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HEMATOPOIETIC ORGANS OF MICE AFTER A SINGLE INJECTION OF HYDROCORTISONE

V. N. Shvets and V. V. Portugalov*

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The method of exogenous cloning of hematopoietic stem cells (colony-forming units — CFU) in the spleen and bone marrow of lethally irradiated recipients was used to study the population kinetics and direction of differentiation of CFU from mice receiving a single dose (5 mg per mouse) of hydrocortisone. Against the background of prolonged involution of the lymphoid tissue changes took place in the population and differentiation of CFU. Meanwhile the CFU concentration in the spleen and femoral bone marrow of the mice remained constant. After administration of the hormone, the powers of differentiation of CFU from spleen and bone marrow changed sharply in opposite directions: Marrow CFU behaved like splenic CFU whereas splenic CFU behaved like marrow CFU of normal mice. It is suggested that these effects are due to redistribution of T lymphocytes and not to the direct cytotoxic action of hydrocortisone on the CFU population.

KEY WORDS: hematopoietic organs; hydrocortisone; stem cells; differentiation.

Injection of corticosteroids into animals and man causes incidental involution of the lymphoid organs, with rapid and profound lymphocytopenia. These changes take place chiefly as a result of destruction of immunologically immature, cortisone-sensitive and a redistribution of immunologically competent, cortisone-resistant T lymphocytes [2-5, 11]. It is therefore natural to suggest that steroid hormones have a direct or indirect effect on the pool of hematopoietic stem cells for, on the one hand, stem cells are morphologically equivalent to lymphocytes and, on the other hand, they are closely interconnected with T lymphocytes [1, 6, 9].

The object of this investigation was to study the possible role of hydrocortisone in regulation of the number and differentiation of stem cells taking place during involution of lymphoid tissues.

*Corresponding Member of the Academy of Medical Sciences of the USSR.

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EXPERIMENTAL METHOD

Experiments were carried out on female F_1(CBA × C57BL) mice aged 2-3 months, divided into two groups: experimental and control. The experimental animals received an intraperitoneal injection of hydrocortisone acetate (17-hydroxycorticosterone) in a dose of 5 mg per mouse. In the mice of both groups the thymus, spleen, inguinal lymph nodes, and femoral bone marrow were removed 1.5 and 5 h, and also 1, 2, 4, 6, 7, 15, 25, 32, and 40 days after a single injection of the hormone. The lymphoid organs were weighed. The spleen, thymus, and bone marrow were suspended to count the total number of nucleated cells. The population of stem cells (CFU) in the bone marrow and spleen was studied by the exogenous cloning method [14] in a lethally irradiated (950-990 R) syngeneic recipient. The recipients were irradiated with ^{137}Cs γ rays with a dose rate of 35 R/min. Nine days after transplantation of a standard number of cells (10^5 bone marrow and 10^6 spleen cells) the spleen and femora were removed from 489* recipients. After fixation in Bouin's fluid, the number of colonies visible macroscopically in the spleen was counted. The spleen and femora were then embedded in paraffin wax; the number of colonies growing in the whole volume of the spleen and femoral marrow was counted in serial histological sections 5-6 μ thick, stained with hematoxyl—ineosin. In some experiments on the first day after injection of hydrocortisone, sedimentation factor (f) was determined by the method of Siminovitch et al. [13]. For statistical analysis of the results the method of confidence intervals ($P \leqslant 0.05$) was used.

EXPERIMENTAL RESULTS

The maximal reduction in weight of the lymphoid organs was observed on the second day after injection of hydrocortisone. The weight of the thymus decreased most significantly, that of the spleen and lymph nodes (inguinal) to a rather lesser degree. The weight of the lymphoid organs remained low for a long time and not until the 15th day of the experiment was slight recovery observed. By the 25th day the weight of all the lymphoid organs was close to its value in the control (Fig. 1A).

Parallel with the decrease in weight of the lymphoid organs the number of nucleated cells in them was reduced. The number of cells in the femoral marrow was almost unchanged (Fig. 1B).

*The repetitiveness of the experiments was two.

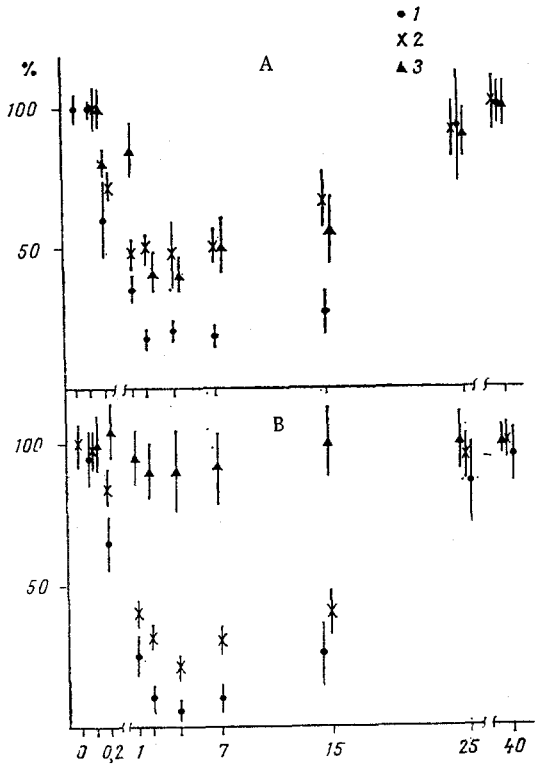

Fig. 1. Dynamic of changes in weight of lymphoid organs (A) and number of cells in them (B). 1) Thymus; 2) spleen; 3) inguinal lymph node (in A), femoral marrow (in B). Initial values: weight of thymus 50 ± 2.5 mg, spleen 80 ± 4 mg, lymph node 4 ± 0.2 mg. Number of cells (in millions) in thymus 142 ± 4, spleen 230 ± 4, bone marrow 22 ± 2. Abcissa, days after injection of hydrocortisone; ordinate, weight (in A) or number of cells (in B) (in % of initial values).

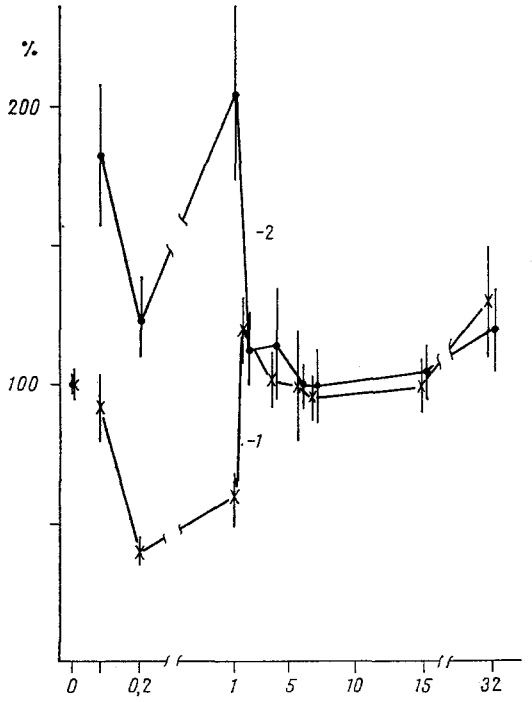

Fig. 2

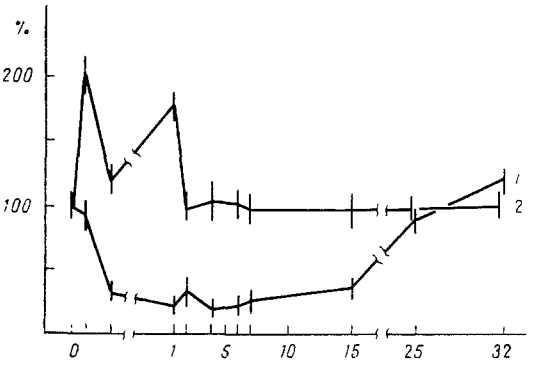

Fig. 3

Fig. 2. Number of colonies found in spleen after transplantation of spleen (1) and bone marrow (2) cells of mice receiving hydrocortisone. Initial number of colonies after transplantation of 10^5 bone marrow cells of intact mice 14.6 ± 0.3, after transplantation of 10^6 spleen cells 10 ± 0.2. Abscissa, days after injection of hydrocortisone; ordinate, number of colonies (in % of initial value).

Fig. 3. Changes in absolute number of CFU in spleen (1) and femoral marrow (2) of mice receiving hydrocortisone, allowing for CFU sedimentation factor in recipient's spleen. For CFU of bone marrow f = 0.2, for splenic CFU f = 0.17. Abscissa, days after injection of hydrocortisone; ordinate, number of CFU (in %).

In the first 24 h after injection of hydrocortisone the relative number of CFU increased in the bone marrow and decreased in the spleen (Fig. 2). Subsequently the number of CFU became stabilized at the normal level. The absolute number of CFU in the bone marrow of the experimental mice was unchanged except after 1.5 and 24 h, when it was significantly increased (Fig. 3). Meanwhile the absolute number of CFU in the spleen fell sharply in the first 24 h after injection of hydrocortisone. During the next four days the number of CFU remained at approximately the same level (Fig. 3), after which it increased slowly. Comparison of these data with the results of counting the spleen cells showed correlation between the kinetics of the change in number of CFU and the total number of nucleated cells.

Changes in the CFU population under the influence of hydrocortisone may be based on a number of mechanisms. The decrease in the number of CFU in the spleen evidently obeyed the same rules as the change in the remaining population of spleen cells. Redistribution of CFU, i.e., their migration from the spleen into the bone marrow, also is possible, or hydrocortisone may have a cytotoxic effect on CFU in the spleen and a proliferative effect on CFU of bone marrow. Finally, under the influence of hydrocortisone the CFU may modify the organ affinity of these cells on their subsequent transplantation into the recipient. In that case it would be expected that sedimentation factor would be increased for injected marrow CFU and reduced for splenic CFU in the recipient's spleen. Meanwhile, as the data in Table 1 show, hydrocortisone did not affect the distribution of bone marrow CFU: The same number of transplanted CFU was retained in the recipient's spleen and bone marrow as in the case of CFU from normal mice. Sedimentation factor for splenic CFU was unexpectedly increased both in the spleen and the bone marrow of the recipient. These results, in conflict with the dynamics of changes in the number of CFU, point to the absence of selective organ affinity of CFU under the influence of hydrocortisone.

TABLE 1. Distribution of CFU in Recipient's Spleen and Femoral Marrow (based on counting "splenic macrocolonies")

Source of CFU	Site of proliferation in recipient	Sedimentation factor (f)	
		normally	24 h after injection of hydrocortisone
Bone marrow	Spleen	0,17—0,24	0,18—0,24
	Bone marrow	0,015—0,018	0,02—0,022
Spleen	Spleen	0,12—0,21	0,36—0,66
	Bone marrow	0,03—0,04	0,05—0,08

Legend. Figures represent combined results of three experiments.

The results thus suggest two ways whereby hydrocortisone can influence the CFU population. It may be directly or indirectly through other target cells. The direct action of hydrocortisone on the CFU pool may be manifested as a cytotoxic or a proliferative effect. Meanwhile, despite the marked decrease in the absolute number of CFU in the spleen and its increase in the bone marrow, the CFU concentration in the two organs was not significantly changed except at certain times. For instance, 1.5 and 24 h after injection of hydrocortisone the number of CFU in the bone marrow increased from 0.1% normally to 0.18-0.21% in the experiment. Meanwhile the CFU concentration in the spleen remained constant at all times of observation except after 5 and 24 h, when it was reduced by about half (from 0.008% normally to 0.003-0.0045% in the experiment). These results indicate that hydrocortisone may have a cytotoxic action on splenic CFU or a proliferative action on bone marrow CFU. However, there is another possible explanation: redistribution of CFU from the spleen into the bone marrow, in the same way as the redistribution of T lymphocytes [3, 4, 11], on account of which the sensitivity of the CFU to the action of hydrocortisone is mediated through T lymphocytes which affect the number and differentiation of the CFU. The T cells are known to have a role in the regulation of the CFU population [6, 10]. Changes in the T lymphocyte population (destruction and redistribution) can evidently lead to changes in the colony-forming properties of the CFU. Under these circumstances redistribution of the T cells and, in particular, their migration from the spleen into the bone marrow [2-5, 11], may prove a more substantial factor than their destruction for CFU proliferation. This shift of the center of localization of T lymphocytes must be accompanied by a change in the potential powers of the CFU in the direction of erythropoiesis, for interaction between thymocytes and CFU leads to stimulation of erythropoiesis [7-9] and to the formation of predominantly erythroid colonies in the bone marrow of a syngeneic recipient [1]. Meanwhile migration of T lymphocytes from the spleen presupposes a reduction in the erythropoietic potential of the splenic CFU, for splenic CFU of normal mice from chiefly erythroid colonies in the recipient's bone marrow [1]. Among the population of spleen cells there are known to be 35% of T lymphocytes [12]. If the suggestion is true that changes in differentiation of CFU are based on cellular interaction between T lymphocytes and CFU, after injection of hydrocortisone the bone marrow CFU ought to behave like splenic CFU and splenic CFU like bone marrow CFU of normal mice. In fact, as a study of differentiation of CFU from mice receiving hydrocortisone showed, changes in differentiation of CFU from the two sources took place in different directions.

It will be clear from Table 2 that the erythropoietic potential of bone marrow CFU was increased, whereas that of the splenic CFU, on the contrary, was reduced. The granulopoietic potential of the bone marrow CFU was reduced whereas that of the splenic CFU was increased. All these changes applied to CFU proliferating in the medullary cavity of the recipients, whereas in the spleen, with the exception of bone marrow CFU, they did not change their character of differentiation. The erythropoietic potential of the latter was increased even in the spleen. Whereas bone marrow CFU of normal mice form predominantly erythroid colonies only in the spleen, and myeloid colonies predominate in the bone marrow, after injection of hydrocortisone these same cells thus formed predominantly erythroid colonies in both the bone marrow and spleen of the recipient. By contrast with bone marrow CFU, the splenic CFU of normal mice form predominantly erythroid colonies in both the spleen and the bone marrow, whereas after injection of hydrocortisone they formed predominantly erythroid colonies in the spleen only. It is easy to see that under the influence of hydrocortisone the potential of

TABLE 2. Ratio of Number of Erythroid to Number of Myeloid Colonies (E/M) for CFU from Different Sources, Forming Colonies in Recipients' Spleen of Femoral Marrow

Time after injection of hydrocortisone	E/M			
	splenic CFU		bone marrow CFU	
	in spleen	in femur	in spleen	in femur
0	5,5	1,4	2,1	0,6
1½ h	4,3	1,0*	2,7	0,6
5 h	4,0	0,4*	3,4	1,6*
1 days	3,0	0,4*	5,2*	1,5*
2 »	3,3	0,7*	5,0*	1,5*
4 »	3,0	1,0	4,0*	1,5*
6 »	3,7	1,4	2,6	1,6*
7 »	4,0	1,8	2,6	0,7
15 »	4,5	1,5	2,0	0,7
32 »	4,7	1,5	2,0	0,5

*Differences statistically significant at 0.05 > P > 0.01 level

splenic and bone marrow CFU to differentiate changed sharply: Bone marrow CFU behaved like splenic CFU whereas splenic CFU behaved like bone marrow CFU of normal mice. The disturbance of CFU differentiation took place only until the fourth to sixth days after injection of hydrocortisone. Subsequently these changes disappeared. It is still too early to draw final conclusions from these observations regarding the disturbance of CFU differentiation, for it is by no means certain that changes of differentiation in fact affect polypotent stem cells. The possibility cannot be ruled out that under the influence of hydrocortisone already commited CFU, more mature than stem cells, may take part in colony formation. Further experimental investigations will provide the answer to the question of whether CFU are self-supporting in the body under the influence of corticosteroids.

It can be concluded from the results described above that a single injection of hydrocortisone leads to a sharp decrease in weight of the lymphoid tissue. The course of recovery of the lymphoid tissue is slow and continues only until the 25th day after injection of the hormone. Changes in the lymphoid tissue are linked with a disturbance of the number and differentiation of the CFU. There is reason to suppose that the changes in differentiation and number of the CFU under conditions of hypercorticism are connected with redistribution of the T lymphocytes and not with the effect of the hydrocortisone itself.

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