes in MA in adult animals become activated. As the result of this change the amplitude and frequency of the fluctuations of MA increased and the level of cell divisions reached a maximum in the morning. By the age of 45 days the diurnal rhythm of mitosis was similar in character to the rhythm in adult animals [2, 4, 9], but it evidently had not yet reached a stable state, as revealed by a considerable variation in MA during the period of increased cell proliferation. In the rats aged three months the diurnal rhythm of mitosis was definitely unimodal in character, as in the adult.

During the postnatal development of rats there is thus a gradual increase in the degree of synchronization of cell division (see the ratio MI_{max}/MI_{min} in Table 1). The slight decrease in the ratio observed on the fourth day of life was evidently due to loss of the synchronizing effect of the mother. Meanwhile, during development of the rats a significant decrease in the general level of cell proliferation, assessed on the basis of the mean values of MA for the 24-h period, was observed.

Administration of thyroxine to animals aged 15 and 45 days led to a decrease in the level of MA at many of the points of investigation during the 24-h period, but this decrease approached significance only in the rats aged 45 days in the interval between 7 P.M. and 1 A.M. ($P = 0.037$–0.059). The increase in MA at 10 A.M. and 10 P.M. found after administration of thyroxine to the 15-day-old rats was not significant. The character of the diurnal rhythm of mitosis was unchanged after injection of the hormone.

The results obtained by this study of the changes in MA in the esophageal epithelium of rats in response to injection of thyroxine thus did not confirm the hypothesis of the G_2-effect of thyroid hormones.

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