# FUNCTIONAL PROPERTIES OF HEMOGLOBIN DURING INCUBATION OF BLOOD IN OXYGEN

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The wide use of oxygen in the treatment of various hypoxic states requires a detailed study of its effect on functional systems of the body, including the blood system itself, for under certain conditions oxygen has a toxic as well as a beneficial action. Experiments on animals indicate that hyperoxia and hyperbaric oxygenation lead to significant changes in the affinity of hemoglobin for oxygen. To understand the mechanism of these changes and regulation of the oxygen-binding properties of the blood, it is important to study the reaction of the principal oxygen carrier, hemoglobin, under conditions of increased concentrations of this gas, after removal of protein from erythrocytes.

Model experiments of this kind can determine to what extent hemoglobin molecules are responsible for modification of affinity for oxygen, the causes of the change in affinity, the intensity of these changes, and so on. The object of the present investigation was to determine the answer to some of these questions.

## EXPERIMENTAL METHOD

Blood from adult noninbred rabbits, taken by puncture of the marginal vein of the ear, was used. Blood and hemoglobin solutions were incubated for 4 h in special glass saturators (Fig. 1), which were filled with 99% oxygen, air, and argon at 21-22°C and at atmospheric pressure. To ensure uniform mixing of blood and hemoglobin solutions the saturators were placed in an automatic flask shaker. Oxyhemoglobin dissociation curves were obtained spectrophotometrically [1], the concentration of 2,3-diphosphoglyceric acid (2,3-DPG) was determined by a nonenzymic method [2], the blood glucose level was determined by the orthotoluidine method as usually adopted for clinical purposes, and the hemoglobin solutions were prepared and the methemoglobin level in the blood determined as described in [3].

## EXPERIMENTAL RESULTS

Control measurements yielded information on affinity of hemoglobin for oxygen and concentrations of 2,3-DPG, glucose, and methemoglobin in rabbit blood in agreement with data in the literature (Table 1). Incubation of whole blood in oxygen at atmospheric pressure significantly ($P < 0.001$) increased the oxygen-binding properties of the hemoglobin: The dissociation curves were shifted to the left. It is generally considered that a shift of the curves to the left is one component of the defensive reaction of the blood system to excessive oxygen loading and it is manifested by a decrease in the oxygen supply to the tissues during hyperoxygenation of the organism. It must be expected that a change in the affinity of hemoglobin for oxygen during incubation in air will be less marked because the partial pressure of oxygen in this case is about 80% lower. However, the shift of the dissociation curves to the left was just the same, and the value of p50 (the oxygen pressure at which hemoglobin is half saturated with oxygen) fell, whereas affinity rose ($P < 0.001$). The oxygen-binding properties of hemoglobin isolated from erythrocytes and incubated in oxygen and air also increased, and the value of p50 fell in the first case to 28.7 ± 0.6 mm Hg ($n = 11$; $P < 0.001$), and in the second case to 30.2 ± 0.6 mm Hg ($n = 11$; $P < 0.001$) compared with the control. The methemoglobin concentration in the blood was unchanged.

In the modern view the principal factor determining affinity of hemoglobin for oxygen is 2,3-DPG. Rabbit erythrocytes are characterized by a very high concentration of this phosphate compared with other species of animals [4]. Hyperoxia leads to a decrease in the blood

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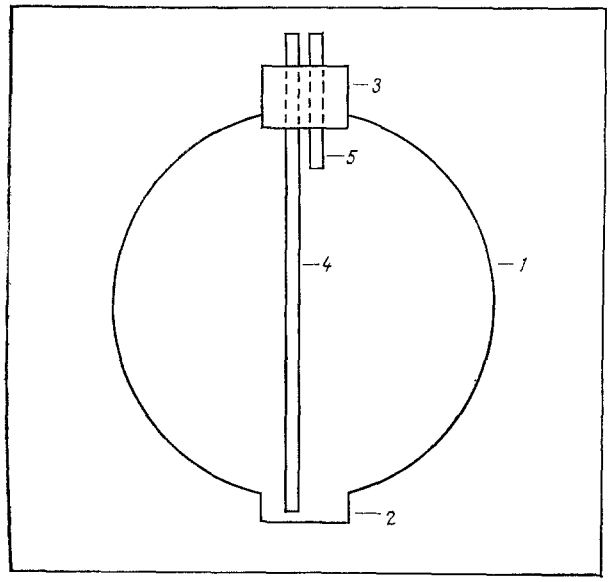


Fig. 1. Diagram of saturator for incubating blood in gaseous media. 1) Body of saturator; 2) cuvette for blood; 3) stopper; 4) gas inlet pipe; 5) gas outlet pipe.

TABLE 1. Indices of Red Blood during Incubation of Erythrocytes in Air, Oxygen, and Argon (M ± m)

| Experimental conditions | p50 mm Hg | n | 2,3-DPG, μmole/ml | n | Glucose, mg % | n |
|---|---|---|---|---|---|---|
| Control | 33,0±0,7 (28,0—36,4) | 43 | 8,61±0,38 (6,83—10,41) | 11 | 20,77±0,83 (15,16—24,85) | 12 |
| Oxygen | 29,8±0,7 (26,0—33,7) | 21 | 6,24±0,68 (4,12—8,63) | 11 | 13,66±0,94 (7,70—19,05) | 12 |
| Air | 29,0±0,9 (25,0—33,5) | 22 | 5,82±0,72 (3,13—8,50) | 9 | 14,80±0,77 (10,0—18,70) | 10 |
| Argon | 31,4±1,1 (26,0—35,3) | 11 | 4,74±0,60 (2,68—8,21) | 8 | 4,97±9,58 (2,0—7,30) | 12 |

Legend. Limits of variations shown in parentheses.

level of 2,3-DPG whereas anoxia, on the other hand, increases it. With a decrease in the 2,3-DPG concentration in the erythrocytes the affinity of hemoglobin for oxygen rises, but in the presence of high concentrations of 2,3-DPG it falls. Incubation of blood in oxygen and air in fact led to a sharp decrease in the concentrations of 2,3-DPG and glucose; the decrease was parallel and amounted on average to 30% ($P < 0.001$ and less than 0.01 respectively). It can be calculated on the basis of these results that a decrease in the 2,3-DPG content in the erythrocytes of 1 μmole would cause the dissociation curve to shift to the left by about 1.5 mm Hg.

In previous experiments to study the oxygen-binding properties of the hemoglobin of rabbits exposed to hyperoxia, affinity of hemoglobin for oxygen was shown [5] to increase by 10-13%. In that investigation *in vitro*, within a comparatively short time an increase in affinity also was demonstrated; the increase was the same regardless of whether blood or hemoglobin solution was incubated, and of whether it was incubated in oxygen or air. This indicates that hemoglobin isolated from erythrocytes, like that present in the erthrocyte, responds equally to an increase in the partial pressure of oxygen in the gaseous medium. Meanwhile, on the basis of the idea of the cascade fall in oxygen pressure in the body, an air medium will be hyperoxic both for cells and for hemoglobin solutions. In the experiments *in vivo*, the effect of an increase in the oxygen-binding properties of hemoglobin could be obtained only at relatively high oxygen pressures (2-4 atm) or as a result of adaptation of the animals to a pressure chamber for up to 30 h [5, 6]. This confirms the view that the properties of blood are regulated *in vivo* by several functional systems, the activity of which is directed against the disturbing action of hyperoxia. *In vitro*, the oxygen-

binding capacity of the blood in the hemoglobin molecule can be regulated only by the medium in which it functions and by the properties of the protein itself. Separation of cells from blood and of hemoglobin from erythrocytes rules out the effect of adaptive reactions of the systems of organs and of the blood system itself in response to hyperoxia. The molecules are compelled to resist, by their own devices, the increased oxygen concentration in the surrounding medium, and this is expressed as a rapid change in affinity and a shift of the dissociation curves to the left. This shift evidently lies within certain limits which, on the basis of the results of the present investigation and data in the literature, amount to 2-5 mm Hg.

Incubation of blood in oxygen and air leads to slowing of glycolysis because of a decrease in the activity of certain enzymes. In a medium of oxygen at a partial pressure of 150 mm Hg, activity of the key enzyme of glycolysis, hexokinase, is known to be blocked [7]. Inhibition of glycolysis takes place through acidification of the internal medium of the erythrocyte, because of an increase in the proportion of oxygenated hemoglobin in the cell. This leads to a decrease in production of 2,3-DPG, binding of which with oxygenated hemoglobin is interfered with, the effect of the phosphate on the protein is reduced, and the oxyhemoglobin dissociation curves are shifted to the left.

Incubation of blood in a medium of argon is of special interest. Under anoxic conditions glycoloysis proceeds more intensively, and by the end of the experiment the content of the initial and intermediate products falls sharply (glucose by 76%, 2,3-DPG by 45%). Meanwhile oxygen must diffuse from the blood to balance the pressure in the liquid and gaseous phases. Because of this reduced hemoglobin can accumulate in the cells, where it binds 2,3-DPG and thereby influences affinity. When the 2,3-DPG is bound with deoxygenated hemoglobin, phosphate has no inhibitory action on 2,3-DPG mutase, and further production of 2,3-DPG is facilitated, but since the quantity of glucose and glycolysis products in the incubation medium is limited, no increase in the phosphate level takes place. Considering that the decrease in the 2,3-DPG content in the blood samples was less than that of glucose, it can be tentatively suggested that under these conditions of incubation, when most of the 2,3-DPG is bound with hemoglobin, the breakdown of this substance is interfered with and its concentration in the cells remains high. Moreover, the final stage of incubation must be accompanied by accumulation of lactic acid, the end product of glycolysis. In this case a direct effect of pH on the affinity of hemoglobin for oxygen is possible, and causes the dissociation curve to shift to the right. As a result the change in affinity may be due to two processes. First, a fall in the 2,3-DPG level in the blood should lead to an increase in the affinity of hemoglobin for oxygen, but second, an increase in the lactic acid concentration should lead to a decrease in affinity. This may take place in experiments under both hyperoxic and hypoxic conditions, but since under hypoxic conditions lactate accumulation takes place more than twice as fast, the fall in the 2,3-DPG level and the increase in hydrogen ion concentration on account of the increase in lactate concentration causes the oxygen-binding properties of the hemoglobin to remain substantially unchanged during incubation of the blood in a medium of argon. On the whole, the mechanism of the shift of the dissociation curves under conditions of complete anoxia is not quite clear and further investigations are needed, more especially because hypoxia *in vivo* causes an increase in the 2,3-DPG concentration in the blood and a decrease in the affinity of hemoglobin for oxygen.

The results of the experiments *in vitro* thus confirm previous observations *in vivo* that the affinity of hemoglobin for oxygen is increased in hyperoxia and they suggest that the defensive reaction of the blood system to hyperoxygenation of the body commences at the molecular level. Hemoglobin isolated from cells responds to an increase in the partial pressure of oxygen by an increase in its affinity for this gas.

## LITERATURE CITED


1. L. A. Bokeriya, V. N. Doroshevich, and A. I. Marin, in: Collection of Scientific Publications by Graduate Students and Junior Staff, First Moscow Medical Institute [in Russian], Moscow (1969), p. 104.
2. I. N. Voronova and V. V. Gladilov, Lab. Delo, No. 11, 662 (1976).
3. V. V. Gladilov, in: Biological Research in the North-East of the European Part of the USSR [in Russian], Syktyvkar (1975), p. 130.
4. V. V. Gladilov, in: The Special and Clinical Physiology of Hypoxic States [in Russian], Part 3, Kiev (1979), p. 6.

5. M. S. Kushakovskii, Clinical Forms of Damage to Hemoglobin [in Russian], Leningrad (1968), p. 63.
6. I. S. Luganova and M. N. Blinov, Lab. Delo, No. 11, 652 (1975).
7. F. Bunn, Science, 172, 1049 (1971).