COMPARISON OF HEMOLGLOBIN DISSOLVED IN PLASMA AND CONTAINED IN RED CELLS FOR MAINTAINING THE CIRCULATION AND RESPIRATION

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UDC 612.235

The basic circulatory and respiratory indices were compared in two series of cats. In the animals of series I the blood was completely replaced by an 8% solution of human hemoglobin (Hb), purified from stroma and procoagulant activity, whereas in the animals of series II the blood was diluted with dextran to an Hb concentration of 8 g%. The solution readily became saturated in the lungs and gave up its oxygen in the tissues, but did so much less readily than Hb contained in red cells. Dissolved Hb did not completely meet the oxygen demand of the body, and the animals developed hypoxia. Moderate anemic hypoxia caused by dilution of the blood with dextran was easily compensated. An Hb solution can be regarded as the basis or first stage in the creation of a blood substitute and oxygen carrier: KEY WORDS: hemoglobin; blood substitutes; hypoxia.

It was shown comparatively recently that hemoglobin (Hb) removed from red cells remains capable of binding with and giving up O_2, and in its molecular weight it closely resembles the plasma albumins and can maintain the colloidal osmotic pressure [3]. However, attempts to transfuse Hb solution have not proved successful because it led to severe kidney damage in the experimental animals. After the discovery that Hb, completely freed from stroma and coagulation factors, was safe for transfusion, the possibility of using an Hb solution as a blood substitute and oxygen carrier began to be studied [6, 7]. Experiments with partial and complete replacement of blood by Hb solution, conducted on dogs, dwarf pigs, and monkeys have shown that this solution can perform a respiratory function in the animal [4, 5, 7, 9]. A Soviet preparation of purified Hb, obtained by Rozenberg et al. [1], has been studied in cats whose blood was completely replaced by a solution containing 7-8 g% Hb. In these experiments, as in others by workers outside the Soviet Union, it was found that the Hb solution is readily saturated with O_2 in the lungs but does not give up sufficient oxygen to the tissues [2].

The object of this investigation was to compare the ability of an Hb solution and of blood containing the same quantity of Hb as the solution to maintain respiration and the circulation.

EXPERIMENTAL METHOD

Two series of experiments were carried out on cats weighing 2-4 kg anesthetized with pentobarbital (30 mg/kg). In series I (six cats) exchange tranfusion of blood was carried out in doses of 10 ml at a time up to a total volume of not less than three times the animal's blood volume, with a solution containing 8 g% Hb, obtained by Rozenberg et al. [1]. By this method of exchange transfusion it is possible to remove all red cells from the animal's blood (hematocrit index 1-3%, red cell count about 500,000/mm^3) without any change in the circulating blood volume or arterial pressure (BP).

In the experiments of series II (seven cats) the same exchange transfusion was carried out, but with dextran until the Hb level was about 8 g%. It was thus possible to compare two series of experiments in which the Hb concentration in the animals was the same: in series I, however, the Hb was dissolved in the plasma, and in series II it was contained in the red cells.

BP was measured in the femoral artery of the animals, the central venous pressure (CVP) was measured at the mouth of the inferior vena cava, the total gas exchange and the blood gases, partial pressure of oxygen (pO_2) and carbon dioxide (pCO_2) in the arterial and venous blood, the Hb level, and hematocrit index were deter-

Laboratory of Pathological Physiology, Central Institute of Hematology and Blood Transfusion, Ministry of Health of the USSR, Moscow. (Presented by Academician of the Academy of Medical Sciences of the USSR N. A. Fedorov.) Translated from Byulleten' Éksperimental'noi Biologii i Meditsiny, Vol. 86, No. 8, pp. 131-133, August, 1978. Original article submitted February 17, 1977.

0007-4888/78/8608-0977S07.50

TABLE 1. Comparison of Ability of Hb Dissolved in Plasma and Contained in Red Cells to Maintain Respiration, M ± m

Index	Dissolved Hb					Hb contained in erythrocytes			
	Stage of investigation								
	initial	Hb 8 g%	1 h	2 h	3 h	initial	Hb 8 g%	2 h	3 h
OC, vols. %	14,5	8,9±0,6*	8,7±0,8*	8,8±1,4*	8,6±1,5*	14,0	9,9±0,8*	9,0±0,8*	9,1±1,1*
CAO_2, vols. %	13,0	8,4±0,5*	8,4±0,5*	7,9±1,2*	8,2±1,6*	12,4	8,7±0,6*	7,7±1,4*	7,8±1,6*
CVO_2, vols. %	8,0	5,5±0,7*	5,5±0,9*	4,9±1,6*	4,8±1,8*	7,8	4,6±0,7*	3,5±1,5*	3,5±1,8*
OA-V, vols. %	5,0	2,9±0,4*	2,0±0,5*	3,0±0,9*	3,4±1,1*	4,6	4,1±0,1*	4,2±0,3*	4,3±0,3*
Hb O_2, %:									
arterial blood	90	93±4	93±4	90±4	89±4	89	88±4	86±4	86±4
venous blood	51	65±3*	63±4*	58±4*	56±4*	55	46±3*	39±4*	31±4*
pO_2, mm Hg:									
arterial blood	93	109±8*	110±10*	113±15*	114±15*	89	91±10	104±16*	108±15*
venous blood	45	122±2*	21±4*	21±5*	23±4*	44	39±3	33±5*	33±6*
pCO_2, mm Hg:									
arterial blood	42	32±3*	28±3*	29±4*	30±5*	39	38±4	32±6*	33±7*
venous blood	48	35±3*	32±4*	33±4*	34±6*	43	41±6	36±6*	36±8*
Coefficient of O_2 utilization	0,37	0,3±0,01*	0,33±0,03*	0,33±0,03*	0,34±0,03	0,38	0,48±0,01*	0,55±0,04*	0,56±0,03*
O_2, demand, ml/(min·kg)	8,3	5,6±1,3*	4,2±1,3*	3,5±1,3*	1,8±1,5*	5,6	6,3±0,6	6,7±0,8	6,5±0,7

*Difference significance compared with initial data ($P < 0.05$).

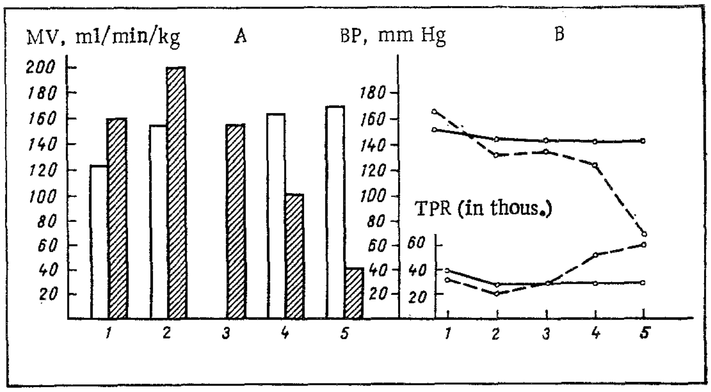

Fig. 1. Changes in MV, BP, and TPR after exchange blood transfusion with Hb solution and dextran. Abscissa, stages of experiment: 1) initial data, 2) immediately after replacement of blood (Hb 8 g%), 3) 1 h, 4) 2 h, 5) 3 h after transfusion. Ordinate: A) MV (in ml/min/kg), unshaded columns represent blood diluted with dextran, shaded columns Hb dissolved in plasma. B) Arterial pressure, in mm Hg, and TPR, in dynes · sec · cm^{-5}); continuous line represents blood diluted with dextran, broken line Hb dissolved in plasma. Difference significant compared with initial data ($P < 0.05$).

mined and the number of residual red cells counted. The minute volume of the circulation (MV) was calculated by the Fick method, the total periphereal resistance (TPR), arteriovenous difference in oxygen concentration (O_{A-V}) and the oxygen utilization coefficient after Krogh were calculated. The indices studied were determined before exchange blood transfusion (initial data), 10-15 min after (Hb 8 g%), and 1 h (in the experiments with Hb solution), 2 and 3 h after exchange transfusion.

EXPERIMENTAL RESULTS

Changes in the basic hemodynamic indices are given in Fig. 1. Immediately after exchange transfusion a small decrease in BP, not outside the limits of physiological variations, was observed in the animals of both series. In the experiments in which the blood was diluted with dextran, no further change in BP took place, but in the experiments with Hb solution it began to fall after 2 h, and fell catastrophically toward 3 h. TPR immediately after exchange transusion fell in the animals of both series, but later in the experiments with Hb solution it began to rise after 1 h, whereas in the experiments with dilution of the blood with dextran it was unchanged. The changes in BP and TPR can be understood if they are compared with the value of MV.

The decrease in TPR immediately after exchange blood transfusion in both series of animals was due to the increase in MV in response to the decrease in the oxygen capacity (OC) of the blood. Later, in the experiments with Hb solution, MV began to fall and BP was maintained for some time by spasm of the vessels and an increase in TPR, i.e., under conditions clearly unfavorable for the organism. By the third hour the compensatory mechanisms had collapsed. MV fell sharply and the increased TPR was insufficient to maintain BP. One of the main causes of the disturbance of the hemodynamics in these experiments was that the Hb remained for too short a time in the blood stream: the half-elimination period of Hb is about 90 min [8].

Changes in the gas composition of the blood and the O_2 demand are shown in Table 1. Since by the third hour of observation of animals with exchange transfusion with Hb solution severe hemodynamic disturbances were observed, it is more interesting to compare the results obtained in the early stages, when the hemodynamics was compensated. Although the same Hb concentration was present in both series of experiments, the O_2 supply to the body was maintained differently. Whereas the indices of the arterial blood were about the same, evidence of the good oxygenation of Hb solution in the lungs, the indices of the venous blood differed considerably. The first feature to be noted was the higher O_2 concentration and the higher O_2 saturation of Hb in the animals in which blood was replaced by Hb solution. One result of this was a lower value of O_{A-V}. Despite the fall in MV, it did not show any compensatory increase until the last moment, but then it was unable to significantly increase the O_2 supply to the tissues. Adequate O_2 reserves thus still remained in the venous blood, but at the critical moment they could not be used. The high O_2 concentration in the venous blood was connected with a shift to the left of the oxygen dissociation curve of free Hb, preventing the giving up of O_2 to the tissues when its pO_2 was low [8]. Characteristically the coefficient of O_2 utilization in animals after replacement of blood by Hb solution was almost unchanged compared with the initial state, whereas in animals whose blood was replaced by dextran, this coefficient rose in response to a decrease in OC. One result of the inadequate utilization of O_2 despite its perfectly satisfactory supply to the tissues by the Hb solution was the development of hypoxia, as shown primarily by the decrease in pO_2 in the venous blood. With this low level of pO_2 it might be supposed that true tissue hypoxia was beginning to develop, i.e., a state of mismatching between the O_2 supply and the O_2 consumption by the tissues. In fact the total O_2 consumption steadily fell during these experiments, whereas in the experiments in which blood was diluted with dextran it was unchanged.

The life span of the animals whose blood was replaced by Hb solution was the same as in the experiments described previously [2]. After total replacement of blood by Hb solution it is of course difficult to expect the animals to survive, for besides Hb, the blood also contains many other vitally important components not present in the solution.

In the report of one investigation Hb solution was actually called an ideal blood substitute in the title of the paper, but this in fact is untrue. Even in the early periods after replacement of blood by such a solution, before the circulation has become disturbed, the dissolved Hb was unable to provide sufficient O_2 to meet the demand, whereas moderate anemic hypoxia due to dilution of blood with dextran was easily compensated. Thus Hb solution can be regarded only as the basis for the first stage in the creation of a blood substitute and oxygen carrier.

LITERATURE CITED

1. G. Ya. Rozenberg, E. P. Vyazova, G. N. Ivanova, et al., Probl. Gematol., No. 11, 25 (1975).
2. N. A. Fedorov, V. S. Yarochkin, and V. B. Kiziner, Byull. Éksp. Biol. Med., No. 10, 402 (1977).
3. N. R. Amberson, Science, 106, 117 (1944).
4. N. R. Kaplan and V. S. Murthy, Fed. Proc., 34, 1499 (1975).
5. G. S. Moss, R. De Woskin, A. L. Rosen, et al., Surg. Gynecol. Obstet., 142, 357 (1976).
6. G. W. Peskin, K. O'Brien, and S. F. Rabiner, Surgery, 66, 185 (1965).
7. S. F. Rabiner, Fed. Proc., 34, 1454 (1975).
8. L. Sunder-Plassmann, R. Dieterle, I. Seifert, et al., Eur. J. Intensive Care Med., 1, 37 (1975).
9. I. M. Unseld, Anaesth., Resusc. Intensive Ther., No. 85, 90 (1974).