Long-term organ cultures of the primordial mouse RT, both intact and isolated from mesenchyme, were thus obtained. As a result, differences were discovered in the growth powers of the proximal and distal portions of RT and it was shown that long-term disturbance of epithelial-mesenchymal interactions leads to cessation of morphogenesis and to a reduction in the growth powers of the epithelium, and also that important deviations in differentiation and bkological properties of the cells arise in epithelium deprived of the normal inducing influence of the mesenchyme, as is shown by the appearance of foci of proliferation of atypical cells in the experimental cultures.

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CORRELATION BETWEEN CIRCADIAN RHYTHMS OF LYMPHOCYTE RECIRCULATION AND cAMP SYNTHESIS

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KEY WORDS: circadian rhythm, lymphocytes, thymectomy, adenylate cyclase, cyclic nucleotides.

One of the principal processes supporting function of the immune system is continuous redistribution of cells, in accordance with a regular spatiotemporal organization that is reflected in the circadian rhythms of the number of cells in the lymphoid organs and the number of lymphocytes in the peripheral blood [3]. Recirculation and migration of individual subpopulations of lymphoid cells are synchronized with each other in a definite manner, and also with cyclic fluctuations of the functional state of the lymphocytes and the associated level of intracellular metabolism [3, 8-10]. In our opinion, the study of correlation between circadian rhythms of recirculation of immunocompetent cells, and the turnover of cyclic nucleotides — universal regulators of differentiation and function of cells [1], in them is a matter of great interest. In particular, it has been shown that differentiation of lymphocytes, mainly T cells, is accompanied by elevation of the intracellular cAMP level, with immediate participation of adenylate cyclase (AC), the enzyme catalyzing conversion of ATP into cAMP [4, 11].

Analysis of correlations between circadian rhythms of the parameters of distribution of lymphocytes and rhythms of AC activity in them could help to elucidate the principles and mechanisms of regulation of temporal coordination between such fundamental processes in the immune system as cellular migration, recirculation, and differentiation. The investigation described below was devoted to a study of these problems.

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EXPERIMENTAL METHOD

Swiss mice, intact and thymectomized at the age of 3 months, were used. The thymectomized mice were investigated 2 months after the operation. The animals were killed at 4 and 10 a.m. and 4 and 10 p.m. during the first day. The number of cells in suspensions of the thymus, spleen, and peripheral blood, taken from the caudal vein, was counted in a Goryaev's chamber. The leukocyte formula of the blood was determined in films stained by the Romanovsky–Giemsa method. AC was detected cytochemically in films from suspensions of lymphoid organs and peripheral blood [2]. Activity of the enzyme was estimated visually, and the number of cells with high (++), average (+), and low (0) enzyme activity in the films was counted. The intensity of the immune response to sheep's red blood cells (SRBC) was estimated from the number of antibody-forming cells (AFC) in the spleen on the 4th day after intraperitoneal injection of the antigen [5]. All the numerical data were subjected to statistical analysis by Student's t test and the coefficient of correlation was determined.

EXPERIMENTAL RESULTS

Determination of the number of cells in the lymphoid organs and the number of lymphocytes in the peripheral blood showed significant circadian fluctuations of these parameters. These fluctuations in the spleen and peripheral blood coincided in phase, but were opposite in phase to the circadian rhythm of the number of cells in the thymus (Fig. 1). The coefficient of correlation between the cell content of the thymus and spleen was –0.810, whereas no correlation was found between the cell content of the thymus and the number of peripheral blood lymphocytes, possibly due to predominance of recirculation processes in the bloodstream and the temporary localization of the process of cell migration from the thymus toward peripheral lymphoid organs. The negative character of correlation betweeen the number of cells in the thymus and spleen probably reflects the order of emigration of the thymocytes and their colonization of T-cell zones of the spleen. To study correlation between the times of lymphocyte recirculation and cAMP synthesis in the lymphocytes, the data on the total number of nucleated cells in the thymus and spleen, the lymphocyte concentration in the peripheral blood, and the relative percentages of cells with high AC activity (AC^{++} cells) among them, were subjected to correlation analysis. Positive correlation of average strength was found between the circadian rhythm of the total number of nucleated thymus cells and the fractions of AC^{++} cells among them (r = 0.489). The data for peripheral blood showed much weaker correlation (r = 0.21), and correlation in the spleen was negative (r = –0.628). These data can be interpreted as evidence that circadian fluctuations of the cellular composition of the thymus are due to cells with a high level of cAMP synthesis. Fluctuations of the intensity of cAMP metabolism in the peripheral blood and of redistribution of the cells are probably shifted in phase and are su-

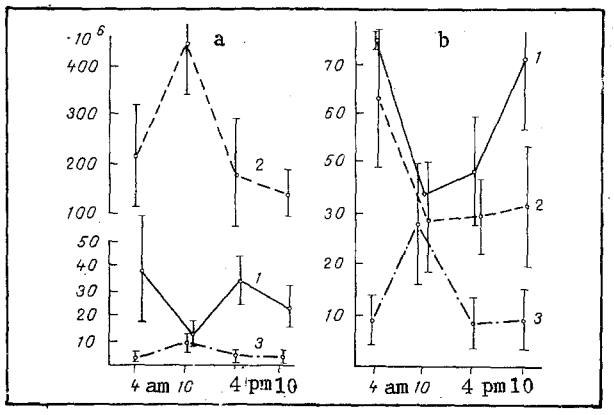

Fig. 1

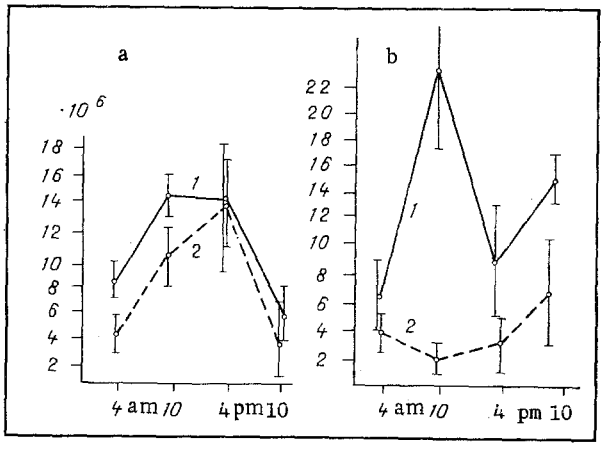

Fig. 2

Fig. 1. Circadian rhythms of number of cells (a) and relative percentage of AC^{++} lymphocytes among them (b) in thymus (1), spleen (2), and peripheral blood (3) of intact mice. Here and in Figs. 2 and 3: abscissa, clock time.

Fig. 2. Circadian rhythm of number of lymphocytes in 1 ml peripheral blood (a) and relative percentage of AC^{++} lymphocytes among them (b) in intact (1) and thymectomized (2) mice.

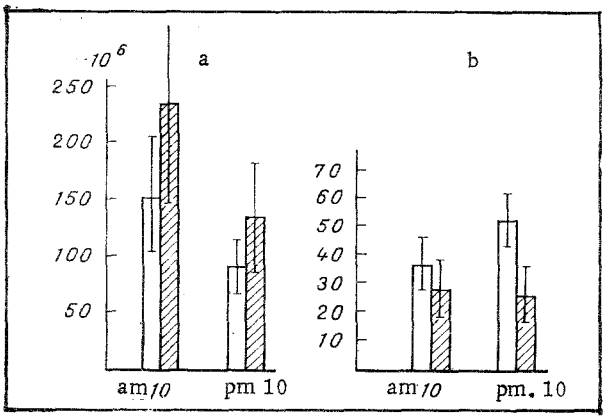

Fig. 3. Circadian fluctuations of the number of cells in the spleen (a) and relative proportion of AC^{++} splenocytes among them (b) in intact (unshaded columns) and thymectomized (shaded columns) mice.

perposed on one another because the pathways of circulation of lymphocytes with different levels of cyclic nucleotide metabolism cross in the bloodstream. In the spleen, however, circadian fluctuations of cell composition take place mainly on account of cells with low enzyme activity. Injection of SRBC into the animals during the daytime, when AC activity in peripheral blood and splenic lymphocytes is high, promotes the generation of more AFC in the spleen than immunization during the evening or at night (187.0 ± 55.0 and 49.0 ± 13.5 plaque-forming cells per 10^6 spleen cells, respectively). The daytime increase in AC activity in the peripheral lymphocyte pool thus probably reflects stimulation of their functional activity at this time of day.

To study the role of the thymus as a possible regulator of biorhythms of immune functions, circadian fluctuations in the above-mentioned parameters were investigated in mice thymectomized at the age of 3 months, 2 months after the operation. Thymectomy did not change the character of the circadian rhythm of the number of lymphocytes in the peripheral blood, but the relative percentage of AC^{++} lymphocytes in the thymectomized mice was lower at all time points of the investigation, and there was no circadian rhythm of this parameter. The greatest difference between AC activity in the experiment and control was observed at 10 a.m., when the number of AC^{++} cells recorded in intact mice reached a maximum (Fig. 2). Negative correlation appeared in the thymectomized animals between the number of lymphocytes in the peripheral blood and the relative percentage of AC^{++} lymphocytes among them ($r = -0.645$). In the spleen the circadian rhythm of the number of cells was unchanged in the absence of the thymus, but the relative proportion of AC^{++} splenocytes among them fell at those times of the day when the value of this parameter recorded in intact animals was maximal (Fig. 3). The coefficients of correlation between the number of cells and enzyme activity in the spleen remained negative, but it was weaker than normal ($r = -0.234$).

These results are evidence that although the thymus has little effect on redistribution of the total pool of peripheral lymphocytes, it does control the circadian rhythm of cAMP in them, possibly through the production of hormonal factors [11]. Disturbance of the time sequence of recirculation and cAMP synthesis in the peripheral lymphocyte pool of the thymectomized mice can be regarded as the earliest manifestation of disturbances of immune homeostasis developing at the later stages after removal of the thymus [6, 7].

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EFFECT OF SYNGENEIC THYMOCYTES ON PROLIFERATION OF THE SMALL INTESTINAL EPITHELIUM IN MICE

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KEY WORDS: intestinal epithelium; proliferation; thymocytes.

The principal function of the lymphoid system is controlling antigen-structural homeostasis [3, 7]. Data have been obtained in recent years to show that lymphocytes can not only eliminate genetically defective cells, but they can also regulate proliferation and differentiation of unchanged somatic cells [1, 2, 6, 12]. The study of the role of the lymphoid system in regulation of physiological regeneration, a process determining the dynamic constancy of the cellular composition of the tissues, is undoubtedly important.

The aim of the present investigation was to study the action of syngeneic thymocytes on proliferation of the epithelium of the mouse small intestine.

EXPERIMENTAL METHOD

Male CBA mice aged 3 months were used. The animals were divided into 4 groups. The mice of group 1 were given an intravenous injection of thymocytes from intact blood donors in a dose of $4 \cdot 10^7$ cells 18-20 h before an injection of ^{3}H-thymidine. Animals of group 2 were injected with hydrocortisone-resistant thymocytes in a dose of 10^7 cells. Two days before sacrifice the cell donors were given an injection of hydrocortisone in a dose of 2.5 mg per mouse. Animals of group 3 were injected with 0.5 ml of medium 199. The mice of group 4 remained intact. All animals were given an intraperitoneal injection of ^{3}H-thymidine in a dose of 1 μCi/g body weight at 9 a.m. and killed 1 h later. A segment of jejunum 2 cm long, taken 1 cm distally to Treitz' ligament, was fixed in Carnoy's fluid. Paraffin sections were coated with type M photographic emulsion, exposed for 2 weeks at 4°C, and developed in amidol developer. Histoautoradiographs were stained with Mayer's hemalum. To study the zone of proliferation, the crypts were selected and divided along their longitudinal axis. The role of enterocytes from the midpoint on the floor of the crypt to the base of the villus was described as a "cryptal column" (CC). The total number of enterocytes and the number of labeled cells in CC was counted and a curve of the distribution of CC plotted on the basis of the number of DNA-synthesizing cells. The index of labeled cells (labeling index — LI) was determined both for the total number of enterocytes in CC and for each cell position. Curves of distribution of LI based on cellular positions of CC were plotted. Intraepithelial leukocytes were excluded from analysis.

The numerical data were subjected to statistical analysis by Student's test at a level of significance of $P \leq 0.02$.

EXPERIMENTAL RESULTS

The animals receiving an injection of medium 199 were indistinguishable from intact mice with respect to all parameters studied. The procedure of intravenous injection evidently does not affect the state of the intestinal epithelium, and these two groups can be regarded as an

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