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STATE OF THE OXYGEN TRANSPORT FUNCTION OF BLOOD IN RABBITS WITH HYPERTHERMIA

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When the mechanisms ensuring a balance between the oxygen demand of an organism and its demand during the development of exogenous hyperthermia are studied, most attention has been paid to the investigation of the circulation and respiration [1, 2]. The study of the acid-transport function (ATF) of blood has been reduced to determination of the partial pressure (pO_2) and concentration (cO_2) of oxygen and the degree of saturation of the arterial and venous blood with oxygen (sO_2) [1, 10]. The affinity of hemoglobin for oxygen has not actually been determined during hyperthermia, although the possible position of the oxyhemoglobin dissociation curve (ODC) and the effect of factors responsible for its shift under these circumstances have been discussed. The state of the ATF of the blood during the first few hours after the end of exposure to heat has received even less study.

The aim of this investigation was to determine parameters characterizing ATF of mixed venous blood, including the affinity of hemoglobin for oxygen (P_{50}), in different phases of hyperthermia.

EXPERIMENTAL METHOD

Exogenous hyperthermia was simulated in 25 noninbred rabbits of both sexes weighing 2.5-3.5 kg. Under ether anesthesia a catheter was introduced through the jugular vein into the right atrium. The position of the catheter was verified after the experiment at autopsy. The rabbit's rectal temperature was measured 1 h after recovery from the anesthetic and an initial blood sample was taken. The animals were placed in a heat-insulated chamber, which was supplied with hot air, but their movements were in no way restricted. Heating took place under conditions of controlled hyperthermia: In the course of 30-45 min the body temperature rose to 42°C, at which level it was maintained for the next 30-45 min, after which the animal

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TABLE 1. Parameters of Blood OTF and Acid-Base State before and after Hyperthermia (M ± m, n = 25)

Parameter	Before hyperthermia	Toward end of hyperthermia	After hyperthermia	
			1 h	4 h
Hemoglobin concentration, g/liter	107,8±3,1	102,9±3,7	96,7±3,2*	93,5±3,0*
p_vO_2 at 37°C, mm Hg	34,7±1,25	26,34±0,88*	27,35±0,89*	33,38±1,34
p_vO_2 at blood t, mm Hg	38,7±1,17	36,8±0,93	34,3±1,36*	39,2±1,55
c_vO_2, vols %	7,06±0,42	5,21±0,53	5,25±0,64	6,77±0,61
OC, vols %	14,79±0,54	13,76±0,43	12,65±0,52	12,39±0,61
s_vO_2, %	47,74±1,51	37,84±1,61*	41,53±1,43	54,67±1,17*
pH at 37°C, units	7,336±0,039	7,379±0,061	7,290±0,041*	7,278±0,058*
pH at blood t, units	7,309±0,012	7,297±0,016	7,231±0,017*	7,248±0,016*
p_vCO_2 at 37°C, mm Hg	36,7±0,78	30,1±0,73*	30,2±0,77*	34,1±0,51*
p_vCO_2 at blood t, mm Hg	40,2±0,83	38,1±1,06	35,5±1,20*	38,1±0,72*
Buffer base deficit, meq/liter	−5,55±0,80	−6,54±0,93	−11,56±0,94*	−10,59±0,82*
Standard bicarbonate, meq/liter	19,84±0,69	19,27±0,84	15,81±0,72*	16,67±0,71*

Legend. Here and in Table 2, asterisk indicates statistically significant differences compared with initial data; t denotes temperature.

TABLE 2. Changes in P_{50} and 2,3-DPG Concentration before and after Hyperthermia (M ± m)

Parameter	Before hyperthermia	Toward end of hyperthermia	After hyperthermia	
			1 h	4 h
P_{50}, mm Hg, at pH 7.4, pCO_2 40 mm Hg, and 37°C (n = 25)	32,4±0,47	29,2±0,51*	28,4±0,48*	28,6±0,47*
P_{50}, mm Hg, at blood pH, pCO_2 40 mm Hg, and 37°C (n = 25)	36,0±0,57	38,8±0,69*	32,5±0,57*	32,4±0,59*
P_{50}, mm Hg, at blood pH and t, pCO_2 40 mm Hg (n = 25)	39,9±0,65	43,5±0,79*	40,4±0,85	38,3±0,66
P_{50}, mm Hg, at real values of pH, pCO_2, and t (n = 25)	39,1±0,64	42,7±0,75*	38,9±0,79	37,1±0,61*
2,3-DPG, mmoles/ml er. (n = 12)	6,31±0,69	4,13±0,62*	3,54±0,59*	4,5±0,44*

was removed from the chamber. The rectal temperature was recorded by means of a UT-1 electrothermometer, which was calibrated periodically. Repeated testing of the mixed venous blood was carried out at the end of exposure to hot air and 1 and 4 thereafter. Values of pO_2, the partial pressure of carbon dioxide (pCO_2), and pH were measured in the blood samples on a Microgas-analyzer ("Radiometer"). Besides these parameters, the oxygen capacity (OC), c_vO_2, and P_{50} also were measured, i.e., pO_2 and sO_2 was 50%. The value of c_vO_2 was calculated from the increase in pO_2 in a blood sample of known volume after displacement of oxygen from oxyhemoglobin with a 0.33% solution of potassium ferrocyanide [14]. The value of OC was measured by the same method, after first completely saturating the blood with oxygen. The value of s_vO_2 [15] was calculated from the ratio c_vO_2:OC. The parameter P_{50} was determined by the mixing method [15]. For this purpose the blood sample was divided into two equal parts (each of 0.6-1 ml) and placed in thermostatically controlled saturators, into one of which was passed a moist oxygenating gas mixture (94.5% O_2 and 5.5% CO_2), into the other, a deoxygenating mixture (94.5% N_2 and 5.5% CO_2). After oxygenation and deoxygenation (pO_2 < 1 mm Hg) and separate parts of blood were mixed in a thermostated syringe in equal proportions and pO_2 was determined, and as a first approximation, with these relations between the oxygenated and deoxygenated blood samples, it was equal to P_{50}. The exact value of s_vO_2 was found by the equation in [15] and was used to calculate P_{50}. For determination of the standard P_{50} (pH 7.4, pCO_2 40 mm Hg, and temperature 37°C) Bohr's constant was taken to be 0.48, the average between the extreme variants described in the literature for rabbit hemoglobin [8, 9]. Values of pO_2, pCO_2, pH, and P_{50} were reduced to values of the animal's body temperature by the use of appropriate coefficients [12]. The concentration of 2,3-diphosphoglyceric acid (2,3-DPG) was determined by the method in [7]. The position of the ODC corresponding to standard and real values of pH and pCO_2, was calculated from data for p_vO_2 and c_vO_2 [12]. Besides these experiments, tests also carried out in vitro, in which blood samples taken from 12 rabbits were incubated for 15, 30, 45, and 60 min at 42°C. Next, P_{50} and the concentration of 2,3-DPG were determined by the methods indicated above. The results were subjected to statistical analysis by the method of indirect and direct differences.

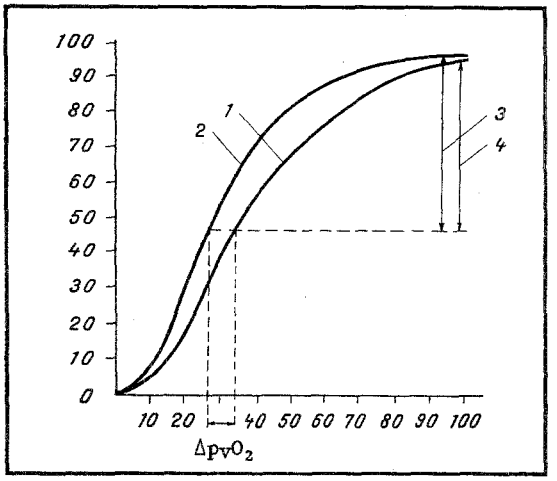

Fig. 1. Oxyhemoglobin dissociation curves calculated by Hill's equation using averaged values of P_{50} and arteriovenous difference in O_2 concentration toward end of exposure to hyperthermia at real (1, 4) and standard (2, 3) values of pH, pCO_2, and temperature. Δp_vO_2) Increase in blood pO_2 due to rise of temperature. Here and in Fig. 2: abscissa, pO_2 (in mm Hg); ordinate, s_vO_2 (in %).

EXPERIMENTAL RESULTS

Values given in Table 1 for parameters of the acid-base state before and after exposure to hot air demonstrate the development of respiratory alkalosis and a corresponding metabolic acidosis. This biphasic picture of changes in the acid-base state is characteristic of an organism exposed to hyperthermia [10]. The magnitude of the metabolic component is evidence of predominance of anaerobic processes in the body. However, despite the developing hypoxia, P_vO_2 at the real blood temperature toward the end of exposure to hot air and 4 h thereafter showed no significant change (Table 1). Toward the end of exposure to heat s_vO_2 and c_vO_2 fell by 10.10 ± 1.161% ($p < 0.01$) and 1.85 ± 0.53 vol. % ($p < 0.05$), and 1 h after the end of exposure by 6.21 ± 1.43% ($p < 0.01$) and 1.81 ± 0.64 vol. % ($p < 0.05$), respectively, evidence of an increased utilization of oxygen from the blood by the tissues. After 4 h, s_vO_2 and c_vO_2 had increased up to 54.67 ± 2.17% and 6.77 ± 0.61 vol. %, respectively. Similar relations between c_vO_2 and P_vO_2 for mixed venous blood indicate a change in P_{50}. The investigations (Table 2) showed that under real conditions of the circulation of the blood, i.e., at the temperature, pH, and pCO_2 of the blood, P_{50} rose by 3.6 ± 0.84 mm Hg toward the end of exposure to hyperthermia ($p < 0.01$). This decrease in P_{50}, moreover, was found when pO_2 was reduced by 2.1 ± 0.81 mm Hg ($p < 0.05$) and with a virtually unchanged pH. Consequently, the increase in P_{50} was due mainly to a rise of temperature (Fig. 1). The value of P_{50}, reduced to t = 37°C, pH 7.4, pCO_2 = 40 mm Hg, was 13.5 ± 0.9 mm Hg below the real value and 3.2 ± 0.8 mm Hg below that corresponding to these standard conditions in the initial state. Thus, by the end of exposure to hot air P_{50} under standard conditions was re-

TABLE 3. Changes in pH, P_{50}, and 2,3-DPG Concentration at Various Times of Incubation of Blood at 42°C (M ± m; n = 12)

Parameter	Control	Incubation time, min			
		15	30	45	60
pH, units	7,191±0,013	7,217±0,016	7,163±0,014*	7,167±0,013*	7,142±0,016*
P_{50}, mm Hg, at blood pH, pCO_2 40 mm Hg, and 37°C	43,6±1,83	42,7±2,16	41,0±1,64	41,7±1,99	40,4±1,63
P_{50}, mm Hg at pH 7.4, pCO_2 40 mm Hg, and 37°C	34,3±0,40	33,9±0,54	31,4±0,57*	31,1±0,26*	30,27±0,26*
2,3-DPG, mmoles/ml er.	6,89±0,45	6,04±0,72	4,89±0,51*	4,69±0,63*	4,25±0,60*

Legend. Asterisk indicates statistically significant differences compared with control data. er) Erythrocytes.

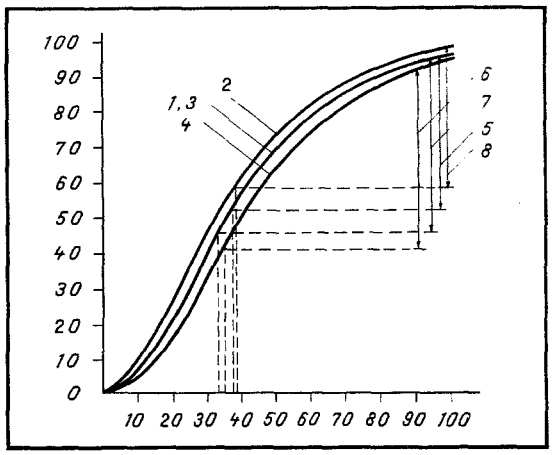

Fig. 2. Oxyhemoglobin association curves calculated by Hill's equation from average values of P_{50}, at real values of pH and pCO_2, and at different temperatures. 1) Initial temperature; 2) temperature toward end of exposure to hyperthermia; 3) 1 h; 4) 4 h after end of hyperthermia; 5, 6, 7, 8) arteriovenous difference in oxygen concentration at corresponding stages of hyperthermia.

duced, and this was confirmed by the observed decrease in the concentration of 2,3-DPG, a modulator of the acid-binding properties of hemoglobin [6], in the erythrocytes, dependent on the state of metabolism in these cells.

Incubation of rabbit blood at 42°C (Table 3) also led to a fall of P_{50}. The decrease in P_{50} started at the 30th minute of incubation and the difference between the initial value and that after incubation for 60 min amounted to 4.1 ± 0.6 mm Hg ($p < 0.01$). Meanwhile the 2,3-DPG concentration decreased inversely proportionally to the incubation times (Table 3).

The results of the experiments in vitro suggest a mechanism for the effect of temperature on P_{50} of erythrocytes during hyperthermia. With a rise of temperature the velocity of glycolysis in the erythrocytes increases, leading to a decrease in the blood pH. By the 60th minute of hyperthermia pH was reduced by 0.05 ± 0.004 unit ($p < 0.01$). Lowering of the pH led to activation of 2,3-DPG-phosphatase and to inhibition of 2,3-DPG-mutase [11]. As a result of this, a decrease in synthesis of 2,3-DPG is accompanied by an increase in its breakdown, leading ultimately to lowering of the 2,3-DPG level and, consequently to an increase in P_{50}.

In vivo, due to a rise of temperature the velocity of metabolic reactions rises not only in the erythrocytes, but also in the remaining tissues, and judging by the changes in parameters of the acid-base balance, this leads to metabolic acidosis, evidence of oxygen deficiency in the tissues. The fall of pH, together with the raised temperature, cause a shift of ODC to the right and, ultimately, to an increase in the reduced hemoglobin concentration, and according to data in [4], this should lead to intensification of glycolysis in the erythrocytes and to 2,3-DPG production. However, this mechanism does not work, and the 2,3-DPG concentration falls, an evident sign of essential disturbances in the turnover of this ligand in the erythrocytes during hyperthermia and, possibly, of its relations with hemoglobin. On alkalification and a rise of temperature, the value of P_{50} decreases in response to 2,3-DPG [5, 13].

When the role of the increase in P_{50} in the oxygen supply to animals exposed to hyperthermia is evaluated, it must be emphasized that it does not ensure better delivery of oxygen to the tissues. Oxygen is supplied to the tissues in this case, not as a result of metabolic modification of the properties of hemoglobin at the erythrocyte level, but of a combination of the influence of the raised temperature and the Bohr effect. Analysis of ODC (Fig. 2) shows how the arteriovenous difference in oxygen concentration changes in the flowing blood at different stages of the experiment. The increase in the arteriovenous difference in oxygen concentration during hyperthermia is brought about by the rise of temperature

at which essential metabolic disturbances are observed, including in erythrocytes. These changes subsequently, at a normal temperature, reduce the ability of the blood to become deoxygenated and do not contribute to the oxygen deficiency formed during hyperthermia.

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