Relationship between Hemoglobin Affinity For Oxygen and Lipid Peroxidation in Fever

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Hemoglobin affinity for oxygen (P_{50} parameter) and the content of malonic dialdehyde (MDA), Schiff's bases, and diene conjugates in the plasma and red cell mass were examined in mixed venous blood of rabbits with pyrogenal fever. Correlation regression analysis of the tested parameters showed that reduction of the actual affinity of hemoglobin for oxygen is conducive to activation of free-radical processes.

Key Words: *fever; hemoglobin affinity for oxygen; lipid peroxidation; oxyhemoglobin dissociation curve; pyrogenal*

The significance of hemoglobin affinity for oxygen is traditionally [8,9] regarded from the viewpoint of its contribution to oxygen delivery to tissues. Our findings [3] indicate a certain relationship between this parameter and the activity of lipid peroxidation (LPO). A shift of the oxyhemoglobin dissociation curve to the left or to the right is associated with, respectively, a reduction or increase in the activity of free-radical processes, that is, hemoglobin with its capacity to bind and release (to a varying degree) oxygen helps maintain a specific prooxidant-antioxidant balance in the body. This hypothesis has been confirmed in some critical states associated with a marked alteration of the oxygen regime (normobaric and thermal hypoxia) but still requires to be experimentally validated.

This research was carried out to elucidate the relationship between the oxygen-binding properties of hemoglobin and LPO activity in pyrogenal fever characterized by significant changes in the energetics of the organism [5] and in lipid metabolism [8].

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MATERIALS AND METHODS

Experiments were carried out with 15 outbred male rabbits weighing 2.4 to 3.1 kg. Fever was induced by intravenous pyrogenal in a dose of 4 LD_0 per kg body weight. Blood was collected with a catheter through the jugular vein from the right atrium before and 2, 3, and 4 h after pyrogenal injection. Blood samples were analyzed for the basic parameters of the oxygen-transporting function of the blood and LPO products. The pO_2 and acid-base balance were assessed with an ABL-330 gas microanalyzer (Radiometer). Hemoglobin affinity for oxygen was assessed from the P_{50} parameter (blood pO_2 corresponding to 50% oxygen saturation of blood), which was determined by the "mixing" method in our modification [4] and corrected using Severinghaus' formulas [14]. The oxyhemoglobin dissociation curve was plotted on the basis of the resultant P_{50} values using Hill's equation. The activity of free-radical oxidation processes was assessed by measuring the primary, secondary, and final LPO products. The content of diene conjugates (DC) was assessed from changes in the forming conjugated diene structures of lipid hydroperoxides [6]. The MDA concentration was estimated

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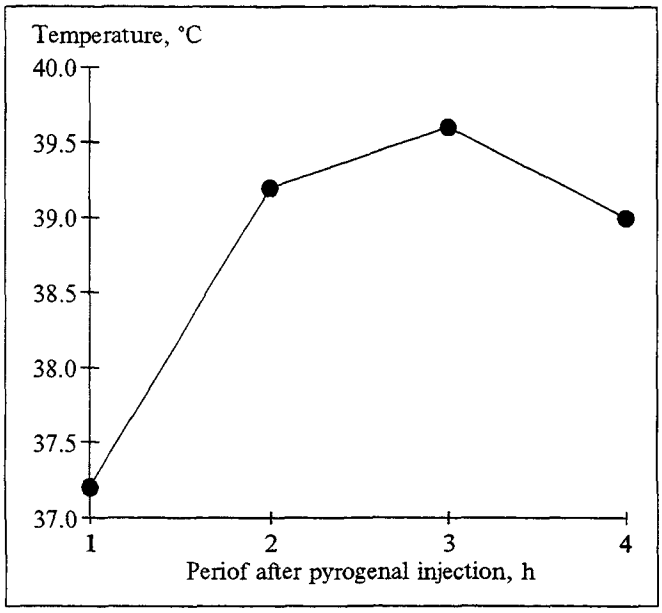

Fig. 1. Changes in rectal temperature after pyrogenal injection.

spectrophotometrically [1]. The level of Schiff's bases was measured with a Hitachi F-4010 spectrofluorometer according to the intensity of the extract fluorescence at excitation wavelength 344 nm and fluorescence wavelength 440 nm [11]. Changes in rectal temperature were recorded with a TPEM-01 electrothermometer. The data were statistically processed with a PC using Statgraphics software.

RESULTS

The pattern of temperature increase in the experimental animals is presented in Fig. 1. The maximal increase of temperature (2.9±0.3°C, $p<0.05$) was observed 3 h after pyrogenal injection. By the end of the fourth hour of pyrogenal fever the rectal temperature was 39.0±0.2°C ($p<0.05$). Table 1 presents data on the status of the major parameters of the acid-base balance: the blood pH increased from 7.317±0.025 to 7.398±0.015 ($p<0.05$) by the end of the third hour; the blood pCO_2 decreased by the end of the second hour from 45.3±1.5 to 33.4±0.8 mm Hg ($p<0.01$) and continued to drop slightly during the next 2 h (32.0±0.8 and 32.8±0.8 mm Hg, $p<0.01$). On the whole such a time course of changes in these parameters was true for the rectal temperature as well. This was paralleled by a reduction in the concentrations of hydrocarbonates and standard bicarbonate and an increase in the excess of buffer bases, indicating the development of compensated respiratory alkalosis evidently caused by thermal dyspnea. Blood pO_2 values did not differ much, and the actual pO_2 was somewhat increased, this indicating, along with changes in the acid-base balance, moderate disorders in the oxygen supply of the organism.

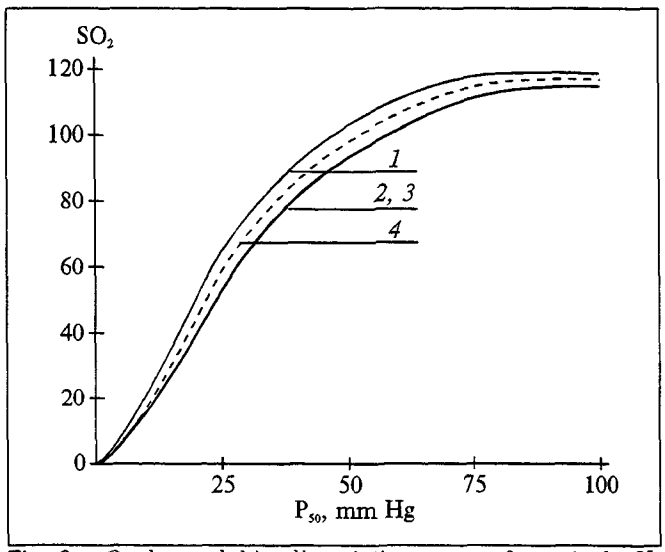

Fig. 2. Oxyhemoglobin dissociation curves for actual pH, pCO_2, and temperature values at various stages of fever.

The P_{50} parameter at standard values (pH 7.4, pCO_2=40 mm Hg, and temperature 37°C) fell from 32.6±0.5 to 29.7±0.5 ($p<0.05$) mm Hg by the end of the second hour of fever and remained so for the subsequent 2 h. The actual P_{50} value in experimental animals with changed pH, pCO_2, and body temperature increased at the peak of fever from 29.8±1.4 to 34.2±0.8 ($p<0.05$) mm Hg, indicating a shift of the oxyhemoglobin dissociation curve to the right under real conditions (Fig. 2). By the end of the fourth hour of fever the actual P_{50} was approaching its initial value.

On the whole, the content of the principal markers of LPO activity in the plasma and red cells had a tendency to increase, (Fig. 3). The level of diene conjugates was virtually unchanged in the plasma; it increased in the red cells at rest from 15.3±1.16 ΔD233/ml to 19.76±1.08 ($p<0.05$) and 18.74±1.13 ($p<0.05$) ΔD233/ml 2 and 3 h after pyrogenal injection. The MDA concentration in the plasma and red cells increased 3 h after pyrogenal injection to 1.47±0.07 and 9.31±0.56 μmol/ml, respectively ($p<0.05$), for initial values of 0.75±0.05 and 6.25±0.32 μmol/ml, respectively. At the peak of fever the concentration of Schiff's bases increased by 156% in the plasma and by 78% in red cells. The increase in these parameters reflects activation of free-radical oxidation of lipids.

Multiple correlation analysis of the relationship between the principal LPO products and the P_{50} parameter (actual and standard) revealed the following values of paired correlation coefficients (Table

TABLE 1. Effect of Pyrogenal on the Principal Parameters of the Blood Acid–Base Balance ($M \pm m$, $n = 15$)

Parameter	Initial	Hour after pyrogenal injection		
		end of 2nd	end of 3rd	end of 4th
pH	7.317±0.025	7.393±0.012*	7.398±0.015*	7.364±0.015
pH, actual	7.321±0.021	7.359±±0.011	7.359±0.015	7.334±0.015
pCO_2, mm Hg	45.3±1.5	33.4±0.8*	32.0±0.79*	32.8±0.8*
pCO_2, actual, mm Hg	44.6±1.2	37.3±0.83	35.8±0.9	35.8±0.6
pO_2, mm Hg	34.6±0.9	33.38±0.8	32.2±1.0	33.1±1.1
pO_2, actual, mm Hg	34.1±1.3	39.2±1.1	38.8±1.2	38.3±1.4
HCO_3, mmol/liter	23.02±0.96	20.33±0.46*	19.57±0.67*	18.5±0.77*
TCO_2, mmol/liter	24.43±0.95	21.36±0.48*	20.68±0.62*	19.55±0.78*
ABE, mmol/liter	−2.65±1.19	−4.02±0.56	−4.42±0.82	−5.93±0.93*
SBE, mmol/liter	−2.48±1.18	−4.24±0.54	−4.59±0.79	−6.04±0.89*

Note. Asterisk shows reliable differences vis–a–vis the initial value.

2). For the actual P_{50}, DC, MDA, and Schiff's bases this value varies from +043 to +055, except for the plasma diene conjugates. This reflects the presence of a moderately expressed correlation between the said parameters. The actual P_{50} value is preferable for analysis of the relationships between hemoglobin affinity for oxygen and LPO, for it more objectively reflects the process of blood desaturation in the capillaries. A shift of the oxyhemoglobin dissociation curve to the right favors the processes of blood desaturation at the level of capillaries [9]. Thus, perfusion of rabbit heart with blood for P_{50} increased by 13 mm Hg results in an increase of oxygen uptake by 30% [10].

The stepped-up oxygen delivery to tissues during a right-side shift of the oxyhemoglobin dissociation curve leads to an increase of pO_2 [9,10], this apparently causing, under certain conditions, an imbalance of various routes of oxygen utilization in the body and, among other things, enhances the processes of single-electron transfer associated with the formation of such reaction-capable intermediates as singlet oxygen, superoxide radical, and hydroxyl radical [15]. The synthesis of these intermediates initiates chain oxidative reactions causing an avalanche-like increase of oxidative destruction of lipids. It is obvious that under such conditions the body's mechanisms of antioxidative defense, which McCord [13] compared to a "house of cards," due to their functional fragility, are incapable of adequately performing their protective function.

Undoubtedly, an increase of the oxygen content in the blood significantly contributes to the

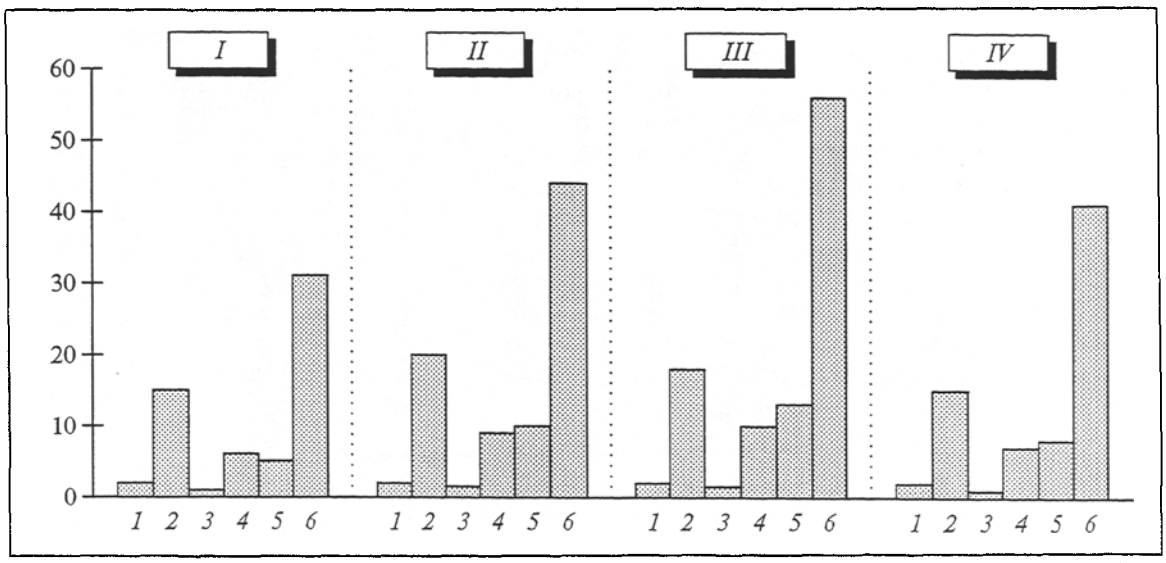

Fig. 3. LPO activity before (*I*) and after pyrogenal injection: by the end of the second (*II*), third (*III*), fourth (*IV*) hours. *1*) DC; *2*) DC in red cell mass; *3*) plasma MDA; *4*) MDA in red cell mass; *5*) Schiff's bases in plasma; *6*) Shiff's bases in red cell mass.

TABLE 2. Matrix of Paired Correlation Coefficients between Characteristics of Hemoglobin Affinity for Oxygen and Major Parameters of LPO in Pyrogenal Fever

Parameter	P_{50}, actual	P_{50}, standard	Plasma DC	Red cell DC	Plasma MDA	Red cell MDA	Plasma SB	Red cell SB
P_{50}, actual	1	0.22*	0.19*	0.51*	0.55*	0.43*	0.48*	0.46*
P_{50}, standard		1	0.13	0.43*	0.034	0.01*	0.16*	0.43*
Plasma DC			1	0.43*	0.29*	0.23*	0.06*	0.43*
Red cell DC				1	0.68*	0.70*	0.73*	0.82*
Plasma MDA					1	0.88*	0.78*	0.72*
Red cell MDA						1	0.78*	0.81*
Plasma SB							1	0.74*
Red cell SB								1

Note. SB: Schiff's bases. Asterisk shows statistically reliable coefficients of paired correlation reflecting moderate and strong correlations between the analyzed parameters.

activation of nonenzymatic oxygenase reactions of LPO, which are known to always proceed in the organism in small volumes due to the presence of activators of free-radical lipid oxidation in certain doses [14]. It is important to stress the increase of the actual pO_2 of venous blood observed in our experiments (Table 1). Hence, a reduction of hemoglobin affinity for oxygen, by increasing the level of molecular oxygen in the blood, creates, under conditions of unbalanced utilization of oxygen, the prerequisites for LPO activation. A similar mechanism of induction of LPO oxygenase reactions is observed in some hyperoxic states due to excess of oxygen, an electron acceptor [14,15]. Our data on changed values of actual P_{50} and LPO products in fever and the presence of a direct correlation between these parameters indicate the prooxidant nature of the hemoglobin affinity for oxygen.

It should be borne in mind that LPO processes are exothermal and entail the release of significant amounts of energy in the form of heat. Thus, a rise of the body temperature by 1°C involves an increase of energy consumption of almost 25% [7]. When assessing the relationship between LPO and changes in the hemoglobin affinity for oxygen from the standpoint of its biological significance, one must regard it as a compensatory adaptive reaction of the body which intensifies thermogenesis according to the "thermal free-radical boiler" mechanism [2] and, as a consequence, raises the body temperature.

Hence, under conditions of pyrogenal fever, hemoglobin, being an inherent part of the hemic component of the oxygen transport system, acts as one of the elements of the organism's multilevel prooxidant-antioxidant defense and, due to its affine properties, redistributes the oxygen quotas, in our case along the oxygenase reactions pathway.

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