EFFECT OF SODIUM CYANATE-MODIFIED AFFINITY OF HEMOGLOBIN FOR OXYGEN ON RESISTANCE OF RATS TO HEAT

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Increased resistance of individuals to the action of high temperature is an important problem. Among the known methods of increasing resistance to heat most are based on optimization of physical, and to a lesser degree, of chemical thermoregulation [7], which is a directly oxygen-dependent process [5]. Since hyperthermia is characterized by significant disturbances of oxygen supply [8], there is good reason to study the state of the various mechanisms responsible for adequacy of oxygen transport to the tissues for subsequent correction of their function and for ensuring adaptation of the body to exposure to heat. Hyperthermia has been shown to cause considerable changes in the properties of the blood, the affinity of hemoglobin for oxygen (AHbO), and the Bohr effect [2]. However, the role of the shift of the oxyhemoglobin dissociation curve (OHbDC) in the mechanisms of adaptation to heat remains largely unexplained.

The aim of this investigation was to study the effect of a deliberate change of AHbO on the thermal resistance (TR) of experimental animals.

EXPERIMENTAL METHOD

Experiments were carried out on noninbred male albino rats weighing 210-240 g, kept for the 4 weeks before the investigation under animal house conditions. The animals as a whole were divided into four groups: 1) intact, for investigation of the basic parameters. The remaining animals were exposed to hot air at a temperature of 41°C. In group 2, a blood sample was taken from the right atrium at the 60th minute of exposure. In groups 1 and 2 the blood gas composition was determined on an ABL-330 microanalyzer ("Radiometer"). The value of P_{50} (pO_2 corresponding to 50% saturation of the hemoglobin with oxygen), used to judge AHbO, was estimated by the mixing method [3] and corrected by the use of Severinghaus' formulas [12]. Animals of groups 3 and 4 underwent similar exposure to heat, and their rectal temperature was measured on a TPÉM-01 electrothermometer, and their heart and respiration rates were determined. TR was determined from the time of cardiac arrest. In the animals of group 4 (24) a shift of the OHbDC to the left was produced, and for 4 weeks these animals were kept on a special diet, with the addition of sodium cyanate to their drinking water [6]. During this period of time the weight of the animals did not change significantly. In six animals of this group the value of P_{50} was determined. All the experiments were carried out on anesthetized animals, into which pentobarbital was injected intraperitoneally in a dose of 5 mg/100 g. The results were subjected to statistical analysis on a personal computer of the IBM PC/AT type.

EXPERIMENTAL RESULTS

The results are given in Table 1. The value of P_{50} in group 2 at standard values of pH, pCO_2, and temperature, during hyperthermia was 33.4 ± 0.5, compared with 35.9 ± 0.4 mm Hg in the control ($p < 0.01$). The time

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TABLE 1. Basic Parameters of Oxygen Transport Function of Blood in Intact and Hyperthermic Animals and in Animals with Increased Affinity of Hemoglobin for Oxygen (M ± m)

Parameter	Intact animals	Hyperthermic animals	Animals with increased affinity of hemoglobin for oxygen
pH, units	7.318±0.013	7.296±0.016	7.281±0.019
pCO_2, mm Hg	53.3±1.9	44.2±1.6*	51.1±2.8
P_{O_2}, mm Hg	19.2±1.5	14.9±1.4*	15.1±3.1
Standard P_{50}, mm Hg	35.9±0.4	33.4±0.5*	23.3±1.3*
P_{50} at real temperature, mm Hg	35.3±0.5	45.8±1.1*	21.4±1.5*
P_{50} at real pH, P_{CO_2}, and temperature, mm Hg	32.2±0.6	40.8±1.3*	18.8±1.6*
Hemoglobin concentration, g/liter	112.3±3.4	124.8±2.8*	123.1±3.2

Legend. Asterisk indicates statistically significant differences.

course of the change in AHbO during hyperthermia was similar to that obtained in rabbits in the same state [2]. The change observed in this parameter led to some worsening of the conditions for deoxygenation of the blood at the capillary level. It was shown, for instance, that an increase in AHbO, i.e., a decrease in the value of P_{50} from 30 to 18 mm Hg, led to a decrease in the volume of oxygen supplied to the tissues by 22% [12]. However, during hyperthermia, taking into account the exothermic character of the interaction between oxygen and hemoglobin, OHbDC was shifted significantly to the right (P_{50} increased, allowing for the real temperature, to 45.8 ± 1.1 mm Hg). This is 10.5 ± 0.8 mm Hg more than the value of this parameter in animals of the control group, with the ordinary body temperature. Allowing for the real pH of the blood, however, P_{50} of the hyperthermic animals was somewhat reduced (40.8 ± 1.3 mm Hg), but the difference compared with the intact group still remained significant (ΔP_{50} = 8.6 ± 1.5 mm Hg). Accordingly, OHbDC at the real value of pH, pCO_2, temperature, shifted to the right during hyperthermia.

To investigate correlation between AHbO and the animal's body temperature during hyperthermia, a correlation-regression analysis of these parameters was undertaken. Values of the coefficients of correlation obtained indicate the existence of quite close positive correlation between rectal temperature and values of P_{50}, standard and real (Fig. 1).

During hyperthermia, the thermodynamic features of interaction of hemoglobin with oxygen thus bring about a considerable increase in AHbO. It follows from the traditional views that such a change in AHbO favors desaturation of the blood in the capillaries. However, normalization of the oxygen supply does not take place under these circumstances, as is confirmed by the decrease in the value of pO_2 of the blood (Table 1). AHbO during hyperthermia is modulated not only by factors such as temperature, pH, and pCO_2, as is confirmed by calculations using the equation in [11], according to which the value of P_{50} theoretically attainable at the discovered values of pH, pCO_2, and temperature, amounts to 43.3 ± 1.4 mm Hg, which is almost 2.5 mm Hg more than the real value in this group. This is evidently connected with a change in the intraerythrocytic metabolic status, namely a decrease in the 2,3-diphosphoglyceric acid concentration [2]. This gives rise to the perfectly reasonable question of the purpose of this change in AHbO. In particular, when discussing the importance of changes in the oxygen-binding properties of hemoglobin during exogenous hyperthermia [6, 7], the authors cited consider that they do not play an essential role in the genesis of the oxygen imbalance. According to our opinion, the increase in AHbO observed in hyperthermia

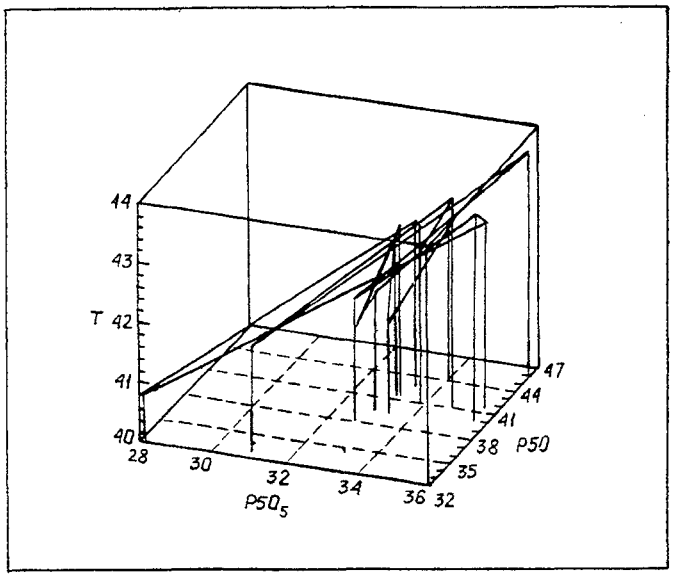

Fig. 1. Relations between affinity of hemoglobin for oxygen: standard (P_{50s}) and real (P_{50r}) and rectal temperature during hyperthermia: $P_{50s} = 2.06 \times T - 54.33$, $r = +77$ ($p < 0.001$); $P_{50r} = 33.05 \times T - 111.87$, $r = +0.76$ ($p < 0.001$).

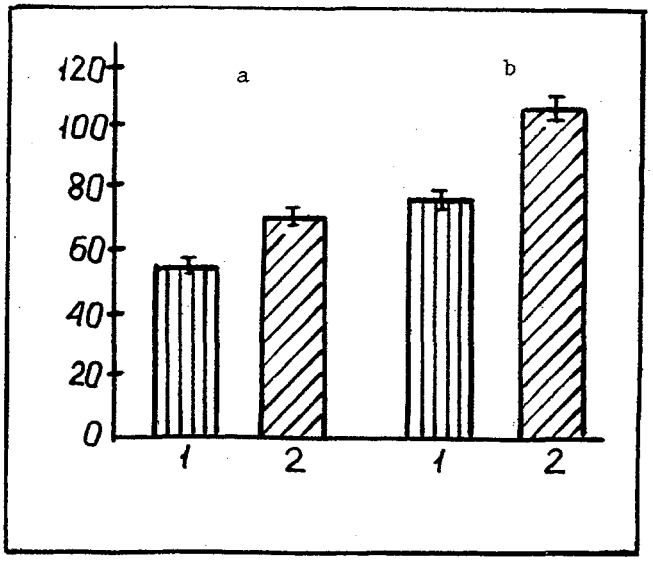

Fig. 2. Time taken for rectal temperature to reach 42°C (a) and time of death (b) of control animals (1) and of animals with increased affinity of their hemoglobin for oxygen (2).

must evidently be regarded as a special case of the general rule [9] of the effect of temperature on the physiochemical properties of molecules, which is directed, despite the change in body temperature, to maintenance of the various functions of the body at a certain optimal level.

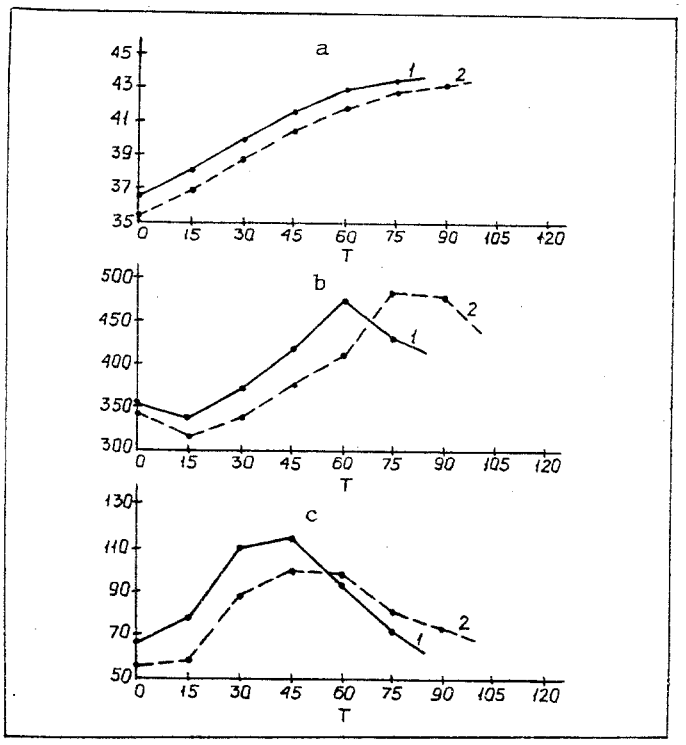

Fig. 3. Character of changes in rectal temperature (a), heart rate (b), and respiration rate (c) in animals with normal (1) and modified affinity of hemoglobin for oxygen (2).

To assess the role of the change in AHbO in adaptation to heat, a series of experiments was carried out involving determination of TR of rats in which an irreversible increase in AHbO had been produced beforehand with the aid of sodium cyanate. Measurement of P_{50} in six animals of this group (Table 1) showed a significant decrease in the value of this parameter (23.3 ± 1.3 mm Hg, $p < 0.05$), or allowing for real values of pH, CO_2, and temperature, an even more significant decrease (18.8 ± 1.6 mm Hg). The difference with respect to this parameter, compared with the control group, is 14.0 ± 1.7 mm Hg, and with the hyperthermic animals, it is even more significant (22.0 ± 2.1 mm Hg).

A rise of body temperature of 4-6°C, such as was observed in the present experiments, because of the characteristics of interaction between hemoglobin and oxygen indicated above, increases the value of P_{50} by 12-16 mm Hg. Correspondingly, in rats with irreversibly increased AHbO, considering the low initial value of P_{50}, in response to a significant rise of body temperature its value was in fact the same as in intact animals (Table 1). As will be seen, the effect of a rise of temperature in these animals virtually disappeared, and this undoubtedly had an effect on the conditions for oxygen diffusion into the tissue.

Possibly because of this, the time taken for the rectal temperature of these animals to reach 42°C and the time of death were considerably greater than in the control group (50.9 ± 1.9 and 65.8 ± 1.9 min, compared with 87.8 ± 4.1 ($p < 0.01$) and 108.8 ± 3.4 min ($p < 0.01$) respectively (Fig. 2).

Data on the dynamics of changes in the heart rate, respiration rate, and rectal temperature in the control and experimental animals are given in Fig. 3. The rate of rise of the respiration and heart rates was more marked in the control group, after 30 and 45 min. The heart rate of animals with irreversibly increased AHbO was reduced by 10.1% ($p < 0.05$) and 9.2% ($p < 0.05$) respectively. The respiration rate in this case was 87 ± 7 and 105 ± 8, which

are also lower than in the control animals (−7.8% and −8.6%, $p < 0.05$). This reflects the less intensive work of the mechanisms of heat loss and, evidently, an optimal level of function of chemical thermoregulation, and of the system of thermoregulation as a whole. At the 75th minute, the relations of these parameters in the control and experimental animals were opposite in character (+11.2% and +11.1%, $p < 0.05$ respectively), evidence of the more effective functioning of these leading mechanisms of heat exchange in rats with increased AHbO, and their decompensation in intact animals. This is confirmed by the earlier death of the latter. The rate of rise of the rectal temperature in the control and experimental groups was virtually identical, but the length of survival of these animals after reaching the 42°C level differed (15.5 ± 1.7 and 29.9 ± 1.9 min, $p < 0.05$ respectively), evidently due to the temperature-dependent features of interaction of hemoglobin with oxygen in the intact animals, and their disappearance in animals with modified AHbO.

It follows from the foregoing facts that it must be accepted that the reversible increase in AHbO, despite some worsening of the conditions for desaturation of the blood in the tissues, increases TR of the animal, and it must be regarded as one stage in the process of thermal adaptation.

When the possible mechanism of the increase in TR of the body in the presence of an increase in AHbO is analyzed, the dissipative character of energy metabolism during hyperthermia must be emphasized; it is manifested as weakening of coupling of oxidative phosphorylation and tissue respiration [5], as a result of which the expenditure of energy necessary for synthesis of one pyrophosphate bond is increased, and this increases also the proportion of the heat losses, i.e., of primary heat. According to the calculations in [5] heat production rises by 50% or more when the coefficient of phosphorylation falls from 3 to 2. An essential role in the process of the increase in heat production is ascribed to lipid peroxidation, activation of which takes place during hyperthermia [10]; this fact, in accordance with the views on the "thermal free-radical boiler," intensifies heat production at the stage of primary heat formation [1]. The latter is a favorable factor in hypothermia, but in exogenous hyperthermia, on the other hand, it limits TR.

The facts described above indicate that chemical thermoregulation functions under stress in hyperthermia, and this cannot be compensated by increased work of the heat loss mechanisms. There is no doubt that an important role in the development of the heat imbalance is played by disturbance of the adequacy of the oxygen supply of the body, realized through failure of the basic bioenergetic processes, lowering of their efficiency, and activation of free-radical reactions. Under these conditions mechanisms of stabilization and optimization of the flow of oxygen into the tissues assume particular importance. The shift of OHbDC to the right favors oxygen transport into the tissues, increasing the load on the thermoregulatory system. Since a shift of OHbDC to the left lowers the concentrations of the main products of lipid peroxidation: conjugated dienes, malonic dialdehyde, Schiff bases [4], modification of AHbO during hyperthermia, by limiting the flow of oxygen into the tissues, possesses an antioxidant, protective action, which may perhaps be more important under these conditions, when it is not the loss of heat from the body but the production of less heat that dominates the picture.

Thus the increase in AHbO observed during hyperthermia is adaptive in character. A goal-directed shift of OHbDC to the left in rats increases their resistance to heat, due to a decrease in heat production by the body and reduction of the load on the mechanisms of physical thermoregulation as a result of the antioxidant character of a shift of OHbDC to the left.

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