THE MECHANISM OF THE DIURNAL PERIODICITY OF MITOSIS

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The changes in mitotic activity in the course of the 24 hours is a particular manifestation of the diurnal periodicity of various physiological processes in the body. The diurnal mitotic regime has been studied in detail in plants, in the protozoa,and in various organs of animals and man. Despite the large number of investigations on the subject of the diurnal periodicity of mitosis in normal and pathological conditions [4, etc], the mechanism of these processes remains far from clear.

From a number of observations it may be deduced that the diurnal variations in mitotic activity are connected with the variations in light [15, 16, 19], with changes in the temperature or with the rhythm of functional activity of the body [9, 10, 15]. In the present work an attempt was made to discover experimentally the significance of these factors in bringing about the diurnal periodicity of mitosis in the animal body.

TABLE 1

Changes in Mitotic Activity in Response to Disorganization of the Diurnal Alternation of Light and Darkness *

Experimental conditions	Number of animals	Time of investigation	Number of mitoses (M ± m) and phase coefficient (K)			
			cornea	skin	tongue	esophagus
Natural alternation of light and darkness	6	Morning	486±24 K — 1,3	22±2 K — 2,6	114±14 K — 1,5	70±8 K — 1,3
	6	Evening	105±23 K — 1,9	9,6±2,5 K — 3,4	43,6±3,6 K — 1,0	6,0±1 4 K — 1,4
Disorganized alternation of light and darkness	6	Morning	119±14 K — 2,0	5,8±1,5 K — 2,0	40±4 K — 2,7	5,1±1,0 K — 1,6
	6	Evening	482±30 K — 1,9	20,6±1,9 K — 2,2	147±20 K — 1,3	66±16 K — 1,7

* In all the groups P = 0.001-0.002.

TABLE 2

Diurnal Variations in Mitosis in Rats after Adrenalectomy

Group of animals	Number of animals	Time of investigation	Number of mitoses (M ± m) and phase coefficient (K)			
			cornea	skin	tongue	esophagus
Control	6	Morning	156±13 K—2,0	136±11 K—0,9	32±7.6 K—1.3	18±3.1 K—1.5
	6	Evening	11,4±2 K—2.5	72±15 K—1.5	2±0,9 K—3.0	8.5±2.4 K—1.4
			P<0,001	P=0,004	P=0,002	P=0,01
After thyroidectomy	6	Morning	105±21 K—2,2	86±15 K—1.0	29±4,1 K—1.2	20±4.9 K—1,1
	6	Evening	8±2.6 K—2.6	96±26 K—1.6	18,2+3.2 K—1.9	12±3.4 K—1.4
			P=0.001	P=0.38	P=0.04	P=0.11

TABLE 3

Diurnal Variations in Mitosis in Rats after Thyroidectomy

Group of animals	Number of animals	Time of investigation	Number of mitoses (M ± m) and phase coefficient (K)			
			intestine	tongue	cornea	skin
"Night"	7	Morning	412±21 K—1,4	146±15 K—1.3	290±29 K—1,5	23±6 K—2,1
	7	Evening	252±13 K—2.2	24±3.5 K—2,0	172±71 K—1,3	7,6±2 K—1,0
			P<0,001	P<0,001	P=0,08	P=0,018
"Day"	6	Morning	314±19 K—1.8	48±3.7 K—1,1	286±36 K—1.6	18±1.6 K—2,0
	6	Evening	420±22 K—1.6	78±6 K—1.9	91±23 K—1.6	11±0.7 K—2.2
			P=0,01	P=0,002	P=0,001	P<0,001

EXPERIMENTAL METHOD

Experiments were carried out on white mice and rats (males) aged 2-3 months. The mitotic activity was determined in the morning (8A.M.) and evening (8 P.M.) in the epithelium of the cornea, skin, tongue, esophagus and intestine and in the lymphocytes of the thymus gland. The magnitude of the mitotic activity was judged by the number of dividing cells per constant area (1.65 mm^2), by the percentages of the individual phases of mitosis and by the phase coefficient.

TABLE 4

Changes in the Mitotic Activity during Organization and Disorganization of the Feeding Program

Group of animals	Number of animals	Time of investigation	Number of mitoses (M ± m) and phase coefficient (K)				
			cornea	skin	tongue	esophagus	thymus
Control	6	Morning	318±34 K—2.4	37±4.2 K—3.1	91±13 K—0.8	36±3.9 K—1.9	117±16 K—3.0
	6	Evening	93±12 K—2.6	10±2.6 K—3.0	60±11 K—0.9	14±5.5 K—1.0	68±19 K—2.2
			P < 0.001	P < 0.001	P—0.06	P—0.003	P—0.04
After adrenalectomy	6	Morning	260±35 K—2.9	20±3.3 K—1.5	107±8.9 K—1.1	40±6.3 K—1.2	105±12 K—2.8
	6	Evening	115±25 K—2.2	12±1.3 K—2.6	70±11 K—1.2	12±2.5 K—1.0	66±14 K—2.7
			P—0.004	P—0.02	P—0.05	P—0.001	P—0.03

In the first series of experiments we studied the diurnal variations in mitoses when the normal periodicity of light was disturbed during the 24 hours. The groups of experimental mice were kept in artificial illumination during the night (9P.M. to 7A. M.) and during the daytime (7 A. M. to 9 P. M.) they were kept in total darkness. Control animals were kept in ordinary conditions of natural alternation of day and night. The experiment was continued for 40 days, for the main guarantee of success in the experiment was its duration.

In a second series of experiments we studied the diurnal variations of mitoses in white rats 8 days after bilateral adrenalectomy.

In the third series of experiments rats were subjected to the operation of thyroidectomy. The mitotic activity was determined in the morning and evening of the 9th day after the operation.

In the fourth series of experiments we studied the mitotic activity when the digestive activity was disorganized. The first group of mice in this series (the "night" group) received food only during the night (from 8 p.m. to 8 a.m.), and the food was withdrawn during the day. The animals of the second group (the "day" group) received food only during the daytime (from 8 A.M. to 8 P.M.) and food was withdrawn during the night. Both groups of mice were kept under identical conditions of illumination (a semi-darkened room). The experiment lasted 2 months. The mice were sacrificed in the morning (7:30 A.M.) and evening (7:30 P.M.). Since in this series we assumed that the general physiological activity of the animal was disorganized as well as the digestive activity, at the beginning of the experiment we periodically introduced a female into the cage with the male mice for 2.3 days: at night with the "night" group of animals and during the daytime with the "day" group. In all the experiments we studied the RNA and DNA content of the tissues as well as determining the mitotic activity. The material was fixed with Helly's mixture. Sections were stained with methyl green and pyronine by Unna's method (control sections being treated with ribonuclease) and by Feulgen's method.

EXPERIMENTAL RESULTS

The results of the first series of experiments showed that disorganization of the alternation of day and night led to complete disorganization of the diurnal periodicity of mitotic activity (Table 1). In animals in natural conditions of diurnal periodicity of light and darkness, the maximum values of mitotic activity in the epithelium of the cornea, skin, tongue and esophagus occurred in the morning, and the minimum values in the evening. In the

animals of the experimental group (illumination at night and darkness by day) the diurnal rhythm of mitosis was completely disorganized. Maximum values for the mitotic activity were observed in the evening and minimum values in the morning. The results of this series of experiments thus showed that one of the important factors determining the diurnal rhythm of mitosis is the periodic alternation of day and night, of light and darkness.

A number of workers have described the stimulation by light of the hypophysis, the sex glands, the thyroid gland and the adrenals, and the changes in the activity of these organs on account of diurnal alternations of light and darkness [3, 8, 9]. The endocrine organs also play an important role in the regulation of cell division [1, etc.]. It has also been found that stimulation of mitoic activity by the supplementary illumination of animals is effected through the thyroid gland [7]. These observations suggest that the effect of the diurnal alternation of light and darkness on the mitotic regime is connected with changes in the activity of the endocrine organs.

Experiments were accordingly carried out in which the diurnal variations in mitosis were studied in animals after preliminary extirpation of the adrenals and thyroid gland. The results of these experiments showed that after adrenalectomy (Table 2) and after thyroidectomy (Table 3) the diurnal variations in mitosis in the morning and evening were maintained. The scale of variation of mitotic activity in the morning and evening was, however, slightly reduced in some cases. This was noted in the epithelium of the cornea and skin after adrenalectomy and in the epithelium of the tongue, esophagus and skin after thyroidectomy. The fluctuations in mitosis in the epithelium of the tongue between morning and evening were completely obliterated. From the results of these experiments it may be postulated that the adrenals and the thyroid gland are of some importance in effecting the diurnal rhythm of mitosis in animals. The role of these organs, however, is evidently not a decisive one, for after their extirpation the diurnal fluctuations in the number of mitoses are maintained. This appears all the more likely because a diurnal periodicity of mitosis is present in protozoa and plants, and the diurnal mitotic regime in different organs of animals is not identical.

In the experiments in which the diurnal alternation of light and darkness was disturbed, attention was drawn to the fact that the changes in the alternation of day and night led at the same time to a complete disorganization of the rhythm of functional activity of the animal. In the mice illuminated during the night (the "night" group of animals) the maximum level of functional activity was shifted to the daytime. These observations suggested that the effect of alternation of day and night on the mitotic regime was brought about primarily by an alternation in the functional rhythm of the different organs. The digestive organs were selected as a test object.

Preliminary experiments (on 41 animals) showed that the intestinal epithelium evidently possesses a different diurnal rhythm of periodicity of mitosis from that of other organs [15]. In any case, we observed no essential difference between the mitotic activity in the morning and evening (morning 326 ± 17, evening 350 ± 11).

The maximum digestive activity of laboratory mice is known to occur during the night, although they periodically ingest food during the day. If the diurnal rhythm of the mitosis were connected with the functional activity of an organ, then it might have been expected that with a modification or complete disorganization of the feeding program, the diurnal rhythm of mitosis would also be changed. A third series of experiments was accordingly carried out, in which some mice (the "night" group) received food at night only, and the others (the "day" group) received food only by day. Although the experiment lasted for two months, we were unable to bring about complete disorganization of the general functional activity in this way. The change in the digestive activity was the result of the experimental environment itself. The results of these experiments (Table 4) showed that feeding animals only at night leads to the appearance of obvious diurnal variations in mitosis in the intestinal epithelium.

With disorganization of the feeding program (in the daytime) the diurnal variations in mitosis became inverse in nature: the higher the level of mitotic activity observed in the evening, the lower the values in the morning. The diurnal variations in mitosis in the epithelium of the tongue were disorganized at the same time. The difference between the mitotic activity in the morning and evening in the "day" group of animals was less than that in those animals in which only the feeding program was disturbed from its normal pattern. The diurnal variations in mitosis in the epithelium of the cornea and skin were essentially unchanged. In two experiments, however, unusually high levels of mitotic activity were observed in the "night" group of animals in the evening. It is possible that, had the experiments been continued longer, changes would have occurred in the general functional activity of the animal and in the mitotic regime in organs unrelated to the digestive system. The rhythm of functional activity of the cells determines the mitotic regime, probably by modifying the metabolic processes with which mitotic division is associated.

From a number of observations, including those in our own laboratory [2, 5], it may be considered that one of the essential links in the biochemical mechanism of mitosis is the nucleoprotein metabolism. From this it may be deduced that diurnal variations will take place in the RNA and DNA contents, corresponding to the variations in mitotic activity. Such changes have been described in the liver cells [14, 6]. Our observations were conducted on the epithelium of the cornea, tongue, intestine and liver and on the lymphocytes of the thymus gland in rats and mice (A. A. Zhirnova). The results of these experiments showed that, corresponding to the diurnal variations in mitotic activity, obvious diurnal variations in the RNA and DNA content of the cells were observed. In the epithelium of the cornea, tongue and liver, and in the lymphocytes a higher content of nucleic acid was found in the morning and a lower content in the evening. The RNA and DNA content of the intestinal epithelium (and the mitotic activity correspondingly) showed no essential difference between morning and evening. When the diurnal alternation of light and darkness was disturbed, obvious changes took place in the nucleic acid content of the cells. When the animals were illuminated at night, the maximum content of RNA and DNA was shifted from the morning to the evening.

The experimental results thus show that the diurnal periodicity of mitosis is connected with various factors. The diurnal alternation of light and darkness, the pattern of functional activity and changes in metabolic reactions are all links in the complex chain of processes responsible for the diurnal rhythm of mitosis. The final links of this chain must evidently be sought in the changes in the nuclein metabolism in the course of the day.

SUMMARY

Inversion of the alternation of light and darkness during the 24-hour period resulted in a complete inversion of the diurnal rhythm of mitotic activity in the epithelium of the skin, cornea, tongue and esophagus. After removal of the adrenal or thyroid glands the diurnal fluctuations of the number of mitoses were preserved, but their range in the morning and evening hours was somewhat reduced. Diurnal fluctuations of mitotic activity, absent in control animals, were noted in mice fed only at night. When mice were fed only in the morning the diurnal fluctuations were changed in the intestinal and tongue epithelium. The diurnal fluctuation of the RNA and DNA content noted in the cells of different organs corresponded to the mitotic rhythm. When the animals were illuminated at night the time of the maximum nucleic acid content changed from the morning to the evening.

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