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EFFECT OF DEGREE OF HEMOGLOBIN POLYMERIZATION ON CUMULATION AND ELIMINATION

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Chemically modified hemoglobin (Hb) and, in particular, pyridoxylated polyhemoglobin (PPHb) is regarded by many investigators as a potential artificial oxygen carrier (AOC) [2, 3, 8, 9]. In experiments to study blood replacement by solutions of such compounds, their ability to maintain life of animals for a long time was demonstrated [3, 10].

Studies of various modified hemoglobins have shown that they can circulate in experimental animals for periods ranging from 2-4 h [5, 11] to 48-72 h [8]. The life span of Hb derivatives in the bloodstream is estimated relative to their half-elimination time. As has been shown, this characteristic is affected by the injected dose of Hb [5] and the degree of its chemical modification [1].

The half-elimination time is determined by measuring the change in concentration of the injected substances in the plasma during its circulation disregarding its excretion with the urine and its possible accumulation in the organs.

The aim of this investigation was the simultaneous estimation of the quantity of PPHb circulating in the plasma, excreted with the urine, and accumulating in the organs during the first few hours after injection. Various times of PPHb, differing in their degree of modification, were studied after intravenous injection into animals in a dose of 1 g/kg body weight.

EXPERIMENTAL METHOD

Modification of Hb with pyridoxyl-5-phosphate and glutaraldehyde was carried out by the method described previously [1]. The Hb concentration in the solutions was measured on a "Co-Oximeter 1L-282" instrument. The content of polymeric components in the samples was determined by high-pressure liquid chromatography on a TSK-250 column ("Bio-Rad," USA) in 0.1 M phosphate buffer, pH 6.5, with detection at 405 nm. The biological experiments were conducted on 42 male Wistar rats

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TABLE 1. Degree of Polymerization of Modified Hemoglobins

No. of Hb compound	PPHb	PPHb-1	PPHb-2	PPHb-3
Degree of polymerization (%)	0	14,4	24,8	53

weighing 240 ± 5 g. Excretion of PPHb, differing in their degree of polymerization, was studied in six groups of animals after intravenous injection of 5% solutions in a dose of 1 g/kg body weight. The urine was collected by catheterization of the bladder. All animals were anesthetized with pentobarbital (35 mg/kg). The plasma and urinary Hb concentrations were determined by the hemoglobin cyanide method, 1, 3, and 6 h after injection. The circulating plasma volume (CPV) was determined by dilution of a 5% sample of PPHb in plasma 1 min after its injection. It was assumed that immediately after the injection TPV increased by 20 ml/kg, corresponding to the volume of the injected solution, and returned to normal by the first hour of observation, as was shown by restoration of the hematocrit. The quantity of PPHb in the plasma was calculated by the equation:

$$PPHb_p = Hb_p \times TPV$$

where Hb_p denotes the concentration of PPHb in the plasma. The quantity of PPHb excreted with the urine was calculated by the equation:

$$PPHb_u = Hb_u \times VU$$

where VU denotes Hb_u the concentration of PPHb in the urine. The difference observed between the quantity of injected PPHb and its concentrations in the plasma and urine has been attributed to accumulation in the body. Morphological investigations were carried out on two groups of animals, with three rats in each group, and which were killed 5 days after injection of the test samples. The intensity and localization of hemosiderin deposits in the liver, spleen, lungs, heart, and kidneys were determined in both groups. Pieces of the organs were fixed with 10% neutral formalin and embedded in paraffin wax. Besides general survey staining with hematoxylin and eosin, Perls' reaction also was used to detect hemosiderin granules. The number of hemosiderin granules per unit area of histological section of the spleen was calculated with the aid of G. G. Avtandilov's grid, followed by statistical analysis of the results.

EXPERIMENTAL RESULTS

In the biological experiments four different series of chemically modified Hb, whose degree of polymerization is shown in Table 1, were studied. As Table 1 shows, the content of various Hb polymers in the synthesized samples varied from 0 to 53%. Our previous studies of similar compounds in a biological experiment [1] indicated a significant difference between the samples studied with respect to their retention time in the bloodstream. The possible differences in behavior of these PPHb in vivo depending on the degree of their polymerization were interesting. On the basis of these results interaction between PPHb and the recipient could be reflected more completely and, in addition, the optimal degree of polymerization of Hb for the creation of AOC on its basis could be selected.

A dose of 1 g/kg body weight was selected for the biological experiment, for this dose enabled PPHb concentrations sufficient for analysis by the methods described above to be obtained in the test samples without disturbing the circulating plasma volume.

The results of measurement of the PPHb concentration circulating in the plasma, excreted with the urine, and accumulating in the organs, are given in Fig. 1. They show that lowering the concentrations of injected samples in the plasma is accompanied by an increase in the quantity of Hb excreted with the urine. The increase in the degree of polymerization of Hb, moreover, led to an increase in the retention time of the injected PPHb sample in the plasma, as our previous results confirmed [1]. For instance, at 6 h of observation the quantity of PPHb-3 with a degree of polymerization of 50% was about 25% of the injected dose in the plasma, whereas for PPHb-1, containing 15% of the polymer, amounted to only 10% in the plasma at this

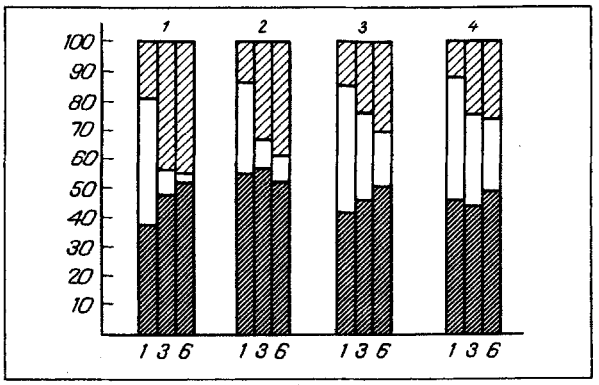

Fig. 1. Quantitative ratios of chemically modified hemoglobins in plasma (unshaded columns), urine (shaded columns), and organs (closely shaded columns) 1, 3, and 6 h after injection into animals' bloodstream. Ordinate, Hb concentration in (in %); abscissa, time of observation 1, 3, and 6 h. 1) PPHb, 2) PPHb-1, 3) PPHb-2, 4) PPHb-3.

time. For all the samples tested, regardless of the degree of polymerization, the fraction of the substance found in the plasma and urine was about 50-60% of the injected dose. These results suggested that about half of the chemically modified Hb injected accumulates in the animals' body. The fraction of accumulating substance is reflected by the lower closely shaded part of the columns, which account for 40 to 50% for all samples of PPHb studied, irrespective of the times of observation.

Accordingly, it was interesting to examine the possible ways of accumulation of PPHb of different composition in order to give rise to these differences. In the pathological investigation no hemosiderin was found in the kidneys, lungs, or heart of the animals after injection of PPHb and PPHb-2. Small granules of hemosiderin were found in individual endotheliocytes of the liver in two rats after injection of PPHb-2. Hemosiderin was found in the spleen of the animals of both groups in macrophages of the red pulp. The number of hemosiderin granules per unit area of histological section of the spleen was the same after injection of these two samples, and exceeded the physiological normal value. According to the literature [4], hemosiderosis may develop after blood transfusions. Deposition of hemosiderin in cells of the mononuclear phagocyte system, observed in the presence of low body iron levels, according to data in the literature [7] has no toxic action on organs and tissues. Thus a target organ for injection both of PPHb and its polymeric derivative, under the biological experimental conditions described above, is the spleen. Hemosiderin distribution in the spleen takes place chiefly in cells of the mononuclear phagocyte system of the trabeculae of the splenic pulp. The intensity of hemosiderosis in this case was independent of the degree of polymerization of the protein.

These investigations of chemically modified hemoglobins, injected into the animals' bloodstream thus showed that in the course of the first 6 h from 25 to 40% of the injected dose is excreted with the urine, depending on the degree of polymerization of Hb. For all the samples studied 50% accumulation of PPHb in the organs was observed, and this was confirmed at autopsy. In this experiment the location and number of hemosiderin granules in the organs was the same after injection of chemically modified Hb differing in their degree of polymerization.

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