DIURNAL RHYTHM OF MITOSIS IN THE CORNEAL EPITHELIUM AFTER SPLENECTOMY

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Splenectomy was performed on CBA mice and the diurnal rhythm of mitotic activity in the corneal epithelium studied 5 days later, after preliminary injection of colcemid into the animals 3 h before sacrifice. In that part of the 24-h period that is characterized by a high mitotic index (from 3 a.m. to 3 p.m.) the index of c-mitoses in the experimental mice was significantly lower than in the control and the peak of mitotic activity was postponed. The duration of the phase of an increase in the number of cell divisions was shortened. The total number of c-mitoses in the control animals in the course of the 24-h period was 72.3% of the control number. The rate of physiological regeneration of the corneal epithelium was reduced in the splenectomized mice. It is suggested that the lymphoid system affects the entry of cells into mitosis in epithelial tissue and the formation of the wave of mitosis during the period of increased mitotic activity.

KEY WORDS: biorhythm; mitosis; epithelium.

The role of the lymphoid system in the regulation of regeneration [1, 2] and of other morphogenetic processes [9] has recently been convincingly demonstrated. However, there is no evidence in the literature of a role of the immune system in the regulation of physiological regeneration.

In the investigation described below an attempt was made to study the diurnal rhythm of cell proliferation and the time of cell renewal in the corneal epithelium of mice after splenectomy. This experimental scheme was chosen because all known populations of lymphoid cells are represented in the spleen, where, moreover, the cells consist mainly of T lymphocytes, functioning in cooperation with B cells [6].

EXPERIMENTAL METHOD

The experiment was carried out on 72 male CBA mice in August, 1977. The spleen was removed from some of the mice under ether anesthesia. A mock operation was performed on the control animals. The control and experimental mice were killed 5 days after the operation so that the time of the operation coincided approximately with the time of sacrifice. The animals of both groups received an intraperitoneal injection of colcemid in a dose of 5 mg/kg body weight at noon, 3, 6, and 9 p.m., midnight, and 3, 6, and 9 a.m., and they were sacrifice 3 h later. In total preparations of each retina 20,000 cells were examined. The colcemid mitotic index (MI_{col}) was calculated in promille.

EXPERIMENTAL RESULTS

The results are given in Table 1 and Fig. 1. The ordinary rhythm of mitosis with high values of MI_{col} in the morning and afternoon and low values in the evening was found in the corneal epithelium of the control mice. MI_{col} reached a maximum between 6 and 8 a.m. and a minimum between 6 and 9 p.m. ($P = 0.0001$). The character of the curve of mitotic activity throughout the 24-h period coincided with the diurnal curve of c-mitoses in the corneal epithelium obtained in experiments on noninbred albino mice [5] and C57BL mice [4]. However, the total number of cells commencing mitosis during the 24-h period was greater (27.8%) in CBA mice than in noninbred (18.0%) or C57BL mice (20.3%). Consequently, the intensity of cell multiplication in CBA mice was higher than in mice of the other lines studied. Regeneration of the liver, it should be noted, also follows a more rapid course in CBA mice than in animals of the C57BL line, which have low reactivity [3]. CBA mice, with a high level of immunologic reactivity, must also possess a higher level of mitotic activity in their epithelial tissue.

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TABLE 1. Diurnal Changes in MI_{col} in Corneal Epithelium after Splenectomy

Time of day	Control (mock operation)		Splenectomy		
	$MI_{col} \pm m$, ‰	P_2	$MI_{col} \pm m$, ‰	P_2	P_{1-2}
Noon-3 p.m.	46,1±2,9	0,001	23,2±2,7	—	0,01
3-6 p.m.	23,0±2,4	0,021	25,7±4,2	0,074	—
6-9 p.m.	11,7±2,7	—	13,8±3,6		—
9 p.m.-midnight	12,5±1,2	0,184	14,5±3,0	—	—
midnight-3	24,0±7,3	—	10,8±1,6	0,172	0,115
3—6 a.m.	47,5±3,5	0,027	18,9±5,0		0,002
6—9 a.m	58,6±1,1		42,5±4,0	0,008	0,008
9 a.m.-noon	54,7±3,1	—	51,8±5,0	0,177	
	Total number of c-mitoses during 24 h period: 278.1 ‰.		Total number of c-mitoses during 24 h period: 201.2‰.		

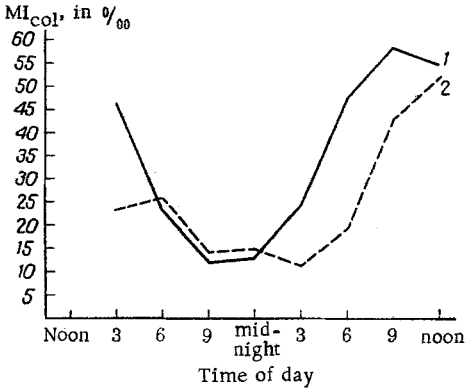

Fig. 1. Diurnal changes in index of c-mitoses in corneal epithelium of mice after splenectomy. 1) MI_{col} in control, 2) MI_{col} in experiment. Abscissa, time of day; ordinate, index of c-mitoses in promille.

Splenectomy led to considerable disturbances of mitosis in the corneal epithelium (Table 1). During that part of the 24-h period that is characterized by an active phase of the rhythm of cell proliferation in control animals, MI_{col} in the cornea of the splenectomized animals was considerably lower than in the control at nearly all times of investigation. Whereas in animals undergoing the mock operation the increase in the number of mitoses occurred on the boundary between the dark and light periods of the day, in the experimental animals it was much later. The peak of the mitotic index in the splenectomized mice also was postponed until later. Rhythmic changes in the morphological and functional parameters of biological systems are known to determine their stability [8]. Any factor which ensures regularity of an oscillatory process must therefore be regarded as an important member of the system of reactions which constitute the temporal organization of the cell. The data on changes in the rhythm of cell proliferation after splenectomy obtained in these experiments indicate that the lymphoid system and, in particular, processes of interaction between cells taking place in the spleen, somehow influence the entry of cells into mitosis in epithelial tissue and the formation of the "wave of mitosis" during the period of increased mitotic activity.

In splenectomized mice the total number of mitoses blocked during the 24-h period by colcemid was 20.1%, or 72.3% of the control value. The renewal time of the corneal epithelium of the mice was correspondingly lengthened as a result of splenectomy – from 3.6 days in the control to 5 days in the experiment. The rate of physiological regeneration was thus slowed. These findings correlate with results [7] showing a decrease in the mitotic activity of hepatocytes in the regenerating mouse liver when the spleen was removed 48 h before partial hepatectomy.

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IMMUNOHISTOCHEMICAL STUDY OF DIFFERENTIATION OF THE CEPHALIC LOBE OF THE CHICK EMBRYONIC ADENOHYPOPHYSIS

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It was shown by the method of indirect immunofluorescence that ACTH appears in the cephalic lobe of the adenohypophysis of chick embryos after the 8th day of development. A new tissue-specific antigen (A3) was found in the adenohypophysis: It is located in the cephalic lobe and appears on the 7th day of embryonic development. It is concluded from the results of quantitative analysis of the distribution of ACTH and antigen A3 in cells of the adenohypophysis of 11-day chick embryos that antigen A3 is present in the corticotropic cells.

KEY WORDS: chick embryonic adenohypophysis; ACTH; tissue-specific antigen; immunofluorescence.

It has been shown by immunologic methods that ACTH in birds is contained in the cephalic lobe of the adenohypophysis [6, 7, 9]. The period of specific differentiation of the adenohypophysis in chick embryos begins with the 6th day of development. ACTH is found immunohistochemically after the 9th day [8] although, as the results of biological tests have shown, corticotropic activity appears on the 8th day [10, 12] and melanocyte-stimulating activity, also characteristic of ACTH, appears on the 5th day [5, 11] or at 6-6.5 days of development [4]. To discover the principles governing differentiation of the embryonic adenohypophysis it is interesting to study the dynamics of appearance not only of hormones, but also of tissue-specific antigens [1, 2]. The object of the present investigation was to make a comparative study of the appearance of ACTH and the tissue-specific adenohypophyseal antigen A3 and their localization in the adenohypophysis of chick embryos.

EXPERIMENTAL METHOD

Adenohypophyses of chick embryos (from Russian White hens) at 6-11, 13, 15, and 18 days of development and 6-day-old chicks were investigated. In some experiments the hypophyses of 8-day quail embryos, rabbits, a 20-week human fetus, and 11-, 15-, and 18-day CBWA mouse embryos also were used. Tissues were fixed in Bouin's mixture and embedded in paraffin wax; serial sections were cut to a thickness of 5 μ and treated by the indirect immunofluorescence method [3].

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