A comparative study of GAPD activity in medium with different pH values showed (Fig. 3) that optimal activity of the enzyme from normal human muscles occurred between pH values of 8.2 and 8.4 (0.08 M Tris-HCl buffer). In atherosclerosis the pH optimum was shifted to the acid side (pH 7.8-8.0).

The experiments thus demonstrated a decrease in the functional activity and changes in the physicochemical properties of GAPD, a very important enzyme of carbohydrate metabolism, in atherosclerosis. The possibility of obtaining crystalline enzymes from autopsy material allows the pathochemical basis of diseases to be studied at the molecular level.

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COMPARATIVE STUDY OF GAS-TRANSPORT CHARACTERISTICS OF EXTRAERYTHROCYTIC OXYGEN CARRIER MODELS

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KEY WORDS: hemoglobin; artificial oxygen carrier; gas transport characteristics.

During the attempt to create an artificial substitute for the erythrocytes of the blood, which has made rapid progress during the last decade, high-molecular-weight derivatives of hemoglobin (Hb) capable of circulating for a long time in the blood stream have been created [1, 6]. One of the main disadvantages of these compounds has been the lower efficiency of oxygen delivery compared with blood, which is also a feature of the original solutions of purified Hb and is due to the loss of 2,3-diphosphoglyceric acid (2,3-DPG) in the process of Hb isolation [7]. This problem was subsequently solved by the covalent addition of pyridoxal-5'-phosphate (PP), a functional analog of 2,3-DPG and a regulator of the affinity of Hb for oxygen, to Hb and its derivatives, so that the affinity of these Hb derivatives for oxygen is brought closer to the characteristic values for human blood [2]. A study of the principal physicochemical and biochemical properties of an Hb polymer (PHb) containing PP (PHb-PP) gave positive results [2] and laid the foundations for an extensive study of the gas-transport properties of the resulting compound.

The object of the investigation described below was to study the oxygen-dissociation curves (ODC) of PHb-PP and Hb-PP, the time course of their binding with oxygen at different Hb concentrations, and also the character of interaction with allosteric effectors under conditions as close to physiological as possible, a matter of the greatest importance when assessing the acceptability of these Hb derivatives as artificial oxygen carriers.

EXPERIMENTAL METHOD

ODC of the test compounds were recorded under physiological conditions (pH 7.4, pCO_2 40 mm Hg, temperature 37°C, Cl^- concentration 0.15 M) on a Blood Gas Laboratory 1L-217 instrument.

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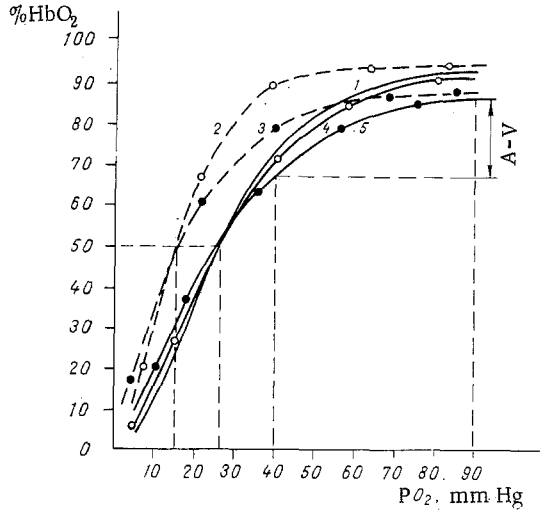

Fig. 1. Oxygen dissociation curves of hemoproteins: 1) blood, 2) Hb, 3) PHb, 4) Hb-PP, 5) PHb-PP. pCO_2 40 mm Hg, pH 7.4, 37°C; Cl^- 0.15 M; concentrations of Hb, PHb, Hb-PP, and PHb-PP 6%.

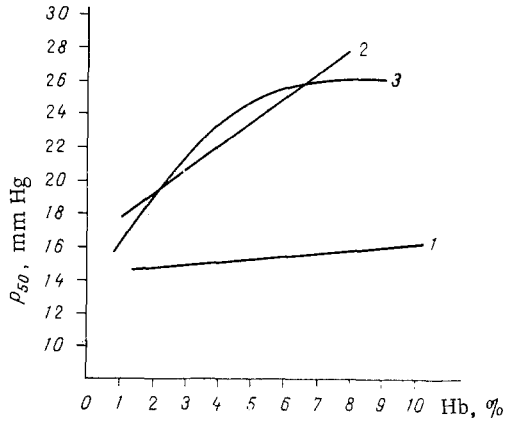

Fig. 2. Effect of concentration of Hb and its derivatives on P_{50}. 1) Hb not containing regulator of affinity for oxygen; 2) mixture of Hb with PP, 3) PHb-PP.

The Bohr alkaline effect was studied within the pH range from 6.8 to 7.5; the pH of the solutions was set by adding Tris (from Serva, West Germany) to them. Interaction between Hb and its derivatives with CO_2 was studied by varying pCO_2 from 20 to 80 mm Hg; the pH was adjusted to 7.4 by the use of Tris or aspartic acid (from Reanal, Hungary).

The effect of the concentration of Hb and its derivatives on their affinity for oxygen was studied by varying the Hb concentration between 2 and 10%, which corresponds to free Hb concentrations that may actually arise in the animal's blood stream.

The numerical results were subjected to statistical analysis.

EXPERIMENTAL RESULTS

For effective gas transport *in vivo* the artificial oxygen carrier must have a definite affinity for oxygen, characterized by the partial pressure of oxygen at 50% oxygen saturation of Hb (P_{50}). For freshly prepared donors' blood this parameter has a value of 26-28 mm Hg, and for purified Hb solutions, because of the loss of 2,3-DPG, it is 15-16 mm Hg (Fig. 1). As Fig. 1 shows, this change in the value of P_{50} leads to a significant decrease in the quantity of oxygen given up during a physiological drop of its partial pressure from 90 mm Hg (in the artery) to 40 mm Hg (in the vein) (Fig. 1, the distance A-V). Addition of PP to Hb and PHb yielded products with an affinity for oxygen practically identical with that of blood (25.79 ± 0.90 mm Hg). Nevertheless, the value of P_{50} is not a natural characteristic reflecting the efficiency of oxygen transport by Hb and its derivatives: To assess this factor, the

TABLE 1. Effect of pH and pCO_2 on Affinity of Hemoproteins for Oxygen

Substance	Number of experiments	$\Delta \log P_{50}/\Delta$ pH—$M \pm 3m$	$\Delta \log P_{50}/\Delta \log pCO_2$ $M \pm 3$ m
Blood	5	0,42±0,03	0,10±0,01
Hb	5	0,25±0,01	0,17±0,01
PH	5	0,26±0,04	0,19±0,03
Hb-PP	5	0,31±0,01	0,07±0,01
PH-PP	5	0,31±0,03	0,06±0,01

form of ODC, especially of its upper part, must be taken into account. For instance, it will be clear from Fig. 1 that a change in the shape of the ODC of Hb as a result of its polymerization (keeping the value of P_{50} unchanged) affects the distance A-V. Calculation of the distance A-V for Hb and its derivatives shows that if A-V for blood be taken as 100%, its value for Hb is 20-22% and for PHb 37-40%; after addition of PP the distance A-V increased to 98-113% for Hb-PP and 86-94% for PHb-PP (relative to blood).

To study the effect of fluctuations of PHb on the gas-transport characteristics of PHb-PP and to assess its role in carbon dioxide transport, interaction of Hb and its derivatives with ligands (H^+ and CO_2) was studied. The results of such investigation also allow certain changes in the structure of Hb, possibly as a result of its modification, to be judged. We know, in particular, that CO_2 competes with 2,3-DPG just as it does with PP [5] for binding with the α-amino groups of valine in the β-chains of Hb. That is why disturbance of the corresponding principles discovered for Hb and blood points to a modification of these regions or to the stereochemical changes which have arisen, making interaction with ligands more difficult. As controls, PHb not containing PP and a solution of native Hb and of Hb-PP were investigated.

As Table 1 shows, the Bohr effect ($\Delta \log P_{50}/\Delta pH$) for solutions of purified Hb and the effect of pCO_2 on the affinity of Hb for oxygen ($\Delta \log P_{50}/\Delta \log pCO_2$) differ significantly from the corresponding characteristics of blood, due to the absence of 2,3-DPG, and in agreement with data in the literature [8]. The results are evidence that the Bohr effects of polymerized and native Hb are virtually indistinguishable ($P > 0.05$). Hence it can be concluded that the groups responsible for the Bohr alkaline effect are not modified in the case we are discussing. The effect of CO_2 also remained at the level of the original Hb ($P > 0.05$), i.e., the α-terminal groups of the α- and β-chains of PHb were not blocked. Addition of PP both to Hb and to PHb led to an increase in the absolute magnitude of the Bohr effect to 0.31 ± 0.1, although this is 26% below the value obtained for blood. Under these circumstances the value of the CO_2 effect likewise occupies an intermediate position between the values for Hb and blood. The α-terminal amino groups of Hb, and also β-146 His and α-122 His are responsible for the CO_2 effect [3]. Considering the fact that not only the CO_2 effect but also the Bohr effect for PHb-PP was less than that for blood, this suggests that PP binds not only with the β-terminal groups of valine [4], but also, perhaps, with the α-terminal amino groups responsible for binding with CO_2, and with the basic groups taking part in interaction with 2,3-DPG.

The dependence of P_{50} of Hb and its derivatives on their concentration in solution also was studied (Fig. 2). For Hb not containing the regulator, this dependence was virtually absent (Fig. 2, curve 1); after the addition of PP to a solution of Hb, P_{50} rose significantly with an increase in concentration; the dependence, moreover, was linear in character (curve 2), in agreement with data in the literature [9]. For PHb-PP the linear character of this dependence was lost at concentrations above 3%; if the PHb-PP concentration was above 7-8%, any change in it had virtually no effect on the value of P_{50}. It can thus be tentatively suggested that the decrease in the PHb-PP concentration in the course of its circulation in the blood stream may have an adverse effect on the efficiency of oxygen transport mainly when the concentration of PHb-PP in the plasma falls below 3-4%.

On the whole this series of investigations has shown that the introduction of the PP molecule into PH as an analog of 2,3-DPG, functioning within the erythrocyte, led to the obtaining of an artificial oxygen carrier with gas transport characteristics close to those of freshly prepared donors' blood. Research into the functional characteristics of PHb-PP under physiological conditions, enabling its behavior in the blood stream to be predicted approxi-

mately, leads to the conclusion that the addition of PP not only lowers the affinity of PHb for oxygen and produces an increase in the quantity of oxygen given up by it practically to the characteristic values for blood, but also increases (although by a rather lesser degree) the efficiency of interaction between the artificial carrier and other ligands (H^+ and CO_2), which are themselves also regulators of gas transport processes *in vivo*.

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LIPID PEROXIDATION IN TISSUES OF NORMAL AND HUNGRY RATS OF DIFFERENT AGES

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According to the free-radical theory [7, 9], aging is associated with the action of free radicals on the genetic apparatus and on the biological membranes of the cell [8]. **Unsaturated acyl** residues of fatty acids of phospholipids, which take part in the process of peroxidation, are most sensitive to their action. Unsaturated fatty acids are formed during oxidative catabolism of lipids. In this connection, investigation of lipid peroxidation (LPO) is important during starvation, when the body switches from the carbohydrate type of metabolism to the lipid type [4].

The results of the study of LPO in the blood serum, adipose tissue, and liver tissue in rats of different ages, under normal conditions and after starvation for 48 h are described below.

EXPERIMENTAL METHOD

Experiments were carried out on young (aged 4-5 months) and old (aged 24-26 months) Wistar rats. The malonic dialdehyde (MDA) concentration was determined by the method in [6]. The background MDA level and its concentration during spontaneous, nonenzymic, and enzymic LPO *in vitro* (incubation for 30 min at 37°C with continuous shaking) were investigated and the mean rate of MDA formation was calculated. The composition of the reaction medium for spontaneous LPO was: 50 mM Tris-HCl, 160 mM KCl (pH 7.4), and 0.1 ml blood serum or 50 mg of finely minced test tissue (liver, epididymal adipose tissue). The final volume of medium was 2 ml. For nonenzymic (ascorbate-dependent) LPO 2.5 μM $FeSO_4 \cdot 7H_2O$ and 0.2 mM ascorbic acid also were added to the medium for enzymic (NADPH-dependent) LPO, 1 mM NADPH, 20 mM nicotinamide, 4 mM ADP, and 2.5 μM $FeSO_4 \cdot 7H_2O$ were added to the medium. The optical density of the solutions was measured on the SF-26 instrument at 532 nm.

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