HEMOGLOBIN PATTERN IN NORMAL AND HYPOXIC RATS

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UDC 612.273:612.111.11:569.323.4

The hemoglobin (Hb) pattern in normal adult and newborn rats and in animals exposed to the action of hypoxia was studied by starch gel electrophoresis. Polymorphism of Hb was demonstrated in the rats. In young rats slow Hb fractions are more marked and its alkali resistance is greater than in adult rats. During adaptation to hypoxia there is a shift in the relative proportions of the fractions toward an increase in one of the slow fractions, but this is not the result of increased synthesis of fetal Hb. The electrophoretic mobility of the fractions was unchanged. The minimal character of changes in Hb composition during hypoxia is an indication of the stability of Hb synthesis in these animals.

KEY WORDS: rats; hemoglobin; hypoxia.

No general agreement has been reached in the literature on the question of changes in the properties of hemoglobin (Hb) during hypoxia. There is information that the hemoglobin spectrum in rats undergoes substantial changes as a result of hypoxia and anemia. The mobility of the fractions is increased, the protein content in some of them increases, and the Hb becomes more resistant to alkaline denaturation [1-3, 5, 17]; under these circumstances many of the changes discovered have been ascribed to increased synthesis of fetal Hb (HbF). However, investigations of changes in the fractional composition of rat Hb following exposure to similar conditions either have not been observed at all or were negligible [10, 11, 16].

The electrophoretic pattern of Hb from rats adapted to hypoxia was studied. Albino rats are a very suitable object for such investigations because their Hb is highly heterogeneous. If the properties of a certain fraction correspond best to the new conditions and if Hb synthesis is sufficiently labile, it can be postulated that the production of that component is increased.

Information in the literature on the fractional composition of Hb in rats is very contradictory. Best agreement between the data is found in studies by starch gel electrophoresis [8, 9, 11, 12, 14, 15]. However, although some workers express the view that this criterion exhibits polymorphism in rats, others have concluded that the Hb formula of these animals is uniform.

EXPERIMENTAL METHOD

Noninbred male albino rats were adapted to a pressure chamber by the method usually adopted in the laboratory [1] and, after the end of the training period (30, 60, and 90 days) they were decapitated along with control animals. The Hb spectrum of normal newborn rats also was determined. Erythrocytes were washed 3 times with physiological saline and then hemolyzed with 10 volumes of water alkalified with ammonia, to prevent crystallization of the Hb. The hemolysate on strips of filter paper was introduced into a thin layer (2 mm) of 13% starch gel. Preliminary tests showed that the presence of the erythrocyte stroma does not interfere with the fractionation of Hb. A continuous buffer system was used [13]. The dc voltage was 80-100 V applied to a plate with the gel measuring 11 × 15 cm. The plate was placed on sponges immersed in electrode dishes. Electrophoresis continued for 2-3 h. The strips of gel were stained with Amido black and washed to remove excess of dye by the usual method. The Hb spectra were photographed and the negatives examined densitometrically. The alkali resistance of the Hb was determined by Badyuk's 1-minute denaturation method.

Laboratory for the Study of Resistance of the Organism and Laboratory of Comparative Biochemistry of Inorganic Ions, I. M. Sechenov Institute of Evolutionary Physiology and Biochemistry, Academy of Sciences of the USSR, Leningrad. (Presented by Academician of the Academy of Medical Sciences of the USSR P. N. Veselkin.) Translated from Byulleten' Éksperimental'noi Biologii i Meditsiny, Vol. 85, No. 1, pp. 18-20, January, 1978. Original article submitted May 11, 1977.

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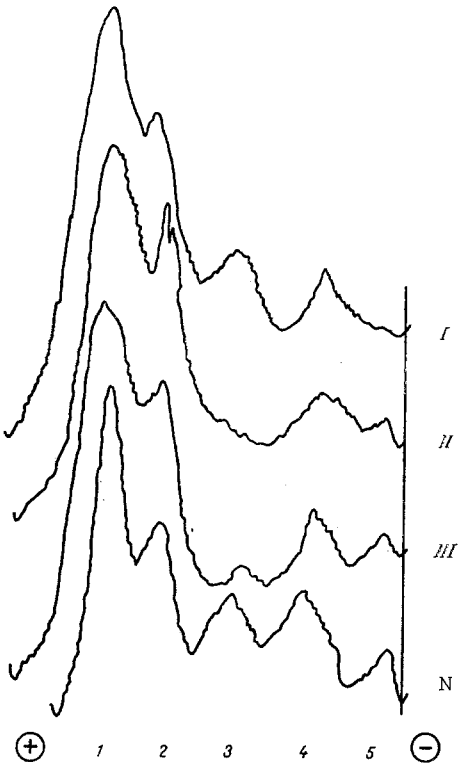

Fig. 1. Densitogram of hemoglobin fractions from normal adult (I, II, III) and newborn (N) rats. 1, 2, 3, 4, 5) Hb fractions. Start on the right.

EXPERIMENTAL RESULTS

In its fractional composition rat Hb was found to be nonhomogeneous (Fig. 1). Altogether five bands located at approximately equal distances apart were identified. In 63 rats (two-thirds of all animals) fractions 1, 2, 3, and 4 were present (type I), and fractions 1, 2, 4, and 5 were present in nine rats (type II). In both groups the intensity of the fractions was directly proportional to their mobility. The last group (type III) consisted of 23 rats in which all five fractions were present; the intensity of fraction 3 varied considerably in this group, but never exceeded the intensity of fraction 4; the protein content in fractions 4 and 5 varied accordingly. Type III was probably hybrid. Some workers [12] also regard fractionation into five bands as hybrid, but it has also been found in inbred rats [14]. The results of the present experiments thus point to polymorphism of Hb in albino rats.

Hypoxia caused slight changes in the relative proportions of the Hb fractions in the rats. The results of investigation of rats with the type I Hb spectrum are given in Table 1 (the data for the other types could not be subjected to statistical analysis because their numbers were too small). In rats trained for 30 days a tendency was found for an increase in fraction 3. After training 90 days this increase became significant, as also did a decrease in fraction 1. No changes in the mobility of the fractions could be found.

An increase in the slow and a decrease in one fast fraction were thus found, in partial agreement with the results of a previous investigation [17] of the action of bleeding on the Hb composition. Since the slow fractions are more resistant to alkali than the fast [4], with a change in the relative proportions of the fractions in favor of slow, the resistance of the rat Hb to alkaline denaturation may be increased. The physical role of this change is not yet clear, for the functional properties of the components are unknown. However, it is quite incorrect to associate the increase in alkali resistance with an increase in the proportion of HbF, for this is absent in rats and the formation of their Hb synthesis during the embryonic period consists of the successive appearance of all the various "adult" fractions [7, 9, 11]. Although some fractions at different periods of intrauterine development predominate quantitatively over the rest, this is no reason to regard them as fetal.

A criterion of alkali resistance can be used to judge the quantity of fetal Hb only in very limited cases. Even if HbF is found in animals of a given species, this does not mean that it is more resistant to alkaline denaturation than adult Hb. According to some data [6], the alkali resistance of Hb in newborn rats is the same

TABLE 1. Fractional Composition of Hb of Rats Adapted for Different Periods to Hypoxia

Training period, days	Group of animals	Number of animals	Hb fractions			
			1	2	3	4
30	Control	10	44,2±1,6	29,7±2,7	18,0±0,7	8,1±1,9
	Experiment	15	42,0±1,7	30,3±4,8	19,5±0,7	8,2±2,4
60	Control	8	39,0±1,6	30,0±1,1	20,0±1,5	11,0±0,9
	Experiment	11	38,8±1,4	30,0±1,1	20,7±0,7	10,5±0,7
90	Control	9	41,1±1,0	28,2±0,7	19,3±0,4	11,5±1,0
	Experiment	10	37,9±1,1	28,8±0,8	21,1±0,7*	12,2±0,9

*Statistically significant differences ($P < 0.05$).

as in adult rats. According to the results of the present experiments, it is 1.5 times higher in newborn rats. However, this is explained not by the properties of HbF, but by the greater representation of slow, more alkali-resistant fractions in newborn animals (Fig. 1). Analysis of the densitograms showed that in newborn rats the total of fractions 3, 4, and 5, as a relative percentage of the total Hb content, is higher than in adults, again by 1.5 times.

The redistribution of the components of Hb in the course of adaptation to hypoxia possibly took place not on account of a change in its synthesis, but as a result of conformational changes of some fractions into others, just as is observed during aging of erythrocytes in vivo and during their prolonged keeping. This phenomenon has been observed in rats with phenylhydrazine anemia [16]. The present experiments confirm the view expressed previously [9, 16] regarding the stability of Hb synthesis in rats. The action of hypoxia during periods commensurate with the life of one individual is evidently insufficient to bring about significant changes in Hb synthesis.

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