Adaptation of the animals was shown to have no significant effect on the character of the dynamics of the plasma 5α-dihydrotestosterone level during the 24-h period. Both in intact and in adapted animals negative correlation was observed between it and the testosterone concentration.

Adaptation of the animals to the experimental conditions led to a very small increase in the mean daily androstenedione level (Table 1). As Fig. 2 shows, there was a moderate decrease in the steroid concentration in the blood of animals adapted for 1 month in the second half of the day, but in the morning its concentration also was a little below the initial level.

The dehydroepiandrosterone level fell steadily during the afternoon in both intact and adapted animals to reach a minimum during the evening. A distinct circadian rhythm of the steroid was found with its acrophase in the early morning.

Thus 5α-dihydrotestosterone, androstenedione, and dehydroepiandrosterone were characterized by a fall in their blood concentration in the evening and a rise in the early morning, whereas in the case of testosterone, one of the principal sex hormones, synthesized in the testes, a circadian rhythm was found with its acrophase in the evening. The clearest circadian rhythms were noted for testosterone and dehydroepiandrosterone.

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IMMUNOCOMPETENT CELLS IN THE BED OF A FULL-THICKNESS SKIN ALLOGRAFT TRANSPLANTED AT DIFFERENT TIMES OF DAY

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KEY WORDS: skin allografting; immunocompetent cells; biorhythms.

The possibility of using natural biological time factors (rhythms) to change the state of a graft has been demonstrated by studies of the chronobiology of skin grafts [10]. The effect of the time of day when the transplantation was done on the degree of destruction of the epithelium of the skin graft and its infiltration by polymorphonuclear leukocytes and lymphocytes, and on the number of viable epitheliocytes was also demonstrated previously [2].

The object of this investigation was to compare the dynamics of the number of immunocompetent cells in the bed of a skin allograft in mice in the course of its rejection, depending on the time of the day of the skin grafting operation.

EXPERIMENTAL METHOD

The organization and conditions of the experiments, the technique of transplantation, the method of obtaining material, and the histological treatment of the grafted skin were all described previously [2]. The number (in units/mm^3) of lymphocytes, immunoblasts, plasmablasts, and juvenile and mature plasma cells was counted in a standard volume of tissue ($648 \cdot 10^{-6}$ mm^3) in the bed of the graft. The significance of differences was determined by the Wilcoxon–Mann–Whitney criterion and also by Student's t test for series with tied pairs.

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TABLE 1. Estimation of Number of Immunocompetent Cells (thousands/mm³) after Transplantation at Different Times of Day

Type of cell	Time of operation, h	Mean value of parameter between		Significance of difference (P) for operation performed at		
		66 and 86 h after operation	136 and 156 h after operation	midnight	8 a.m.	4 p.m.
Lymphocytes	midnight	1,8	4,1		0,05	0,05
	8 am	0,50	6,7	0,05		—
	4 pm	0,14	0,40	0,05	0,05	
Immunoblasts and plasmablasts	midnight	0,10	0,44		—	—
	8 am	0,10	0,48	0,05		0,05
	4 pm	0	0,17	—	0,05	
Plasma cells	midnight	1,2	0,38		0,05	0,05
	8 am	0,28	1,5	0,05		0,05
	4 pm	0	0,07	0,05	0,05	

Legend. P above main diagonal denotes after 66-86 h, below it — after 136-156 h.

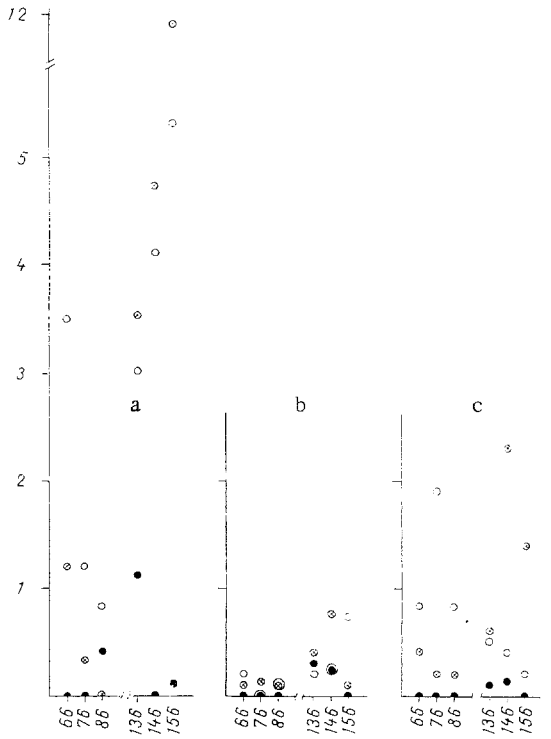

Fig. 1. Number of immunocompetent cells in bed of skin graft transplanted at different times of day. Abscissa, time after operation (in h); ordinate, number of cells per unit volume of tissue (in thousands/mm³). Empty circles — transplantation at midnight; circles with crosses — at 8 a.m.; filled circles — at 4 p.m. a) Lymphocytes; b) immunoblasts and plasmablasts; c) plasma cells.

EXPERIMENTAL RESULTS

Individual values of the parameters studied are given in Fig. 1 and the statistical significance of their differences in Table 1.

Differences in the number of lymphocytes in the graft bed after operations performed at midnight and at 8 a.m., and at midnight and at 4 p.m., counted 66, 76, and 86 h after transplantation, were statistically significant (Fig. 1a). The largest number of lymphocytes was recorded after transplantation at night and the smallest after transplantation in the afternoon. In previous experiments, the number of lymphocytes in the connective-tissue part of the graft and epidermis fell between 66 and 86 h after an operation performed in the afternoon, but an increase in the number of these cells was soon (after 10-20 h) observed in the grafted skin [3, 4], indirect evidence of the possible migration of lymphocytes (probably T killer cells) from the bed directly into the tissue of the grafted cells, for processes of partial regeneration could be observed in them at this period. Differences in the number of immunoblasts and plasmablasts in this period were not statistically significant (Fig. 1b). The greatest number of plasma cells was observed after transplantations done at midnight (Fig. 1c) but differences in the number of plasma cells after operations done at 8 a.m. and 4 p.m. also were statistically significant (the number was least at 4 p.m.). Differences in the number of these immunocompetent cells were seen more distinctly 136, 146, and 156 h after transplantation. The number of lymphocytes in tissues of the graft bed after an operation performed at 8 a.m. was statistically significantly greater than the number after operations at midnight and 4 p.m., and the number after grafting at midnight also was greater than after an operation at 4 p.m. (Fig. 1a). The number of less differentiated blast forms differed statistically significantly after transplantations done at 8 a.m. (when the number was greatest) and at 4 p.m. (Fig. 1b). The maximal number of juvenile and mature cells during this period was observed after operations at 8 a.m., and differences in their number when results of transplantation at midnight and 4 p.m. were compared also were statistically significant (Fig. 1c). The data showing the number of immunocompetent cells in the bed after operations done at 4 p.m. agree with the time course of the number of lymphocytes infiltrating the epidermis and dermis of the graft, which is characterized by a decrease in their number between 136 and 156 h after the operation [4]. These also broaden our views on quantitative criteria reflecting cellular and tissue reactions in skin grafts [1, 12].

The statistically significant decrease in the number of immunocompetent cells found after transplantations in the afternoon is comparable with observations on circadian rhythms in mice, in which ACTH production by the anterior pituitary is maximal at about 10 a.m., whereas production of corticosterone, which is an immunodepressant, by the adrenals and its concentration in the blood serum are maximal at 4-5 p.m. [9].

These data, as well as observations on regeneration of different tissues and organs [5-8, 11] and the chronobiology of skin grafting [10] are evidence of a significant effect of the circadian phase of the state of tissue and organ systems on the course and intensity of cell and tissue reactions during reparative regeneration and after transplantation. Considering previous results of a study of tissue reactions during rejection of a skin allograft transplanted at different times of day [2], it can be postulated provisionally that 8 a.m. is a less favorable time for skin allografting in mice, whereas 4 p.m. is a more favorable time. This indicates progress toward the choice of optimal time of day for transplantation in accordance with the state of donor and recipient at that time.

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EFFECT OF CHRONIC STRESS ON CELL DIVISION IN THE CORNEAL AND LINGUAL EPITHELIUM

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A single exposure or five exposures to stress (moderate contact hypothermia, fixation stress, injections of pyrogenal) can inhibit mitotic activity by lengthening the G_2 phase. The level of pathological mitoses (PM), index of labeled nuclei (ILN) and the intensity of labeling (IL) were unchanged under these circumstances [7, 8]. On repeated exposure to severe stress (sublethal hyperthermia, hypoxia) DNA synthesis was activated and mitotic activity stimulated [1, 5].

The object of this investigation was to study the character of the effect of prolonged exposure to stress on epithelial proliferation.

EXPERIMENTAL METHOD

Experiments were carried out on male albino rats weighing 180-200 g. Chronic stress was simulated by cooling the animals for 1.5 h daily to 30-28°C for 28 and 35 days by the method described previously [6]. The animals were sacrificed at 4 p.m. (48 h after the last exposure to cold). The rats received an injection of [^{3}H]thymidine in a dose of 0.6 μCi/g 1h before sacrifice. The corneas were incubated for 1 h at 37°C in medium 199 with [^{3}H]-thymidine (concentration 2 μCi/ml). In one series of experiments, 2 h before sacrifice the rats were injected with colchicine in a dose of 2 μg/g, after which the index of colchicine-blocked mitoses (MIC) was determined. The mitotic index (MI), and the levels of PM, MIC, ILN, and IL were determined by the method described previously [2]. MI and MIC were expressed in promille, PM as a percentage of the total number of mitoses, and IL as the mean number of tracks above the labeled nucleus. To prove the development of a stress reaction, the animals' body weight, thymus index, and index of weight of the adrenals were determined. The adrenalin concentration in the adrenals was measured by the method in [4]. Altogether 96 animals were used in the experiments. The results were subjected to statistical analysis by Student's method.

EXPERIMENTAL RESULTS

The results showed that exposure to contact hypothermia for 28 days causes the development of a stress reaction. This was confirmed by the decrease in the animals' total body weight and the index of weight of the thymus, by an increase in the index of weight of the adrenals, and a decrease in the adrenalin concentration in the adrenals (Fig. 1). A study of cell division showed that exposure to stress for 28 days led to activation of DNA synthesis in the corneal and lingual epithelium. ILN in the cornea and tongue of the experimental animals was increased compared with the control by 1.5 and 1.4 times respectively. IL in the cornea also was increased by 1.5 times. However, neither in the tongue nor in the cornea did MI undergo any significant changes (Table 1). In rats exposed to hypothermia for 28 days, and receiving colchicine 2 h before sacrifice, preventing any change in the time of mitosis, MIC in the cornea and tongue was 2.3 and 1.8 times higher respectively than the control values. Since the experiments with colchicine were conducted asynchronously with

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