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EFFECT OF ESTRONE ON FORMATION OF A FOCUS OF HETEROTOPIC HEMATOPOIESIS

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The considerable reduction in the cell content of the bone marrow in patients with myelofibrosis and in animals with an experimental model of that condition [1, 4] is generally associated with mechanical displacement of hematopoietic cells by newly formed bone tissue from the medullary cavity. To test this hypothesis, a model of estrone-induced myelofibrosis has been developed [5], in which relations between bone tissue and hematopoiesis in a heterotopic hematopoietic focus beneath the renal capsule in mice, where there is no closed medullary cavity, is assessed.

METHODS

Female (C56Bl × CBA)F_1 mice were used. Heterotopic transplantation of syngeneic bone marrow beneath the renal capsule was carried out on recipients anesthetized with hexobarbital, 7 days after ovariectomy. Myelofibrosis was induced by giving the experimental animals an injection of an oily solution of estrone in a dose of 0.5 mg daily for 4-6 weeks. The first injection of estrone was given one day after bone marrow transplantation. Control animals were given injections of peach oil in accordance with a similar scheme.

The newly formed focus of ectopic hematopoiesis was removed after one month. The dried bony capsule was weighed, and the number of myelokaryocytes counted in the focus and in the femur. The effect of estrone on formation of the focus of heterotopic hematopoiesis also was studied morphologically.

RESULTS

After a 4-week course of estrone injections active proliferation of bone trabeculae of the metaphysis was observed in the femur, accompanied by thickening of the diaphysis. A morphologically similar pattern was observed during the formation of a focus of heterotopic hematopoiesis after transplantation of syngeneic bone marrow beneath the renal capsule of experimental animals. Whereas the bony capsule in the focus in the control animals consisted of a comparatively thin lamina of bone tissue with very few trabeculae, lying only at the edges of the focus facing the kidney tissue, four injections of estrone caused the active formation of cancellous bone, the trabeculae of which filled the whole space of the newly formed organ. Hematopoietic tissue cells were located between the trabeculae (Fig. 1).

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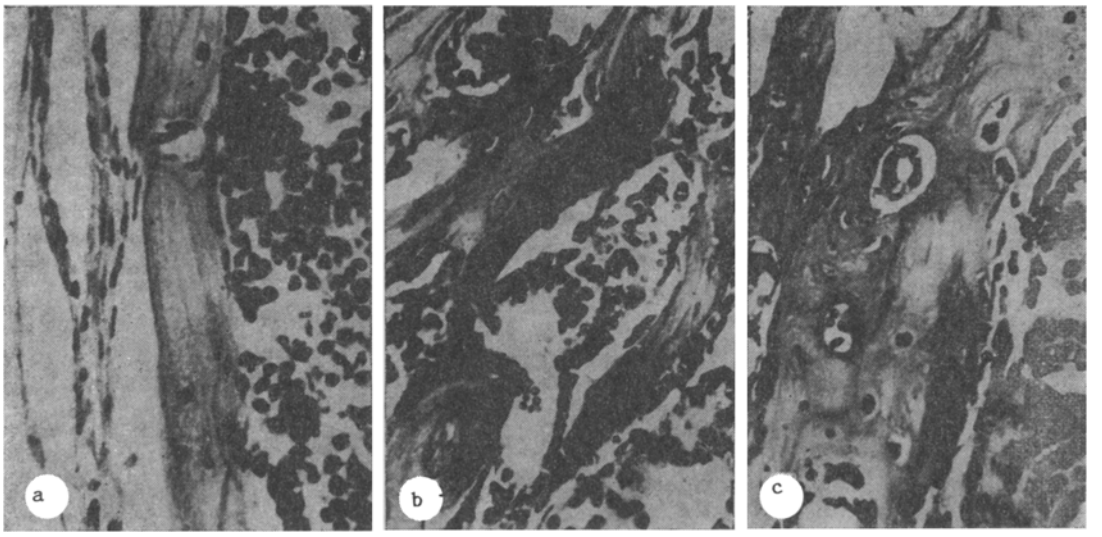

Fig. 1. Changes in ratio of hematopoietic and bony components in a focus of heterotopic hematopoiesis in a mouse. a) Normal focus (4 weeks); b) focus after 4 injections of estrone; c) focus after 6 injections of estrone. 140×.

TABLE 1. Effect of Estrone on Weight of Femur and Bony Capsule and Number of Cells in Femur and Ectopic Focus

Experimental conditions	Weight of		Cell content of bone marrow	
	femur	bony capsule	femur	ectopic focus
Control	11,2±0,2 (20)	0,95±0,1 (15)	10,2±0,3 (20)	5,4±0,4 (15)
Intervals between injections, h				
	14,3±1,7 (8)	1,45±0,4 (14)	4,6±0,3* (8)	1,2±0,4* (14)
	24,0±1,0* (9)	12,2±2,9* (8)	0,3±0,2* (7)	0,3±0,2* (8)

Legend. Number of heterotopic foci and femora studied given in parentheses. Asterisk indicates significant difference from control.

After injection of estrone the volume of the hematopoietic component decreased both in the focus itself and in the femur (Table 1). A tendency (not statistically significant) was observed for the weight of the bony capsule to increase. A marked increase in the number of dilated blood vessels also must be noted.

Six injections of estrone into the animals led to virtually complete filling of the medullary cavity with bone tissue both in the femur and in the focus of heterotopic hematopoiesis (Fig. 1). The cell content of the femoral marrow and of the ectopic focus was sharply reduced, whereas the weight of the femur and of the bony capsule increased significantly (Table 1). Activation of myelopoiesis also took place in the spleen.

The almost complete absence of hematopoiesis in the femur and in the heterotopic focus in response to injection of estrone, revealed by this investigation, cannot evidently be explained by the inhibitory effect of this hormone on proliferation of hematopoietic cells, for stimulation of extramedullary hematopoiesis took place in the spleen of these same animals. In addition, as other workers have found [6], the number of polypotent hematopoietic stem cells was increased both in the spleen and in the peripheral blood of mice receiving estrone. The reduction in the volume of hematopoietic tissue connected with bone during the development of osteogenic myelofibrosis likewise cannot be reduced simply to mechanical factors, for no closed medullary cavity is formed in a heterotopic transplant beneath the kidney capsule. Excessive development of bone tissue under the influence of estrogen probably leads to damage

to the hematopoietic microenvironment in the medullary cavities and to a disturbance of its construction in a heterotopic focus of hematopoiesis.

This investigation thus showed that the disturbance of hematopoiesis, at least in one type of experimental myelofibrosis (estrogen-induced) cannot be explained by mechanical displacement of hematopoietic cells from the bone marrow. However, several new questions arise: what is the mechanism of dissociation of osteogenesis and hematopoiesis, and of disturbance of the hematopoietic microenvironment; what is the cellular substrate in the development of fibrosis of bone marrow under the influence of estrogens? The examination of these questions requires special investigation.

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