HEMOGLOBIN POLYMERS IN THE ANIMAL CIRCULATION

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One of the main demands which must be satisfied by a blood substitute and oxygen carrier, based on hemoglobin (Hb), is its long life in the blood stream. It is rapid elimination of Hb from the vascular system through the renal tubules [2, 3] that serves as the stimulus for creation of a polymer on the basis of Hb which, because of its increased molecular weight, would be retained longer in the circulation [1, 5, 7]. The aim of these investigations was to determine the half-elimination time of modified hemoglobins, which varied within quite wide limits because of differences both in the experimental models used, and in the characteristics of the compounds chosen for study.

The aim of the present investigation was to study the effect of molecular weight of various Hb polymers (PH) on the length of their life in the blood stream of animals, which would help to explain the mechanism of elimination of the oxygen carrier from the vascular system, and also to study changes in the fractional composition of the circulating polymer.

EXPERIMENTAL METHOD

Modifications of Hb were carried out by methods described previously [1, 4], with the aid of glutaraldehyde and pyridoxal-5'-phosphate. The Hb concentration in solutions and samples of blood plasma and also their content of the met-form were determined on a CO Oximeter IL-282. Values of pH of specimens for analysis were measured on the IL-305 pH-meter. Gel-penetrating chromatography of specimens of plasma and polymers was carried out on columns packed with sepharose CL-6B, and also by high-pressure liquid chromatography on a TSK-250 column (Bio-Rad, USA), and ion-exchange chromatography on the anion-exchange resin DEAE-Toypearl-650, with detection of the eluate on an LKB multichannel absorptiometer (Sweden) at 408 nm. Isoelectric focusing of the plasma samples was carried out in polyacrylamide gel within the pH range 3.5-9.5, with current strength of 25 A and power of 25 W. The experimental study of native and polymerized Hb was undertaken on 32 clinically healthy rabbits of both sexes, weighing 1.8-2 kg. About 20 ml of the test solution, in a dose of 1 g/kg body weight, was injected into the rabbit's auricular vein. Blood samples were taken after 5 min and 2, 4, 6, 18, and 24 h. The data were subjected to statistical analysis on the HP 85 computer.

EXPERIMENTAL RESULTS

The effect of the degree of polymerization of Hb on the duration of its stay in the animals' circulation was studied in the case of four series of modified Hb with different degrees of polymerization, the basic characteristics of which are summarized in Table 1.

It will be clear form Table 1 that the concentrations of all samples studied in solutions balanced as regards electrolyte composition were closely similar. Values of affinity of the modified Hb for oxygen were identical with one another, thus ruling out, in this investigation, the hypothesis [4] that this parameter may affect the life of modified Hb

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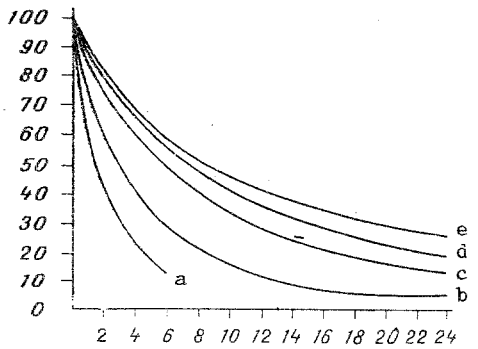

Fig. 1. Elimination of modified Hb from the animals' blood stream. Abscissa, time (in h); ordinate, concentration of modified Hb in plasma (in % of initial quantity); a) original Hb; b) PH-1, c) PH-2, d) PH-4, e) PH-3.

TABLE 1. Basic Characteristics of Modified Hb

Sample	Characteristics of injected solution				P_{50}, mm Hg	Composition of modified Hb	
	g/dl	met-Hb (%)	K^+	Na^+		% PH	% of unpolymerized Hb
			mmoles/liter				
PH-1	9,5	5,0	1,0	150	22,7	—	100
PH-2	10,0	3,8	3,5	160	27,8	29	71
PH-3	9,5	2,6	1,5	145	28,0	45	55
PH-4	9,4	2,2	2,0	152	25,0	62	38

in the blood stream. It was thus the direct influence of the degree of polymerization on the half-elimination time from the vascular system of the experimental animals that could be observed, excluding any influence of other factors.

It will be clear from Fig. 1 that the half-elimination time of the Hb polymers depended on the fractional composition: the higher the degree of polymerization, the longer the half-elimination time. The half-elimination time of the original unmodified Hb was about 2 h.

However, despite the different shapes of the elimination curves of the modified Hb, a certain quantity of the oxygen carrier continued to circulate in the blood stream 24 h later, even if the sample injected did not contain polymer, as in the case of PH-1.

Investigation of samples of blood plasma for the presence of met-Hb demonstrated the sufficiently high stability of the Hb polymers and showed that the average increase in the amount of met-form during 24 h of observation was about 15%. Thus throughout the period of observation there was no statistically significant change in the pH values in the samples tested, which varied between 7.35 and 7.45.

Gel-chromatograms obtained by elution of samples of plasma containing PH-3 on sepharose CL-6B (Fig. 2) are evidence that during the first 6 h of circulation preferential elimination of the low-molecular-weight fraction takes place, and in the next 12 and 18 h of circulation the rate of elimination of all the fractions is the same.

The method of high-pressure liquid chromatography on a TSK-250 column, by means of which the molecular-weight composition of the circulating oxygen carrier could be estimated quantitatively (Table 2) was used for analysis of the fractional composition of plasma samples containing other poly-Hb. In the process of circulation of all modified Hb, the appearance, followed by an increase in the content of fractions with mol. wt. of over 300 kilodaltons (kD) was observed, possibly due to elimination of low-molecular-weight products and also to the formation of complexes of the injected samples and plasma proteins.

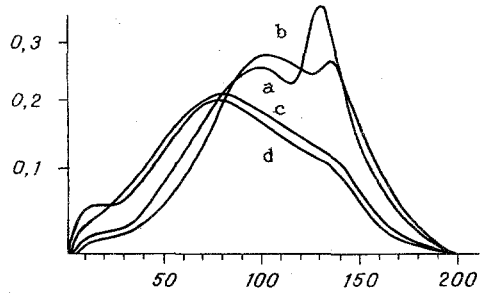

Fig. 2

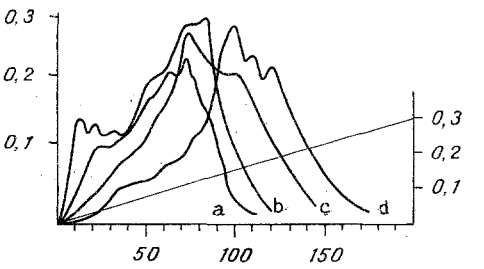

Fig. 3

Fig. 2. Gel-chromatograms of samples of blood plasma containing PH-3 at different circulation times. Abscissa, time (in h); ordinate, optical density at 408 nm ($OD_{408\ nm}$); a) 0 h of circulation; b) 6 h; c) 18 h, d) 24 h. Carrier: sepharose CL-6B; K24/100. Rate of elution 20 ml/h, eluant 0.85% NaCl + 0.02% NaN_3; weight of sample 50 mg.

Fig. 3. Gel-chromatograms of plasma samples containing PH-3 at different circulation times. Carrier) DEAE-Toypearl-650, K 19/27. Rate of elution 100 ml/h. Eluant 0.05 M Tris (pH 8.6) + 0.3 M NaCl. Weight of sample 50 mg. Legend as in Fig. 2.

TABLE 2. Content of Fractions of Modified Hb (with mol. wt. of over 300 kD) during Circulation in Animals' Blood Stream (in %)

Sample	Circulation time, h			
	0	6	18	24
PH-1	—	8	86	100
PH-2	—	8	84	98
PH-3	13	42	75	86

The use of ion-exchange chromatography to study the composition of plasma samples led to the discovery of changes in the total charge of polymers circulating in the plasma. The reuslts of chromatography of plasma samples containing PH-3 at different times of observation are given in Fig. 3. It was found that during circulation the charge of the fractions shifts toward lower values, as shown by an increase in the retention time of samples taken at later periods of observation on the carrier. During circulation the peak corresponding to unmodified Hb completely disappeared (Fig. 3: 1, 4), in agreement with data given above on the molecular-weight distribution of the plasma samples.

The results of this study of Hb polymers in the course of their circulation in the blood stream confirmed the hypothesis that they interact with plasma proteins, in agreement with data in the literature [3, 5, 6] on solutions of native Hb.

The length of life of modified Hb in the blood stream is therefore determined not only by their molecular weight, but also by other factors and, in particular, by their ability to interact with plasma proteins.

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