identified in the present investigation in the composition of the anlage of the heart and the limb buds. The remaining CFU_{bm} evidently were located in other regions of the embryo.

The results thus indicate that the region of the anlage of the heart and limb buds of the quail embryo can serve as the main source for HSC populating both intraembryonic and extraembryonic hematopoietic organs. It can also be postulated that the anlage of the blood, which initially is located in a restricted region of the visceral layer of the mesoderm (in the caudal part of the developing embryo), in the course of morphogenesis is dispersed and distributed in the mesenchyme of the anlagen of the embryonic and definitive hematopoietic organs.

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STEM CELLS AND T AND B LYMPHOCYTES IN ACUTE HYPOXIA

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KEY WORDS: antibody-forming cells; hypoxia; colony-forming cells; hematopoietic stem cells.

The effect of hypoxia on functional systems of the body is currently being intensively studied [1, 2, 5]. However, the immunologic reactivity of the body during hypoxia has hardly been studied at all [3, 11]. Analysis of immunologic reactivity at the present-day level must include a study of the basic cell processes responsible for immunogenesis [7, 8].

The object of this investigation was to study colony-forming stem cells (CFU) in the bone marrow, spleen and peripheral blood, and the migrating ability of CFU and also of T and B lymphocytes of mice exposed to graded rapid acclimatization to hypoxia.

EXPERIMENTAL METHOD

$(CBA \times C57BL)F_1$ hybrid mice obtained from the "Stolbovaya" Nursery of Laboratory Animals, Academy of Medical Sciences of the USSR, were used in the experiments. Female animals age 3-4 months, weighing initially 18-20 g, were used.

Graded rapid acclimatization to hypoxia was carried out in a pressure chamber; the animals were "raised to an altitude" of 4000, 5000, 6000 and 8000 m at the rate of 50 m/sec, they were kept at each of the above "altitudes" for 1 min, and they were then "lowered" in the course of 2 min 40 sec. Raising and lowering under these conditions were repeated 15 times. Training was given on three successive days, and the animals were investigated during the 3-4 h after the last training session. The control (intact) group of mice was kept in an atmosphere with a normal oxygen concentration.

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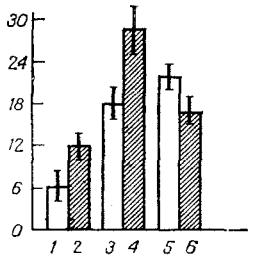

Fig. 1. Number of hematopoietic stem cells in blood (1, 2), bone marrow (3, 4), and spleen (5, 6). 1, 3, 5) Control; 2, 4, 6) after graded rapid acclimatization to hypoxia for 3 days. Ordinate, number of colonies.

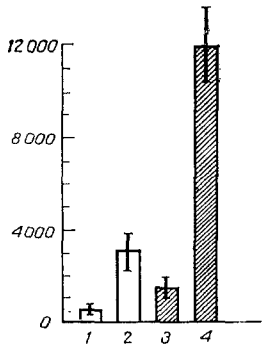

Fig. 2

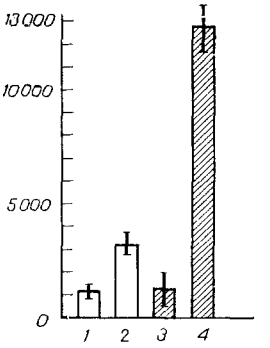

Fig. 3

Fig. 2. Migration of B lymphocytes from bone marrow into spleen after graded rapid acclimatization to hypoxia. 1, 3) Two days after screening of bone marrow sheep's red blood cells ($2 \cdot 10^8$) were injected; 2, 4) Two days after screening of bone marrow, lymph node cells of normal mice ($2 \cdot 10^6$) were transplanted and sheep's red blood cells ($2 \cdot 10^8$) injected. Here and in Fig. 3: unshaded columns – control mice; shaded columns – experimental mice. Ordinate, number of AFC in spleen.

Fig. 3. Migration of T cells from thymus into spleen after graded rapid acclimatization to hypoxia for 3 days. 1, 3) Two days after screening of thymus sheep's red blood cells ($2 \cdot 10^8$) were injected; 2, 4) two days after screening of the thymus bone marrow cells were transplanted from normal mice and sheep's red blood cells ($2 \cdot 10^8$) were injected.

To investigate the number of CFU in the bone marrow, peripheral blood, and spleen the method of exogenous cell cloning [12] was used. For this purpose, bone marrow, peripheral blood, and spleen cells were taken from intact animals and from mice exposed to acute hypoxia, and injected intravenously into syngeneic recipients, which were irradiated in a dose of 850 R 3-4 h before transplantation of the cells. The number of exogenous colonies in the spleen was counted after 8 days.

Migration of stem cells from the bone marrow into the spleen was studied in irradiated (800 R) control and experimental mice with a screened part of the bone marrow (the right hind limb to mid-calf level). On the 8th day after irradiation the number of colonies on the surface of the spleen formed from migrating stem cells was counted [6].

The effect of the conditions of training described above on migration of B lymphocytes from the bone marrow was determined as follows [9]. Mice were irradiated in a dose of 800 R with the hind limbs screened up to the level of the knee (the source of B cells). After two days the mice were given an injection of $2 \cdot 10^6$ lymph node cells from normal syngeneic mice and of $2 \cdot 10^8$ sheep's red blood cells, and on the 8th day the number of antibody-forming cells (AFC) in the spleen was counted by Jerne's method [13].

To study migration of T cells from the thymus [10], mice were irradiated in a dose of 800 R with the region of the thymus screened, and two days later they received injections of $10 \cdot 10^6$ bone marrow cells and $2 \cdot 10^8$ sheep's red blood cells, and the number of AFC in their spleen was counted 7 days later.

EXPERIMENTAL RESULTS

The study of the number of hematopoietic stem cells in the peripheral blood, bone marrow, and spleen of the control animals and of animals exposed for three days to graded rapid acclimatization to an "altitude" of 8000 m showed that the number of CFU in the blood and bone marrow of the acclimatized animals was increased compared with their number in the control animals. It is interesting to note that the number of CFU in the spleen of animals acclimatized to hypoxic hypoxia was considerably reduced (Fig. 1). These results are evidence that during exposure to hypoxia redistribution of hematopoietic stem cells evidently takes place.

The results of the next investigations showed that the process of migration of CFU from the bone marrow is intensified in mice exposed to hypoxia for three successive days. For instance, in intact mice, in which part of the bone marrow of the hind limb up to mid-calf was screened during lethal irradiation, 15 ± 2 CFU migrated, compared with 22 ± 2 CFU which migrated from the same region of bone marrow in the experimental mice. Similar results were obtained during the study of the effect of acute hypoxia, caused by exposure of the mice in the pressure chamber to an "altitude" of 6700 m for 12 h [4].

A very small number of AFC was formed in the spleen of mice irradiated with part of the bone marrow screened, not receiving T cells, and immunized with sheep's red blood cells (Fig. 2). This was connected with the deficiency of T helper cells in these mice. Injection of T cells from lymph nodes and of sheep's red blood cells two days after irradiation led to the accumulation of a considerable number of AFC. This was the result of cooperative interaction between B lymphocytes, migrating from the bone marrow, and transplanted T cells. As Fig. 2 shows, four times more AFC were formed in the spleen of the screened, irradiated experimental mice, subsequently receiving T cells, than in the spleen of the control screened irradiated mice receiving T cells. These observations are evidence that in a state of acute hypoxia migration of precursors of AFC from bone marrow is intensified. In mice exposed to acute hypoxia, increased migration of T cells from the thymus into the spleenwas observed (Fig. 3). For instance, the number of AFC formed in the spleen of experimental mice irradiated with the region of the thymus screened, and receiving an injection of bone marrow cells and sheep's red blood cells two days later, was three times greater than the number of AFC which accumulated in the spleen of the control mice.

The results are thus evidence that during graded rapid acclimatization of mice to hypoxia the number of colony-forming cells in the bone marrow and peripheral blood is increased, whereas in the spleen it is reduced. Intensified migration of hematopoietic stem cells of the bone marrow and of T and B lymphocytes to the periphery was observed. The changes thus discovered in the number of stem cells in the course of cell migration can be taken as evidence of a redistribution of the hematopoietic and lymphoid cell populations as a result of exposure to hypoxic hypoxia.

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