

DIURNAL RHYTHM OF PROLIFERATIVE ACTIVITY OF TRACHEAL EPITHELIAL CELLS

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UDC 612.215.2.014.3:612.6"52"

Diurnal rhythms of mitotic activity and in the number of DNA-synthesizing cells were found in the stratified ciliated epithelium of the trachea by autoradiography with thymidine- H^3 . The maximal number of mitoses and labeled cells was found during the morning. The mean daily mitotic index (0.4%) and the mean daily index of labeled nuclei (0.40%) were found. Only the basal cells were able to divide.

The diurnal rhythm of the indices of proliferative activity – the mean daily mitotic index (MI) and index of labeled nuclei (RI) – have been established for most tissues [2, 4, 9, 10]. However, no data on the diurnal rhythm of proliferative activity of the stratified ciliated epithelium of the air passage could be found in the accessible literature. This is all the more surprising because mitotically dividing basal cells were described as long ago as in 1879 [8]. According to Bertalanffy and Lau [5], 2% of the cells in the tracheal epithelium divide every day. According to calculations by Wells [13], MI for the tracheal epithelium of young rats is 0.13%. Determination of the percentage of cells labeled with thymidine- H^3 by autoradiography has been the subject of more numerous investigations [6, 7, 12, 13].

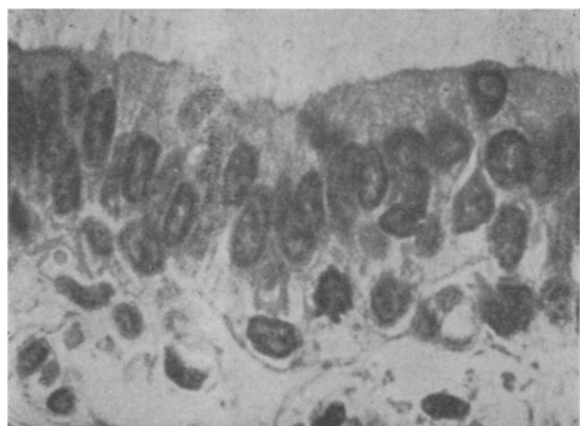


Fig. 1

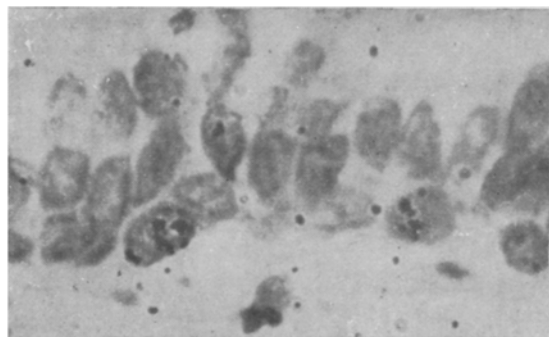


Fig. 2

Fig. 1. Metaphase of basal cell of stratified ciliated epithelium of the rat trachea. Here and in Fig. 2: Hematoxylin-eosin.

Fig. 2. Autoradiograph of stratified ciliated epithelium of rat trachea 1 h after injection of thymidine- H^3 .

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TABLE 1. Diurnal Changes in Proliferative Activity of Stratified Ciliated Epithelial Cells from the Rat Trachea

Time	No. of animals	M1 (in %)	R1 (in %)
10 a.m.	3	0,16±0,33	0,24±0,04
12 noon	4	0,66±0,21	0,34±0,04
2 p.m.	5	0,18±0,10	0,34±0,04
4 p.m.	2	0,33	0,17
6 p.m.	3	0,22±0,06	0,19±0,06
8 p.m.	3	0,22±0,05	0,12±0,03
10 p.m.	2	0,16	0,46
Midnight	3	0,11±0,10	0,32±0,03
2 a.m.	3	0,22±0,12	0,26±0,05
4 a.m.	3	0,66±0,27	0,91±0,19
6 a.m.	4	0,88±0,35	0,56±0,14
8 a.m.	4	1,08±0,26	0,66±0,008
10 a.m.	3	0,28±0,30	0,27±0,05
Mean data for the 24-h period		0,40±0,19	0,37±0,06

TABLE 2. Data in Literature for Number of Labeled Tracheal Epithelial Cells 1 h after Injection of Thymidine- H^3

Authors	Year	Animals used	Wt. of animals (in g)	Type of labeled cells	No. of labeled cells (in %)
Schorter, Titus, Divertie [12]	1964	Mice	15—20	All types	1—4
Blenkinsopp [7]	1967	Rats	200	Chiefly basal	1,1—1,2
Bindreiter, Schappler, Stokinger [6]	1968	Newborn rats		Only basal	2,3
Wells [13]	1970	Rats	40—100	The same	0,7—1,2
T. V. Kamenetskaya	1973	"	190—200		0,12—0,91 (Mean for 24-h period 0,40)

This paper describes an investigation to discover whether mitotic activity in the tracheal epithelium has a diurnal rhythm.

EXPERIMENTAL METHOD

Experiments were carried out on 65 adult rats weighing 190-250 g on April 24-25, when according to the weather service, the duration of daylight in Leningrad is 17 h. The rats were kept under identical conditions with natural illumination and were fed daily at 8-10 a.m. The rats were killed five at a time at intervals of 2 h. Thymidine- H^3 was injected into the rats 1 h before sacrifice in a dose of 0.5 μ Ci/g body weight. The material was fixed in Bouin's fluid. Autoradiographs were prepared from paraffin sections, 7 μ in thickness, by the usual method [3] with type R emulsion. Mitoses and labeled nuclei (with not less than five grains above them) were counted in 6000 epithelial cells only in those animals in whose tracheal mucous membrane no marked inflammatory changes could be found. The numerical results were subjected to statistical analysis.

EXPERIMENTAL RESULTS

Mitoses were found only in the basal cells of the tracheal epithelium (Fig. 1), confirming other observations [1, 8]. The results of counting mitoses and labeled nuclei are given in Table 1, examination of which reveals the considerable scatter of the individual values. According to Wells [13] this is due to the high incidence of chronic respiratory diseases among rats.

Analysis of the material by the Fisher-Student method showed a statistically significant difference between the highest values of mitotic activity at 8 a.m. and the minimal values between 6 p.m. and midnight ($0.02 < P < 0.05$). No significant difference was found between the highest values of mitotic activity

from 4 to 8 a.m. ($P > 0.1$). Further analysis by the nonparametric method, using the U-test (Wilcoxon; Mann-Whitney) showed that the difference between the mean maximal values from 4 to 8 a.m. and the minimal values from 2 p.m. to midnight were significant ($P < 0.001$). MI at noon differed significantly from the neighboring minimal values at 10 a.m. and at 2 p.m. to midnight ($P = 0.5$ and $P < 0.01$, respectively). On the whole, the diurnal changes in mitotic activity can be represented as a unimodal curve with a small additional peak. The maximal value of the index of mitotic activity (0.07-0.11%) agreed with that obtained by Wells (0.13%) for younger animals.

Labeled nuclei also were found only in the basal cells (Fig. 2). Analysis of the figures for the number of labeled cells by the Fisher-Student method showed that it reached a maximum in the early morning, from 4 to 8 a.m., between which limits there were no significant differences ($0.05 < P < 0.1$). The minimal values were recorded between 4 and 8 p.m. and the differences between these limits likewise were not significant ($P > 0.1$). On the other hand, the difference between the maximal and minimal values was statistically significant ($0.02 < P < 0.5$). Statistical analysis by the U-test gave much more information. The mean maximal numbers of labeled cells between 4 and 6 a.m. differed significantly from the minimal values at 4-8 p.m. ($P < 0.001$). The values of the intermediate peak between noon and 2 p.m. differed significantly from the neighboring minimal values ($P < 0.001$). On the whole, therefore, the diurnal changes in the number of cells labeled with thymidine- H^3 should also have fallen on a unimodal curve with a small additional peak.

The results obtained by the autoradiographic method were compared with data in the literature (Table 2). When these results are examined, attention is drawn to differences between the results obtained by Schorter et al. [12] and those of other workers for the type of cells synthesizing DNA. Unfortunately, the authors cited give no photomicrographs in their papers and it is difficult to judge the reasons for this difference; all that can be conjectured is that these workers analyzed their autoradiographs in tangential as well as frontal sections. The mean daily percentage of labeled cells calculated in the present investigation was close to that obtained by Wells, especially if allowance is made for the morning and evening fluctuations. Bindreiter et al. [6] evidently obtained somewhat higher values because of the age of their animals.

It thus follows that only the basal cells divide in the intact stratified ciliated epithelium of the trachea. The diurnal changes in mitotic activity and in the number of DNA-synthesizing cells can be represented by unimodal curves and with a small supplementary peak.

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