DIURNAL RHYTHM OF MITOSES IN THE EPITHELIUM OF THE MOUSE TONGUE

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A distinct diurnal rhythm of mitoses is found in the cells of the stratum basale and stratum spinosum of the epithelium of the lower surface of the mouse tongue, identical in both strata. The number of mitoses reaches a maximum at 7-10 a. m. and a minimum at 4-10 p.m. The mean mitotic index for the 24 h is 6.55% for the stratum basale and 1.55% for the stratum spinosum. No significant differences are found in the relative number of the various phases of mitosis in the cells of the stratum basale and stratum spinosum.

Several workers have studied the principles governing the diurnal periodicity of mitoses and the rate of physiological regeneration of stratified squamous epithelium of the tongue [7]. In most cases, however, the tissue cells were investigated without regard to the degree of their differentiation.

The object of the present investigation was to compare the diurnal periodicity of mitotic cell division in the stratum basale and stratum spinosum of the lingual epithelium in mice.

EXPERIMENTAL METHOD

Male C57Bl mice weighing 20 g were used. The animals were sacrificed in the course of 24 h at intervals of 3 h, 6-8 mice at each time. The tongue was fixed in Carnoy's fluid and transverse sections (6 μ) were cut starting a short distance from the tip of the tongue and stained with hematoxylin. Mitoses were counted separately in the stratum basale and stratum spinosum of the epithelium of the lower surface of the tongue, from 4000 to 6000 cells in each layer being examined. The mitotic index (MI) was expressed in promille relative to the total number of cells in the corresponding stratum of epithelium.

EXPERIMENTAL RESULTS

As Table 1 shows, the number of cell divisions varied with the time of day or night. In the stratum basale of the epithelium the number of mitoses reached a minimum between 4 and 10 p. m. By 1 a. m. the MI had started to rise, reaching its maximum by 7 a.m. By 10 a.m. the MI had started to fall, but the decrease between 7 and 10 a.m. is not significant ($P = 0.16$). A significant decrease in mitotic activity was found by 1 p.m. The largest number of cell divisions (about 85% of the total number of mitoses observed) occurred between 4 a.m. and 1 p.m. The mean MI for the 24 h in cells of the stratum basale was 6.55 ‰.

During certain time intervals (for example, 10 p. m. to 1 a. m., 4 to 7 a.m.) the differences in the mean values of MI were close to significant. This was usually observed when sharp changes took place in mitotic activity and they were accompanied by considerable (from 7 to 8 times) individual differences in the number of mitoses. This indicated that changes in the diurnal rhythm of cell divisions took place at different times in individual animals.

Diurnal changes in the number of mitoses in cells of the stratum spinosum were similar in character although less marked. The period from 4 to 10 p. m. was characterized by minimal, and from 7 to 10 a. m. by maximal mitotic activity. The differences between MI at 7 a.m. (3.97 ‰) and 7 p.m. (0.15 ‰) are statistically significant ($P = 0.0001$). The mean value of MI for the 24 h in cells of the stratum spinosum was 1.55 ‰, and that for both strata 4.13 ‰. Similar results for the diurnal rhythm of mitoses in the epithelium of the upper surface of the tongue have been obtained in rats [1, 5]. However, the mean values of MI at all times of investigation were higher in rats than, according to the present findings, in mice.

In the lingual epithelium as a whole 1655 mitoses were found, of which 280 (17%) were in the stratum spinosum. This corresponds closely to the values obtained by L. Ya. Zhorno and L. P. Ovchinnikova [4],

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TABLE 1. Mitotic Index in Epithelium of Lower Surface of Mouse Tongue

Time of sacrifice	Mitotic index (in $^0/_{00}$)					
	stratum basale	P	stratum spinosum	P	total	P
10	11,47	0,03	3,33	0,21	7,41	0,048
13	6,64	0,001	1,62	0,01	4,20	0,008
16	1,00	—	0,43	—	0,73	—
19	0,78	—	0,15	—	0,49	—
22	0,44	0,05	0,43	0,18	0,43	0,099
1	6,09	—	1,27	—	3,81	—
4	7,90	0,05	1,53	0,04	4,64	0,04
7	18,37		3,97		11,50	

TABLE 2. Number of Individual Phases of Mitosis in Cells of the Stratum Basale and Stratum Spinosum

Phase	Number of mitoses	
	stratum basale	stratum spinosum
Prophase	232 (16,9%)	41 (14,6%)
Metaphase	731 (51,3%)	116 (41,4%)
Anaphase	250 (18,2%)	82 (29,3%)
Telophase	162 (11,8%)	41 (14,6%)

who found that about 13% of the total number of mitoses in the lingual epithelium of guina pigs occurs in the stratum spinosum.

The question of the ability of cells of the stratum spinosum to embark upon the mitotic cycle has not yet been sufficiently elucidated. O. S. Frankfurt [6] found no incorporation of thymidine-H^3 in cells of the stratum spinosum of the mouse forestomach and postulated that DNA synthesis takes place only in cells of the stratum basale. Consequently, in the stratum spinosum the only cells capable of dividing are those which doubled their DNA content while they were in the stratum basale.

L. Ya. Zhorno and L. P. Ovchinnikova [4], using cytophotometric methods of determining the DNA content, discovered not only diploid, but also triploid and tetraploid nuclei in cells of the stratum spinosum of the lingual epithelium of guinea pigs, indicating that processes of DNA synthesis take place in them.

The possibility that cells which have already started on mitosis can migrate from the stratum basale into the stratum spinosum was examined by comparing the number of individual phases of mitosis in the two strata (Table 2).

The percentage of late phases of mitosis (anaphases and telophases) in the stratum basale was rather less than in the stratum spinosum, but the difference is not significant ($P = 0.132$). Consequently, no data were obtained to support the view that dividing cells migrate from the stratum basale into the stratum spinosum.

Examination of the data obtained for the relative percentages of the various phases of mitosis revealed predominance of the absolute number of early phases (prophases and metaphases) in the morning, although on account of considerable individual variations these differences were not statistically significant. The absolute number of late phases of mitosis showed a smaller change in the course of the 24 h.

A distinct diurnal rhythm of mitoses was thus demonstrated in the stratum basale and stratum spinosum of the epithelium of the lower surface of the mouse tongue, similar in both strata. The mean mitotic activity for the 24 h in the stratum basale is more than four times higher than in the stratum spinosum. The results described do not finally settle the problem of the origin of the dividing cells, but they do indicate that the cells of the stratum spinosum can embark upon mitosis independently.

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