RELATIONSHIP BETWEEN DIVISION AND FUNCTIONAL ACTIVITY OF THE CELLS OF THE ADRENAL CORTEX

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Translated from Byulleten' Éksperimental'noi Biologii i Meditsiny, Vol. 54, No. 9,
pp. 87-91, September, 1962
Original article submitted October 12, 1961

The relationships between the division and functional activity of cells have not yet been fully explained. Meanwhile, according to some writers [1, 5] the functional activity of the cell is one of the factors determining the natural rhythm of mitosis during the 24 h period. During the study of the functional and mitotic rhythms in various organs (intestine, salivary glands, pancreas, liver, kidney, epithelium of the skin, and mucous membranes) an inverse relationship was found between the working and division of the cell [4]. The maximum of mitotic activity coincided with the minimal functional activity of the organ. Notwithstanding essential differences in the 24 h rhythms of the different organs, as a rule in nocturnal animals (mice and rats) high indices of mitotic activity (and correspondingly, minimal functional activity) were observed during the hours of morning, and low values at night. Meanwhile, in mice and rats the maximum of mototic activity of the adrenal cortex was observed at night [7, 9], i.e., at a time of high functional activity of the animals.

It was necessary to discover whether the same relationship between the division and work of the cell as was found in the other organs also held good in the case of the adrenal cortex.

EXPERIMENTAL METHOD

Experiments were carried out on albino mice aged 2.5 months. The degree of mitotic activity of the adrenal cortex was judged by the number of dividing cells in an area of 3.3 mm^2 and by the relative percentages of the individual phases of mitosis.

In the first series of experiments we studied the variations of mitotic activity in the course of the 24 h. Animals were sacrificed (in batches of 5-6) every 3 h for 24 h. In addition to the mitotic activity, the eosinophils of the blood were counted and the ascorbic acid in the adrenal cortex was demonstrated by Backhus's method. The blood leukocytes were determined in a counting chamber and the leukocyte formula obtained from blood films. The motor activity of the mice was recorded before the animals were sacrificed. The recordings were made in a special cage with an electrical contact and a counter, so that every time the animal ran this was recorded. Two such groups of experiments were carried out. The difference between these two groups was that in the first group of experiments the animals received fresh food at 9 P.M. and in the second group at 9 A.M.

In the second series of experiments we studied the changes in the mitotic activity of the adrenals and the blood eosinophil count during a stress reaction induced by cooling the animals. The mice were placed in cold water (5°) for 10 min. The animals were sacrificed 1.5 and 4 h after cooling.

In the third series of experiments the adrenals were studied after a single (2.5 units 2 and 4 h before sacrifice) and repeated (1 unit twice a day for 10 days) injection of ACTH.

EXPERIMENTAL RESULTS

The experimental results showed that the mitotic activity of the adrenal cortex underwent regular changes in the course of the 24 h. In the first series of experiments the maximum of mitotic activity was observed at 2 A.M. and the minimum at 2 P.M. and 8 P.M. (Fig. 1). The differences between the maximal and minimal indices were well defined ($P = 0.001$). During the day (at 5 P.M.) a second but insignificant increase in mitotic activity was observed ($p = 0.04$); this was also observed in the second group of experiments ($P = 0.02$-0.03). In contrast to other investigations of organs, high indices of the mitotic activity of the adrenals were observed at night–at a time of high general activity of mice (11 P.M.).

The criterion of the functional activity of the adrenals was their concentration of ascorbic acid and the level of the blood eosinophils. The ascorbic acid concentration in the adrenal cortex underwent no sharp changes in the first hours of the day, although a slight increase in the intensity of the reaction was observed at night. The comparatively small differences which could be detected only between the extreme periods of time were evidently associated with the relative specificity of Backhus's reaction. More definite results were obtained from the study of the blood eosinophil count, for this index may be used as an indirect sign of the function of the adrenal cortex. The maximal eosinophil count was observed at night or in the early morning (2-8 A.M.) and the minimal during the afternoon. This maximal eosinophil count was slightly displaced and was observed after the maximum of mitotic activity. Bearing in mind the slight variations in the blood eosinophil count, it would be difficult to expect that the very slight diurnal increase in mitotic activity would be reflected in the curve of the 24 h changes in the eosinophils. However, a comparison of the general character of the curves shows that the changes in the two indices are approximately parallel.

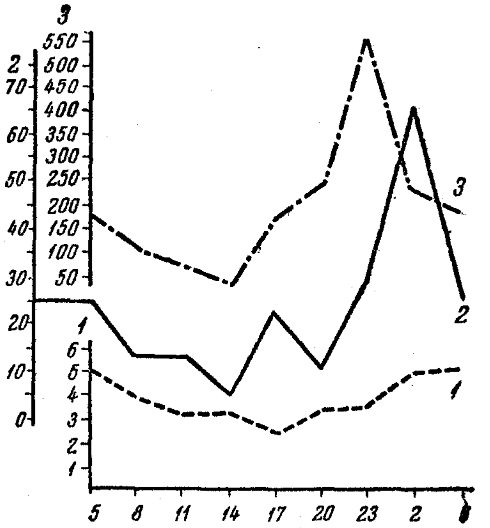

Fig. 1. 24 h rhythm of mitotic activity of the adrenal, eosinophil count, and motor activity of mice. Animals fed at 9 P.M. 1) Eosinophils (%); 2) number of mitoses; 3) number of runs.

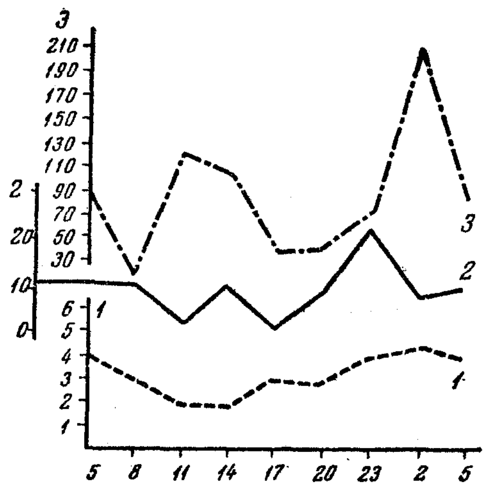

Fig. 2. 24 h rhythm of mitotoc activity of the adrenal, eosinophil count, and motor activity of mice. Animals fed at 9 A.M. Legend as in Fig. 1.

Similar results were obtained in the second group of experiments of this series (Fig. 2). The level of mitotic activity of the adrenal cortex in the animals of this group was lower, but the character of the 24 h rhythm differed only very slightly. The maximum of mitotic activity was observed at 11 P.M. and the minimum at 11 A.M. and at 5 P. M. The changes in the blood eosinophil count were almost parallel, although their maximum was also slightly displaced. In this group of experiments, in contrast to the last group, the animals received fresh food in the morning and not in the evening. This resulted in the appearance of an additional peak period of general activity of the animals during the hours of daylight. It was previously shown that this alimentary increase in activity, related to the feeding time of the animals, is reflected in the daily rhythm of mitosis of the digestive organs, but does not affect the mitotic activity in the epithelium of the skin and cornea [4]. The 24 h rhythm of mitosis in the adrenal, like that of the integumentary epithelium, is more resistant and is not significantly modified by changes in the pattern of feeding and motor activity created artificially in the laboratory.

Because the 24 h variations in the eosinophil count observed in both groups of experiments differed from those reported in the literature [5, 7, 8, and others], we carried out further experiments. Repeated determinations of the eosinophil count in mice (in groups of 5-6) gave closely similar results (Table 1). Despite the considerable individual variations and certain differences in isolated experiments, higher eosinophil counts were usually observed at 2-8 A.M. and lower counts during the afternoon.

Comparison of the 24 h variations in the mitotic activity of the adrenal and in the blood eosinophil count in the two groups of experiments showed that the lowest numbers of mitoses were observed when the eosinophil count was low. An increase in the eosinophil count in the blood was observed at a time of high mitotic activity. Consequently, we may postulate that a decrease in the number of mitoses in the adrenal cortex coincides with high functional activity of this organ. This hypothesis was confirmed by the results of determinations of the 24 h fluctuations in the concentration of corticosterone in the blood. In mice, the maximum of corticosterone concentration was observed at 4 P.M. and the maximum of mitotic activity at midnight [7]. In rats the corticosterone concentration was maximal during the afternoon and evening [8].

TABLE 1. 24 h Variations in the Blood Eosinophil Count in Mice.

Time of day	No. of eosinophils in mm^3 Blood	
	absolute	%
5 A.M.	513	2
8 A.M.	405	1.7
11 A.M.	309	1.6
2 P.M.	418	1.5
5 P.M.	185	1.2
8 P.M.	161	0.9
11 P.M.	174	0.9
2 A.M.	341	1.5

This inverse relationship between division and function of the cell was confirmed by the second series of experiments. The stress reaction and associated functional activity of the adrenal cortex led to a decrease in the number of dividing cells (Table 2). Half an hour later the mitotic activity of the adrenal had fallen to less than half its previous level. Meanwhile a decrease in the number of first stages of division was observed, indicating a delay in the onset of mitosis in the cells. Four hours later, the intensity of cell division was still lower. A parallel decrease took place in the blood eosinophil count. In both groups of experiments in 4 of the 6 animals the eosinophils fell from 4% in the controls to 1.8-2%.

In the third series of experiments we studied the changes in the mitotic activity of the adrenal after administration of ACTH. Single and repeated injections of ACTH led to a decrease in the intensity of the reaction for ascorbic acid. Activation of the adrenal cortex was accompanied during the first hours by a decrease in mitotic activity (Table 3). Two hours later the number of dividing cells had fallen to one third of the control figure. Four hours later it remained at a low level, but in individual animals it had regained the control figures.

TABLE 2. Changes in the Mitotic Activity of the Adrenal Cortex During a Stress Reaction

Mice	No. of animals	Time after exposure (in h)	No. of mitoses	Relative percentage of phases of mitosis				P
				P	M	A	T	
Control	6	–	27	47	41	2	10	
Experimental . .	6	$1^1/_2$	10.6	37	29	5	29	0.04
" . .	6	4	5.4	37	46	0	17	0.01

TABLE 3. Changes in Mitotic Activity After Administration of ACTH

Group of animals	No. of animals	Time after injection (in h)	Adrenal						Cornea		
			no. of mitoses	relative percentage of phases of mitoses				P	no. of mitosis	phase coefficient	P
				P	M	A	T				
Control	8	–	20	41	34	3	22		176	1.2	
Receiving 2.5 units ACTH . . .	8	2	6.5	63	27	0	10	0.001	171	1.1	0.4
Control	7	–	20.6	48	34	3	15		96	1.3	
Receiving 2.5 units ACTH . . .	7	4	13.4	38	42	0	20	0.09	107	1.4	0.3
Receiving repeated doses of 1 unit ACTH. . .	7	4	64	46	33	4	17	0.001	88	1.6	0.4

The delay in cell division after administration of ACTH was evidently of short duration, for within 2 h an increase in the relative proportion of prophases was observed. After the prolonged administration of ACTH a further decrease in the intensity of the reaction for ascorbic acid and a simultaneous and considerable increase in mitotic activity were observed. The intensity of cell proliferation was increased threefold and reached unusually high figures for this organ. Hypertrophy of the adrenal cortical cells was observed at the same time. Changes in mitotic activity after administration of ACTH were observed only in the adrenals, and they did not extend to the corneal epithelium.

Hence, in the course of the natural 24 h cycle and after temporary stimulation of the function of the adrenal cortex, an inverse relationship was observed between the division and work of the cell. Despite the unique functional rhythm of the adrenal, this organ showed the same general principles as were observed in other organs.

However, the inverse relationship was not confined to the division and working of the cell. From our point of view, ideas of the "antagonism" between function and mitosis [2,10,11] are too categorical and give a one-sided picture of the complex relationship between these processes. Whereas in cases of moderate functional stress an inverse relationship was in fact observed between the functional activity and division of the cell, the more prolonged stimulation of function (for example, as a result of repeated injections of ACTH) led to increased mitotic activity. Similar changes also take place in connection with the compensatory hypertrophy of the organ. At the same time the possibility cannot be excluded that the stimulation of cell division, which is usually observed after an organ has finished its work, is the result of this work. In any case, the relationships between the functional activity of the cell and its division are among the more important factors determining the mitotic pattern of the different organs.

SUMMARY

A study was made of the 24-hour mitotic activity rhythm of the adrenal cortex, of the ascorbic acid content in it, of the number of blood eosinophils and of the motor activity of mice. The results of experiments have demonstrated that the minimum mitotic activity coincides with the maximum functional activity of the adrenal gland. Inverse relation between the motor activity and cellular division was confirmed by experiments with stimulation of the adrenal gland function. The mitotic activity was reduced in conditions of stress reaction or after a single ACTH administration. The relation between the functional activity of the cell and mitosis is not limited to their inverse ratio. The cellular proliferation intensity increased with prolonged stimulation of the adrenal gland function (repeated ACTH administration). The relation between the motor activity and cellular division is one of the important factors determining the mitotic regimen of the organ.

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