# EFFECT OF ADRENALECTOMY ON DIURNAL RHYTHM OF NUMBER OF DNA-SYNTHESIZING AND DIVIDING CELLS IN THE MOUSE LINGUAL EPITHELIUM

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Diurnal changes in the number of DNA-synthesizing cells and in the number of mitoses were studied in the lingual epithelium of intact and adrenalectomized mice. After adrenalectomy partial desynchronization of mitotic cell division, a decrease in the amplitude of variations in the mitotic index during the 24-h period, and lengthening of the period of increase in the number of mitoses began to occur. The mean diurnal mitotic index in the stratum basale of the lingual epithelium was the same in the control and the experimental series. The rhythm of DNA synthesis in the adrenalectomized mice was the same as in the control. Adrenalectomy thus disturbs the diurnal rhythm of the number of mitoses in the lingual epithelium but does not affect the rhythm of DNA synthesis or the level of proliferative activity.

KEY WORDS: *lingual epithelium; diurnal rhythm; DNA synthesis; mitosis.*

The hormones of the adrenal cortex take part in the regulation of cell division [1, 2, 5]. According to some authorities, they also affect diurnal changes in mitotic activity [4, 6, 7]. However, the problem of the role of glucocorticoid hormones in the formation of the diurnal rhythm of cell proliferation requires further study. In particular, diurnal changes in cell division under conditions of a deficiency of glucocorticoid hormones in the body have hardly been investigated. It is interesting to compare the temporal dynamics of the number of DNA-synthesizing and dividing cells under these conditions, in view of reports in the literature that the mechanisms of cell synchronization before the S phase of the mitotic cycle and before mitosis can function independently [4].

In this investigation changes in the number of mitoses and in the number of DNA-synthesizing cells in the lingual epithelium of mice were studied before and after adrenalectomy.

## EXPERIMENTAL METHOD

Experiments were carried out on 95 noninbred male albino mice weighing 20-25 g. Bilateral adrenalectomy was performed on some of the animals under pentobarbital anesthesia (0.03 ml of a 2% solution of pentobarbital/10 g body weight). The adrenalectomized mice received 1% NaCl solution to drink. The experimental (four to five a time) and control animals (six to eight at a time) were decapitated 8 days after adrenalectomy at 10 a.m., 1, 4, 7, and 10 p.m., and at 1, 4, 7, and 10 a.m. the next day. The animals of each group received an intraperitoneal injection of thymidine-$^3$H in a dose of 1 μCi 1 h before sacrifice. The tongue was fixed in Carnoy's fluid. The number of mitoses and the number of labeled nuclei per 4000-6000 cells in the stratum basale and stratum spinosum of the epithelium of the lower surface of the tongue were counted on autoradiographs. The index of labeled nuclei (ILN) and the mitotic index (MI) were calculated in promille. The numerical results were subjected to statistical analysis by the Fisher–Student method.

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TABLE 1. Diurnal Change in Number of Cells Synthesizing DNA and of Number of Mitoses in the Stratum Basale of the Epithelium of the Lower Surface of the Tongue in Normal Mice and after Adrenalectomy (M ± m)

| Time | Control | | | | Adrenalectomized | | | |
|---|---|---|---|---|---|---|---|---|
| | ILN | P | MI | P | ILN | P | MI | P |
| 10 a.m. | 68,3±5,5 | 0,845 | 4,2±1,0 | 0,158 | 101,4±30,1 | 0,148 | 20,5±6,2 | 0,059 |
| 1 p.m. | 71,4±9,8 | 0,04 | 2,3±0,6 | 0,097 | 48,6±10,2 | — | 6,2±0,9 | — |
| 4 p.m. | 118,5±20,1 | 0,0001 | 3,4±1,0 | 0,258 | 55,5±9,6 | 0,001 | 4,4±1,0 | — |
| 7 p.m. | 226,3±22,6 | 0,258 | 6,4±4,1 | 0,562 | 203,5±29,6 | 0,352 | 5,6±1,8 | 0,115 |
| 10 p.m. | 204,8±23,0 | 0,148 | 9,4±2,3 | 0,009 | 167,3±22,4 | — | 13,3±3,5 | 0,099 |
| 1 a.m. | 152,0±20,6 | — | 32,1±7,5 | 0,308 | 171,4±26,0 | 0,116 | 22,5±2,8 | 0,099 |
| 4 a.m. | 178,5±12,7 | 0,0001 | 41,2±4,7 | 0,0001 | 240,0±21,6 | 0,0001 | 33,5±2,8 | 0,275 |
| 7 a.m. | 44,4±10,1 | 0,442 | 12,0±3,1 | 0,120 | 72,1±9,2 | 0,005 | 22,5±6,7 | — |
| 10 a.m. | 51,6±10,1 | | 5,7±1,9 | | 110,3±3,2 | | 21,4±2,3 | |
| Mean | 133,0 | | 15,4 | | 132,5 | | 16,1 | |

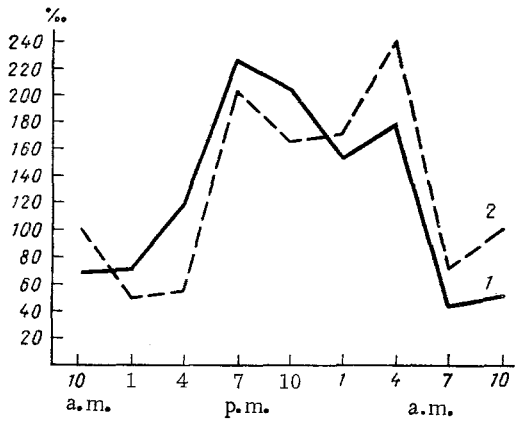


Fig. 1

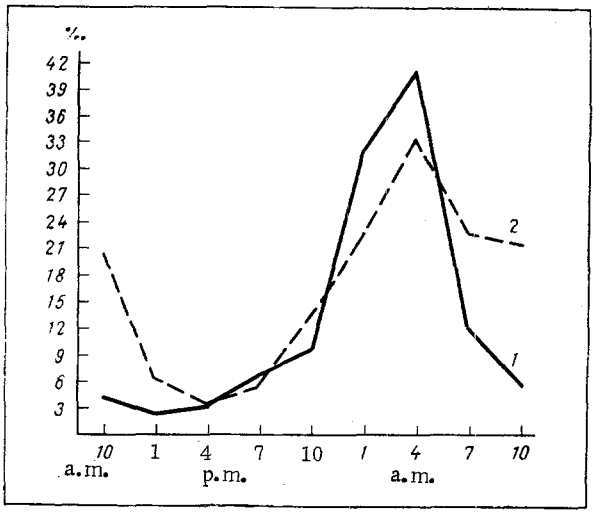


Fig. 2

Fig. 1. Diurnal changes in ILN in stratum basale of epithelium of inferior surface of normal (1) and adrenalectomized (2) mouse tongue. Abscissa, time of day; ordinate, ILN (in °/oo).

Fig. 2. Diurnal changes in MI in stratum basale of epithelium of inferior surface of normal (1) and adrenalectomized (2) mouse tongue. Abscissa, time of day; ordinate, MI (in °/oo).

## EXPERIMENTAL RESULTS

The experimental results are presented in Table 1 and Figs. 1 and 2. The number of cells synthesizing DNA in the stratum basale of the lingual epithelium of the control animals was small at 7 a.m.-1 p.m., it rose to 4 p.m. (the index of significance of differences between the results for 10 a.m.-1 p.m. and for 4 p.m.; P = 0.006) and, in particular, to 7 p.m., after which it remained high without significant fluctuations until 4 a.m. (Fig. 1). The mean value of ILN in the stratum basale between 4 p.m. and 4 a.m. was 176.7°/oo, and between 7 a.m. and 1 p.m. 62.6°/oo (P = 0.0001). In earlier investigations the diurnal changes in the number of DNA-synthesizing cells in the lingual epithelium of C57BL mice were similar in character. However, the mean diurnal values of ILN differed considerably in these two experiments. In the present experiment the mean diurnal ILN was 133.0°/oo, but in the previous experiment it was 58.1°/oo. The rate of physiological regeneration observed in the animals in the different experiments may therefore differ widely, and this must be taken into account when the action of particular factors on cell multiplication is assessed.

The rhythm of DNA synthesis in the stratum basale of the lingual epithelium in adrenalectomized animals was similar on the whole to its rhythm in the control animals (Fig. 1).

There was no difference likewise in the mean diurnal values (Table 1). On the eighth day after adrenalectomy neither disturbances of the rhythm of DNA synthesis in the lingual epithelium nor changes in the number of DNA-synthesizing cells in the course of the 24-h period were thus observed in the mice.

Changes in the number of mitoses in the basal cells of the lingual epithelium (Fig. 2) of the control animals can be represented graphically as a monomodal curve with a maximum at 4 a.m. and minimal values at 1-4 p.m. ($P = 0.0001$). In the adrenalectomized mice a monomodal rhythm of mitosis also was observed but the amplitude of the diurnal fluctuations in the number of mitoses was reduced. In the control the highest value of MI was 17.9 times greater than the minimal, whereas in the experimental series the difference was only 7.6 times. The period of day during which MI in the experimental animals exceeded the mean diurnal value was longer in duration than in the control: In the control mice a high value of MI was observed from 1 to 4 a.m., but in the experimental animals from 1 to 10 a.m. These changes were evidently the result of a decrease in the synchronization of the entry of the cells into mitosis at a time of a marked decrease in the blood glucocorticoid level in the mice. The mean diurnal values of MI were indistinguishable in the control and experimental animals.

In the more highly differentiated stratum spinosum of the epithelium DNA-synthetic and mitotic activity of the cells was sharply reduced. However, the cells capable of proliferating still were able to respond to hormonal mechanisms of regulation. In the control and experimental animals the rhythm of DNA synthesis and of mitosis in the stratum spinosum repeated the diurnal fluctuations of these indices in the stratum basale. The mean diurnal ILN in the stratum spinosum of the control animals was $21.2^{\circ}/_{oo}$ and the mean diurnal MI was $2.4\ ^{\circ}/_{oo}$. In the experimental mice the mean diurnal values of ILN and MI were 22.9 and $2.6^{\circ}/_{oo}$, respectively. Consequently, adrenalectomy did not affect proliferation in the stratum spinosum.

Adrenalectomy in mice thus does not change the mean diurnal level of proliferative activity of the lingual epithelium but leads to partial desynchronization of mitotic cell division during the 24-h period, to a decrease in amplitude of the diurnal variations in MI, and to lengthening of the period of an increased MI. Since adrenalectomy causes changes in the diurnal rhythm of the number of mitoses but not in the diurnal rhythm of DNA synthesis, another important conclusion to be drawn from these results is that the mechanisms of synchronization of the cells before the S phase and before mitosis differ in their sensitivity to glucocoriticoids and that, consequently, in principle these mechanism may function separately.

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