

# THE CHARACTER OF THE 24-HOUR PERIODICITY OF MITOSIS IN THE CORNEAL EPITHELIUM OF VARIOUS LABORATORY ANIMALS

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In the last few years the attention of many investigators has been devoted toward studying the role of factors of the external environment in the phenomena involved with the 24-hour rhythm of cell division processes that take place in the tissues of an organism [2, 5, 6, 7, 9, 10].

It has been established that 24-hour periodicity of mitotic activity is characteristic of the majority of tissues in the organism. Despite the fact that the question of 24-hour periodicity of mitotic activity has been dealt with in a considerable number of works, up until now there has not been performed a comparison of the 24-hour rhythm of mitosis in the same tissue of different species of animals. Along with this, a comparative approach to the study of this phenomenon would be able to aid the understanding of the mechanism of cell multiplication and the conditions that provide for one or another intensity in the proliferation of cellular elements.

The purpose of this investigation was to study the 24-hour periodicity of mitosis in the corneal epithelium of animals of different species at different times of the year.

## METHOD

The investigation was carried out on male white mice (18-20 g in weight), white rats (130-150 g in weight), and guinea pigs (300-350 g in weight). The animals were sacrificed in the course of a 24-hour period at 6-hour time intervals. The larger portion of the experiments were begun at 8:00 in the evening, while several of the experiments were started at 8:00 in the morning, the purpose of this being to test the influence of the time of the experiment's beginning on the character of the 24-hour rhythm of mitotic activity observed.

The experiments were performed in the various seasons of the year: spring (in March), summer (in June and August), fall (in September) and winter (in December and January).

Before setting up the experiments the animals were kept under standardized conditions for 6-7 days. All 24-hour experiments were carried out in periods of maximum quiet within the laboratory rooms (on Saturday evening and Sunday). In order to reduce to a minimum the effect of various secondary external factors on the 24-hour regime

of mitosis, we fed the animals and took samples of the cells at the same time in the 24-hour period. In addition, sacrifice of the animals was carried out in a neighboring room, and not in the experimental chamber.

Following fixation of the cornea in Carnu's solution and subsequent staining with hematoxylin by the method of Karachcha, total preparations were made from it. For the determination of mitotic activity we investigated the entire epithelium of the cornea and compiled a chart of the distribution of mitoses, recording the stage of cell division. For the mitotic index we used the number of mitoses per 100 visual fields, which, using the diaphragm we selected, corresponded to 1 mm<sup>2</sup> in corneal area. The index was calculated the same way, whether for the entire cornea as a whole, or for isolated zones within it (peripheral and central).

Eighteen series of experiments were set up, on 360 animals. At each interval of the investigation 5 animals were sampled.

## RESULTS

The data on the mitotic regime of the corneal epithelium, during the course of 24 hours, in mice, rats, and guinea pigs, are presented in the figure. The trial described was carried out in August.

As is apparent from the data presented, 24-hour periodicity of the mitosis in the corneal epithelium of mice was clearly manifested in the form of a monophasic curve. The smallest number of cell divisions in the corneal epithelium of the white mice was observed in the evening hours. The mean mitotic index at that time of the 24-hour period was, on the average, 45 mitoses per 100 visual fields.

At 2:00 A.M. the number of cell divisions in the investigated tissue clearly increased (198 mitoses) and attained a maximum in the morning hours (437 mitoses).

A marked fall in the mitotic activity of the corneal epithelium was already observed at 2:00 P.M. (140 mitoses).

The curve of the changes in mitotic activity of the corneal epithelium in white rats over the course of 24 hours (see figure) repeated the tracing of the 24-hour curve for the white mice. However, in the corneal epithelium of the white rats the range of the 24-hour fluctuation in the num-

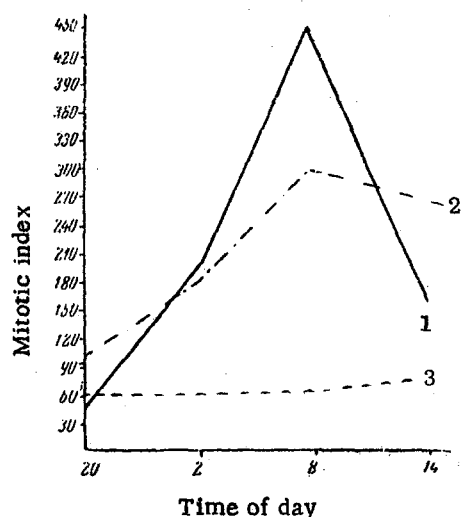


Figure. The 24-hour rhythm of mitosis in mice (1), rats (2), and guinea pigs (3).

ber of dividing cells was demonstrated to a lesser degree.

In the corneal epithelium of the white rats, as in the case of the white mice, the number of mitoses in the evening hours was always minimal; on the average it was equal to 95 mitoses. In the night hours the number of dividing cells increased; the mean mitotic index at that time reached 179.

The maximum number of cell divisions in the corneal epithelium of the white rats was noted in the morning hours (297 mitoses). In the afternoon hours (2:00 P. M.), as a rule there occurred reduction in the total number of dividing cells of the corneal epithelium; the mean mitotic index was lowered to 260.

In contrast with the data obtained from the white mice and white rats, no 24-hour rhythm of mitosis was observed in the corneal epithelium of the guinea pigs. The magni-

tude of the mean mitotic indices in their case did not essentially change in the course of the 24-hour period. The mitotic index throughout the full 24 hours ranged within the borders of 63-76 mitoses (see figure).

The results of the experiments on the study of the 24-hour rhythm of mitotic activity in the corneal epithelium of white mice, white rats, and guinea pigs during the various seasons of the year are presented in the table.

The character of the 24-hour changes in mitotic activity in the experimental animals was maintained as approximately the same in all seasons of the year.

In the mice and rats the maximum number of mitoses were always observed in the morning hours, and the minimum, in the evening. The reliability of the discrepancy between the maximum and minimum indices was sufficiently high in all cases.

As far as the mitotic activity of the corneal epithelium in the guinea pigs is concerned, regular shifts in the 24-hour curve were not observed, as a rule, during the various seasons of the year. However, in the summertime (June) a certain increase was detected in the number of dividing cells during the evening hours as compared with the remaining intervals of the investigation. Statistic treatment of the material showed that this difference was close to being statistically significant ( $P = 0.03$ ).

The distribution of the dividing cells in the separate zones of the corneal epithelium at different hours of the day was not the same. The increase in the mean mitotic index of the corneal epithelium in the white mice during evening time occurred as a result of the increase in the number of dividing cells in the peripheral zone of the tissue. At 2:00 A. M. there was an average of 3-5 times more mitoses in the periphery of the cornea than in the central zone. The reduction in the over-all mitotic index for the corneal epithelium of the white mice during the daytime hours of the 24-hour period also occurred primarily at the expense of the peripheral zone. The number of cell divisions in the perip-

The Mitotic Activity of the Corneal Epithelium in the Course of 24 hours During the Various Seasons of the Year<sup>1</sup>

Species of animal	Time of day	Months					
		January	March	June	August	September	December
Mice	8:00 P.M.	45±4	22±2	39±11	45±19	21±5	55±9
	2:00 A.M.	194±42	221±31	210±39	198±38	247±39	199±45
	8:00 A.M.	347±25	448±23	438±48	437±22	392±25	301±28
	2:00 P.M.	121±18	152±11	169±21	140±21	129±19	72±20
Rats	8:00 P.M.	153±32	76±18	185±11	95±13	45±18	90±32
	2:00 A.M.	279±20	171±39	181±26	179±16	182±29	241±19
	8:00 A.M.	272±26	362±25	354±21	297±10	261±62	311±22
	2:00 P.M.	272±20	191±21	217±17	260±16	340±22	207±27
Guinea pigs	8:00 P.M.	85±6	96±18	87±7	63±7	69±5	75±5
	2:00 A.M.	109±26	81±1	71±9	63±2	82±5	69±6
	8:00 A.M.	108±17	85±8	100±4	67±8	84±5	78±9
	2:00 P.M.	111±8	84±6	85±6	76±7	100±7	82±6

<sup>1</sup>The mean values of the mitotic indices are presented in the table.

hery of the cornea at 2:00 P. M. was, on the average,  $1\frac{1}{2}$  times smaller than in the central zone.

Summing up the material presented above, it can be noted that there exist species-specific differences in the mitotic regime of the corneal epithelium of laboratory animals.

They are concerned with both the intensity of proliferation and the character of the 24-hour dynamics of mitotic activity. The mean 24-hour mitotic index for the corneal epithelium of white mice and rats was higher than in guinea pigs.

The most clearly demonstrated 24-hour periodicity of mitosis appeared in the mice.

In mice the number of mitoses in the morning was greater than in the evening by an average of 12 times, while in rats it was greater by only 3 times. Our data on the 24-hour periodicity of mitosis in the corneal epithelium of white rats corresponded to the data of M. T. Gololobova [1].

No regular shifts in the 24-hour rhythm of mitosis were observed in the corneal epithelium of the guinea pigs. In connection with this, it should be noted that the dynamics of the motor activity of guinea pigs are characterized by the absence of a regular 24-hour rhythm [7].

Guinea pigs differ from mice and rats in that they manifest a less dynamic mitotic activity in their corneal epithelium [2, 3].

#### SUMMARY

The 24-hour periodicity of mitosis was studied in the corneal epithelium of white mice, white rats and guinea pigs. There were species-specific differences both in the intensity of the cellular multiplication, and in its 24-hour rhythm. The 24-hour periodicity of mitotic activity is clearly manifested in mice and rats, the maximal amount of mitoses being observed during morning hours, and the

minimal—in the evening. The curve, characterizing the 24-hour periodicity of mitoses is uniform during all the seasons of the year. The amplitude of the 24-hour variation of mitotic indices in mice is on the average 3 to 4 times greater than in rats. In guinea pigs the 24-hour mitotic periodicity of the corneal epithelium is not manifested so clearly.

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\*See C. B. translation.