ADP-induced aggregation. According to published data, defensin molecules contain lipophilic regions and can accomplish passive diffusion through the membrane lipid bilayer by hydrophobic interactions [5]. As demonstrated [4], defensins of human neutrophils act as inhibitors of protein kinase C which, in turn, mediates platelet aggregation, secretion from granules, and activation of arachidonic acid metabolism [6]. Without questioning the importance of this observation, we are inclined to believe, however, that the decrease in platelet functional activity in the presence of defensin revealed in the present study may be accounted for by a direct membrane-protective effect of the peptide.

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Myoglobin as an Indicator of Stress-Induced Damage

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Key words: *myoglobin; hypoxia; stress; adaptation*

Significantly elevated activity of cytoplasmic and lysosomal enzymes in the blood (i.e., enzymemia) is generally regarded as an important criterion for judging the severity of stress-associated damage to the body [4]. It has also been shown that elevated blood myoglobin is the earliest marker of damage sustained by the heart muscle in acute myocardial infarction [2, 13], which is known to be accompanied by a stress reaction [14]. However, the questions of whether myoglobin is released into the blood in the event of stress-associated damage and to what extent myoglobinemia may be relied upon as a criterion of such damage and of the efficacy of protective measures against stress have remained open. In view of this, the present study was undertaken to evaluate the impact of a relatively short single exposure of animals to stress on their blood content of myoglobin and to see how adaptation to periodic hypoxia, which is known to afford protection against stress [10-12], might affect myoglobinemia in the stressed animals.

MATERIAL AND METHODS

The experiments were carried out on four groups of male Wistar rats weighing 300±50 g: intact rats (group 1, control), those exposed to stress on a single occasion (group 2, stressed), those adapted to periodic hypoxia (group 3, hypoxia-adapted), and those exposed to such stress after adaptation to periodic hypoxia (group 4, hypoxia-adapted + stressed). The rats were stressed by being kept immobile in the supine position for 1 h. For adaptation to hypoxia, they were kept in a pressure chamber during 4 h daily over 40 days, starting with an "altitude" of 1000 m above sea level

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on day 1 and then increasing the "altitude": stepwise to 4000 m by day 4. The rats were sacrificed by decapitation immediately after the exposure to stress in group 2, on the second day after the last hypoxia adaptation session in group 3, and immediately after the exposure to stress on the second day following the last adaptation session in group 4. After decapitation, blood was rapidly collected into a test tube, kept there for 1-2 h at room temperature, and then centrifuged for 15 min at 3000 rpm; the supernatant (serum) was stored at -20°C until analyzed for myoglobin. This was determined by radioimmunoassay with the radionuclide ^{125}I using the commercially available kit "RIA-Myoglobin ^{125}I [3]. The results were processed statistically by Student's *t* test.

RESULTS

The 1-hour exposure to stress without prior adaptation to hypoxia led to a more than 2.5-fold rise of the serum myoglobin concentration (Table 1). The adaptation to hypoxia, while not affecting the myoglobin level in itself, did significantly limit its stress-induced elevation. Thus, as shown in the table, the hypoxia-adapted rats exhibited a myoglobin elevation that was only two-thirds of that experienced by the nonadapted animals (31.63±2.3 ng/ml *vs* 43.37±2.5 ng/ml). These findings indicate that a relatively short-term exposure to immobilization stress causes myoglobin to be released into the circulation, and that adaptation to hypoxia can diminish stress-induced myoglobinemia in addition to exerting the previously reported stress-alleviating effects [10-12]. In considering the present results it should be appreciated that myoglobin rises in the blood as a result of its liberation from muscle cells consequent to their death or the disrupted integrity of cell membranes. In myocardial infarction, for example, myoglobinemia is associated with damage to or death of cardiac myocytes in the ischemic zone, and, in addition, myoglobin may be released from such cells in the nonischemic areas of the heart that have been damaged as a result of the stress reaction accompanying the infarction. Indeed, the abnormalities of myocardial distensibility and contractility in rats with left ventricular infarction [7] are similar to those seen in animals experiencing emotional pain stress [1].

TABLE 1. Serum Myoglobin Levels in Stressed, Hypoxia-Adapted, and Hypoxia-Adapted + Stressed Rats

Group	Myoglobin, ng/ml
Control (n = 10)	16.87±1.25
Stressed (n = 8)	43.37±2.52*
Hypoxia-adapted (n = 8)	16.11±1.22
Hypoxia-adapted + stressed (n = 8)	31.63±2.30**

*Significant difference from the control group.
**Significant difference the stressed group.

In the study reported here the stress myoglobinemia may be thought to have occurred as a result of myoglobin release from the myocardium as well as from other muscles because the cell membranes had been made more permeable by the stress reaction-related damaging factors such as activated lipid peroxidation, toxic effects of excess catecholamines, and activation of lysosomal enzymes [4, 5]. The less severe stress myoglobinemia observed in the hypoxia-adapted rats can apparently be explained by two circumstances: first, by the mitigation of the stress reaction and of its damaging effects through the adaptation-induced activation of the stress-limiting regulatory systems of the body [8, 9]; and second, by the adaptation-induced increased resistance to damage of the cell membranes themselves by virtue of the development, during adaptation, of the so-called "phenomenon of adaptive stabilization of cellular structures" [6, 8].

In general, the results of our study suggest that elevated blood myoglobin may be regarded as a new indicator of stress-induced damage and of the efficacy of protective measures against stress. Moreover, since the myoglobin molecule has a much lower mass than the marker enzymes, myoglobinemia is likely to provide an earlier warning of damage than enzymemia. This possibility is supported by the finding [13] that myoglobin reaches its peak level in the blood 6 to 12 h after the onset of myocardial infarction, whereas creatine kinase activity peakes as late as 24 h after its onset.

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Influence of Polyhemoglobin Composition on Erythrocyte Aggregation and Circulation Time

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Investigations in the area of oxygen-carrying blood substitutes have resulted in the development of a variety of preparations meeting the principal requirements of a blood substitute, namely a sufficient intravascular persistence [1, 7] and effective oxygen transport [3, 5].

Works dealing with the circulation of such substances in an intravascular system faced major problems of toxicity [4], clearance mechanism [1, 6] as well as changes in the fractional composition of poly-Hb [2]. In addition, the concern about the effect of poly-Hb on the state of the formed elements of the blood is of great value in evaluating the optimum composition of poly-Hb solutions.

On this account the purpose of this study was to evaluate changes in the erythrocyte aggregation *in vivo* and in vitro in a model of 50% blood loss using chemically modified hemoglobin solutions with a varying content of the polymerized form.

TABLE 1. Main Characteristics of Chemically Modified Hemoglobins

Sample	Poly-Hb content,%	Oxygen affinity, mm Hg	Mn, thousand	Mv, thousand
PG-PF-1	2-3	28-30	-	-
PG-PF-2	25-30	26-31	100	90
PG-PF-3	40-45	27-29	230	200
PG-PF-4	70-75	25-28	600	650
Blood		26-29	-	-

MATERIAL AND METHODS

Hemoglobin modifications were prepared as previously described by the reaction with glutaraldehyde and pyridoxal-5-phosphate [1]. The hemoglobin concentration in the solutions and in the plasma samples as well as the methemoglobin concentration were measured on an IL 282 Co-Oximeter (Instrumentation Laboratory, USA). Compositions of samples of modified Hb were determined by high-performance liquid chromatography on a TSK-G3000 SW column (7.5 x 300 mm) (LKB, Sweden) in a 0.01 M phosphate buffer at pH 6.5; flow rate was 0.5 ml/min. Absorbance was measured at 280 nm. The percentage of poly-Hb was calculated using a 2220 Recording Integrator (Hewlett-Packard, USA). (M_n) and (M_w) were determined by sedimentation analysis using an analytical ultracentrifuge (Beckman Instruments, USA).

For a study of erythrocyte aggregation, samples of modified Hb were mixed 1:1 with samples of canine blood, and cells were counted in a Goryaev chamber: free, incorporated in aggregates, and total amount. Erythrocyte sedimentation rate was measured in capillaries by the standart technique: poly-Hb solution was added in a 1:1 volume ratio to fresh donor blood to simulate 50% blood loss compensation.

The experiments on dogs were carried out in a model of acute lethal (50 mg/kg body weight) blood

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