Reports

Survival at Extreme Altitude: Protective Effect of Increased Hemoglobin-Oxygen Affinity

Abstract. *Decreased hemoglobin-oxygen affinity is thought to be of adaptive value to humans and nonindigenous animals at high altitude. To test this, hemoglobin-oxygen affinity was modified by carbamoylation of hemoglobin in rats. Exposure of control (low oxygen affinity) and experimental (high oxygen affinity) animals to a pressure equivalent to high altitude revealed that increased, rather than decreased, hemoglobin-oxygen affinity will permit survival at greatly reduced environmental oxygen pressures.*

Man responds to various hypoxic stimuli through a decrease in hemoglobin-oxygen affinity (a "right shift" of the oxygen dissociation curve). Acutely, this may occur through physiological acidosis, whereas chronically the response stems primarily from an increase in the concentration of red cell 2,3-diphosphoglycerate (DPG) (*1*, *2*). A right shift of the oxygen dissociation curve in hypoxic states in humans is almost always an appropriate response, in that it functions to increase oxygen unloading in the tissues (*2*). If, however, greatly diminished environmental oxygen pressures are the cause of hypoxia, arterial hemoglobin-oxygen saturation will decline and a right shift of the hemoglobin-oxygen dissociation curve will only exacerbate this arterial desaturation. In the extreme case (at very high altitude), low arterial oxygen pressures, in combination with decreased hemoglobin-oxygen affinity, should lead to severe hypoxemia and eventual death. Our study was undertaken to investigate the possible protective effects of artificially increased hemoglobin-oxygen affinity during acute exposure of rats to very low environmental oxygen pressures.

Twenty-eight male Sprague-Dawley rats (average initial weight approximately 250 g) were randomly assigned to two groups of 14 animals each. Experimental animals were given drinking water containing 0.5 percent NaOCN (sodium cyanate), an agent known to irreversibly carbamoylate hemoglobin amino groups, thereby increasing hemoglobin-oxygen affinity (*3*). Controls were given water with 0.488 percent NaCl to equalize sodium intake in the two groups. Two weeks after the animals were placed on these regimens, blood was drawn via cardiac puncture; hemoglobin-oxygen saturation at an oxygen partial pressure, p_{O_2}, of 27.1 mm-Hg was measured; and the oxygen partial pressure at which 50 percent of the hemoglobin is oxygenated, p_{50}, was extrapolated from this (*4*). Red cell DPG concentrations (*5*), hemoglobin, and hematocrit value were also measured. The percentage of carbamoylated hemoglobin in the experimental animals was determined according to the technique of Berger and Eaton (*6*). Following cardiac puncture, the surviving animals (10 control and 12 experimental) were placed on plain drinking water for 24 hours to correct possible fluid imbalances. Each was then exposed in a hypobaric chamber to an atmospheric pressure of 223 mm-Hg (equivalent to an altitude of 9,180 m or 28,000 feet). The animals were held at this pressure for 78 minutes following a 12-minute period required to gradually attain it (total time, 90 min-

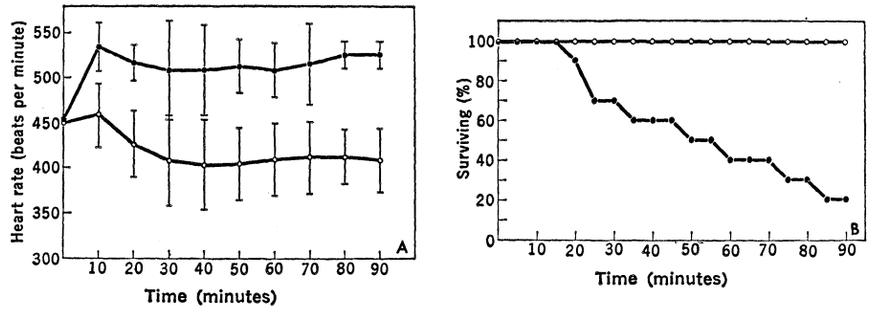

Fig. 1. Results of exposure of 12 experimental rats (open circles) and 10 control rats (closed circles) to a pressure equivalent to an altitude of 9180 m. (A) Average heart rates. Vertical lines through the individual points represent 1 standard deviation. Control values were averaged exclusive of the progressive bradycardia which characteristically developed 5 to 10 minutes before the death of each control animal. The significance of the differences (Student's *t*-test, two-tailed) was: 10 to 20 minutes, $P < .001$; 30 to 50 minutes, $P < .01$; 60 minutes, $P < .02$; and 70 to 90 minutes, $P < .05$. (B) Percentage of rats surviving in each group.

Table 1. Hematologic indices of experimental and control rats. Values are means ± 1 standard deviation. Abbreviations: hemoglobin, Hb; weight, w; volume, v. Carbamoylated Hb is measured as the percentage of total reactive amino groups.

Animals	Carbamoylated Hb (%)	p_{50} at pH 7.40 (mm-Hg)	Hb (% w)	Hematocrit value (% v)	Red cell DPG (μmole per gram Hb)
Control ($N = 10$)		37.3 ± 1.56	15.0 ± 0.67	42.7 ± 1.78	27.7 ± 1.83
Experimental ($N = 12$)	82.0 ± 11.4	21.0 ± 1.51*	14.9 ± 1.09	42.5 ± 1.93	21.0 ± 1.51†

* $t = 22.7$; $P < .001$. † $t = 9.89$; $P < .001$.

Scoreboard for Reports: In the past few weeks the editors have received an average of 68 Reports per week and have accepted 12 (17 percent). We plan to accept about 12 reports per week for the next several weeks. In the selection of papers to be published we must deal with several factors: the number of good papers submitted, the number of accepted papers that have not yet been published, the balance of subjects, and length of individual papers.

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utes). Heart rate was continuously monitored through two electrodes implanted subcutaneously and bilaterally on the chest and read out on an oscilloscope.

Approximately 80 percent of the reactive hemoglobin amino groups in the experimental animals had been carbamoylated after 2 weeks of ingestion of the water containing 0.5 percent NaOCN (Table 1). Simultaneously, p_{50} was reduced from 37.3 mm-Hg in the controls to 21 mm-Hg in the experimental animals (Table 1). No significant differences between the two groups were observed in hemoglobin or hematocrit value, but red cell DPG concentrations were somewhat lower in the experimental animals. Exposure of the rats to the reduced atmospheric pressure revealed highly significant differences in both heart rate (Fig. 1A) and survival (Fig. 1B). Throughout, experimental animals consistently displayed a much slower heart rate than did surviving control rats, which probably reflected less severe hypoxemia in the experimental animals. Furthermore, all of the treated animals survived the 90-minute trials, whereas eight of ten control animals died. The differences between the two groups in percent survival after 90 minutes is highly significant ($\chi^2 = 11.28$ with Yates's correction for continuity, $P < .001$). All experimental animals had an uneventful recovery from the exposure and remained alive until they were killed at a much later date. Because cyanate will react with the amino groups of many proteins, we recognize the possibility that the functional characteristics of other proteins may have been altered. However, it is most likely that the protective effect of cyanate which we have found is specifically due to a cyanate-induced alteration of hemoglobin-oxygen affinity.

Hemoglobin-oxygen affinity is decreased both in humans indigenous to high altitude areas (*7*) and in newcomers after exposure for about 12 to 24 hours (*8*). Although appropriate to most forms of hypoxia encountered by man at lower altitudes, this diminished oxygen affinity may be of no adaptive value in high altitude hypoxemia. At an altitude of 4,540 m (14,900 feet), arterial saturation in healthy human males is approximately 80 percent (arterial $po_2 = 45$ mm-Hg) and venous saturation is roughly 65 percent (*7*). In this "steep" portion of the oxygen dissociation curve, any increase in oxygen delivery to tissue gained by decreased hemoglobin-oxygen affinity will be accompanied by an almost equal loss in arterial oxygen saturation. Our results demonstrate that increased, rather than decreased, oxygen affinity is an effective mode of short-term adaptation to markedly reduced environmental oxygen pressures. This may prompt a reevaluation of the idea (*7–9*) that decreased hemoglobin-oxygen affinity is of adaptive value to humans at high altitudes.

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Bacteriophage Structure: Determination of Head-Tail Symmetry Mismatch for Caulobacter crescentus Phage φCbK

Abstract. *Electron micrographs of negatively stained bacteriophage φCbK have been analyzed by Fourier methods. Computer-calculated Fourier transforms that contain phase as well as magnitude information have established fivefold rotational symmetry for the head and threefold rotational symmetry for the tail. These results indicate that a symmetry match is not necessarily required between separate structural components of a bacteriophage.*

Simple viruses are constructed from multiple copies of one or a small number of protein subunits. These proteins bind noncovalently in a process analogous to crystallization to form a three-dimensional shell that functions to contain and protect the viral nucleic acid. The angles and positions of bonding contracts which any one of these proteins can make with another are quite specific and, thus, symmetry considerations are of great importance in determining the size and shape parameters of the protein coat or capsid for simple viruses and may also control the self-assembly in vivo. For isometric or "spherical" viruses, Caspar and Klug (*1*) proposed a scheme for packing of the subunits into a capsid with icosahedral point group symmetry. This scheme, which places constraints on the number of packing units making up the viral structure, has been repeatedly verified for many spherical viruses. The rod-shaped viruses, such as the plant viruses, and the filamentous bacteriophages are constructed from structural proteins organized with helical symmetry and are apparently limited as to size by the length of the nucleic acid which they encapsulate.

The tailed bacteriophages are one step further in structural complexity from the simplest viruses and contain structurally differentiated components with functionally distinct roles. Again the separate components of the structure are assembled from many copies of one or a few identical subunits, and symmetry, very likely, plays an important part in their interaction. However, it is not known what symmetry principles may be involved in the interaction of structurally distinct com-