

# DIURNAL PERIODICITY OF MITOTIC DIVISION OF THE ADRENAL CELLS OF ALBINO RATS

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Translated from *Byulleten' Éksperimental'noi Biologii i Meditsiny*, Vol. 54, No. 9,

pp. 91-96, September, 1962

Original article submitted December 12, 1961

Many histological observations have shown that the number of mitoses in the various zones of the mammalian adrenal cortex differs. Most workers have demonstrated considerable mitotic activity in the zona glomerulosa and lesser activity in the zona fasciculata and zona reticularis [1, 6, 8]. However, few investigations have included a quantitative determination of the mitotic activity in the cortical layer of the adrenal gland, and as a rule this activity has been judged from counts of the number of mitoses in sections through the gland or in a conventional unit of area of a histological section [1, 2, 10]. Yet the dimensions of the gland and of its different zones are inconstant, and are dependent on the age of the animals and the functional state of the organ [1, 2, 6, 8].

There is an almost complete absence of numerical data in respect of the mitotic activity of the mammalian adrenal medulla. The reason for this is evidently the widespread notion that in the cells of this tissue mitoses are found only in young animals, while in adult animals they are either very rare or absent altogether [8]. Nevertheless, Messier and Leblond [13], who injected thymidine- $H^3$  into adult rats (weighing about 200 g), found a high radioactive index (0.48-0.84%) in this tissue.

The diurnal periodicity of mitoses in the mammalian adrenal has also been incompletely investigated. Mühlemann and co-workers [14] showed that the number of mitoses found in the adrenal cortex of young rats (weight 135 g) was greater in the morning than in the evening. These results were confirmed [15, 16] by investigations of the adrenals of adult rats weighing about 250 g. Halberg and co-workers [12] found the same relationship between the number of mitoses in the adrenal cortex of 5-day old mice. Messier and Leblond [13] investigated the diurnal periodicity of incorporation of thymidine- $H^3$ , administered 6 h and 3 h before sacrifice, into the cortical cells and found a higher radioactive index in the adrenals fixed at 10 A.M.

We have been unable to find details of the mitotic activity of the adrenal medulla at different times of day.

In view of the foregoing facts and of the important role of the adrenal hormones in the regulation of the rhythmic changes in the various body functions [11], we deemed it important to make a more detailed investigation of the mitotic activity of the adrenal glands in fully grown animals.

## EXPERIMENTAL METHOD

Adult male albino rats weighing 160-180 g were used in the experiment. The animals were kept in natural conditions of illumination (July). Food pellets and water were given once a day at 10-11 A.M. The animals were sacrificed at intervals of 3 h for a period of 2 days, ten rats at each time. The adrenals were fixed in Zenker's fluid and embedded in paraffin wax, and sections 8  $\mu$  thick were stained with Carazzi's hematoxylin.

Mitoses and cells were counted under a binocular microscope (objective 90 $\times$ , eye-piece 7 $\times$ ), the eye-piece of which contained a square diaphragm measuring 8 $\times$ 8 mm. The mitotic coefficient (MC) was calculated pro mille, and all the numerical results were treated statistically by the Fisher-Student method.

The mitotic activity was investigated separately in the zona glomerulosa, zona fasciculata, and zona reticularis of the cortex and in the medullary substance of the adrenal glands.

Counts of the cells or, more accurately, of their nuclei in 100 fields of vision of each of these layers showed that the mean number of cells per field of vision in the zona glomerulosa was 178, in the zona fasciculata and zona

reticularis 124, and in the medulla 85. Since when investigating the zona glomerulosa in each case we examined 150-175 fields of vision, it follows that the mitotic activity was determined in 27,000-32,000 cells of each gland, in the case of the zona fasciculata and zona reticularis (150-200 fields of vision)—in 18,000-22,000 cells, and in the case of the medulla (100-125 fields of vision)—in 8500-10,000 cells.

TABLE 1. Diurnal Changes in the Mitotic Activity of the Cells of the Zona Glomerulosa of the Adrenal Glands of Rats

Time of fixation	1st day			2nd day		
	MC (promille)	P	$\frac{EP+P+M}{A+T+LT}$	MC (promille)	P	$\frac{EP+P+M}{A+T+LT}$
10 A.M.	0.14		2.20	0.34		1.10
1 P.M.	0.29	0.015	2.50	0.26	0.430	0.75
4 P.M.	0.21	0.127	1.40	0.33	0.210	1.45
7 P.M.	0.34	0.03	1.45	0.51	0.027	2.18
10 P.M.	0.55	0.003	1.15	0.54	—	1.00
1 A.M.	0.30	0.0001	1.35	0.31	0.019	0.90
4 A.M.	0.28	—	0.73	0.27	—	1.12
7 A.M.	0.38	0.434	0.93	0.30	—	1.10

Note. EP) Early prophases; P) prophases; M) metaphases; A) anaphases; T) telophases; LT) late telophases.

TABLE 2. Diurnal Changes in the Mitotic Activity of the Cells of the Zona Fasciculata and Zona Reticularis of the Adrenal Glands of Rats

Time of fixation	1st day			2nd day		
	MC (promille)	P	$\frac{EP+P+M}{A+T+LT}$	MC (promille)	P	$\frac{EP+P+M}{A+T+LT}$
10 A.M.	0.10	—	0.73	0.07	—	0.85
1 P.M.	0.13		2.87	0.10		0.72
4 P.M.	0.04	0.014	0.50	0.03	0.004	1.00
7 P.M.	0.05	—	0.43	0.03	—	0.50
10 P.M.	0.05	—	0.60	0.04	—	0.28
1 A.M.	0.02	0.208	3.00	0.04	—	2.00
4 A.M.	0.02	—	1.00	0.08	0.021	0.45
7 A.M.	0.09	0.05	1.37	0.05	0.420	1.50

#### EXPERIMENTAL RESULTS

The results of the examination of the zona glomerulosa of the adrenals are given in Table 1 and illustrated graphically in Fig. 1.

During the morning and afternoon (10 A.M.-4 P.M.) of the first day of the investigation the mitotic activity of the cells of the zona glomerulosa was slight. After 4 P.M. it rose sharply to reach a maximum at 10 P.M. The mean mitotic coefficient at 10 P.M. was significantly higher ( $P=0.0001$ ) than the mean mitotic coefficient at each of the preceding hours of daylight. From 10 P.M. to 1 A.M. the mitotic activity fell significantly ( $P=0.0001$ ). From this time until 4 P.M. next day the variations in the number of mitoses were insignificant. At 7 P.M., and still more so at 10 P.M., on the second day the mitotic activity again reached its maximal value. This increase was significant both during the interval from 1 to 7 P.M. ( $P=0.003$ ) and from then until 10 P.M. ( $P=0.0001$ ). Subsequently, as also during the first day, a decrease in mitotic activity was observed from 10 P.M. until 1 A.M., and a still greater decrease until 1 A.M. ( $P=0.019$  and  $0.001$ , respectively).

Hence, in the zona glomerulosa of the adrenals a diurnal periodicity of mitotic division of the cells was clearly observed: The relatively few cell divisions observed throughout the night and during the early part of the day (1 A.M. to 4 P.M.) gave way to a considerable increase in the number of mitoses at 7-10 P.M., which was again followed by

a decrease in mitotic activity. The changes in the number of mitoses during the second day of the investigation almost coincided with the changes seen during the first day. The differences between the value of the mitotic coefficient at 10 A.M. on the first and second days ( $MC = 0.14$  and  $0.34$  pro mille) were not statistically significant ( $P = 0.107$ ). The mean mitotic coefficient for the first day of the investigation was  $0.31$  pro mille, and for the second day— $0.35$  pro mille.

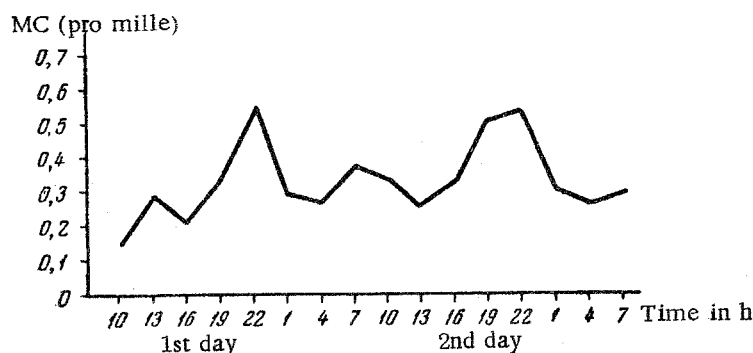


Fig. 1. Changes in mitotic activity of the cells of the zona glomerulosa of the adrenal cortex for a period of 2 days [hours numbered from 1 (1 A.M.) to 24 (12 P.M.)—Publisher's note].

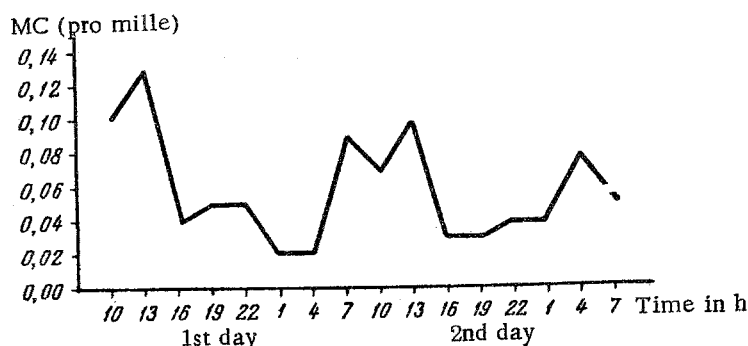


Fig. 2. Changes in mitotic activity of the cells of the zona fasciculata and zona reticularis of the adrenal cortex for a period of 2 days [hours numbered from 1 (1 A.M.) to 24 (12 P.M.)—Publisher's note].

The relative frequency of each phase of mitosis, calculated as a percentage of the total, averaged 15.5% for the early prophases, 5% for the prophases, 34.5% for metaphases, 5.2% for anaphases, and 39.8% for telophases and late telophases.

The character of the diurnal periodicity of mitosis was different in the zona fasciculata and zona reticularis of the adrenal (Table 2, Fig. 2). In the first half of the day (10 A.M. to 1 P.M.) many mitoses were present; by 4 P.M. the number had fallen and it remained at a low level until 4 A.M. At 7 A.M. the number of mitoses increased and reached a maximum at 1 P.M. This increase in the period from 4 A.M. to 1 P.M. was statistically significant ( $P = 0.0001$ ). At 4 P.M. on the second day there was another considerable fall in mitotic activity, which remained at a low level until 4 A.M. Consequently, in these zones a diurnal periodicity of mitoses was also observed, the numbers being maximal during the mornings and afternoons and minimal during the late evenings and nights.

It should be noted that the mitotic activity in the zona fasciculata and zona reticularis was much lower than in the zona glomerulosa. The mean mitotic coefficient during the first day of the investigation was  $0.06$  pro mille, and during the second day  $0.05$  pro mille. In these zones slightly different relative proportions of the different phases of mitosis were found: early prophases 6.9%, prophases 1.6%, metaphases 40.2%, anaphases 5.3%, and telophases and late telophases 46.6%. This increase in the relative proportions of metaphases and telophases may be explained by the fact that cells starting mitosis in the zona glomerulosa are moved to the zona fasciculata, where they complete

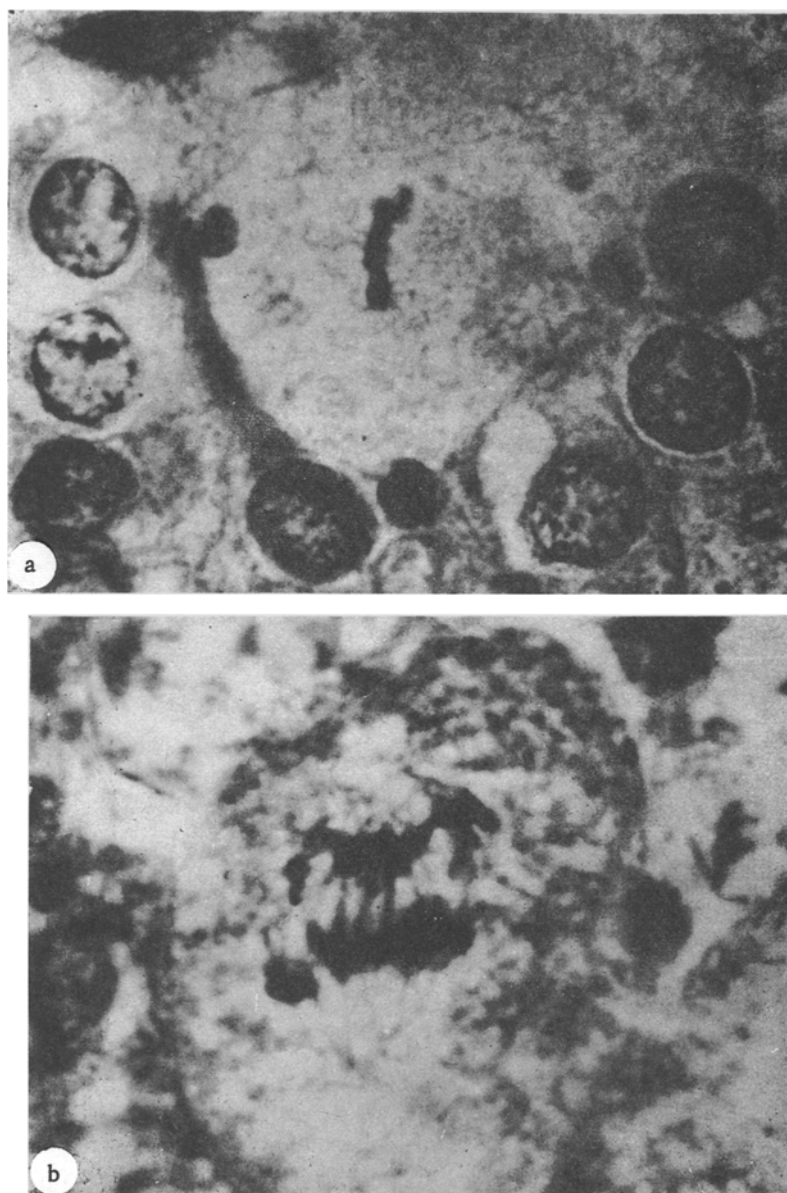


Fig. 3. Mitoses in the cells of the adrenal medulla of a rat. a) Meta-  
phase; b) anaphase.

mitosis. However, counts of the number of mitoses in the outer and inner halves of the zona fasciculata and zona reticularis showed no significant differences in either the number or the incidence of the phases of mitosis. The relative proportions of the phases of mitosis differed at different times of the investigation, but these differences were not consistent in character, as shown by variations in the ratio between the early and late phases of mitosis (see Table 2).

TABLE 3. Diurnal Changes in the Mitotic Activity of the Cells of the Adrenal Medulla of Rats

Time of fixation	1st day	2nd day
	MC(pro mille)	MC(pro mille)
10 A.M.	0.16	0.45
1 P.M.	0.33	0.30
4 P.M.	0.16	0.32
7 P.M.	0.19	0.40
10 P.M.	0.37	0.41
1 A.M.	0.27	0.20
4 A.M.	0.38	0.37
7 A.M.	0.35	0.33

A more intensive mitotic activity was found in the adrenal medulla than might have been expected from the reports in the literature. During the first day of the investigation the mean mitotic activity over the 24 h was 0.28 pro mille, and during the second day 0.35 pro mille, i.e., almost the same as in the zona glomerulosa.

Of the 155 adrenals investigated, in only nine were no mitoses found, in 30 cases the mitotic activity was 0.3-0.4 pro mille, in 35 cases 0.4-0.6 pro mille, in 10 cases 0.6-0.7 pro mille, and in 7 cases over 0.7 pro mille.

As a rule all the phases of mitosis were readily distinguishable (Fig. 3).

The average incidence of the different phases of mitoses was as follows: early prophase and prophase 41.9%, metaphases 19%, anaphases 1.2%, and telophases and late telophases 37.9%.

It was more difficult to draw any conclusion regarding the character of the diurnal periodicity of the mitoses in the adrenal medulla (Table 3).

It is clear from Table 3 that the changes in mitotic activity during the first and second days of the investigation did not duplicate each other. Further research is required, however, before conclusions can be drawn regarding the character of the diurnal changes in the number of mitoses in this tissue or before the absence of any general principle governing these changes can be confirmed.

According to Messier and Leblond [13], the radioactive index of the adrenal medulla in rats varies sharply in the course of the 24 h, although no significant differences have been established in relation to any particular times of day.

Hence, the diurnal periodicity of the mitoses in the cells of the zona glomerulosa may be expressed by a unimodal curve with a maximal number of cell divisions in the late evening and a minimal number during the night or early part of the day. This character of periodicity of mitoses has not hitherto been demonstrated in any other mammalian tissues.

The diurnal periodicity of the mitoses in the zona fasciculata and zona reticularis may also be expressed by a unimodal curve, but with a maximal number of mitoses during the morning and afternoon and a minimal number during the afternoon and evening.

It must be pointed out that the periodicity of the mitoses was found to be of the same character in certain other tissues of these rats, namely the corneal epithelium and the cortical layer of the kidneys [3], the parenchymal cells of the liver [4], and the epithelium of the esophagus [5].

The question of the special features of the diurnal periodicity of the mitoses in the different zones of the adrenal gland requires further study. In this connection some interesting results have recently been obtained by N. V. Miltitsyna [6], who has shown that the zona glomerulosa is mainly under parahypophyseal control while the zona fasciculata and zona reticularis are mainly under transhypophyseal control.

#### SUMMARY

An inquiry was made into the daily periodicity of mitotic activity in various adrenal gland zones of adult male rats (weighing 160-180 gm) for a period of 48 hours. The average daily mitotic activity in the zona glomerulosa of adrenal glands constituted 0.33 pro mille. The maximal number of mitoses was revealed at 7-10 P.M., the minimal during the early and late morning hours. The average daily mitotic activity in the zona fasciculata and zona reticularis was 0.05 pro mille. The maximal number of mitoses was present at the early and late morning hours (from 7 A.M. to 1 P.M.), the minimal from the afternoon to 1-4 A.M. Marked mitotic activity was revealed in the medulla of adrenal glands - the average daily coefficient being 0.32 pro mille. There were no regular 24-hour changes in the number of mitoses in this tissue.

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