# MITOTIC ACTIVITY IN WHITE RATS IN A STATE OF DRUG-INDUCED SLEEP

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It has been shown that excitation of the body by several different stimuli leads to a lowering of the mitotic activity of certain tissues [4].

The problem of the influence of inhibitory processes in the central nervous system on cell division has not been adequately studied.

Some authors [5, 7] have observed that the tissues of animals which were in a resting state before death were characterized by a higher level of mitosis than those of animals which were in a state of excitation before death. We are aware of the work of Bullough [6] on the effect of barbiturates on the mitotic activity of the epidermis of the ear in white mice. In his opinion, animals in a state of drug-induced sleep show a higher degree of mitotic activity. It must be pointed out, however, that there were several technical defects in this research which might arouse doubts about the results obtained: the counting of the mitotic activity was carried out on several successive pieces of tissue taken from the same animal.

In the present research we endeavored to explain the influence of drug-induced sleep of long and short duration on cell division in several germinating areas of the body.

## EXPERIMENTAL METHOD

Investigations were carried out on male rats weighing 100-150 g. The experimental animals were injected subcutaneously with a 2% solution of sodium amytal, in a dose of 0.5 ml/100 g body weight. The control rats were injected with the same volume of physiological saline.

In order to define the state of sleep which usually developed after 5-10 minutes, the corneal reflex of the experimental rats was studied, and the position of the body was noted. The animals were killed by decapitation at the moment of waking; the control rats were killed at the same times.

For histological investigation the tissues were fixed in Zenker's solution, embedded in paraffin wax and stained with hematoxylin by Carazzi's method. The counting of the mitoses was done in every case under the same magnification (objective 90×, ocular 10×). On the cover slips of total preparations of the cornea two perpendicular lines were drawn in ink, crossing at the center of the cornea. The cells and mitoses were counted along these lines. In each case an average of 95 fields of vision were examined. An area of small intestine (10 cm from the outlet from the stomach) was embedded in paraffin wax, and sections were cut from it to a thickness of 8$\mu$. The mitoses were counted in each case in 50 crypts, followed by counting of the cells.

The mitoses in the epidermis of the ear of the rats were counted in serial sections 8$\mu$ in thickness (alternate sections). In each case 6000-8000 cells were examined altogether. The mitotic coefficient per 1000 was calculated in relation to the total number of cells. By comparison of the mitotic coefficients the probability (P) of random difference in their values was determined by the Fisher-Student method [3].

## EXPERIMENTAL RESULTS

As may be seen from Table 1, after a single injection of a hypnotic drug during the morning and afternoon (10 A.M. to 2 P.M.) there was no change in the mitotic coefficient in the cornea, skin and intestine.

Under these circumstances, however, considerable individual variations in the value of the mitotic coefficient were observed. These variations were evidently due to the fact that animals in which the sleep had lasted for different lengths of time were killed at the same time of day (from 11 A.M. to 6 P.M.). It is known that the mitotic activity in rats in the morning and evening hours is different [2].

We accordingly carried out an additional experiment in which a single injection of the hypnotic drug was given at a time when the mitotic activity was low (5 P.M.). The animals were killed at 8-9 P.M. In this experiment the mitotic coefficient was determined in the corneal epithelium alone. The results of the experiment (Table 2) showed that in the evening hours after a single injection of the hypnotic drug causing induced sleep, the mitotic coefficient in the experimental animals was slightly increased by comparison with that of the controls. This increase was, however, small and was not statistically significant.

In order to ascertain what changes took place in the mitotic activity after prolonged, continuous drug-induced sleep, we carried out a second series of experiments. Sleep lasting 10-11 hours was produced by repeated injections of sodium amytal. Each successive injection was given at the moment when the animal was emerging from sleep. The control animals were given a single injection of physiological saline in order to avoid the possibility of a lowering of the mitotic activity in response to the needle. According to the number of injections the animals were divided into 4 groups.

TABLE 1

Mitotic Coefficient (per 1000) in the Epithelium of the Cornea, the Epidermis of the Ear and the Crypts of the Small Intestine of White Rats after a Single Injection of Sodium Amytal during the Morning Hours

| Duration of sleep (in hr) | Group of rats | Number of animals | Mitotic coefficient | | |
|---|---|---|---|---|---|
| | | | cornea | skin | intestine |
| 1 | Experiment | 6 | 11.6 | 3.5 | 28.4 |
| | Control | 6 | 10.1 | 3.1 | 27.8 |
| 2 | Experiment | 10 | 7.5 | 1.8 | 25.5 |
| | Control | 6 | 8.5 | 3.6 | 27.9 |
| 3 | Experiment | 8 | 9.7 | 1.6 | 24.1 |
| | Control | 8 | 7.4 | 2.1 | 26.9 |
| 4 | Experiment | 6 | 6.2 | 2.4 | 26.9 |
| | Control | 4 | 5.8 | 2.5 | 30.9 |

The results of this experiment, given in Table 3, show that after drug-induced sleep lasting continuously for 10-11 hours, a significant increase was observed in the number of cell divisions in the corneal epithelium of the experimental animals by comparison with the controls (in all the groups P varied between 0.04 and 0.001). This stimulation of mitotic activity did not depend on the number of injections of sodium amytal given in the course of the experiment.

From the counting of the mitoses in the epithelium of the ear no such difference was revealed, although the experimental animals showed a certain tendency to increase of the mitotic coefficient by comparison with

the controls. No changes were observed in the mitotic activity of the epithelium of the crypts of the small intestine after prolonged, continuous drug-induced sleep.

Irrespective of the duration of the sleep, the differing depth of the sleep in the experimental animals was evidently accounted for by their typological peculiarities. On these grounds it was possible to subdivide all the experimental animals of the second series into two groups: the first included rats in which a state of deep sleep was observed, and the second rats with only superficial sleep. It must be pointed out that the sleep of longest duration occurred in the animals after the second injection of the hypnotic drug. After subsequent injections the duration of sleep decreased, presumably because habituation developed. The results of this experiment are shown in Table 4.

TABLE 2

Mitotic Coefficient (per 1000) in the Corneal Epithelium of White Rats after a Single Injection of Sodium Amytal during Evening Hours

| Duration of sleep | Experiment | | Control | |
|---|---|---|---|---|
| | number of animals | mitotic coefficient | number of animals | mitotic coefficient |
| 1 hour 35 minutes | 4 | 8.80 | — | — |
| 2 hours 30 minutes | 17 | 4.91 | 9 | 4.23 |
| 3-4 hours | 10 | 6.15 | 10 | 5.72 |
| Mean | — | 6,6 | — | 5.0 |

As may be seen from Table 4, the mitotic activity of the corneal epithelium of the group of animals in deep sleep was almost twice as high as in the corneal epithelium of the control rats ($P < 0.0001$). In the group of animals with superficial sleep the mitotic activity in the cornea was also higher than in the control animals, but this increase was not significant ($P = 0.57$). On the other hand, by comparison of the mitotic coefficients of the corneal epithelium of the rats in these two groups, a statistically significant difference was found in the number of mitoses ($P = 0.0007$). The mitotic coefficient of the epidermis of the ear was higher in the animals with deep sleep than in those with superficial, and it differed from that in the control animals but this

TABLE 3

Mitotic Coefficient (per 1000) in the Corneal Epithelium, the Epidermis of the Ear and the Epithelium of the Crypts of the Small Intestine of White Rats after the Repeated Injection of Sodium Amytal during Evening Hours

| No. of injections | Cornea | | | | Skin | | | | Intestine | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | experiment | | control | | experiment | | control | | experiment | | control | |
| | number of animals | mitotic coefficient | number of animals | mitotic coefficient | number of animals | mitotic coefficient | number of animals | mitotic coefficient | number of animals | mitotic coefficient | number of animals | mitotic coefficient |
| I | | | 24 | 5,3 | | | 23 | 2,1 | | | 22 | 25.7 |
| II | 4 | 8.9 | | | 4 | 2,5 | | | 3 | 24.9 | | |
| III | 13 | 7.7 | | | 13 | 2.4 | | | 13 | 28.8 | | |
| IV | 16 | 8.6 | | | 16 | 2,9 | | | 16 | 21.6 | | |
| V | 7 | 8.6 | | | 7 | 2.5 | | | 7 | 26.8 | | |

increase was not statistically significant (P = 0.19). No changes in the mitotic activity in relation to the depth of sleep were observed in the epithelium of the crypts of the small intestine.

Counting of the mitoses by phases showed predominance of the earlier phases of mitosis in the experimental animals of this series by comparison with the controls. The ratio between the numbers of early and late phases in the cornea of the control animals, for instance, was 1.0, and in the experimental animals 1.3-1.5, irrespective of the number of injections of hypnotic drug and the depth of sleep.

The findings described thus show that sleep of short duration after a single injection of hypnotic drug, whether in the morning (10 A.M. to 1 P.M.) or evening (5 P.M.) hours, had no effect on the level of mitotic activity in the corneal epithelium of white rats.

Prolonged, continuous drug-induced sleep, after repeated injections of sodium amytal, caused an increase in the mitotic coefficient in the epidermis of the ear and had no effect on the mitotic activity of the epithelium of the crypts of the small intestine. The increase in the number of cell divisions in the corneal epithelium depended on the depth of the animal's sleep; in the epidermis of the ear this relationship was expressed to a lesser degree, and in the intestine it was not found.

The greater increase in mitotic activity in the corneal epithelium than in the other germinating areas (skin, intestine) was evidently due to its greater reactivity to the most diverse agents. It is also known that the cornea responds by a considerable lowering of its mitotic activity to the injection of adrenalin, whereas the intestine

TABLE 4

Mitotic Coefficient (per 1000) in the Corneal Epithelium, the Epidermis of the Ear and the Epithelium of the Crypts of the Small Intestine of White Rats after Emerging from a State of Deep and Superficial Drug-Induced Sleep

| Test object | Control | | Sleep | | | |
|---|---|---|---|---|---|---|
| | | | superficial | | deep | |
| | number of animals | mitotic coefficient | number of animals | mitotic coefficient | number of animals | mitotic coefficient |
| Cornea | 24 | 5.3 | 20 | 7.0 | 20 | 9.7 |
| Epidermis | 23 | 2.1 | 20 | 2.3 | 20 | 2.9 |
| Intestine | 22 | 25.7 | 20 | 28.2 | 19 | 26.1 |

does not react to this drug [1, 4]. The epidermis showed the same tendency to increase of mitotic activity as did the corneal epithelium, but the corresponding changes were expressed to a lesser degree. It is not ruled out that the dose of hypnotic causing changes in the cornea was inadequate for the epidermis.

It may be postulated from the results obtained that changes in the intensity of cell division are dependent on the fundamental processes taking place in the central nervous system.

## SUMMARY

Single administration of hypnotics in the morning or in the evening induces no changes in the mitotic activity of the cornea, skin and crypts of the small intestine. Prolonged continuous drug-induced sleep for a period of 10-11 hours causes a rise of the mitotic activity in the cornea, slightly increases the mitotic activity in the ear epidermis, but leaves it unchanged in the crypts of the small intestine. The rise of mitotic activity in the corneal epithelium depends on the profoundness of the animal's sleep.

## LITERATURE CITED

[1] N. V. Bogoyavlenskaya and V. N. Dobrokhotov, Byull. Éksptl. Biol. i Med., 46, No. 8, 104-108 (1958).*

[2] M. T. Gololobova, Byull. Éksptl. Biol. i Med., 46, No. 9, 118-122 (1958).*

[3] V. I. Romanovskii, The Use of Mathematical Statistics in Experimental Work, Moscow-Leningrad, 1947 [In Russian].

[4] G. S. Strelin and V. V. Kozlov, Proceedings of the Sixth All-Union Congress of Anatomists, Histologists and Embryologists, pp. 537-539, Khar'kov, 1958 [In Russian].

[5] I. A. Utkin et al., Byull. Éksptl. Biol. i Med., 41, No. 4, 62-66 (1956).*

[6] W. S. Bullough, Proc. Roy. Soc., Ser. "B", London, 1948, v. 135, p. 233-242.

[7] J. S. Friedenwald and W. Buschke, Am. J. Physiol., 1944, v. 141, p. 689-694.

*Original Russian pagination. See C.B. Translation.