Oxygen Dissociation Curves of Mammalian Blood in Relation To Body Size

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ABSTRACT

SCHMIDT-NIELSEN, KNUT AND JAMES L. LARIMER. Oxygen dissociation curves of mammalian blood in relation to body size. Am. J. Physiol. 195(2): 424-428. 1958.—Oxygen dissociation curves were determined in mammalian blood at the CO₂ tension of the organism, without the addition of buffers, dilution of the blood, or other alterations. It appears that the dissociation curve is related to body size in such a way that the blood of smaller animals has a higher unloading tension for oxygen. This finding is discussed in relation to the higher metabolic need for oxygen of the smaller animal. It is suggested that, in addition to the higher capillary density in the small animal, a higher unloading tension for oxygen also contributes to the steepness of the diffusion gradient for oxygen from the capillary to the tissue cells.

It is well known that the hemoglobin molecule of various animals is species specific. Among the differences that are known to exist it is possible to recognize clear adaptations to environmental needs or living conditions. One such adaptation is the relative displacement to the left of the dissociation curve of animals that normally live in an environment of relatively low availability of oxygen. For example, this is the case in the llama and certain other mammals living at high altitudes (1). The shift in the dissociation curve permits the hemoglobin to become fully saturated with oxygen at a partial pressure at which the blood of other mammals is only partially saturated. A more familiar example is the displacement to the left of the dissociation curve for fetal blood as compared to that of the maternal organism, as originally pointed out by Barcroft (2). This obvious adaptation to the needs of the fetus permits the fetal blood to reach a high degree of oxygen saturation at a relatively low oxygen tension. Similar relationships have been described for the developing chick embryo (3) and for the larva of the bullfrog as compared to the adult (4, 5). All these animals have in common an environment of relative oxygen scarcity, which is being met by a blood adapted to take on a full load of oxygen at a lower oxygen pressure.

While these adaptations to the environmental requirements are well known and lend themselves to easy interpretation, the adaptation to the metabolic need of the animal has received only little attention. It has been established and amply documented that the metabolic rate of an animal is related to its body size (6-9). In mammals the quantitative relationship between body weight and the metabolic rate per gram of tissue can be expressed by the equation: \( O_2 = 0.0633 \times BW^{0.77} \), where \( O_2 \) is oxygen consumption in milliliters per gram of tissue per minute, and BW is body weight in grams (10). Using some familiar animals as examples one finds that a 20-gm mouse can be expected to have a metabolism per gram of tissue (28.2 \( \mu l \) O₂/gm/min.) about 15 times as high as that of a 700-kg horse (1.72 \( \mu l \) O₂/gm/min.). Hence, oxygen must be supplied to the tissues of a mouse at a rate 15 times that in the horse. It has been pointed out by Schmidt-Nielsen and Gjønnes (11) that the oxygen dissociation curves of various mammals are related to the body size in a manner which seems adjusted to this higher need for oxygen in the tissues of the small animal.

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While our previous discussion of the significance of the dissociation curve in the supply of oxygen to the tissues of mammals of various body sizes was based on data compiled from the work of numerous investigators who had used widely varying techniques (11), the present paper reports our findings on mammalian blood investigated under comparable conditions and with a uniform technique. Our data fully substantiate the previous compilation and lend support to the generalization that there is an important relationship between the facility with which the blood of an animal gives up its oxygen and the metabolic rate of the animal.

MATERIALS AND METHODS

Blood samples were obtained from four or more individuals each of 17 different species of mammals, ranging in body size from 21 to 635,000 grams. For the three species that have a body weight of less than 100 grams (cotton rat, white mouse and deer mouse) it was necessary to pool samples obtained from several individuals in order to obtain enough blood for a reliable determination of the oxygen dissociation curve. In all other species a single sample was used for each determination of the oxygen dissociation curve. Coagulation was prevented with heparin.

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The oxygen dissociation curve was determined immediately after drawing the sample, and was completed in 6 to 7 hours. The blood was stored at 3°C, and samples were removed for equilibration at 37°C with five different gas mixtures all containing 40 mm CO₂. The oxygen capacity of the sample was determined by equilibration with atmospheric air. All equilibrations were carried out in a 20-ml syringe which was slowly rotated in a water bath at 37°C and supplied with a constant stream of the appropriate gas mixture saturated with water vapor at the same temperature.

The degree of oxygen saturation at each equilibration pressure was determined gasometrically with a Kopp-Natelson microgasometer in Holaday's modification (12). In our early work the results were plotted in the special coordinate system suggested by Brown and Hill (13). However, we subsequently found it more satisfactory to plot the data in the usual manner in a linear co-ordinate system and draw the best fitting line through the established points. All curves presented in this paper are drawn in this way.

RESULTS

The oxygen dissociation curves that we have determined are plotted in figure 1. The general trend among these curves is a gradual shift of the curve to the right with diminishing body size of the animal. It is important to emphasize that all of these determinations were made on whole, undiluted and unbuffered blood at 40 mm CO₂ pressure. Thus, the blood was kept as close to the conditions existing in the living animal as practicable under experimental conditions, except for the inevitable aging of the sample during the several hours that elapsed for the determination of the dissociation curve. Aside from this time factor the curves as given in figure 1 represent, as closely as possible, the physiological conditions of the blood in a living animal.

The trend in the curves given in figure 1 is quantitatively expressed in figure 2, where the half saturation pressures (p₅₀) have been plotted against the body weights of the animals.

A number of physiological functions are re-
lated to body size in such a way that if plotted on a logarithmic scale they are represented by a straight line. Such relationships can therefore be expressed by an exponential equation of the general form $y = ax^b$, the same type of equation as that which relates metabolic rate to body size. Such heterogonic equations are of great value in the treatment of quantitative data. If one calculates the best fitting straight line for the data given in figure 2 (method of least squares), this curve is represented by the equation $p_{90} = 50.34 \; \text{BW}^{-0.054}$ ($p_{90}$ in mm Hg, body weight in grams). The exponent ($-0.054$) of the equation indicates the slope of the straight line.

DISCUSSION

The affinity of the hemoglobin for oxygen is of possible significance in two processes, a) the uptake of oxygen in the lungs, and b) the unloading of oxygen in the tissues. Those examples of oxygen dissociation curves displaced to the left that were mentioned in the introduction constitute specific adaptations to a low oxygen tension available in the environment, and are therefore related to the processes of formation of oxyhemoglobin in the lungs (or placenta). However, the characteristic shift of the curve to the right with diminishing size of the animal seems to be an adaptation to the metabolic need for oxygen and therefore directly related to the unloading of oxygen in the tissues.

If we select for consideration the two extreme body sizes represented in our data, the white mouse (22.8 gm) and the horse (544,000 gm), it can be calculated that the average oxygen consumption per gram tissue must be about 15 times as high in the mouse as in the horse. In order to supply oxygen to the cells at this higher rate it is necessary that the diffusion gradient from capillary to cell also be 15 times as high. The diffusion gradient is composed of two variables, the diffusion distance (distance from capillary to cell) and diffusion head (difference in oxygen tension at the capillary and at the cell). This gradient can be increased in two ways: a) a shorter diffusion distance, and b) a higher diffusion head.

It is our suggestion that both these factors are of significance in supplying oxygen to the tissues at the high rate required in the small animal.

The capillary distance and its relation to the oxygen supply was studied by Krogh (14) who used an equation developed by Erlang to calculate the oxygen pressure necessary to supply a tissue cylinder around a capillary with oxygen when the radius of the tissue cylinder equals one-half the average distance between the capillaries. Krogh's measurements on capillary densities in muscle unfortunately were made only on a few species of mammals. He found a density of 1350/mm² in the horse, 2630 in the dog and 3000 in the guinea pig. Krogh estimated a density of over 4000 capillaries/mm² of muscle in the mouse. Our own calculations, using Krogh's figures and assuming an exponential regression line, leads to an assumed capillary density of about 4200/mm² in the mouse. Krogh's few observations of capillary densities are sufficient to indicate that the decrease in diffusion distance alone cannot provide the oxygen at the necessary high rate to the tissues of small animals. If one assumes that the diffusion around the capillary has a cylindrical geometry (disregarding longitudinal concentration differences) one can use Krogh's equation for a calculation of the necessary oxygen diffusion head:

$$T_o - T_r = \frac{100p}{d} \left( 1.15 R^2 \log \frac{R}{r} - \frac{R^2 - r^2}{4} \right)$$

($T_o$ and $T_r$ = oxygen pressures in the capillary and at the periphery of the cylinder, $p$ = oxygen consumption in milliliters O₂ per minute per milliliter of tissue, $d$ = diffusion constant for O₂ in muscle as defined by Krogh, and $R$ and $r$ = radius of the tissue cylinder and the capillary, respectively.)

In this equation the radius of the diffusion cylinder ($R$) cannot be established with sufficient accuracy. As mentioned above, the capillary densities in muscles of various animals are not well known, and the geometry of their distribution around the muscle fiber adds a further difficulty. In order to arrive at a workable approximation for the necessary diffusion head, $T_o - T_n$, we will assume an average distribution of six capillaries around each muscle fiber, and using the values for capillary density listed above, we find the maximum diffusion distance to be 24.4 µ for the horse and 13.5 µ for the mouse. (The arrangement of capillaries around the muscle...
fibers is usually not as regular as assumed here, and may vary from animal to animal. If six capillaries are associated with each fiber, this results in a triangular diffusion pattern around each capillary. Krogh's equation, however, assumes a uniform capillary distribution and a cylindrical diffusion pattern. Our observations on capillary distribution indicate that the assumed diffusion pattern approaches the true anatomical condition. The use of Krogh's equation is therefore only justified as an approximation and should be followed by a more careful study of diffusion geometry.

The radius of the capillary (r) has been taken as 3.5 μ for the horse as well as the mouse, because the erythrocytes of the two animals have essentially the same diameter.

The average tissue oxygen consumptions in the horse and the mouse are 1.72 μl O₂/gm/minute and 28.2 μl O₂/gm/minute, respectively. The maximum demands on the rate of diffusion of oxygen occurs when the muscle works maximally, and under these conditions, the largest number of capillaries are open. We have assumed that the rate of oxygen consumption under these conditions is 20 times the average resting tissue metabolism given above.

If the calculations are carried out with the use of these approximations one finds that the necessary diffusion head, \( T_o - T_r \), should be 6.8 mm O₂ for the horse and 26.8 mm for the mouse. In other words, it should take about three times as high diffusion head to supply the mouse muscle with oxygen at the rate required by its higher metabolic rate.

It is now possible to compare the estimated diffusion head for oxygen with the unloading tensions as expressed in the lower part of the oxygen dissociation curve. The \( p_{50} \) for the horse was found to be 24 mm O₂, and for the mouse 48.5 mm O₂; and the \( p_{25} \) (75% of the oxygen unloaded) 14 mm O₂ and 25 mm O₂, respectively. These figures, although not identical to those calculated for the diffusion head, are of the same order of magnitude. Furthermore, the demonstrated ability of the mouse blood to give up its oxygen at higher pressures is consistent with the interpretation that this higher unloading tension is necessary for establishing a sufficiently steep diffusion gradient for oxygen.

It should be emphasized that the characteristic ability of the blood to unload oxygen at a higher pressure in small animals is observed only in the normal, undiluted and unaltered blood at the CO₂ tension normally present in the organism. Dilution of the blood and the addition of buffers do not represent the physiological condition of the blood as it functions in the organism and, as expected, such treatment masks or otherwise alters this relationship. Likewise, a comparison of the isolated hemoglobins in dilute solution at pH 7.4 (15) has failed to show the characteristic relation to body size, but Foreman (16) working at pH 6.8, found a tendency in the same direction as we have pointed out in this paper.
Several variables, such as the number of open capillaries, the arrest of circulation in the muscle capillary during contraction, the functional diameter of the capillary, the longitudinal gradient of oxygen tension, etc. have been omitted because of the complexity of their role and the uncertainty of existing information. Another factor that has not been adequately considered in our discussion is the possible influence of a difference in the Bohr effect in large and small animals. Foreman (16) concluded that, in the species he examined, a “decreasing oxygen affinity is accompanied by increasing Bohr effect.” Further work on this subject has recently been done by Riggs and Tyler (17) who found that the smaller the animal the greater is the sensitivity of its blood to pH.

Although the preceding discussions are based on a series of assumptions, and therefore cannot be regarded as conclusive, the fact remains that a higher unloading tension for oxygen should be favorable to a rapid diffusion of oxygen in tissues, thus meeting the requirement of a high metabolic rate.

REFERENCES