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Hemoglobin and Oxygen: Affinities in Seven Species of Sciuridae

Abstract. Studies of the respiratory function of the bloods of seven species of squirrels suggest that, in the evolution and adaptive radiation of this group, the oxygen affinity of hemoglobin has changed in a manner to better adapt the various species to different ways of life and different habitats. These changes are shown by the relative positions of the oxygen-dissociation curves of bloods of several species having dissimilar habits and environments.

Rodents are the most numerous of mammals, in both number of individuals and number of species. Rodents are also old in evolution, having their origin in early Eocene. One well-defined family of rodents is the Sciuridae, or squirrels, which are almost worldwide in distribution. One of the interesting features in the natural history of squirrels is that they show considerable adaptive radiation. Some show burrowing habits, some are arboreal, some terrestrial; some can move by "gliding." Some species are solitary in habit, while others live in great colonies. Some are diurnal in habit while others are nocturnal. These features make the group an interesting one for physiological studies.

Seven species of squirrel became available during a study of rodents' blood: the common American marmot, *Marmota monax*; the antelope ground squirrel, *Amnospermophilus harasii*; the round-tailed ground squirrel, *Spermophilus tereticaudus*; the thirteen-striped ground squirrel, *S. tridecemlineatus*; the gray squirrel, *Sciurus carolinensis;* the prairie dog, *Cynomys ludovicianus*; and the flying squirrel, *Glaucomys volans*. Respiratory functions of blood of prairie dogs had been previously studied (*1*).

Blood samples were drawn by direct cardiac puncture; animals were tranquilized by small intraperitoneal injections of Nembutal, and minimal amounts of heparin were used as an anticoagulant. Blood samples were kept cold pending transfer to tonometers; all tonometric analyses were completed within 2 hours of blood withdrawal. In all instances mature adult animals in good physiological condition were used; no gross pathology was apparent at autopsy. An abbreviated description of the methods of blood equilibration (*1*) follows: Both oxygen and carbon dioxide were measured by use of 0.5-ml pipettes; the procedure used for these combined analyses has been described by Van Slyke and Neill (*2*). After blood was delivered to the Van Slyke apparatus, the remaining volume was extruded into a volumetric flask (50-ml) containing Drabkin's reagent. The pipette volume up to the lower mark was rinsed several times with this reagent; this solution was used for determination of total hemoglobin; the factor of 1.34 times the gram percentage of hemoglobin was used to give the total oxygen capacity of hemoglobin. Total oxygen capacity of a sample of blood was also determined by saturation of the blood with a pO_2 of 200 mm-Hg. Both methods employed for determination of oxygen capacity agreed within the errors of the analytical methods. Measurement of oxygen capacity on the same sample of blood as used in the Van Slyke analysis has obvious advantages.

Gas used in tonometric equilibration was delivered from large cylinders of mixed gases which had been analyzed in my laboratory. Gases were completely saturated with water vapor at the temperature of equilibration; this becomes very important when small samples of blood are equilibrated in open tonometers. Equilibrations of all samples were for 20 minutes at 37°C.

Table 1. Constituents of bloods of various species of squirrel. Numbers of specimens, followed by average body weights (g), appear in parentheses.

Hemoglobin (g/100 ml)	Hematocrit (% erythrocytes)	Erythrocytes (10^6-mm^3)
Marmot (4/2840)		
13.2	38.9	6.31
Gray squirrel (5/505)		
14.4	44.2	7.73
Prairie dog (9/1280)		
15.1	47.3	9.50
Round-tailed ground squirrel (5/282)		
12.6	40.5	7.31
Antelope ground squirrel (5/100)		
13.2	42.2	8.39
Thirteen-striped ground squirrel (3/153)		
14.9	45.8	10.67

Determinations of hemoglobin, hematocrit, and red blood count were also made. Hematocrit values were found by use of a microhematocrit centrifuge. Blood counts were made by the orthodox method of dilution and counting on a Neubauer slide. Hemoglobin values were determined with an Evelyn photocolorimeter; hemoglobin was read as metcyanhemoglobin.

Oxygen-dissociation curves of blood from seven species of squirrels are shown in Fig. 1; the curves are drawn through the average values obtained. No attempt was made to determine oxygen saturation below 15 percent or above 85 percent. Oxygen pressure to attain 50-percent saturation of the blood, where the ratio of hemoglobin to oxyhemoglobin was 1:1 at *p*H 7.40 and 37°C, had a standard deviation

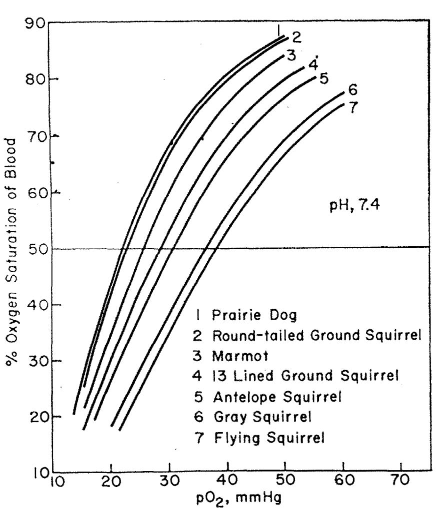

Fig. 1. Oxygen-dissociation curves of whole bloods of seven species of squirrel.

of less than 1.2 mm-Hg for each species tested (Table 1).

Oxygen-dissociation curves of blood from these seven species of squirrels differed considerably between species but were almost identical in individuals of the same species. The partial pressure of oxygen required for half-saturation of blood at *p*H 7.40 varied only about 1 mm-Hg for blood from the same species, but ranged from 22 mm-Hg for the prairie dog to 39 mm-Hg for the flying squirrel. In fact, it would be possible to identify any one of these species, apart from the round-tailed ground squirrel and the prairie dog, by determining the position of the dissociation curve. The prairie dog and round-tailed ground squirrel have similar habits and habitats; their curves differ from each other by less than 2 mm-Hg.

The curves shown in Fig. 1 represent the oxygen affinities of hemoglobin within the confines of erythrocytes and in the chemical environment of the circulating blood; they do not indicate the behavior of these hemoglobins in some other physicochemical environment, or any definitive characteristic of the hemoglobin molecule.

It is well established that the hemoglobin molecule of mammalian blood varies with the species and that its relative oxygen affinity is the most characteristic variant. The position of the oxygen-dissociation curve is related to environmental limitations and to the metabolic requirements of the species. The adaptive value of hemoglobin must be considered from both its ability to combine with oxygen in the lungs and its ability to release this oxygen to the functioning cells of the body.

Schmidt-Nielsen and Larimer (*3*) have shown that the dissociation curve of blood of several mammals is related to body size. This correlation is to some extent apparent in studies of the blood of squirrels when the larger and smaller forms are compared. The curve furthest to the right is that for the flying squirrel, while curves for the prairie dog and marmot are far to the left. Squirrels, like many other groups of animals, exhibit great diversity of types, which appears to be correlated with diversity of ecological habitats as well as with habits of the various species; this has been produced by adaptive radiation during the long evolutionary history of this group. Prairie dogs live in deep burrows where oxygen tensions may become low; their oxygen environment may resemble that at high altitude. The marmot is a hibernating animal, and it too appears to have low metabolic requirements. The gray squirrel and flying squirrel are at the other extreme; they live in the "wide-open spaces" and are active. The gray squirrel, however, is diurnal in habit, while the flying squirrel is nocturnal (*4*). The natural history of these animals is described by Walker (*4*).

How and when changes in the respiratory function of the blood originated during evolutionary development of the species are subjects for speculation, but that these changes did occur and that they are advantageous in the struggle of the various species for existence seem evident. This is the picture presented by seven species of squirrels.

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Control of Enzyme Activity in Growing Bacterial Cells by Concerted Feedback Inhibition

Abstract. *Amino acids of the aspartic acid family are synthesized in bacteria through a multistep branched pathway. In the photosynthetic bacterium* Rhodopseudomonas capsulatus, *the enzyme that catalyzes the first step is specifically inhibited by a combination of two end-product amino acids. Evidence is presented for operation of this kind of "concerted" feedback control as a regulatory device in growing cells.*

The biochemical conversions through which aspartic acid gives rise to L-lysine, L-threonine, L-isoleucine, and L-methionine are believed to be identical in all bacteria (*1*) (Fig. 1). Studies with different bacterial species, however, have revealed the existence of at least three alternative schemes (*2*) of regulation of biosynthesis of these amino acids. One scheme, discovered (*2, 3*) in the photosynthetic bacterium *Rhodopseudomonas capsulatus*, is partly based on control of activity of the first enzyme (β-aspartokinase) by the "concerted" action of two end products, each derived from a separate branch of the pathway. The activity of the cell-free β-aspartokinase is markedly inhibited when L-threonine and L-lysine are present simultaneously; binding of both modifiers to the kinase presumably causes a specific conformational alteration of the protein to a form with greatly diminished catalytic activity. We now present evidence for the operation in vivo of concerted feedback inhibition of β-aspartokinase activity as a regulatory device in *R. capsulatus*.

From the control scheme suggested by experiments (*2*) with cell-free enzyme preparations, excessive accumulation of threonine and lysine in the growing cell should lead to inhibition of activity of β-aspartokinase and homoserine dehydrogenase and, consequently, to a decreased supply of common intermediates required for methionine synthesis. A suboptimum rate of methionine formation should, in turn, lead to depression of the growth rate. This prediction can be tested experimentally by increasing the intracellular concentrations of L-threonine and L-lysine, through addition of the amino acids to the growth medium. Growth experiments (Fig. 2) show the expected effect and also that additional supplementation with methionine causes a reversal of the inhibition due to threonine plus lysine.

When both threonine and lysine are added to the synthetic "basal" medium (containing glutamate as the nitrogen source), growth of *R. capsulatus* is severely inhibited. In the experiments of Fig. 2*A*, supplementary amino acids were added before inoculation of the cultures, and under these circumstances the inhibitory effect of threonine plus lysine is relieved to a significant degree, after a rather extended lag period, when methionine is also present. Addition of lysine to the basal medium results in a slight, but definite, stimulation of growth whereas appreciable inhibition is observed with threonine. The latter amino acid would be expected to interfere with homoserine synthesis through feedback inhibition of homoserine dehydrogenase activity (*2*). The probable reason for the depres-