on the body must be taken into account. The fact is that in rats receiving phentolamine the adrenals were found to be increased in weight by 44.9%: They weighed 28.58 ± 1.18 mg % compared with 19.72 ± 1.56 % in the control. Also it was in the rats of this group that the greatest decrease in the glycogen content took place in the regenerating liver. These changes must be interpreted as the result of the stressor action of phentolamine. Accordingly the writers are inclined to conclude that the inhibitory effect of phentolamine on regeneration in the liver is not merely the result of α-adrenergic receptor blockage, i.e., abolition of the stimulating action of adrenalin, but is also to some degree the result of the stressor effect of this blocking agent.

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EFFECT OF REGENERATION OF THE SPLEEN AND BONE MARROW ON NUMBER OF HEMATOPOIETIC COLONIES IN THE MOUSE SPLEEN

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Two thirds of the spleen (group 1) or the bone marrow from the right tibia (group 2) was removed from sexually mature male CBA mice. On the eighth day after lethal irradiation and injection of $1 \cdot 10^6$ nucleated cells from the intact spleen the number of hematopoietic splenic colonies was counted. A significant increase in the number of colonies was observed in the animals of both experimental groups compared with the control intact mice. The authors suggest that this increase may have been caused both by the local effect of the regenerating splenic stroma and by a certain stimulating factor secreted by the regenerating hematopoietic tissue.

KEY WORDS: *Regeneration of the spleen; regeneration of bone marrow; hematopoietic splenic colonies.*

The stroma of hematopoietic organs has been shown [7, 10, 12] to be the decisive factor in the choice of the way of differentiation of stem cells, and the number of exogenous colonies formed per spleen in irradiated mice depends entirely on the number of donor cells injected into them. The possibility cannot be ruled out that under certain conditions (regeneration, for example) the stroma may also affect the number of splenic colonies.

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TABLE 1. Number of Exogenous Colonies per Intact and Regenerating Spleen

Experimental conditions	Expt. No.	Dose of irradiation, P	No. of animals	Weight of spleen at autopsy	Number of splenic colonies
Intact spleen, irradiation, injection of $1 \cdot 10^6$ cells	1	900—1000	10	23,4±0,42	6,8±0,76
	2	800—900	9		2±0,17
Removal of two-thirds of spleen, irradiation, injection of $1 \cdot 10^6$ cells	1	900—1000	12	13,6±0,22	12,5±1,4
	2	800—900	16		5,25±0,2
Removal of bone marrow, irradiation, injection of $1 \cdot 10^6$ cells	2	800—900	15	22,6±0,89	6,5±0,27

In this investigation the possibility of a change in the number of splenic colonies under the influence of regeneration of hematopoietic organs was studied.

EXPERIMENTAL METHOD

Experiments were carried out on sexually mature male CBA mice weighing 18-20 g, divided into two groups. Two thirds of the spleen was removed from the animals of group 1 as described previously [6]. Bone marrow from the right tibia was removed from the mice of group 2. Intact mice served as the control. Splenic colonies were obtained by the method of Till and McCulloch [10]. Two days after the operation the animals of groups 1 and 2 and the control mice were irradiated with ^{135}Cs γ rays in a dose of 900-1000 R on the "Stebel' 3A" apparatus with a dose rate of 900 R/min (experiment 1) or with x rays on the "RUM-15" apparatus in a dose of 800-900 R with a dose rate of 50 R/min (experiment 2). Intact spleen cells were injected intravenously into the animals 3-6 h after irradiation in a dose of $1 \cdot 10^6$ nucleated cells (in 1 ml Hanks' medium) per mouse. On the eighth day after irradiation the animals were killed and the number of colonies on the surface of the spleen was counted.

Besides exogenous colonies, the formation of endogenous colonies also was analyzed. For this purpose, 10 or 15 animals were left in each of the first and second experimental groups and the control, into which no suspension of intact spleen cells was injected after irradiation.

The results were subjected to statistical analysis. The significance of differences was estimated by means of Student's criterion, which in all cases was 0.95 or above.

EXPERIMENTAL RESULTS

The results of the statistical analysis of the data are given in Table 1. By the 15th day after resection of two thirds of the unirradiated spleen its mass was 63% of the weight of the whole spleen [6]. The present experiments showed that the spleen of lethally irradiated animals still retained much of its power of regeneration after partial resection and injection of exogenous stem cells. By the 11th day after the operation the weight of the residual fragment of the spleen reached 58.2% of the mass of the whole irradiated organ.

Strelin [5], who studied regeneration of the liver, muscle, bone, and nerve, observed some depression of regeneration in animals irradiated in a dose of 1000 R. The present writers showed previously that the partially resected spleen irradiated in a dose of 650 R contains more DNA-synthesizing cells than the intact spleen. After sublethal irradiation, even without injection of stem cells, regeneration of the spleen is thus possible.

In the experimental group with resection of the spleen the number of hematopoietic colonies was 1.8 times (experiment 1) or 2.6 times (experiment 2) greater than in the control. The difference between the results of experiments 1 and 2 can be explained by the different biological action of γ rays and x rays [11]. Transplanted stem cells in this case came into the neighborhood of the proliferating stroma of the spleen. However, the increase

in number of hematopoietic colonies was possibly caused not only by the local effect of the regenerating stroma, but also by some factor secreted by the regenerating tissue. There is some evidence [4, 8] to show that the cells of regenerating tissues secrete biologically active substances.

To exclude the local effect of the regenerating splenic stroma and to discover the effect of regeneration of hematopoietic organs on the number of colonies, the bone marrow was removed from one limb of the mice of group 2, thereby causing regeneration of the bone-marrow tissue. In this case the number of hematopoietic colonies per intact spleen was increased to 3.25 times the number in control intact animals. This suggests that the increase in the number of splenic colonies in the experiments with regenerating hematopoietic organs (bone marrow and spleen) also took place through the action of some factor secreted during regeneration of these organs.

There may perhaps be some common immunological factor, such as lymphocytes, which are somehow modified in animals with regenerating organs [1] and acquire the property of influencing the survival and multiplication of colony-forming units (CFU). In the regenerating bone marrow experiment the number of hematopoietic colonies per intact spleen was significantly greater than per regenerating spleen. The results of the experiments with the regenerating spleen were perhaps a little on the low side. It has been shown [9] that the CFU population factor for the whole intact spleen is 17%, i.e., only 17% of stem cells injected give rise to colonies. There is no information on any change in this value depending on the size of the spleen. It could thus only be tentatively suggested that more than half of the hematopoietic stem cells can settle in the whole intact spleen, i.e., that the number of colonies per regenerating spleen could be twice the number shown in Table 1.

Not a single colony was found in the control series to check the formation of endogenous colonies in the single surviving mice in experiment 2. The results obtained in experiment 1 showed that more endogenous colonies were formed per regenerating spleen (0.6) than per intact spleen (0.01). The reason for this could be that the regenerating spleen contains more stem cells than the intact spleen. For instance, it has been shown [2, 3] that the number of stem cells in the spleen is increased 2 days after partial hepatectomy.

It seems probable that the increase in number of both endogenous and exogenous colonies obtained in animals with regenerating hematopoietic organs is caused by the stimulating action of a factor secreted by the regenerating tissue, which affects the survival and multiplication of CFU, as well as by the direct action of the regenerating stroma.

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