passed synchronously through MC during the 1st day played a considerable part in the formation of acrophases of MI on subsequent days (experiments of series I). This suggests that some cells of the synchronous population preserved relatively high proliferative ability for several days. This, in turn, can be explained on the grounds that the duration of MC of these cells was comparable with the period of the rhythm [2], and for that reason these cells could be restimulated to pass through the next MC after completing the previous cycle. It must be noted that some cells which passed synchronously through the first MC probably then passed into the G_0 phase, in which they remained for 2-3 days, and were capable of responding to the synchronizing stimulus on the 3rd-4th day of the experiment.

Meanwhile the contribution of the population proliferating asynchronously for 24 h to the formation of acrophases of the circadian rhythm of MI was very small (experiments of series II). However, this population also remained capable, to a certain degree, of responding later (on the 3rd day) to the action of the synchronizer.

Investigation of ILM also showed that an approximately equal fraction of the cells of the labeled populations of the two series takes part in proliferation during periods of low mitotic activity in the circadian rhythm For instance, in the interval from 2 p.m. to 2 a.m. every day 10-17% of mitoses were labeled. Consequently, the population of cells proliferating synchronously during the 1st day of the experiment took part on subsequent days in the formation both of acrophases and of troughs of MI. Meanwhile the asynchronously proliferating population, which played virtually no part in acrophase formation, proliferated sufficiently actively during troughs of MI.

It can be concluded from the facts described above that the kinetics of populations passing through MC at different times of the 24-h period differs significantly.

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CHANGES IN RESPONSE OF CIRCULATING COLONY-FORMING UNITS TO ACTH IN THYMECTOMIZED MICE

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With increased concentrations of endogenous glucocorticoids the number of hematopoietic stem cells circulating in the peripheral blood decreases and migration of colony-forming units (CFUs) from a region of bone marrow screened during irradiation is inhibited [1, 2]. There is experimental evidence also of the role of T lymphocytes and thymus hormones in regulation of the function of hematopoietic stem cells, including in their recirculation *in vivo* [5-9, 11].

The object of this investigation was to study responses of circulating CFUs to elevation of the endogenous glucocorticoid level in thymectomized mice.

EXPERIMENTAL METHOD

Experiments were carried out on (CBA × C57Bl)F_1 mice of both sexes weighing 22-28 g. To determine the number of circulating CFUs blood was collected in a heparinized vessel from five decapitated donor mice and the number of leukocytes in it was counted. The blood thus obtained was diluted with medium 199 in the ratio of 1:1 and injected in a volume of 0.4 ml into the caudal vein of syngeneic lethally irradiated recipients. The number of macro-

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TABLE 1. Number of Circulating CFUs 2 h after Injection of ACTH at Various Times after Thymectomy (TE)

Experimental conditions	Time after TE	Number of CFUs in blood	
		per 1 ml blood	per 10^6 leukocytes
Mock TE + physiological saline	2 weeks	57,0±4,5 (9)	22,6±1,8
Mock TE + ACTH		23,0±6,0*(10)	12,0±3,1*
TE + physiological saline		51,5±5,0 (10)	18,1±1,8
TE + ACTH		43,5±4,5 (11)	15,2±1,8
Mock TE + physiological saline	5 weeks	40,5±5,0 (10)	12,1±1,5
Mock TE + ACTH		21,5±3,5* (9)	7,3±1,2*
TE + physiological saline		42,0±7,5 (8)	15,1±2,7
TE + ACTH		41,5±11,5 (7)	20,0±5,5
Mock TE + physiological saline	2 months	41,0±5,5 (10)	15,8±2,1
Mock TE + ACTH		25,5±3,0*(10)	8,9±1,0*
TE + physiological saline		31,0±6,5 (9)	8,4±1,8
TE + ACTH		36,5±7,5 (10)	8,1±1,7
Mock TE + physiological saline	3 months	35,0±5,0 (10)	15,0±2,1
Mock TE + ACTH		16,5±3,0* (9)	6,4±1,2*
TE + physiological saline		17,0±2,5 (8)	4,6±0,7
TE + ACTH		17,0±3,0 (9)	5,3±0,9
Mock TE + physiological saline	5.5 months	37,0±4,5 (10)	8,6±1,0
Mock TE + ACTH		17,0±2,5* (10)	5,0±0,7*
TE + physiological saline		30,0±4,5 (9)	13,2±1,9
TE + ACTH		53,0±3,5*(9)	13,0±0,9

TABLE 2. Blood 11-HCS Level and Response to ACTH in Mice after Thymectomy (TE) and in Control Mice

Experimental conditions	Time after TE	11-HCS concentration, μg %	
		initial	2 h after injection of ACTH
Mock TE	5 weeks	9,3±1,3 (7)	75,6±2,7 (7)
TE	5 weeks	5,4±1,3 (7)	83,9±4,4 (7)
Mock TE	3 months	13,2±2,5 (5)	Not determined
TE	3 months	10,8±1,0 (5)	The same

scopic colonies in the spleen of the recipients was determined 8 days later [13]. Thymectomy and a mock operation were performed on mice aged 6 weeks [12]. To create temporary hypercorticism, the donor mice were given an intraperitoneal injection of ACTH in a dose of 0.05 unit/g body weight (corticotrophin, produced by the VNIITKGP, Moscow), and 2 h later the 11-hydroxycorticosteroid (11-HCS) concentration in the blood plasma was determined [10]. Animals of the control groups each received an injection of 0.5 ml physiological saline. The tests were carried out at intervals between 2 weeks and 5.5 months after thymectomy. The significance of differences was calculated by Student's test.

EXPERIMENTAL RESULTS

It will be clear from Table 1 that the level of circulating CFUs 2 and 5 weeks after thymectomy did not differ from the control values. After 2 and 3 months this parameter fell, as described previously [5]. After 5.5 months the number of CFUs did not differ significantly from the control. The blood 11-HCS concentration showed a sharp increase 2 h after injection of ACTH into mice undergoing mock thymectomy (Table 2) and a regular decrease in the number

of CFUs was found, when calculated both per milliliter of blood and per 10^6 leukocytes. The number of leukocytes in the blood under these circumstances fell moderately (by 21-23%) or remained virtually unchanged. These results suggest that an increase in the concentration of endogenous glucocorticoids leads to a true fall in the number of stem cells circulating in the blood. Similar results were obtained by the writers previously on intact mice [4]. So far as thymectomized mice are concerned (Table 1), 2 weeks after the operation injection of ACTH no longer led to a decrease in the number of circulating CFUs (a decrease of 16%, which was not significant). At all subsequent times after thymectomy (from 5 weeks to 5.5 months) the response of the circulating CFUs to injection of ACTH was completely absent. The character of the changes in the leukocyte count in the peripheral blood after injection of ACTH was the same in general as in the control animals. Only after 5.5 months did injection of ACTH cause a significant increase (by 79%) in the leukocyte count in the experimental mice, which was accompanied by an increase in the number of CFUs, calculated per milliliter. Abolition of the response to CFUs to ACTH in the thymectomized animals was evidently not connected with a decrease in adrenocortical reactivity: Five weeks after thymectomy the rise in the 11-HCS level after injection of ACTH was the same as in mice undergoing the mock operation; 3 months later the initial 11-HCS concentration in the experiment also was practically identical with that in the control (Table 2).

The experiments thus showed that the fall in the level of circulating CFUs on account of an increase in the concentration of endogenous glucocorticoids is a thymus-dependent process. On the basis of modern views on the character of interaction between the lymphoid system and hematopoietic stem cells [11], it can be concluded that abolition of the response of the blood CFUs to ACTH in thymectomized animals is connected with elimination of the short-living subpopulation of T lymphocytes (T_1), which are known to disappear from the body within 2-8 weeks after removal of the thymus in adult mice. Restoration of the lowered rate of migration of CFUs in thymectomized mice after transplantation of syngeneic thymus or lymph node cells into them [5] also indicates close dependence of stem cell recirculation on T cells. The effect of endogenous glucocorticoids on circulation of CFUs is evidently mediated through interaction of the hormone with T_1 lymphocytes. Probably the presence of thymus-dependent lymphocytes in the body is essential for maintaining the normal number of CFUs in the peripheral blood and for exertion of the regulating influence of glucocorticoids. It can also be assumed that abolition of the response of the circulating CFUs to ACTH may be connected with a decrease in the mobile pool of bone-marrow stem cells in thymectomized mice and with absence of T_1-CFUs interaction at bone marrow level. The problem of the possible role of thymic hormone deficiency in this phenomenon requires experimental study.

In recent years the view has been formed that functional activity of stem cells is to some degree under control of the T cell system [6, 11] and the pituitary-adrenal system [3, 4]. The data described in this paper provide a link between these two control mechanisms.

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