Hemoglobin Heterogeneity under Conditions of Abnormal Erythropoiesis

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Translated from *Byulleten' Eksperimental'noi Biologii i Meditsiny*, Vol. 135, No. 6, pp. 660-663, June, 2003
Original article submitted December 27, 2002

Experiments on rats showed that changes in the hemoglobin profile during hypoxia are determined by switching of erythropoiesis from the "basic" to "emergency" mode. The "basic" mode of erythropoiesis is typical of the mature organism under normal conditions. The "reserve" or "emergency" mode is realized in fetuses, senile animals, and during hypoxia. This mode is characterized by production of large erythrocytes with high content of fetal hemoglobin.

Key Words: *hemoglobin subtypes*; *modes of erythropoiesis*; *hypoxia*; *hyperoxia*; *ageing*

Hemoglobin is a heterogeneous protein presented by several isoforms. Hemoglobin profile (the number and relative content of hemoglobin subtypes) varies during ontogeny and depends on the oxygenation regimen. Thus, primitive forms of hemoglobin dominate in human embryos, hemoglobin F in fetuses, and hemoglobin A during postnatal ontogeny. However, high levels of fetal hemoglobin can be observed also during postnatal life under conditions of hypoxia, in hemoglobinopathies, and in some forms of leukemia [3,4]. Specific features of human fetal hemoglobin are higher affinity to oxygen, more pronounced Bohr effect compared to hemoglobin A, and high resistance to acids and alkalies [1]. Thus, modification of hemoglobin composition during ontogeny and under extreme conditions plays an important physiological role.

Similar changes in hemoglobin profile were also observed in other mammalian species, *e.g.* in rats. Human fetal hemoglobin is similar to fractions 5 and 6 of rat hemoglobin. The content of these fractions increased in the antenatal period or in mature rats during hypoxia [3].

Despite a large body of evidence on changes in hemoglobin profile, the mechanisms underlying this phenomenon remain unclear. Some authors suppose that changes in fraction composition of hemoglobin are primarily associated with variations in the proportion of erythrocyte subpopulations differing in the content of individual hemoglobin isoforms, and increased content of some fractions can be explained by expansion of some erythroid clones [4]. According to other concepts, changes in the synthesis of some hemoglobin subtypes are determined by inhibition of specific genes, acceleration of proliferative processes, shortening of the cell cycle [8]. A possible reason why the casual mechanisms of changes in the hemoglobin profile remain unclear is that most scientists studied only the proportion between various hemoglobin fractions, evaluation of intrinsic mechanisms of these processes requires complex analysis of red blood cells. Our aim was to study the heterogeneous hemoglobin system in relation with changes in other parameters of red blood cells under conditions of changed erythropoiesis.

MATERIALS AND METHODS

Experiments were carried out on male random-bred albino rats. Hypoxic hypoxia was modeled in an altitude chamber (daily 6-h sessions for 7 days, 40.98 kPa). Normobaric hyperoxia was modeled in the same chamber filled with 100% O_2 at the pressure of 1013.2 GPa (daily 6-h sessions for 7 days). The state of red

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0007-4888/03/1356-0563$25.00

blood cells was determined at the end of each 7-day stimulation course. Rat age was estimated on the basis of a linear correlation between the age and body weight [2].

Hemoglobin fractions were analyzed by polyacrylamide gel electrophoresis. The amount of cells containing acid-resistant forms of hemoglobin was evaluated by Betke-Kleihauer acid elution test [6]. Myelograms were obtained and analyzed by conventional methods. The total blood count was determined using a MICROX hematologic analyzer (Hoffman la Roche). Erythrocyte diameter was measured using an ocular micrometer, the concentration of red blood cells of a particular size was determined.

Blood samples enriched with erythrocytes were obtained using two approaches. First, simulation of hemolytic anemia by 3 injections of phenylhydrazine in a dose of 5 mg/100 g body weight with an interval of 2 days. Blood samples were collected on day 3 after the first injection. Reticulocyte concentration in these samples usually attained 31.24±1.55%. The second method was separation of erythrocytes by their floating density (J. Murphy method) [7]. The low-density fractions of red blood cells were enriched in reticulocytes (33.84±1.55%).

The data were statistically analyzed by parametric methods using Microsoft Excel software. The descriptive statistics included calculation of means, standard deviations, and coefficients of variation for the studied parameters. Significance of differences was analyzed by Student's *t* test.

RESULTS

Analysis of changes in hemoglobin system during hypoxic hypoxia and normobaric hyperoxia showed that fractions 5 and 6 of rat hemoglobin are most sensitive to these factors. The direction of changes in their content strictly corresponded to variations in oxygen regimens. The absolute concentrations of these fractions in the blood increased during hypoxic hypoxia and decreased during normobaric hyperoxia (Table 1).

Changes in the heterogeneous hemoglobin system were accompanied by changes in the cytometric characteristics of erythrocytes. During hypoxia, the count of large cells increased, while the content of small cells decreased (Fig. 1). The mean volume of erythrocytes increased from 52.93±0.15 to 61.60±0.57 μ^3 ($p<0.001$). By contrast, the relative count of large cells decreased during hyperoxia (Fig. 1). This parallelism of changes in the contents of fractions 5 and 6 hemoglobin and large red blood cells suggests that cell volume and hemoglobin profile of erythrocytes are closely related.

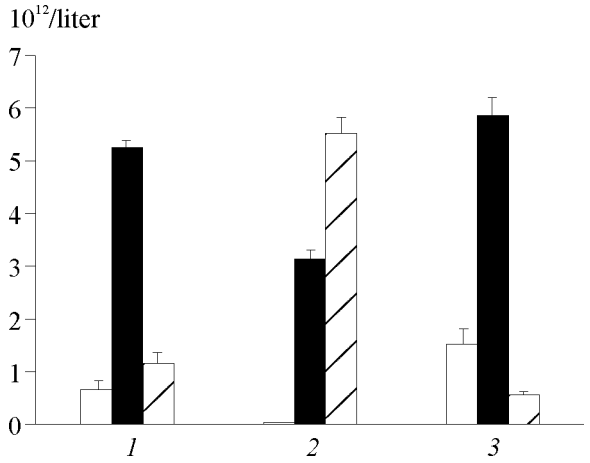

Fig. 1. Changes in blood content of erythrocytes of different size during various modes of oxygenation in rats. *1*) intact rats; *2*) hypoxia; *3*) hyperoxia. Cell diameter: <5.1 μ (light columns); 5.1-6.1 μ (dark columns); >6.1 μ (dashed columns).

For verification of this hypothesis we measured the size of cells containing acid-resistant hemoglobin (fractions 5 and 6 [3]) in blood smears stained by the method of Betke-Kleihauer [6]. It was found that cells containing acid-resistant hemoglobin were larger (6.51±0.08 μ) than cells containing eluted hemoglobin (5.8±0.1 μ, $p<0.001$). Thus, fractions 5 and 6 are contained mainly in large cells.

Analysis of myelograms showed a significant increase in the population of erythroid cells in the bone marrow during hypoxic hypoxia from $8.02\pm0.73\times10^6$ to $10.91\pm0.55\times10^6$/100 g body weight ($p<0.05$). Hyperoxia considerably reduced the content of bone-marrow erythroid cells from $8.02\pm0.73\times10^6$ to $3.19\pm0.10\times10^6$/100 g body weight ($p<0.001$). Hypoxic hypoxia cau-

TABLE 1. Hemoglobin Profile during Various Oxygenation Regimens in Rats

Oxygenation regimen	Fraction, g/liter					
	1	2	3	4	5	6
Intact	14.99±0.70	22.36±1.11	58.95±1.7	24.90±0.74	12.16±0.52	3.28±0.24
Hypoxia	16.5±0.9	39.48±1.25*	61.80±1.89	28.96±1.25*	20.88±0.93*	8.95±0.61*
Hyperoxia	21.66±0.91*	26.68±1.12*	61.96±1.06	25.60±0.89	7.34±0.86*	1.26±0.29*

Note: *$p<0.05$ compared to intact rats.

TABLE 2. Hemoglobin Profile of Erythrocytes As a Function of Reticulocyte Content in Blood Sample (%)

Reticulocyte		Fraction, g/liter					
		1	2	3	4	5	6
Intact rats							
Without separation	2.46±0.21	10.37±0.4	16.46±0.7	43.3±0.61	18.34±0.33	9.07±0.39	2.46±0.18
Heavy cells	1.29±0.24	8.05±0.25	16.17±0.24	42.67±0.7	20.15±0.57	9.26±0.36	3.69±0.25
Light cells	33.84±1.51	8.13±0.35	16.24±0.49	41.25±0.61	20.76±0.46	9.89±0.29	3.72±0.21
Rats with phenylhydrazine-induced anemia							
Without separation	31.24±1.55	8.06±0.17*	14.25±0.26*	32.8±0.48*	22.74±1.06*	14.47±1.06*	7.67±0.25*

sed peripheral blood hyperreticulocytosis (1020±60 g/liter, compared to 179±19 g/liter in the intact rats, $p<0.001$). By contrast, during hyperoxia reticulocyte counts decreased to 33±4 g/liter ($p<0.001$).

It is well known that erythrocytes became smaller with age [5]. Hence, increased content of hemoglobin fractions 5 and 6 and enlargement of erythrocytes can result from activation of erythropoiesis and rejuvenation of red blood cells during hypoxia.

For evaluation of possible age-related heterogeneity of erythrocytes by hemoglobin profile, we studied the ratio of hemoglobin isoforms in red blood cells taken from rats of different ages. Examination of blood samples enriched with reticulocytes by the method of J. Murphy [7] revealed no differences in the hemoglobin profile of young and mature erythrocytes (Table 2). However, in reticulocyte suspension obtained from animals with phenylhydrazine-induced anemia the content of hemoglobin fractions 5 and 6 increased and content of fraction 3 in the reticulocytes decreased compared to those in intact rats (Table 2). Thus, reticulocytes produced under normal conditions and during stimulated erythropoiesis differ in certain hemoglobin fractions. Our findings suggest that the observed modifications in hemoglobin profile can result from changes in the intensity of proliferative processes. This agree with the hypothesis stated previously [8].

For verification of this idea we studied changes in the hemoglobin profile and cell volume of erythrocytes during age-related inhibition of erythropoiesis. Indeed, in old rats (>3 years), the population of erythroid cells in the bone marrow decreased from $8.99\pm0.27\times10^6$ (middle age) to $6.49\pm0.58\times10^6$/100 g body weight in old rats ($p<0.001$), which reflects inhibition of erythropoiesis. In parallel to these changes we observed an increase in the content of hemoglobin fraction 5 (from 11.28±0.63 to 17.72±0.74 g/liter, $p<0.001$) and fraction 6 (from 2.87±0.24 to 5.51±0.70 g/liter, $p<0.001$) in the blood and in the mean erythrocyte volume (from 53.00±0.24 to 56.27±0.62 μ^3, $p<0.001$). Thus, qualitative changes in erythropoiesis consisting in production of large erythrocytes containing acid-resistant fetal hemoglobin isoforms can occur in the absence of activation of proliferative processes in the erythron system. This view is confirmed by the evidence that hemoglobin F and the mean volume of erythrocytes increase during cytostatic therapy in humans and monkeys [9]. Consequently, qualitative rearrangements in the erythron system including the pathways of red blood cell regeneration can serve a possible mechanism of shifts in the hemoglobin profile.

There are several pathways of erythropoiesis in the erythron system. The basic route is typical of adult organism under the normal conditions. The "reserve" or "emergency" mode is realized in fetuses, senile animals, and during hypoxia. It is characterized by production of large cells with increased content of acid-resistant fetal hemoglobin. Hypoxia stimulates switching of the red blood cell regeneration process to the reserve pathway leading to changes in hemoglobin composition in the peripheral blood.

REFERENCES

1. M. V. Borisyuk, *Uspekhi Fiziol. Nauk*, **14**, No. 1, 85-101 (1983).
2. I. P. Zapadenyuk, V. I. Zapadenyuk, E. A. Zakharia, and B. V. Zapadenyuk, *Laboratory Animals. Breeding, Keeping, and Handling in Experiments*, Kiev (1983).
3. N. F. Statrodu and V. I. Nazarenko, Heterogeneous System of Hemoglobins.

Structure, Properties, Synthesis, and Functional Role, Kiev (1987).

4. B. P. Alter, *Exp. Hematol.*, **7**, 200-209 (1979).
5. F. H. Bosch, J. M. Werre, B. Roerdinkholder-Stoelwinder, *et al.*, *Blood.*, **79**, No. 1, 254-260 (1992).
6. E. Kleihauer, T. E. Tang, and K. Betke, *Acta Haematol.*, **38**, 264-272 (1967).
7. J. R. Murphy, *J. Lab. Clin. Med.,* No. 2, 334-341 (1973).
8. G. Stamatoyannopoulos, R. Veith, R. Galanello, and T. Papayannopoulou, *Ann. N.Y. Acad. Sci.*, **445**, 188-197 (1985).
9. M. H. Steinberg, *Semin. Hemoglobin.*, **34**, No. 3, 8-14 (1997).