be explained by the different duration of anemia development (in children over several months, in adults up to 10 years), the wide age range of the adults examined (from 17 to 63 years), and the variation of accompanying diseases in them.

A close correlation (r=-0.93) was discovered between EP activity and the hemoglobin concentration in children suffering from IDA (see Fig. 1). The level of EP activity in the examined patients correlated clearly with the indicators of iron metabolism depicting the degree of deficiency. Thus, the correlation coefficient between the level of EP activity and the concentration of the total iron-binding capacity of the blood serum in the case of IDA in children was +0,74. The same close correlation was revealed between the ferritin content and the activity of EP (r=-0.71). The results obtained give evidence that an adequate response to developing anemia is provided by EP production in young children.

It seems to us that the use of recombinant erythropoetin for the correction of IDA at an early age is hardly justified.

LITERATURE CITED

1. V. P. Bisyarina and L. M. Kazakova, Iron-Deficiency Anemia in Young Children [in Russian], Meditsina, Moscow (1979), p.174.
2. V. I. Gudim, L. L. Eremenko, V. S. Ivanova, *et al. Gematol. Transfuziol.*, No. 7, 3-5 (1991).
3. T. E. Manakova, and N. A. Setkov, *Byull. Eksp. Biol. Med.*, No. 3, 21-24 (1989).
4. A. J. Erslev, *New Engl. J. Med.*, **316**, 101-113 (1987)
5. A. J. Erslev, S. J. Schuster, and J. Caro, *Eur. J. Haemat.*, **43**, 367-373 (1989).
6. J.W. Eschbach, J. C. Egrie, M. R. Downing, *et al. Lancet*, **11**, 1175-1178 (1986).
7. D. S. Halperin, P. Wacker, G. Lacourt, *et al., J. Pediatrics*, **116**, 779-786 (1990).
8. S. B. Krantz, *Blood*, **77**, 419-434 (1991).
9. G. Krystal, *Exp. Hematol.*, **11**, 649-660 (1983).
10.F. Pizarro, P.Vip, *et al.,J.Pediatrics*, **118**, 687-692 (1991).
11. N. W. Tietz and P. R. Finley, Clinical Guide to Laboratory Tests, W. B. Saunders Company (1983).
12. M. R. Tyndal, D. F. Teitel, and W. A. Lutin, *J. Pediatrics*, **110**, 538-544 (1987).

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Erythropoietic Activity in The Serum of Mice during Postnatal Ontogeny

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During postnatal development of mice, changes occur both in the cellular composition of the hematopoietic tissues and in the hematopoiesis-regulating system. Neonatal mice develop transient anemia [5] that lasts for 6 weeks and is accompanied by variations in parameters of the peripheral blood [1] and functional characteristics of the bone marrow [2, 4] and by a rise in the level of the factor stimulating erythropoiesis in the plasma [9, 15]. Several authors have described hematopoietic disturbances in old mice [8, 14]. It has been suggested that the erythropoiesis-regulating mechanisms in young mice are distinct from those in older animals [6]. Examination of various hematological parameters throughout the period of postnatal ontogeny is therefore necessary if the mechanisms regulating hematopoiesis in general and erythropoiesis in particular are to be understood.

The purpose of the present study was to examine changes in erythropoiesis in mice with age by comparing some peripheral blood parameters and serum erythropoietic activity in neonatal (aged 2-25 days), sexually mature (aged 1-12 months), and old (aged 15-18 months) animals.

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MATERIAL AND METHODS

In these experiments, Swiss and (CBA x C_{57}BL)F_1 mice were used as the test animals. Erythropoietic activity was measured in their serum and erythrocyte and reticulocyte counts and hemoglobin concentration were determined in the peripheral blood.

Blood was collected after decapitating the test mice at ages of 2 to 25 days, 1 to 12 months, and 15 to 18 months (10 to 80 animals for each of these three periods). Erythropoietic activity was determined by an in vitro bioassay [7] in the modification described by Manakova and Setkov [3]. BALB/c mice aged 8-12 weeks were made anemic by giving them two intraperitoneal injections of phenylhydrazine hydrochloride at 60 mg/kg body weight on two successive days; on the third day after the second injection, a cell suspension comprising, in the main, cells of the erythroid series was prepared from their spleens. For cell culture, α-MEM medium (Flow Laboratories) was used, supplemented with L-glutamine (2%), 2-mercaptoethanol (5 x 10^{-4} M), kanamycin sulfate (2%), HEPES buffer (4%), and embryonal calf serum (40%); the cell concentration was 4 x 10^7 cells/ml. The suspension was seeded into flat-bottomed 96-well plates (Linbro), 50 µl per well, adding 50 µl of the test material (sera from test mice or the erythropoietin standard). The sera from test mice were inactivated for 30 min at 56°C. For each age point, a dose-effect curve was plotted (3 wells per point). As the erythropoietin standard, plasma from the anemic mice with an activity of 16 units/ml calibrated against the International Erythropoietin Standard was used.

The cells were cultured in an incubator for 20-22 h at 37°C and 100% humidity in the presence of 5% CO_2. Thereafter, ^{3}H-thymidine (1 µCi in 10 µl of α-MEM medium per well) was added to the culture and the cultivation was continued for another 2 h. The cells were then transferred to filters with a harvester (Millipore) and fixed and dried after washing off the free isotope. The radioactivity of each sample was measured in a liquid scintillation β-counter. The level of erythropoietic activity was calculated by comparing linear regression equations obtained for the test sera (through titration) with those obtained for the erythropoietin standard.

Erythrocyte counts and hemoglobin concentrations were determined in a K-2MP photoelectric concentration calorimeter. Reticulocyte counts were made in supravitally stained peripheral blood smears.

RESULTS

During postnatal ontogeny, alterations in hemoglobin concentration and in erythrocyte and reticulocyte counts in the peripheral blood of mice were observed, as were fluctuations of erythropoietic activity in their sera.

In the neonatal period (days 2-25 after birth), hemoglobin remained at a low level and the erythrocyte counts were also relatively low (Fig. 1, a and b), whereas the reticulocyte counts rase steeply between days 3 and 5 and then fell sharply by day 20 (Fig. 1, c). Serum erythropoietic activity was very low (less than 7 mIU/ml) during the first 2 days after birth, but then began to rise, reaching the highest level (145±31 mIU/ml) on day 15, followed by a drastic decline; at the age of 1 month, it did not exceed 7 mIU/ml (Fig. 2).

The young sexually mature mice (1 to 12 months old), showed a fairly stable erythropoietic activity

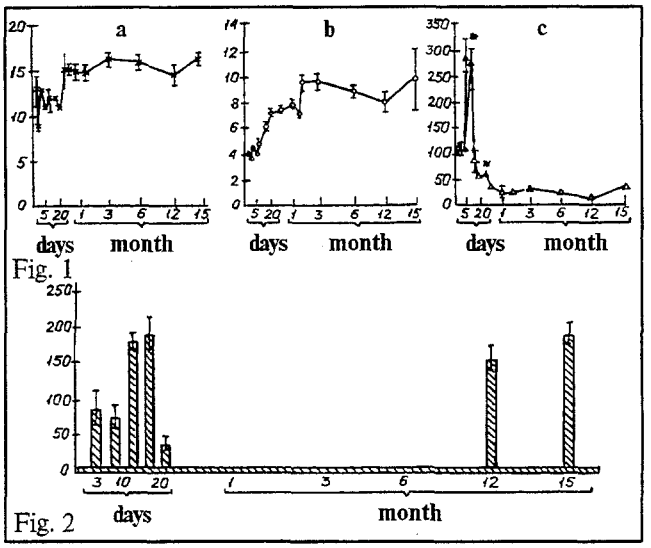

Fig. 1

Fig. 2

Fig. 1. Hematological parameters in the peripheral blood of mice during postnatal ontogeny. a) Hemoglobin level; abscissa (here and elsewhere): age of mice (days and months). b) Erythrocyte counts; ordinate: cell numbers (x 10^{12}/liter). c) Reticulocyte counts; ordinate: percentage of cells. The asterisk indicates values for which $p<0.01$.

Fig. 2. Erythropoietic activity in sera from mice of various ages. Abscissa: age of mice (days and months). Ordinate: erythropoietin concentration (mIU/ml).

accompanied by high hemoglobin levels, high erythrocyte counts, and low reticulocyte counts in the peripheral blood (Fig. 1) and low erythropoietic activity in the serum (below 7 mIU/ml Fig. 2).

Old mice (aged 12-18 months) did not differ significantly from young ones in terms of hemoglobin concentration and erythrocyte and reticulocyte counts, but the erythropoietic activity in their sera was high (Fig. 2).

This study has thus shown that erythropoiesis undergoes certain changes in mice during postnatal ontogeny. The so-called physiological anemia in neonatal mice is characterized by low hemoglobin levels, reduced total erythrocyte counts, and elevated reticulocyte counts in the peripheral blood and by high erythropoietic activity in the serum. Toward the end of the first month, these peripheral blood parameters all return to their normal values.

Our comparison of hematological parameters in young and old mice indicated that their hematopoiesis

is relatively stable, and no signs of anemia described by some authors [8, 14] could be observed in old mice. These findings agree with the results reported by other authors who, too, found basal erythropoiesis to be unchanged in old mice [10, 12].

Old mice, on the other hand, showed impaired humoral regulation of erythropoiesis, manifested in elevated serum erythropoietic activity as compared to young animals. Such high activity is characteristic for neonatal mice [9] in which, however, it is associated with the anemia that accompanies the rapid growth of tissues and is a reflection of their deficient oxygen supply. In contrast, the high erythropoietic activity in the sera of old mice could not be due to the impact on erythropoiesis of factors other than erythropoietin given that this activity, when measured in the murine serum by the in vitro bioassay we used, depends almost entirely on the presence of erythropoietin rather than on growth factors [11].

In old mice, the altered ratios of formed elements and plasma in the blood [8], the accelerated proliferation of erythroid cells, and the elevated activities of certain enzymes involved in erythropoiesis [10] may be consequences of possible disorders in bone marrow functions. In addition, the existing evidence regarding abnormalities in the cellular composition and functional activity of the hematopoietic microenvironment in old mice [13] suggests that the mechanisms whereby bone marrow cells and the hematopoietic microenvironment interact undergo changes with advancing age.

LITERATURE CITED

1. A.K. Baskuryan and A.G. Kartashov, *Gematol. Transfuziol.*, No. 6, 46-49 (1986).
2. E.I. Domaratskaya and O.D. Pryanishnikova, *Ontogenez*, **21**, No. 1, 81-88 (1990).
3. T.E. Manakova and N.A. Setkov, *Byull. Eksp. Biol. Med.*, No. 1, 21-24 (1989).
4. E.A. Hall, S.G. Shinpock, and J.W. Goodman, *Exp. Hematol.*, **12**, 549-557 (1984).
5. K. Halvorsen and S. Halvorsen, *Arch. Dis. Childhood*, **48**, 842-849.
6. D.E. Harrison, *J. Exp. Med.*, **157**, 1496-1503 (1983).
7. G.A. Krystal, *Exp. Hematol.*, **11**, 649-660 (1983).
8. M. Loeffler and K. Pantel, *Exp. Hematol.*, **14**, 487 (1986).
9. A. Meberg, P. Haga, and M. Johansen, *Br. J. Hematol.*, **45**, 569-574 (1980).
10. M. Magnani, L. Rossi, and V. Stochi, *Mech. Ag. Dev.*, **42**, 37-47 (1988).
11. T. Sanengen and S. Halvorsen, *Pediat. Res.*, **21**, No. 2, 149-151 (1987).
12. R. Schofield, R. Dexter, B.I. Lord, and N.G. Testa, *Mech. Ag. Dev.*, **34**, 1-12 (1986).
13. A.V. Sidorenko, L.F. Andrianova, T.V. Macsyuk, and G.M. Butenco, *Mech. Ag. Dev.*, **54**, 131-140 (1990).
14. K.B. Udupa and D.A. Lipschitz, *J. Lab. Clin. Med.*, **103**, 581-584 (1984).
15. J.A. Widness, T. Sanengen, P. Haga, *et al.*, *Acta Physiol. Scand.*, **136**, 527-533 (1989).

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Enterosorption Normalizes the Level of Biogenic Amines in Experimental Bronchospasm

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Recent studies have demonstrated beneficial effects of enterosorption on the clinical course of bronchial asthma. Sorbents of the new generation (Polyphepan, OKN-P, AUVM-Dnepr-MN, and others) administered *per os* have been found to enhance the efficacy of drug treatment in cases of exacerbated asthma and to accelerate the onset of remission [2, 3, 5, 10].

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