HETEROGENEOUS HEMOGLOBIN SYSTEM OF ANEMIC RATS

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UDC 612.111.11-019:599.323.4

The fractional composition of hemoglobin from the peripheral blood, spleen, and bone marrow of intact and anemic rats was studied by electrophoresis in polyacrylamide gel. Anemia was produced by injecting phenylhydrazine hydrochloride into the animals. The basic principles of formation of the heterogeneous hemoglobin system, depending on the source from which it was obtained and on the state of the animals, were established. The possible causes of the observed reorganization of the fractional composition of hemoglobin and its biological significance are discussed.

KEY WORDS: *Hemoglobin; fractions; anemia; rat.*

The assortment of proteins to be synthesized is known to depend substantially on the external conditions or stress which the organism experiences. One such factor acting on the organism is hypoxia caused by anemia. Changes in the fractional composition of hemoglobin in anemia have been investigated in sheep [14, 20], goats [13], ducks [12], mice [15], and cats [16]; as a result some common principles and specific differences in the response of the heterogeneous system of this protein to hypoxia have been established. Rat hemoglobin is a heterogeneous protein but structural and functional features of its individual components have not hitherto been explained [6]. The writers previously studied certain physicochemical properties of hemoglobin and its fractions in rats [8-11] and also demonstrated the basic disturbances of the heterogeneous system caused by the action of ionizing radiation and other factors on the hemoproteins both *in vitro* [5] and *in vivo* [7].

This paper gives the results of an investigation of the state of the heterogeneous hemoglobin system in rats with phenylhydrazine anemia. This evidence is essential for the elucidation of the molecular nature of the heterogeneity of this protein and also for an understanding of the mechanisms responsible for its changes under extremal conditions.

EXPERIMENTAL METHOD

Male albino rats weighing 120-140 g, in which phenylhydrazine anemia was produced as described previously [5], were used. Hemoglobin was isolated [18] from the peripheral blood, spleen, and bone marrow and fractionated in a polyacrylamide gel film [5]. Quantitative analysis of the fractions after electrophoresis was carried out with an MK-2 (Joyce) densitometer, after preliminary staining with Amido Black or Coomassie. The results were subjected to statistical analysis [3].

EXPERIMENTAL RESULTS AND DISCUSSION

By electrophoresis in a film of polyacrylamide gel hemoglobin of rat peripheral blood was separated into six fractions [5]. These same fractions also were discovered in hemoglobin isolated from the spleen and bone marrow. The quantitative proportions of the various hemoglobin fractions of intact and anemic animals are given in Table 1. The difference in relative proportions of hemoglobin fractions in peripheral blood, the spleen, and bone

Institute of Molecular Biology and Genetics, Academy of Sciences of the Ukrainian SSR, Kiev. (Presented by Academician of the Academy of Medical Sciences of the USSR N. N. Zhukov-Verezhnikov.) Translated from Byulleten' Éksperimental'noi Biologii i Meditsiny, Vol. 82, No. 11, pp. 1328-1330, November, 1976. Original article submitted April 20, 1976.

TABLE 1. Content of Hemoglobin Fractions in Red Blood Cells from Peripheral Blood, Spleen, and Bone Marrow of Intact and Anemic Rats

Fraction	Peripheral blood	Spleen		Bone marrow	
	$M \pm m$	$M \pm m$	P	$M \pm m$	P
Intact animals					
1	7,1±0,65	11,0±0,43	<0,001	13,7±0,62	<0,001
2	19,2±0,99	30,4±0,70	<0,001	36,2±1,19	<0,001
3	46,1±1,17	36,2±0,50	<0,001	22,5±0,70	<0,001
4	15,5±0,52	13,7±0,51	>0,5	12,1±0,45	<0,001
5	8,2±0,32	5,9±0,33	<0,001	8,1±0,31	>0,5
6	2,9±0,13	2,4±0,22	>0,05	7,1±0,34	<0,001
Anemic animals					
1	9,0±0,55	14,5±0,67	<0,001	14,2±0,86	<0,001
2	18,5±0,88	36,5±1,25	<0,001	36,4±0,94	<0,001
3	38,5±1,22	24,6±0,75	<0,001	24,8±0,52	<0,001
4	18,0±0,44	12,6±0,38	<0,001	12,9±0,68	<0,001
5	11,0±0,29	6,8±0,39	<0,001	8,1±0,45	<0,001
6	4,7±0,16	2,6±0,14	<0,001	5,4±0,50	>0,2

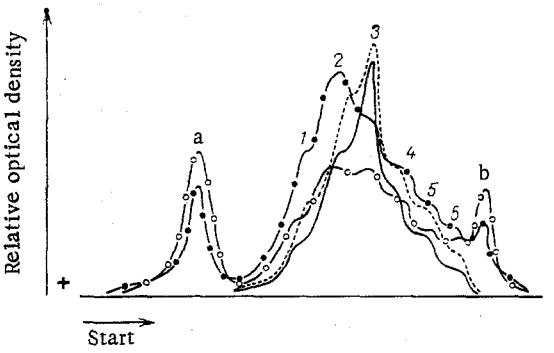

Fig. 1. Densitograms of hemoglobin isolated from red blood cells of peripheral blood (——), (– – –) and bone marrow (—○—○—) (—●—●—) of intact and anemic rats: 1-6, a, b) hemoglobin fraction.

marrow will be noted. In hemoglobin from the spleen and bone marrow the content of fractions 1 and 2 was higher but of fraction 3 lower than in hemoglobin from peripheral blood. In bone marrow there was also more hemoglobin of fraction 6 but less of fraction 4 (in the spleen the content of fraction 5 was reduced). Electrophoresis of bone marrow hemoglobin revealed one extra fraction quite clearly in the anodic and cathodic zones (Fig. 1).

In the heterogeneous hemoglobin system, regardless of the source from which it was isolated, the content of fractions 1 and 2 was increased in phenylhydrazine amemia, but at the same time the level of fraction 3 was lowered. Further, in hemoglobin from peripheral blood the content of fractions 4-6 was increased, whereas in hemoglobin obtained from the spleen and bone marrow their content was unchanged or was lower (but not significantly) than in intact animals. The quantitative content of the two additional fractions in bone marrow hemoglobin was reduced in anemia. It will also be noted that in the hemoglobin of the spleen and bone marrow of the anemic animals the content of fractions 3-6 was lower, but that of fractions 1 and 2 was higher than in peripheral blood hemoglobin.

The results indicate that the fractional composition of hemoglobin from the peripheral blood and hematopoietic organs differs significantly and that the differences are probably only quantitative in character; they also confirm observations [8] that during ontogeny of the red blood cell the relative proportions of the fractions change. The two additional fractions in bone marrow hemoglobin are evidently a free reserve of α- and β-chains, the presence of which has been demonstrated previously in erythroid bone marrow cells and reticulocytes [19]. Evidence in support of this view was given by the marked decrease in the protein content in these fractions during anemia, when synthesis of the polypeptide chains and their assembly into tetramers are sharply intensified. The electrophoretic mobility of

the additional fractions relative to the other components corresponded to the mobility of the separate α- and β-chains obtained by dissociation of rat hemoglobin in an acid medium with the aid of p-chloromercuribenzoate [11]. In phenylhdrazine anemia the content of the minor components in rat hemoglobin, just as in the hemoglobin of other animals [13-20], is increased and at the same time the content of the component which accounts for the largest part of this protein is reduced. The character of the changes as described above is evidently a general feature of the reaction of hemoglobin to hypoxia and is characteristic both of man and of animals [1, 2, 4, 6].

The high content of the two fractions with the greatest mobility in hemoglobin from the hematopoietic organs compared with hemoglobin of the peripheral blood has also been described [18]. This fact and the increase in the content of the two hemoglobin fractions with the highest mobility in anemia evidently reflect the overall pattern of processes including changes in biosynthesis and conformational changes in the individual hemoglobin fraction [8]. The possibility cannot be ruled out that the increase in the content of the two fast-migrating fractions took place as a result of conformational changes not only affecting fraction 3 [5]. In this context interesting results have been obtained [17] to show that functioning hemoglobin differs in structure from hemoglobin just synthesized. Their sensitivity to conformational changes probably also differs, as has been observed for hemoglobin of erythrocytes and reticulocytes [5]. All the facts described above suggest that the character and degree of difference between the content of fractions 3 to 6 for all hemoglobin of intact and anemic rats may to some extent be influenced by the increase in the first two fast-migrating fractions.

In conclusion it must be noted that the changes in the heterogeneous hemoglobin system of rats with phenylhydrazine anemia are similar to changes in the fractional composition of hemoglobin in postradiation anemia [7]. One of the responses of the heterogeneous hemoglobin system to anemia of whatever nature is probably an increase in the content of the minor fractions with a simultaneous decrease in the content of the major fractions. It must be assumed that the functional properties of these fractions differ substantially and that the biological significance of the changes taking place is the normalization of the oxygen supply to the tissues under hypoxic conditions.

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