It can accordingly be concluded from the results of cytological analysis that if PEO-400 is added to the culture medium before the beginning of culture in final concentrations of 5% or 10%, blood cells stimulated by PHA remain capable of transformation and of mitotic division, despite a significant decrease in the mitotic activity of the experimental cultures compared with the controls.

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MECHANISM OF ACTION OF BREAKDOWN PRODUCTS OF GRANULOCYTES ON GRANULOCYTOPOIESIS

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The leukopoietic activity of blood serum was studied after injection of the breakdown products of 12 and 60 million homologous granulocytes per 100 g body weight into intact Wistar rats, and the character of the action of the serum on proliferation and differentiation of hematopoietic stem cells was determined in the spleen of lethally irradiated mice. The accumulation of granulopoietins in the blood under the influence of the granulocyte breakdown products was greater after injection of material from 12 million granulocytes. Granulocytopoietins stimulate the proliferative activity of stem cells and their differentiation into granulocytes in the spleen of lethally irradiated mice. It is concluded that granulocytes breakdown products have a stimulating action on hematopoiesis through granulocytopoietins.

KEY WORDS: granulocyte breakdown products; granulocytopoietin; stem cell; leukopoietic activity of blood.

It was stated in previous communications [1, 2] that granulocyte breakdown products stimulate granulocytopoiesis in intact animals and that the intensity of this stimulation depends on the number of granulocytes injected. The most marked stimulating effect was given by a dose of 12 million lysed granulocytes per 100 g body weight of the rat: this dose increased the proliferative activity of the granulocytes and the absolute number of myelokaryocytes, mainly on account of granulocytes, as well as the number of granulocytes in the peripheral blood. Injection of 60 million lysed granulocytes led to some degree of inhibition of granulocytopoiesis in the first 3 days, followed by moderate activation.

To study the mechanism of action of granulocyte breakdown products on granulocytopoiesis, in the present investigation their effect was examined on the leukopoietic activity of the serum and the character of the action of serum on proliferation and differentiation of hematopoietic stem cells was determined in the spleen of lethally irradiated mice.

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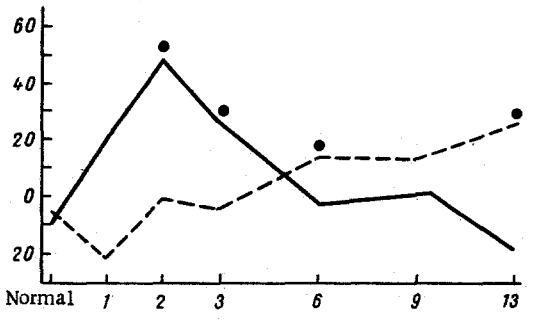

Fig. 1

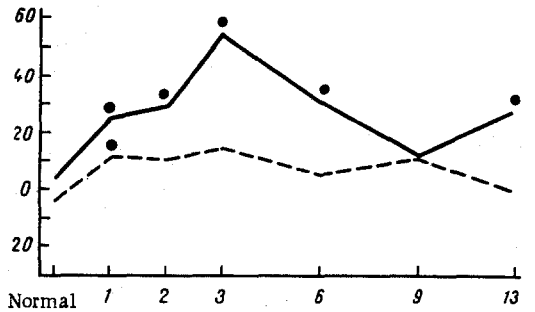

Fig. 2

Fig. 1. Leukopoietic properties of rat serum at different times after injection of granulocyte breakdown products. Abscissa, time of taking serum (in days); ordinate, number of immature granulocytes (in % of initial). Continuous line – serum taken after injection of 12 million lysed granulocytes; broken line – serum taken after injection of 60 million lysed granulocytes; black dots indicate $P < 0.05$.

Fig. 2. Number of macrocolonies in spleen of lethally irradiated mice after injection of bone marrow with addition of serum. Abscissa, time of taking serum (in days); ordinate, number of colonies (in % of control value). Remainder of legend as in Fig. 1.

EXPERIMENTAL METHOD

The leukopoietic activity of serum taken from rats 1, 2, 3, 6, 9, and 13 days after injection of homologous granulocytes, disintegrated by proteolysis, in a dose of 12 or 60 million cells/100 g body weight, was studied by the method of Kakhetelidze and Dolgina [3] on the basis of changes in the morphological composition of the bone marrow of the recipient rats. Serum was injected into the femoral vein of rats in a single dose of 1 ml/100 g body weight. Control animals received the same volume of normal serum. The bone marrow was obtained by femoral puncture before and 3 days after injection of the serum (at the time of maximal changes in hematopoiesis). Films were fixed with methyl alcohol and stained with azure II–eosin. Leukopoietic activity was estimated (in %) from the change in the number of immature granulocytes in the bone marrow after injection of the test serum compared with the corresponding level in the same animals before injection of serum. Each test sample was injected into a group of 6-12 animals and the mean values were calculated.

The effect of serum obtained at different times after injection of 12 and 60 million lysed granulocytes into the rats on proliferation and differentiation of the stem cells was studied by the method of Till and McCulloch [6]. Both donors and recipients of bone marrow were (CBA × C57BL) mice aged 10-12 weeks. Bone marrow was obtained by flushing out the femora with medium 199 and addition of Na_2-EDTA as stabilizer in a dose of 20 mg/100 ml medium. The cell concentration in the suspension was adjusted to 100,000 cells/ml. Each animal received a dose of 50,000 cells in 0.5 ml medium. The test serum was added in a dose of 1 ml/100 g body weight. Each sample was injected into a group of 8-12 mice. The control animals received an injection of a suspension of bone marrow cells in the same concentration. Normal serum was injected into another group of control animals. The mice were irradiated with ^{137}Sr γ-rays in a dose of 1200 rad 2 h before transplantation of the bone marrow. They were killed on the 9th day and their spleens were fixed in Bouin's fluid, and then in 70° alcohol. The number of colonies was counted macroscopically. Serial histological sections of the spleens were cut and stained with hematoxylin–eosin for the microscopic study of the composition of the hematopoietic colonies. The ratio between the types of hematopoietic colonies in the control and experimental groups of animals was determined in percent. Experiments were carried out on 154 rats weighing 80-100 g and on 449 mice.

EXPERIMENTAL RESULTS

The results of the study of changes in the serum leukopoietic activity of the rats treated with breakdown products of 12 and 60 million granulocytes are given in Fig. 1.

Serum taken 1 day after injection of breakdown products of 12 million granulocytes caused an increase in the number of immature granulocytes in the bone marrow of the recipient rats by 21%, and serum taken after 2 and 3 days gave increases of 48 and 26% respectively ($P < 0.02$, $P < 0.05$). The number of immature granulocytes returned to its initial level after 6 days.

TABLE 1. Histological Types of Hematopoietic Colonies (in %) in Spleen of Lethally Irradiated Mice under Influence of Rat Serum Taken at Different Times after Injection of Breakdown Products of 12 and 60 Million Granulocytes

Types of colonies	Control	Normal serum	Time of taking serum after injection of breakdown products, days					
			1	2	3	6	9	13
Injection of 12 million								
Erythrocytic	52	46	46	39	48	54	57	62
Granulocytic	21	23	25	35	34	28	25	22
Megakaryocytic	5	7	9	12	10	9	3	4
Mixed	12	11	13	14	9	9	14	8
Undifferentiated	10	13	6	—	—	1	1	4
Number of spleens	14	9	9	5	11	10	10	5
Injection of 60 million								
Erythrocytic	54	47	54	47	44	65	64	58
Granulocytic	17	22	25	25	23	18	15	19
Megakaryocytic	11	3	8	9	11	5	9	5
Mixed	17	18	11	19	22	13	8	17
Undifferentiated	1	10	2	—	—	—	4	1
Number of spleens	9	9	9	9	9	5	9	9

The increase in the leukopoietic activity of the blood serum coincided with or preceded the maximal changes in the morphological composition of the bone marrow in the donor rats and was probably responsible for the stimulation of granulocytopoiesis.

Serum obtained 24 h after injection of breakdown products of 60 million granulocytes caused a decrease of 22% in the number of immature granulocytes in intact rats. The serum leukopoietic activity began to rise after 6 days and reached a maximum after 13 days (+25%; $P < 0.001$). Normal serum had no leukopoietic activity.

Under the influence of serum taken from rats 24 h after receiving an injection of 12 million lysed granulocytes the number of macrocolonies in the spleens of the lethally irradiated mice increased by 25% ($P < 0.01$) compared with the control (injection of bone marrow without serum), after 2 days it was increased by 30% ($P < 0.001$), and after 3 days by 55% ($P < 0.001$), and it remained significantly increased until the end of the period of observation. Under the influence of serum from intact rats the number of macrocolonies was not significantly increased.

Serum taken from rats after injection of breakdown products of 60 million granulocytes had a weaker effect on proliferation of the stem cells. The maximal increase in the number of macrocolonies was observed under the influence of serum taken 3 days after injection of granulocyte breakdown products, and amounted to only 14.5% ($P > 0.1$) (Fig. 2).

The results of the histological investigations are given in Table 1.

Under the influence of serum obtained after injection of the breakdown products of 12 million granulocytes an increase in the relative percentage of colonies of granulocytes was observed at all times of observation: the increases after 2 and 3 days were 35 and 34% respectively (21% in the control).

In the course of 6 days there was some increase in the relative percentage of colonies of megakaryocytes and a decrease in the percentage of erythrocytic and undifferentiated colonies. Most cells in colonies of mixed type were granulocytes.

Under the influence of serum obtained 1, 2, and 3 days after injection of the breakdown products of 60 million granulocytes the relative percentage of granulocyte colonies also was increased: by 25, 25, and 23% respectively (17% in the control). The percentage of mixed colonies also was increased after 2 and 3 days: by 19 and 22% respectively (17% in the control).

Granulocyte breakdown products thus cause the accumulation in the blood of substances capable of stimulating granulocytopoiesis in intact rats. The intensity of their accumulation depends on the dose of lysed granulocytes injected: injection of 12 million lysed granulocytes causes a more rapid and marked accumulation of granulocytopoietins. Serum taken 24 h after injection of 60 million lysed granulocytes causes some decrease in the number of immature granulocytes in intact animals, followed by a moderate increase. The decrease in the granulocytopoietic activity of the serum associated with an increase in the dose of lysed granulocytes injected, as well as the inhibition of granulocytopoiesis in the donor rats after injection of this dose are probably due to an increase in the quantity of an inhibitor of leukopoiesis contained in mature granulocytes [4, 5].

Granulocytopoietins stimulate proliferation of stem cells in the spleen of lethally irradiated mice and their differentiation into granulocytes. The mechanism of action of granulocytopoietins on the stem cell requires special study.

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RESPONSE OF ANTERIOR PITUITARY CELLS AFTER ACUTE COOLING IN RATS

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The anterior lobe of the pituitary (ALP) of rats exposed to general cooling to −10°C for 6 min was investigated by histological and autoradiographic methods. The diurnal cycle of changes in the functional state of the glandular cells of ALP, the number of mitoses, and the number of labeled cells synthesizing DNA was examined 3, 6, 12, 18, and 24 h after cooling. Marked activation of thyrotrophs and doubling of the number of mitoses were observed 12 h after cooling, with no change in the index of labeled cells in the course of 24 h. The results showed that the stressor response to cold in rats is characterized by potentiation of the thyrotrophic function and by acceleration of mitosis in the cells of ALP.

KEY WORDS: cold stress; thyrotrophs; DNA synthesis; mitosis.

The stressor response consists of a basic essential nonspecific component, in the form of activation of corticotrophs, to which a selective and marked increase in thyrotroph function may be added depending on the type of stressor. During a study of the ultrastructure of the anterior lobe of the pituitary (ALP) in rats exposed to acute cooling, marked activation and an increase in the number of thyrotrophs in the gland were observed by the present writers during the first 24 h [2]. In the investigation described below the effect of acute cold stress on DNA synthesis and reproduction of the cells of ALP was studied in rats and the diurnal cycle of changes in the state of the thyrotrophs was examined. Changes in these functions were judged on the basis of previous information [1] on the diurnal rhythm of DNA synthesis, reproductive activity, and the functional morphology of the glandular cells of ALP in intact rats. There are indications in the literature of an increase in the corticosterone concentration in the blood of rats after short-term general cooling [3] and also of an increase in the thyrotrophin concentration under these conditions [4, 5].

EXPERIMENTAL METHOD

Fifteen male rats weighing 100-120 g were exposed to acute cooling in a chamber at −10°C for 6 min. The pituitary was removed 3, 6, 12, 18, and 24 h after cooling from 3 animals at each time. Meanwhile the pituitary gland of 15 control rats was investigated. DNA synthesis in the glandular cells was investigated by an autoradiographic method with [^{3}H]thymidine with a specific activity of 5.6 Ci/mmole and in a dose of 1 μCi/g body weight. [^{3}H]Thymidine was injected intraperitoneally 1 h before sacrifice. The pituitary glands

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