thelium must be assessed. The final verdict on the connection between the metaphase block of the tubular epithelium of the allografted kidneys and the appearance of pathological C mitoses and immunodepressive therapy will be reached after a series of experiments, which the writers have planned on dogs with transplanted kidneys to which various doses of immunodepressants and cytostatic agents will be administered, have been carried out. So far as the actual phenomenon of the appearance of mitoses in the allografted dogs' kidneys is concerned, it must probably be attributed to postischemic mobilization of the proliferative pool as described in the literature.

LITERATURE CITED

1. V. N. Blyumkin and A. E. Ivanov, Byull. Éksp. Biol. Med., No. 12, 78 (1973).
2. A. E. Ivanov and V. N. Blyumkin, in: Immunodepression after Transplantation of Organs [in Russian], Moscow (1973), pp. 176-182.
3. I. D. Kirpatovskii and N. A. Bykova, Transplantation of the Kidney [in Russian], Moscow (1969).
4. A. A. Makarov, A. B. Tsypin, G. V. Burdina, et al., in: Transplantation of Organs and Tissues [in Russian], Minsk (1974), pp. 83-84.
5. M. E. Marshak, in: Modern Methods of Investigation of the Functions of the Cardiovascular System [in Russian], Moscow (1963), pp. 179-188.
6. B. V. Petrovskii et al., Transplantation of the Kidney [in Russian], Moscow (1969).
7. H. Cain and S. Fazekas, Arch. Path. Anat., 337, 33 (1963).
8. O. Klinge, Beitr. Path. Anat., 130, 352 (1964).
9. C. E. McGreight, Anat. Rec., 130, 336 (1958).

DIURNAL RHYTHM OF MITOSIS AND IN NUMBER OF DNA-SYNTHESIZING CELLS AFTER ADRENALECTOMY IN MICE

S. G. Mamontov and L. N. Ivanova

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Diurnal changes in the number of DNA-synthesizing cells and in the number of mitoses were studied in the corneal epithelium and liver of intact and adrenalectomized mice. The curve of diurnal changes in the number of labeled nuclei in the corneal epithelium of the mice after adrenalectomy became bimodal and the amplitude of fluctuations in the mitotic index in the course of the 24-h period increased sharply compared with the control. The rhythm of DNA synthesis in the liver was similar in the control and experimental series, and the rhythm of mitosis in the experimental animals became biphasic in character. Adrenalectomy thus disturbs the phase structure of the rhythms of DNA synthesis and of mitosis in the tissues studied.

KEY WORDS: diurnal rhythm; adrenalectomy; DNA synthesis; mitotic activity.

Glucocorticoid hormones have a powerful effect on cell division in mammalian tissues. This phenomenon is manifested differently in different tissues and the role of adrenocortical hormones in the regulation of cell division is not yet fully clear.

There are data in the literature indicating a possible effect of glucocorticoids on the entry of cells into the phase of DNA synthesis [7]. If this is so, a fall in the blood glucocorticoid level could cause disturbance of synchronization of the entry of cells into the S period of the mitotic cycle.

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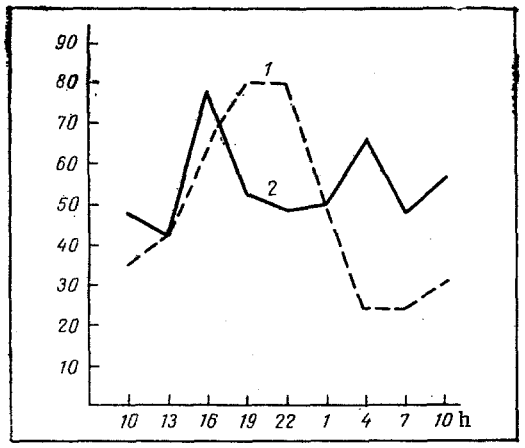

Fig. 1. Diurnal changes in number of labeled nuclei in stratum basale of corneal epithelium of normal and adrenalectomized mice. Here and in Fig. 2: abscissa, time of day or night; ordinate, ILN (in $^{0}/_{00}$). 1) ILN in control; 2) in experimental series.

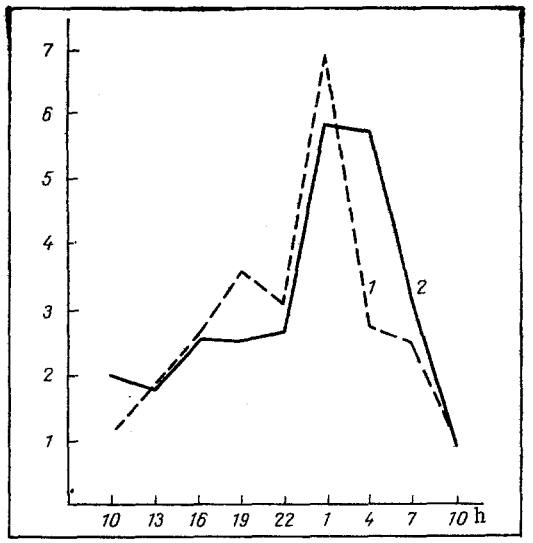

Fig. 2. Diurnal changes in number of labeled nuclei in liver of normal and adrenalectomized mice. Legend as in Fig. 1.

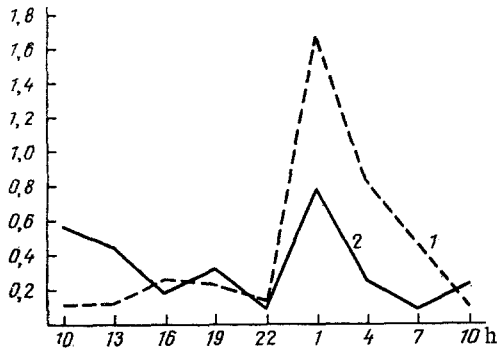

Fig. 3. Diurnal changes in number of mitoses in the liver of normal and adrenalectomized mice: 1) MI in control; 2) in experiment. Abscissa and ordinate: as in Fig. 1.

TABLE 1. Diurnal Changes in Number of Mitoses in Stratum Basale of Corneal Epithelium in Normal and Adrenalectomized Mice

Time of day (in h)	Control		Experimental	
	MI (in $^{0}/_{00}$)	P	MI (in $^{0}/_{00}$)	P
10	7,1	0,626	17,3	0,001
13	6,3	0,291	5,6	—
16	4,4	—	6,6	—
19	4,9	0,561	7,1	—
22	6,7	0,189	6,8	—
1	9,6	0,192	5,4	0,016
4	6,3	—	9,0	—
7	7,6	—	12,6	—
10	6,2		13,7	

The object of this investigation was to study diurnal changes in the number of DNA-synthesizing cells and in the number of dividing cells in the corneal epithelium and liver of mice after bilateral adrenalectomy.

EXPERIMENTAL METHOD

Noninbred male albino mice weighing 20-25 g were used. Bilateral adrenalectomy was performed under nembutal anesthesia (0.03 ml of a 2% solution/10 g body weight). The adrenalectomized mice were given isotonic sodium chloride solution to drink. Eight days after adrenalectomy the experimental (four or five at each time) and control mice (six or eight at each time) were killed by decapitation at 1, 4, 7, and 10 a.m. and 1, 4, 7, and 10 p.m. The animals of each group received an intraperitoneal injection of thymidine-^{3}H, 1 h before sacrifice, in a dose of 1 μCi/g body weight. The eyes and pieces of liver were fixed in Carnoy's fluid. Histological sections 5-7 μ in thickness were coated with photographic emulsion of type M and R. After staining with Meyer's hematoxylin, the number of mitoses and labeled nuclei were counted in the stratum basale and stratum spinosum of the epithelium in the autoradiographs in 4000-6000 nuclei in each layer, and in 10,000-12,000 nuclei in the liver. Nuclei were regarded as labeled if they contained five or more grains of silver. The numerical results were

analyzed by the Fisher–Student method and with the aid of the Wilcoxon–Mann–Whitney nonparametric criterion. The index of labeled nuclei (ILN) and mitotic index (MI) were expressed in promille.

EXPERIMENTAL RESULTS

As Fig. 1 shows, in the stratum basale of the corneal epithelium of the control animals the largest number of DNA-synthesizing cells was observed from 4 to 10 p.m. A similar character of the rhythm of DNA synthesis in the mouse corneal epithelium was described by the writers previously [4]. After adrenalectomy the curve of diurnal changes in the number of labeled nuclei became bimodal, with maxima at 4 p.m. and 4 a.m. The mean diurnal ILN in the experimental group (51.7 °/₀₀) was virtually indistinguishable from the control value (49.3 °/₀₀). The MI in stratum basale of the corneal epithelium of the control animals was minimal at 4-7 p.m., it increased toward 10 p.m., and reached a maximum at 1 a.m. (Table 1). During the 24-h period it was possible to distinguish a stage of increased mitotic activity (from 10 p.m. to 10 a.m.) and a period when the number of dividing cells was small (1-7 p.m.). The small difference will be noted between the maximal (9.6 °/₀₀) and minimal (4.4 °/₀₀) values of MI during the 24-h period ($P = 0.01$). Most workers observed a higher amplitude of diurnal variations of MI in the corneal epithelium [1, 3]. The smoothing of the curve for the rhythm of mitosis in the corneal epithelium obtained in the present investigation could perhaps be due to the time when the experiments were carried out (December). Sokolova et al. [6], who studied mitotic activity in the corneal epithelium of rats during the winter, found a diurnal rhythm of mitosis very similar in character to that now described. This tentative explanation is supported by data showing that with a shortening of the period of daylight there is a reduction in the amplitude of fluctuations in protein synthesis during the 24-h period [8]. It has also been shown experimentally that the regime of photoperiodicity and illumination plays an essential role in the formation of the phase dynamics of diurnal rhythms of cell division [5].

After adrenalectomy the amplitude of fluctuations in MI during the 24-h period in the corneal epithelium of the mice rose sharply. The mean diurnal MI also increased from 6.5 to 9.6 °/₀₀ ($P = 0.0001$).

The study of the effect of adrenalectomy on the diurnal dynamics of DNA synthesis and on cell division in the liver is of great interest. The liver is known to be the target organ for glucocorticoids, an organ sensitive even to a low concentration of adrenocortical hormones. The results obtained show that the rhythms of DNA synthesis in the control and experimental series (Fig. 2) mainly repeat one another, but the phase structure of the rhythm of mitosis after adrenalectomy was disturbed (Fig. 3). The rhythm of mitosis in the liver of the experimental mice became biphasic with maxima at 10 a.m. and 1 p.m. The number of hepatocytes synthesizing DNA and the number of mitoses were the same in the control and experimental series (mean diurnal ILN 2.72 °/₀₀ in the control and 2.83 °/₀₀ in the experimental series; mean dirunal MI 0.4 and 0.33 °/₀₀, respectively).

The results of these experiments thus show that adrenalectomy does not lead to a change in the level of proliferation in the corneal epithelium or in the liver of the mice. Data in the literature on this problem are contradictory. In the thymus [2], the epithelium of the tongue, and the epidermis of the ear [9] an increase in the number of dividing cells has been described after removal of the adrenals. On the other hand, in the epithelium of the lens and cornea after adrenalectomy the number of dividing cells was unchanged or was actually reduced at certain times after the operation [10]. In the tissues investigated in the present experiments ILN for the adrenalectomized animals remained at the control level and MI was increased in the corneal epithelium but unchanged in the liver. An attempt could be made to explain the contradictory character of the existing data by differences in the response of the tissues to a fall in the blood glucocorticoid level and the different times after the operation at which other workers made their observations. However, besides these factors, in the writers' view the following circumstance may play an important role. Investigations of cell proliferation after adrenalectomy have been carried out as a rule on one occasion only and at a certain time of day. It follows from the results of the present experiments that a fall in the blood glucocorticoid level leads to disturbance of the phase structure of the rhythms of cell proliferation. A single investigation of mitotic activity after adrenalectomy cannot therefore give a complete picture of the role of glucocorticoid hormones in the regulation of cell division.

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LITERATURE CITED

1. V. N. Dobrokhotov and A. G. Kudyrumova, Dokl. Akad. Nauk SSSR, 141, 208 (1961).
2. I. I. Zabalueva and A. K. Ryabukha, in: Regeneration and Cell Division [in Russian], Moscow (1968), pp. 139-144.
3. S. G. Mamontov, in: Some Current Problems in Biology and Medicine, No. 1 [in Russian], Moscow (1968), pp. 152-154.
4. S. G. mamantov and L. N. Ivanova, in: Diurnal Rhythms of Physiological Processes of the Organism [in Russian], Moscow (1972), pp. 44-47.
5. Yu. A. Romanov and V. P. Rybakov, in: Biological Rhythms in Mechanisms of Compensation of Disturbed Functions [in Russian], Moscow (1973), pp. 146-163.
6. L. V. Sokolova, A. G. Mustafin, V. N. Dobrokhotov, et al., Byull. Éksp. Biol. Med., No. 12, 64 (1973).
7. O. S. Frankfurt, in: The Cell Nucleus and Its Ultrastructure [in Russian], Moscow (1970), pp. 113-115.
8. L. I. Chekulaeva and V. I. Bulgak, Tsitologiya, No. 7, 863 (1974).
9. E. B. Laurence and E. R. Hansen, Virchows Arch. Abt. B. Zellpath., 9, 271 (1971).
10. M. J. Voaden, Exp. Eye Res., 12, 337 (1971).

DIFFERENCES IN REGENERATION OF THE SKIN IN DIFFERENT SPECIES OF MAMMALS

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The healing of full-thickness skin wounds measuring 1.2 cm^2 was studied in mink and sable. In both species of animals the skin defect closed mainly through contraction of the wound. Hairs and sebaceous glands were found in the small area of regenerating skin formed in the center of the primary defect. It is postulated that these hairs developed from bulbs of "old" hairs which migrated into the regenerating zone together with the lower layers of the dermis adjacent to the wound.

KEY WORDS: contraction of skin wounds; regeneration of skin; sable; mink.

Regeneration of the skin in mammals consists of the following series of regenerative processes: epithelization of the wound defect, the formation of young connective tissue and its reorganization, contraction of the wound, and intercalated growth of the skin around the wound. The role of these processes in regeneration of the skin depends on the site of injury and the species of animal. For instance, the closure of full-thickness skin defects in parts of the mammalian body covered with relatively mobile skin takes place mainly through contraction of the wound [5]. Consequently, intact skin moves into the defect and a very small zone of regeneration is formed from the young tissues in the center of the defect. The focus of regeneration may consist of a connective-tissue scar covered with young epithelium, or skin of atypical structure in which, in some cases, hairs and glands can be found [1, 2, 5, 7, 8]. The origin of the hairs and glands found in regenerating skin differs: these structures may develop from "old" hairs and glands injured during the operation or carried in by contraction of the wound together with individual layers of dermis adjacent to the wound [5, 9]. Finally, hairs and glands may be formed *de novo* from intrusions of the regenerating epithelium into

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