Effect of Dexamethasone upon Circadian Mitotic Rhythm in Rat Cornea[1] (36649)

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(Introduced by W. F. Cantrell)

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The understanding of the mechanisms which control the circadian rhythms would represent a significant step toward the understanding of normal growth processes as well as disturbances of growth resulting in neoplasia, since deviations and/or absence of circadian rhythms characterize several experimental and human neoplasms (1–5). As evidence has been presented indicating that glucosteroids might play an important role in the overall mechanisms which control circadian rhythms in the tissues of the host (6–9), an attempt was made to alter or displace circadian mitotic peaks by the administration of dexamethasone at either 0700, 1500 or 2300 hr. The results obtained and presented in this communication indicate that dexamethasone profoundly alters the general circadian mitotic pattern, with displacements of peaks and troughs of mitotic activity in the cornea of rats.

Materials and Methods. Male rats of the Holtzman strain, with initial weight ranging from 130 to 200 g were used in all experiments. All animals were kept in the animal quarters for 1–2 weeks prior to their use in the experiments. The animals were standardized for periodicity analysis—12 hr of light (from 0600 to 1800) alternating with 12 hr of darkness. They were fed a commercial complete diet *ad libitum*. Dexamethasone[2] (0.4 mg) was administered by the ip route at either 0700, 1500 or 2300 hr of the day. The rats were sacrificed at different hours of the day (time groups) as indicated in the tables and figures. The eyes were removed, fixed, and stained as previously described (7). Mitoses were counted with the use of a microscope (ocular 10×, objective 100×) with a micrometer grid disc inserted in the ocular piece. One hundred grid fields were counted in each cornea. All phases of mitosis were included with exceptions of those cells in early prophase and late telophase.

Results. Two types of experiments were carried out: (1st daily doses of dexamethasone): 0.4 mg doses were given at 1500 hr for 8 consecutive days; (2nd alternate days schedule for dexamethasone administration): 0.4 mg doses repeated every other day for a total of five doses.

Daily doses of dexamethasone. Guided by the results obtained with changes in the light/dark schedules (8–9) we felt that a chronic treatment course would be required to effectively alter the circadian mitotic rhythm of dexamethasone-treated rats. All rats were treated with single daily doses of 0.4 mg of dexamethasone at 1500 hr, repeated every day for a total of 8 days. Sacrifice of the animals was started at 0700 hr of the day following the last dose of dexamethasone. This treatment regimen led to weight loss (20–25%) along with a pronounced depression in the levels of cell division over the 24 hr period succeeding the administration of the last dose of dexamethasone. The results representing dexamethasone-treated and control animals are presented in Table I. The control values represent the means for the respective time groups obtained in several experiments over a span of 2 years, including the actual con-

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TABLE I. Effect of Daily Administration of Dexamethasone at 1500 hr upon Mitoses in the Cornea of Rats.

Groups	Motoses/100 fields ± SE[a]; Hr of day[b]					
	0700	1100	1500	1900	2300	0300
Dexamethasone[c]	67 ± 14 (5)[d]	65 ± 8.5 (4)	42 ± 17 (5)	51 ± 17 (4)	23 ± 3.3 (5)	50 ± 5 (6)
Controls	194 ± 5.1 (51)	126 ± 5.5 (37)	69 ± 5.7 (31)	23 ± 2.2 (30)	29 ± 3.4 (31)	66 ± 5.7 (28)
p for control, × dexamethasone	$< .001$	$< .001$	$< .02$	$> .05$	$> .05$	$< .05$

[a] SE = standard error of the mean values.
[b] Hour of day in which the different groups of rats were sacrificed.
[c] Dexamethasone (0.4 mg) administered every day (ip route) at 1500 hr.
[d] Number of animals per time groups is given in parentheses.

trols for this dexamethasone-treated group of rats. As shown, the depression of mitotic activity was limited to those hours of the day in which high levels of mitotic activity were normally present. Comparison of the control observations with those of respective time groups of dexamethasone-treated rats, reveal significant differences for the 0700, 1100, 1500 and 0300 time groups of rats.

In view of 20–25% weight loss suffered by the animals (while controls gained 25%) toxicity was thought to be playing a major role in the observed effect.

Alternate day schedule for dexamethasone administration. Considering the poor tolerance of the animals to the daily dose regimen of dexamethasone administration, we decided to modify the protocol and administer 0.4 mg of dexamethasone, at either 0700, 1500 or 2300 hr, every other day for a total of 5 doses. This alternate day administration of drug was well tolerated by the animals. Through the experiment, the percentage changes (range) in total body weight (TBW) observed in the animals treated at either 0700, 1500 or 2300 hr were, respectively, −6 to +21%, +2 to +21% and −5 to 5%. Saline controls gained 20 to 50% in TBW from beginning to end of the experiments. For the purpose of assessing overall toxicity through weight changes following the alternate day regimen, some groups of dexamethasone- and saline-treated animals were not sacrificed. Within 2 weeks after the experiments, no significant differences in TBW could be determined between saline controls and dex-

TABLE II. The Effect of Dexamethasone Administration at 0700 hr on Alternate Days upon Mitoses in the Cornea of Rats.

Groups	Mitoses/100 fields ± SE[a]; hr of the day[b]						
	1500	1900	2300	0300	0700	1100	0700
Dexamethasone[c]	95 ± 14 (11)[d]	25 ± 2 (12)	18 ± 5 (11)	84 ± 8 (11)	122 ± 5 (12)	48 ± 17 (7)	112 ± 4 (8)
Saline	74 ± 15 (9)	24 ± 6 (11)	31 ± 5 (16)	89 ± 10 (8)	186 ± 11 (26)	106 ± 8 (16)	211 ± 13 (6)

[a] SE = standard error of the mean values.
[b] Hour of the day in which the different time groups of rats were sacrificed.
[c] Dexamethasone (0.4 mg) administered every other day (ip route) at 0700 hr.
[d] Number of animals per time group. The sacrifice of the different and successive time groups started at 1500 hr, 8 hr after the last dose of dexamethasone.

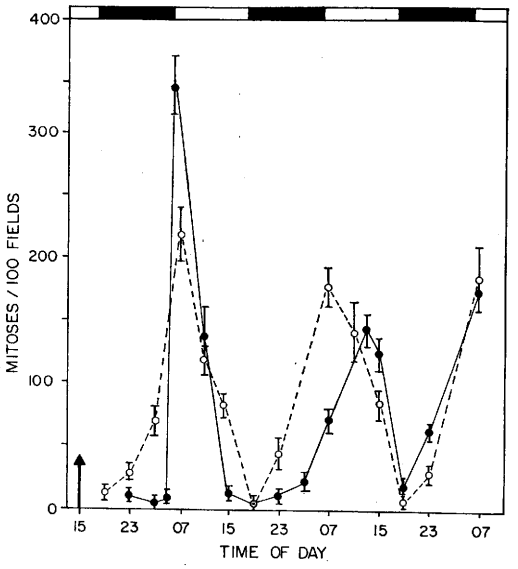

FIG. 1. The effect of dexamethasone administration at 1500 hr, on alternate days, upon mitoses in the cornea of rats. The results represent the number of mitoses ± standard error of the mean values per 100 microscopic fields (ordinate). The number (N) of rats in each of the successive groups of dexamethasone-treated animals (●—) were: 8, 10, 24, 18, 20, 13, 10, 4, 10, 9, 8, 4, 14, and 21. N for the saline-treated groups (○ - -) were 4, 5, 5, 6, 6, 5, 5, 5, 12, 5, 4, 5, 6, and 6. The open and full rectangles (top) indicate, respectively, the 12:12 hr cycles of Light and Darkness. Single daily doses of either dexamethasone (0.4 mg) or saline (0.1 ml) were administered at 1500 hr (arrow), every other day for a total of 5 doses.

amethasone-treated animals. The alterations observed in the circadian mitotic rhythm of 0700, 1500 and 2300 hr treated rats are presented in Figs. 1, 2 and Table II.

With dexamethasone administration at 1500 hr (Fig. 1) on alternate days, a relative synchronization of mitosis around 0700 to 1100 hr was obtained. Levels of mitosis at 0700 hr were 55 to 100% higher ($p < .01$ when compared with controls) than those of untreated control animals. At 1900, 2300, 0300 and 1500 hr of the immediate day following the fifth dose of dexamethasone, significantly lower levels of mitotic activity were found, when compared with untreated controls. In the second day following the fifth dose of the steroid, the mitotic peak was delayed and depressed. The inhibition of this mitotic peak is statistically significant ($p < .01$) when compared with the respective saline peak. Additional experiments will be needed to establish more closely the extent of the above delay.

With the administration of five doses of dexamethasone at 2300 hr, on alternate days, pronounced changes also resulted. These changes shown in Fig. 2 were as follows: (a) delay from 0700 to 1100 hr of the mitotic peak which follows the last dose of dexamethasone, with maintenance of high levels of mitotic activity (comparable to controls) at 0700 hr; (b) suppression of the 24 hr mitotic cycle with resulting low levels of mitotic activity from 1900 of the immediate day after treatment to 2300 hr of the second day after treatment; (c) increased levels of mitotic activity at 0700 hr of the third day after treatment. This "rebound" phenomenon is thought to be related to the suppression of the preceding mitotic peak. The extended periods of low mitotic activity found both in the 1500 and 2300 hr dexamethasone-treated

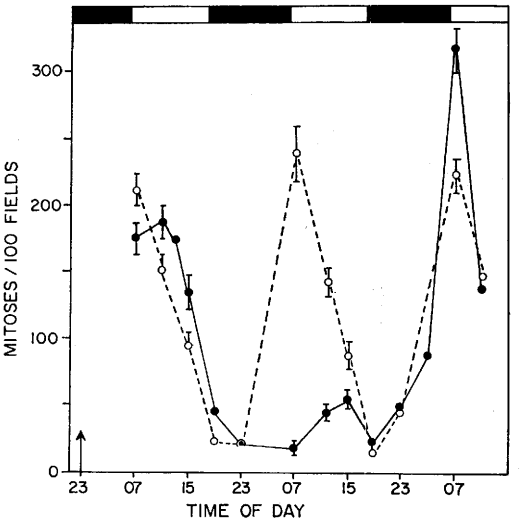

FIG. 2. The effect of dexamethasone administration at 2300 hr on alternate days, upon mitoses in the cornea of rats. See Fig. 1, for the overall design of the experiment. Dexamethasone and saline treatments were at 2300 hr. N for the dexamethasone groups (●—) were: 21, 26, 6, 23, 20, 16, 32, 21, 25, 9, 5, 11, 16, and 5. N for the saline groups (○ - -) were: 13, 15, 7, 10, 10, 12, 19, 17, 12, 11, 11, and 4.

groups, may be in part related to suppression of the hypothalamus–pituitary–adrenal axis function. The return of function of the pituitary (inhibited by dexamethasone) and the adrenal glands, with accompanying reestablishment of the normal circadian pattern for corticosterone in the blood (both in terms of total blood levels and circadian rhythm) is being investigated at the present time, with the hope that it will help to interpret some if not all of the above described changes.

With dexamethasone administration at 0700 hr of the day, the overall levels of mitotic activity were found to be depressed in the 2 immediate days following the last dose of dexamethasone: The mitotic peaks (at 0700 hr) were significantly inhibited when compared with those of untreated controls. On the other hand, the general circadian mitotic pattern in dexamethasone-treated animals was comparable to that of controls.

Discussion. For both animals and man the environment acts as an external conditioner of numerous metabolic reactions. External cues such as light will condition or modulate certain distinctive rhythms in the animals, through as yet, poorly defined systems (8, 9). The endocrine organs seem to play an important role in the mechanism which permits or enables the organism to respond to environmental changes (4–9).

The adrenal–pituitary axis itself seems to respond to environment's major cyclic changes, *i.e.*, those occurring within the span of 1 full day. With the use of laboratory animals this fact is easily demonstrated with artificial establishment of rigid periods of alternating light and darkness. Thus, rats accommodated to a daily cycle of light from 0600 to 1800 hr and darkness from 1800 to 0600 hr have distinct 24 hr (circadian) corticosterone and mitotic cycles. A change or inversion of this light cycle, *i.e.*, lights from 1800 to 0600 hr and darkness from 0600 to 1800 hr, will result in a 12 hr displacement, or inversion, of the peak levels of corticosterone and mitosis (8, 9).

Thus we felt that it would be interesting to determine whether a direct relationship exists between the circadian corticosterone and mitotic rhythms of the rat. For this purpose pharmacological cycles of glucosteroid were produced by the administration of dexamethasone either on a daily or on an alternate day schedule. The striking differences found, especially with the alternate day of drug administration, at either 0700, 1500 or 2300 hr, lend support to the idea that such a direct relationship does exist, between steroid and mitotic cycles. Thus, the administration of dexamethasone at 1500 hr (natural corticosterone peak) accentuates the natural circadian synchronization of cell division, while its administration at the time coinciding with predicted downslope (animals on L:D 12:12 L 0600–1800 L) of corticosterone, shifts the circadian mitotic peak from 0700 to 1100 hr. The same dose of the steroid administered at the predicted circadian upslope of corticosterone (0700 hr) reduced the overall amplitude of the circadian mitotic peak. Thus, it appears that the critical period of the day for enhancement of normal pattern of circadian mitotic events is that which coincides with the natural daily peak of plasma corticosterone (1500 to 1700 hr, in animals exposed to lights from 0600 to 1800 hr).

In conclusion, our results demonstrate a dependence of the dexamethasone effect upon the circadian phase of the animals (possibly hypothalamus:pituitary:adrenal status as well as circadian mitotic cycle) at the time of its administration. The possibility that the observed dexamethasone effect upon corneal epithelium is also manifested in other tissues is being currently investigated. If proven to be a general phenomenon, the parallel displacement of the normal circadian mitotic rhythm in critical tissues of the host leading either to enhanced synchronization or to the establishment of a 48 hr rhythm, might prove itself of interest in: (a) mapping of the circadian mitotic cycle; (b) study of the controlling mechanism(s) which regulate the circadian mitotic rhythm; (c) comparative studies between normal and those neoplastic tissues with abnormal or absent circadian mitotic rhythm (1, 2); (d) synchronization of normal and neoplastic cell populations in such a way as to establish their respective mitotic peaks distinctly separated in time from each other (10).

Summary. Data are presented to indicate that dexamethasone administration to rats produces dramatic changes in the circadian distribution of mitotic activity. They also indicate that the type of response observed is related to the time of the day (circadian system phase) of dexamethasone administration. Thus, either depression, relative synchronization or the establishment of a 48 hr mitotic rhythm were observed, following the administration of the steroid at 0700, 1500 and 2300 hr of the day, respectively.

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