

# COMPARISON OF CIRCADIAN PERIODICITY OF MITOTIC ACTIVITY IN ORGANS OF ALBINO RATS OF BOTH SEXES

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The circadian periodicity of mitotic activity in the corneal epithelium of sexually mature and immature rats is the same regardless of sex. In sexually mature females the maximal number of mitoses in the zona glomerulosa, zona fasciculata, and zona reticularis of the adrenal cortex is found in the early morning, while in males the maximum in the zona glomerulosa occurs in the evening or night, and in the zona fasciculata and zona reticularis in the morning and afternoon.

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A circadian periodicity of cell division has been found in almost all mammalian tissues [1, 3-7, 9, 10, 13]. It has also been shown that the character of this circadian periodicity of mitosis can be influenced by the animals' age, and their feeding and lighting conditions [2, 3, 8, 11, 12]. In many investigations the need for using animals of both sexes in experiments has been stressed. However, no information is available to indicate possible differences between the character of circadian periodicity of mitotic division in the same tissue from animals of different sexes.

The object of the present investigation was to compare changes in mitotic index in the corneal epithelium and adrenal cortex during the 24-h period in albino rats of both sexes and of various ages.

## EXPERIMENTAL METHOD

The experiments were performed in spring on the following groups of rats: young rats of both sexes aged 30 days, young sexually mature females taken on the 10th day after opening of the vagina (mean weight 150 g), adult females (mean weight 200 g), and adult males (mean weight 160-180 g). The animals were kept under natural lighting conditions and with free access to food. The corneal epithelium and the zona glomerulosa of the adrenal cortex, and the zona fasciculata and zona reticularis of the adrenal cortex, taken together, were investigated. Total preparations were made from the cornea and serial histological sections from the adrenal, and these were stained with Carazzi's hematoxylin. Mitoses were counted in 15,000-20,000 cells of the corneal epithelium, 6000-14,000 cells of the zona glomerulosa, and 5000-27,000 cells of the zona fasciculata and zona reticularis of the adrenal cortex for each animal. The rats were killed by decapitation every 3 h during the 24-h period, 10 animals at each time. Statistical analysis of the results was carried out by the Fisher-Student method.

## EXPERIMENTAL RESULTS

Corneal Epithelium. In sexually immature rats aged 30 days the circadian periodicity of mitotic activity in both males and females followed a unimodal curve (Fig. 1) with the number of mitoses reaching a maximum in the morning and minimum in the evening or night. Slight differences were observed only in the level of mitotic activity: the mean mitotic index for the 24 h was 12.4% for males but only 10.5‰ for females.

In the experiments on young female rats the number of mitoses reached a maximum between 4 and 10 A.M. (Fig. 1) and a minimum at 10 P.M. The increase in number of mitoses at 7 P.M. is not statistically significant. In rats of this age the phases of the sex cycle (estrus, diestrus, metestrus) had no sig-

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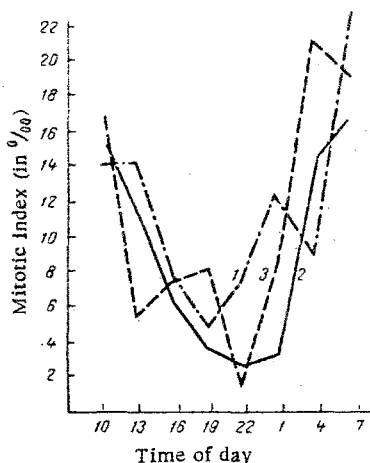


Fig. 1. Circadian periodicity of mitotic activity in corneal epithelium of rats aged 30 days (1), males (2), and females weighing 150 g (3).

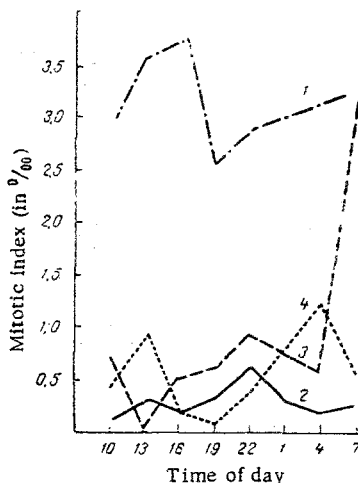


Fig. 2. Circadian periodicity of mitotic activity in zona glomerulosa of adrenal in rats aged 30 days (1), males (2), and females weighing 150 g (3) and 200 g (4).

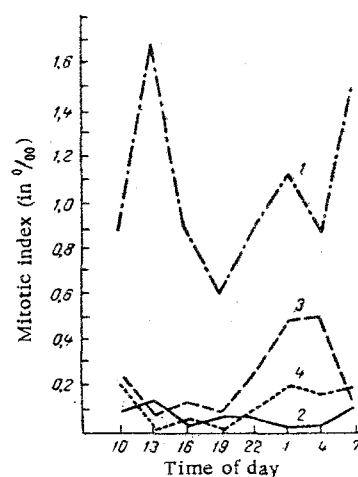


Fig. 3. Circadian periodicity of mitotic activity in zona fasciculata and zona reticularis of adrenal of rats aged 30 days (1), adult males (2), females weighing 150 g (3) and 200 g (4).

nificant effect on the time of occurrence of the maximum or minimum of the number of mitoses in the corneal epithelium.

In the sexually mature male rats the character of the circadian periodicity of mitosis was similar to that found in the corneal epithelium of sexually mature female rats: starting from 10 A.M. the number of mitoses fell rapidly to reach a minimum at between 7 and 10 P.M. By 7 A.M. the number of mitoses had risen by a statistically significant degree (Fig. 1). The mean mitotic activity for the 24-h period in sexually mature female and male rats was 11 and 9.2‰ respectively.

Hence, in the corneal epithelium of sexually immature and mature rats of both sexes the character of the curves for the circadian periodicity of mitosis was uniform: the number of cell divisions reached a maximum in the morning and a minimum during the evening or night. In our earlier investigations [2, 11] we found that the curves of the circadian changes in number of mitoses in the corneal epithelium of rat fetuses and young rats aged 1 and 7 days were of a different character. In rats aged 17 days, whose sex could not be determined and which had lived another 2 days with the palpebral fissure open at the time of sacrifice, the circadian periodicity of mitosis was the same as that found in the present investigation in 30-day old rats and sexually mature females and males.

Zona Glomerulosa of the Adrenal. The number of mitoses in the zona glomerulosa of rats aged 30 days reached a maximum at 1-4 P.M.; a second and less marked increase in the number of mitoses at 7 A.M. not being statistically significant (Fig. 2). At 7 P.M. the number of cell divisions reached a minimum. The mean mitotic index for the 24-h period was 3.6‰.

In young sexually mature female rats the number of mitoses in the zona glomerulosa was maximal at 7 A.M. and minimal at 1 P.M. (Fig. 2). The mean mitotic index for the 24-h period was 0.92‰.

In adult male rats a large number of mitoses was observed in the zona glomerulosa at 10 P.M., while at other times the mitotic activity was slight (Fig. 2). The mean mitotic index for the 24-h period was 0.35‰.

Because of these differences in the character of circadian periodicity of cell divisions in sexually mature female and male rats in the zona glomerulosa of the adrenal, an additional series of experiments was carried out on old adult rats (mean weight 200 g).

The results of these experiments (Fig. 2) showed a significant increase in the number of mitoses in the zona glomerulosa at 4 A.M., but the increase in mitotic index at 1 P.M. is not significant. The mean mitotic index for the 24-h period was 0.6‰. The results of the experiment on young sexually mature fe-

males, indicating sex differences in the character of circadian periodicity of the number of cell divisions in the zona glomerulosa of the adrenal in sexually mature females and males were thus confirmed once more: whereas in males the number of mitoses reached a maximum during the evening and night, in the females this occurred during the morning hours.

Zona Fasciculata and Zona Reticularis. In these zones of the adrenal of young rats aged 30 days (Fig. 3) the number of cell divisions reached a maximum at 1 P.M., the increase in number of mitoses at 7 A.M. not being statistically significant. The mean mitotic activity for the 24-h period was 1.1‰.

In young sexually mature female rats the maximal number of cell divisions in the zona fasciculata and zona reticularis (Fig. 3) was observed at 1-4 A.M., and mitotic activity was minimal during the afternoon. The mean mitotic activity for the 24-h period was 0.26‰. Phases of the sex cycle (estrus, diestrus, metestrus) had no significant effects on the time of onset of the maximum and minimum of the number of mitoses.

In adult male rats numerous mitoses were observed during the morning and afternoon (7 A.M.-1 P.M.), reaching a minimum in the late evening and night (4 P.M.-4 A.M.). The mean mitotic index in this combined zone for the 24-h period was very low, namely 0.06‰. The character of the circadian periodicity of mitoses in the zona fasciculata and zona reticularis of the adrenal thus differed in sexually mature rats of the two sexes.

In the experiment on older females (mean weight 200 g, Fig. 3) the results obtained were the same as in the experiment on young sexually mature females, with the only difference that the time of maximal mitotic activity was more prolonged (from 1 to 10 h).

In the zona glomerulosa and the combined zona fasciculata and zona reticularis of the adrenal the circadian periodicity of mitosis in sexually mature female rats thus differs from the periodicity observed in males. Whereas in males the number of mitoses in the zona glomerulosa reaches a maximum during the evening and night, and in the zona fasciculata and zona reticularis in the afternoon, in females the number of mitoses was maximal in all zones of the adrenal cortex during the morning, in agreement with the time of maximum of the number of mitoses found in other tissues (4, 5, 7, 12, 13).

These observations indicate that circadian changes in the number of mitoses in the adrenal cortex of sexually mature female and male rats are dissimilar, while in the corneal epithelium they are similar. The character of the sex cycle perhaps influences the circadian periodicity of mitoses in the adrenal cortex of female rats.

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