A Method for Measuring the Rate of Oxygen Release From Single Microvessels

Norihiko Tateishi, Nobuji Maeda, and Takeshi Shiga

A system determining the rate of oxygen release from erythrocytes flowing in single microvessels was constructed with an inverted microscope by connecting 1) a scanning/grating spectrophotometer equipped with a photon-counting detector through a thin light guide, to obtain the visible absorption spectrum of a spot (5 μm in diameter) focused on a microvessel, 2) two photomultipliers (connected to a microcomputer via an analog-to-digital converter) through two light guides, to determine the flow velocity of erythrocytes by calculating the cross correlation between the light-intensity changes of two spots (3 μm in diameter, 5 μm apart from each other) focused on the microvessel, and 3) an image processor through a video camera, to estimate the diameter of microvessel from the digitized video images. The rate of oxygen release from single microvessels 7–25 μm in diameter in rat mesentery was measured under the superfusion of deoxygenated solution: 1) The maximal rate was obtained in capillaries, and the rate in arterial microvessels was larger than that in venous microvessels, when similar diameters were compared. 2) The rate was maximum at pH 7.0–7.2, and it decreased in more acidic and alkaline pH values. 3) The rate decreased with a decrease in temperature. The reliability of the measurement using the present apparatus was tested in detail. (Circulation Research 1992;70:812–819)

KEY WORDS • erythrocytes • microvessels • oxygen release • spectrophotometry • photon counting • flow velocity

Quantitative observation of oxygen release from erythrocytes in single microvessels to tissue is an ultimate problem not only for understanding the oxygen transport of blood to tissues but also for interpreting the rheological role of blood (especially of erythrocytes) in oxygen transport. In 1919, the transfer of oxygen from microvessels to tissue was analyzed theoretically by Krogh.1 Because of the difficulties in determining the oxygen tension in both tissues and microvessels, in vivo experimentation has been used to support theoretical analyses.2–5

We have recently constructed a system by which the concentration of hemoglobin and the degree of oxygenation can be calculated; this system is composed of a scanning spectrophotometer and an inverted microscope and is used to record the absorption spectrum of erythrocytes flowing in single microvessels in living tissues.6,7 Furthermore, the flow velocity of erythrocytes in the microvessel has been determined with a novel device by adopting the method of dual-spot cross correlation, and the diameter of the microvessels has been measured by using the digitized video image with an image processor.6,7 The rate of oxygen release from the microvessel can be estimated on the basis of 1) the change of the absorption spectra obtained at two points on a microvessel, 2) the flow velocity of erythrocytes, and 3) the diameter of the microvessel.

This article deals with 1) the constitution and the characteristics of an apparatus for measuring the rate of oxygen release from erythrocytes flowing in single microvessels to tissues, 2) the comparison of the rate of oxygen release from single microvessels (arterioles, capillaries, and venules) of various diameters in rat mesentery, and 3) the effects of pH and temperature on the rate of oxygen release.

Materials and Methods

Microscopic Observation of Microvessels in Rat Mesentery

Wister rats (male, 6–9 weeks old, 180–250 g) were cared for in the Laboratory Animal Center, Ehime University, School of Medicine. This study was carried out in compliance with the guide for animal experimentation and with permission from the Committee of Animal Experimentation, Ehime University, School of Medicine.

Rats were anesthetized with phenobarbital (50 mg/kg i.p.). After laparotomy, each rat was placed on an acrylic plate (with temperature controlled by circulating water) equipped on the stage of an inverted microscope; then, a portion of mesentery was lightly fixed on a cylindrical platform (10 mm in diameter and 5 mm in height, made of transparent acrylic) attached to the acrylic plate and was covered with another transparent acrylic plate (see Figure 1). A peristaltic pump (SJ-1215, Atto Co., Tokyo) was used to superfuse the
mesentery between two acrylate plates with isotonic phosphate-buffered saline containing (mM) NaCl 100, KCl 5, sodium phosphate buffer 45, and d-glucose 5.5 (pH 7.4, 285 mosm) at a flow rate of 1 ml/min through the covering plate at 30°C. The oxygen tension of the superfusing solution was changed by bubbling with air (for the oxygenated state) or by bubbling with nitrogen in the presence of 20 mM sodium dithionite (for the deoxygenated state). The oxygen release from erythrocytes in microvessels was also examined by superfusing with phosphate-buffered saline of different pH values and different temperatures. The rectal temperature was monitored continuously.

**Spectrophotometric Determination of Hemoglobin Concentration and Oxygenation**

The schematic diagram of the apparatus for measuring the oxygen release from single microvessels is shown in Figure 1. The apparatus for the determination of hemoglobin concentration and the degree of oxygenation consisted of an inverted microscope (model IMT-2, Olympus Optical Co., Tokyo) equipped with a rotating cylindrical mirror with three eyepieces, a scanning/grating spectrophotometer (model USP-410, Unisoku Co., Osaka, Japan) equipped with a sensitive photon-counting detector, and a computer (model PC9801 VM2, Nippon Electric Co., Tokyo). The light intensity of a halogen lamp (12 V, 50 W, Halo Star, Osram, FRG) in the microscope was controlled by a current stabilizer (model NLO 18-10, Takasago Manufacturing Co., Tokyo), and an objective lens (model ULWD CDPlan 40PL, Olympus Optics) with a magnification of ×40 and with a long working distance was used for the observation. The spectrophotometer was connected to an eyepiece of the microscope through a thin light guide (0.4 mm in diameter) made of quartz. A spot in the visual field of the microscope was marked on a display through a video camera (model DXC-325, Sony, Tokyo) attached to the vertical eyepiece by projecting a light through the light guide from the spectrophotometer side. The microvessel to be measured was moved onto the mark by moving the stage of the microscope and/or by rotating the cylindrical mirror. The spectrophotometer was operated by the computer: the grating could be scanned by 500 steps in a desired wavelength range (within 200–900 nm), and the detector could count 1 x 10\(^4\) to 3 x 10\(^4\) photons/sec. The visible spectrum from a spot 5 μm in diameter on the visual field of the microscope could be recorded when an objective lens with a magnification of ×40 was used.

Actually, the spectrophotometric analysis of erythrocytes flowing in microvessels of rat mesentery was carried out over the range of 500–600 nm in 250 steps with a gating time of 100 msec/step. One spectrum from a microvessel could be recorded within 40 seconds.

**Determination of optical density.** The optical density (OD) at a wavelength is calculated by the equation

\[
OD = \log_{10} \left( \frac{I_0}{I} \right)
\]

where I is light intensity through the microvessel and \(I_0\) is reference light intensity determined for a portion of mesentery near the measured point of a microvessel (the dark current was subtracted from both I and \(I_0\)). For obtaining the absorption spectrum, a numerical filtering with a moving average of seven successive values in the scanning step\(^*\) was applied to improve the signal-to-noise ratio.

**Correction for light scattering.** According to Pittman and Duling,\(^*\) OD of erythrocytes flowing in a microvessel is expressed at a wavelength by the equation

\[
OD = [c \cdot d] + B
\]

where \(c\) is the molar extinction coefficient of hemoglobin, \([c \cdot d]\) is hemoglobin content, \(c\) is concentration of hemoglobin, \(d\) is optical path length (i.e., the diameter of erythrocyte flow in the present experiment), and \(B\) is a correction term for light scattering (details are given in “Discussion”). A typical absorption spectrum is shown in Figure 2, left panel. ODs at five isosbestic points between oxyhemoglobin and deoxyhemoglobin (506.5, 522, 548, 569, and 580)\(^*\) are plotted against their millimolar extinction coefficients; \([c \cdot d]\) is determined from the slope of the regression line, and \(B\) is the intercept on the ordinate. A was regarded as a constant in the wavelength range of 400–700 nm using the
present apparatus. It should be noted that OD at an isosbestic point was obtained by taking the moving average of seven successive values in the scanning process; thus, some 30 points were involved in the regression line obtained from ODs at five isosbestic points.

**Determination of the oxygenation of hemoglobin.** A spectrum from a single microvessel originates from both oxyhemoglobin and deoxyhemoglobin and light scattering; thus, OD at a wavelength is expressed by the equation

\[
OD = (\varepsilon_{\text{oxy}} S + \varepsilon_{\text{deoxy}} (1-S))(c \cdot d) + B
\]

where \( S \) is the fraction of oxyhemoglobin (i.e., the degree of oxygenation of hemoglobin) and \( \varepsilon_{\text{oxy}} \) and \( \varepsilon_{\text{deoxy}} \) are millimolar extinction coefficients of oxyhemoglobin and deoxyhemoglobin, respectively. To improve accuracy, \( S \) values obtained at six wavelengths (540, 542, 555, 560, 577, and 580 nm, where large differences in light absorption between oxyhemoglobin and deoxyhemoglobin are observed) were averaged. The millimolar extinction coefficients for human hemoglobin\(^{11} \) were adopted throughout, because the spectra of rat hemoglobin were almost identical to those of human hemoglobin in both oxygenated and deoxygenated states.

**Determination of the Flow Velocity of Erythrocytes**

Ten fine light guides (0.2 mm in diameter and 0.5 mm apart from each other) were aligned and fixed in a cylindrical metal block, which was inserted into another eyepiece of the microscope. On the visual field of the microscope, each light guide caught photons from a spot 3 \( \mu \)m in diameter, and two adjacent light guides monitored points 5 \( \mu \)m apart from each other. By moving the stage of the microscope and/or rotating the cylindrical mirror as described above, two light guides monitored the light changes from two spots on a straight portion of the microvessel; these spots were conducted to a pair of photomultipliers. The output currents from these two spots were encoded with a high-speed analog-to-digital converter (64 kilobytes in memory, 10 \( \mu \)sec for speed of data transmission, model ADM-1198 BPC, Microscience Co., Tokyo). According to Baker and Wayland,\(^{12} \) the cross correlation between the changes of light intensity from two channels was calculated with the microcomputer by varying the time delay for erythrocyte flow between two points. The flow velocity of erythrocytes (\( v \)) was estimated by dividing the distance between two spots (usually 5 \( \mu \)m) by the time delay, giving the maximum correlation.

**Measurement of the Microvessel Diameter**

The diameter of the microvessel was determined from the video image. A pointer on the image processor (model PIP-4000, ADS Tech. Co., Nara, Japan) was set on one side of the microvessel wall as an origin; then the pointer was successively moved to two points on the other side of the microvessel wall to form a triangle. A perpendicular from the origin on the line between the other two points gives an estimate of vessel diameter.

**Calculation of the Rate of Oxygen Release From Single Microvessels**

When erythrocytes flow from the upstream point to the downstream point (distance \( L \), actually 0.5–3 mm) in a certain section of microvessel, the rate of oxygen release is estimated by measuring the concentration of hemoglobin (\( c \)), the degree of oxygenation of hemoglobin (\( S \), where \( 0 \leq S \leq 1 \)), the velocity of erythrocyte flow (\( v \)), the diameter of erythrocyte flow (\( d \)), and the diameter of the microvessel (\( \phi = 2r \), where \( r \) is the radius) in both the upstream point (subscript 1) and downstream point (subscript 2), as follows:

The averaged concentration of hemoglobin (\( \bar{c} \)) in the measured microvessel is given by

\[
\bar{c} = 1/2[(c_1 \cdot d_1)/(\phi_1) + (c_2 \cdot d_2)/(\phi_2)]
\]

The flow volume (\( Q \)) was calculated at both upstream and downstream points, and the averaged value (\( \bar{Q} \)), used for accuracy, is given by

\[
\bar{Q} = 1/2(\pi r_1^2 v_1 + \pi r_2^2 v_2)
\]

The hemoglobin content passed through the microvessel of length \( L \) is \( c \cdot Q \). The rate of oxygen release from the unit area of microvessel wall, \( V(O_2) \), is given by the equation

\[
V(O_2) = \bar{c} \cdot \bar{Q} \Delta S/(2 \pi r L)
\]

where \( \Delta S = S_1 - S_2 \) and \( \bar{r} = 1/2(r_1 + r_2) \).

**Results**

**Reliability of the Measurements Using the Present Apparatus**

**Spectrophotometric determination of hemoglobin concentration of erythrocyte suspension and the oxygenation state.** The accuracy of the spectrophotometric measurement with the present apparatus was tested by flowing hemoglobin solution and erythrocyte suspension through rectangular glass tubes (50, 100, 200, 300, and 400 \( \mu \)m in thickness, Microslide, Vitro Dynamics Co., Rockaway, N.J.) on the stage of the microscope, using a peristaltic pump (model SJ-1215, Atto Co., Tokyo) with velocity of 1–10 mm/sec. For the test, human hemoglobin and erythrocytes were used, because 1) rat hemoglobin is easily crystallized after hemolysis\(^{13} \) and 2) rat erythrocytes induce echinocytic transformation after drawing blood.\(^{14} \)

The absorption spectrum of human hemoglobin obtained with the present apparatus corresponded with the reported spectrum.\(^{11} \) When hemoglobin solution was used, the spectrophotometer could detect OD of 0.1–2.5 in the wavelength range of 400–650 nm. The signal-to-noise ratio became quite large at OD of <0.1, and the transmitted light became weak at OD of >2.5. Actually, visible spectra of hemoglobin in the wavelength range of 450–650 nm could be recorded when the product of hemoglobin concentration \( c \) (mM) and path length \( d \) (cm) was in the range of 0.001–0.15 (mM \( \cdot \)cm). Good linearity between hemoglobin concentration \( c \) and calculated \([c \cdot d]\) was obtained for hemoglobin solution in the rectangular glass tubes with various values of \( d \).

For flowing erythrocyte suspension, the linearity between hematocrit (\( Ht \)) and calculated \([c \cdot d]\) was also observed, as shown in Figure 3a, varying the thickness of rectangular tubes. However, when \([c \cdot d]\) was >0.1
mM·cm, data deviated from the linear relation. With increasing [c·d], light scattering B was also augmented (see below). The linearity between light path d and the slope of each linear relation ([c·d]/Ht) is shown in Figure 3b. Actually, the measurement was possible for microvessels of diameter φ > 7 μm and of Ht > 20%.

The degree of oxygenation of hemoglobin measured with the present apparatus using a rectangular glass tube (200 μm in thickness) was in good agreement with those measured with a recording spectrophotometer (model 100-50, Hitachi Manufacturing Co., Tokyo).

**Determination of flow velocity of erythrocytes.** The reliability of the present method for the determination of the flow velocity of erythrocytes was evaluated by moving an erythrocyte-smeared plate linearly with an actuator at various velocities. Good correspondence between true velocity given by the actuator and measured velocity assessed by the present method was shown in the velocity range of 0–10 mm/sec in Figure 4. Experimental error >10 mm/sec was probably due to the shortened time delay of light intensity changes between the upstream and the downstream points.

**Oxygen Release From Erythrocytes Flowing in Single Microvessels**

Deoxygenation of flowing erythrocytes with decreasing oxygen tension. The representative process of deoxygenation of flowing erythrocytes by lowering the oxygen tension on the surface of rat mesentery tissue was observed for three microvessels different in diameter, as shown in Figure 5. Under superfusion of air-saturated solution, the flowing erythrocytes in all of these microvessels were fully oxygenated (S = 1). When nitrogen-saturated solution containing 20 mM sodium dithionite was superfused, flowing erythrocytes were deoxygenated, with accompanying deoxygenation of tissues, and a steady state in the oxygenation of flowing erythrocytes was attained a few minutes later. The oxygenation states were different in each microvessel, probably because of factors such as the distance between the microvessels and the main vessels, the thickness of mesentery tissue on the microvessels, and the flow velocity. Therefore, it was clear that the gradient of oxygen tension was formed between the inside of the microvessel and the surface of the mesenteric tissue. After deoxygenation,
the flowing erythrocytes could be oxygenated again by superfusion with air-saturated solution.

Typical results of the spectral change of erythrocytes flowing in a single microvessel are shown in Figure 6. The absorption spectrum was monitored at two points with a distance of 1 mm. Under superfusion with air-saturated buffer (top panels), the degree of oxygenation of flowing erythrocytes was decreased 13% during 1 mm of flow, probably because of the oxygen consumption by the mesenteric tissue around the microvessel. After superfusion with nitrogen-saturated buffer containing 20 mM sodium dithionite (bottom panels), the degree of oxygenation of flowing erythrocytes decreased 60% to 14%, because of the additional oxygen consumption with sodium dithionite (an amount of oxygen corresponding to 46% in oxygenation of erythrocytes was released during 1 mm of flow in this microvessel).

Effect of diameter of microvessels. Flow velocity of erythrocytes was measured for microvessels of various diameters. The relation between the diameter of microvessels and the flow velocity of erythrocytes is shown in Figure 7, top panel. As the diameter of the microvessels increased, the flow velocity of the erythrocytes markedly increased in arterial microvessels, whereas the velocity in venous microvessels slightly increased but was almost constant in diameters >10 μm. These results agreed with those of Lipowsky et al.15

The rate of oxygen release from erythrocytes flowing in microvessels of various diameters is shown in Figure 7, bottom panel. As the diameter of microvessels decreased, the rate of oxygen release increased markedly in both arterial and venous microvessels. Popel et al16 have also observed the fast oxygen release from arterioles of 23.5 μm in diameter.

Effect of pH. Rat mesentery was superfused with the nitrogen-saturated solution (at various pH levels) containing 20 mM sodium dithionite. The rate of oxygen release from erythrocytes flowing in microvessels is compared at various pH levels in Figure 8, in which the rates are expressed by the relative value to the rate at pH 7.24–7.40 (the measurement in this pH range was always performed for microvessels of different diameter as a control). The rate was maximum at pH 7.0–7.2, and the rate decreased at more acidic and alkaline pH levels.

Effect of temperature. By superfusing the deoxygenated solution at 14.8±2.2°C (monitored around the observing area), the rate of oxygen release from flowing erythrocytes was compared with those at 30.0±1.8°C (the rectal temperature was maintained at ~35°C for both experiments). The measurement was performed for microvessels with different diameters in different animals (n=4). After lowering the surface temperature
of rat mesentery from 30°C to 15°C, the rate of oxygen release decreased by 27.3±20.5%.

Discussion

To assess the oxygen transfer from erythrocytes flowing in microvessels to tissues, oxygen tension in tissues and/or oxygen consumption by tissues have been calculated as an averaged value by spectrophotometric measurement of myoglobin and cytochromes by near-infrared spectroscopy. Whalen et al. have developed a microelectrode technique for measuring the intracellular oxygen tension, and the electrode has been used for the measurement of oxygen consumption in tissues. However, this technique injures the tissues. Pittman and Duling have measured the oxygenation of blood in microvessels by adopting a spectrophotometric technique (at three wavelengths) using the visual field of a microscope. This technique has been successfully used for the determination of the rate of oxygen release from erythrocytes flowing in microvessels >15 μm in diameter but not in capillaries. Recently, Ellsworth et al. have measured the oxygenation of erythrocytes flowing in capillaries by a spectrophotometric method (at two wavelengths), in addition to the determination of flow velocity of the erythrocytes. However, their measurement was adopted for a special condition in which individual erythrocytes flow separately in the capillary.

In the present study, for measuring the rate of oxygen release from erythrocytes flowing in single microvessels 7–25 μm in diameter, an optical system for recording the absorption spectrum of a single microvessel was constructed by combining an inverted microscope and a sensitive spectrophotometer through a light guide. The apparatus for the simultaneous determination of the flow velocity of erythrocytes was connected to the inverted microscope through light guides, and the diameter of the microvessel was measured with an image processor. By using this apparatus, the rate of oxygen release in various microvessels (arterioles, capillaries, and venules) in rat mesentery was determined; the rate was also determined for the tissues exposed to different pH values and temperatures.

Spectrophotometry of Erythrocytes in Single Microvessels

Spectrophotometric measurements of the oxygenation of blood in microvessels have been carried out previously by several investigators, but the rate of oxygen release from microvessels <15 μm in diameter had not been adequately determined because of poor signal-to-noise ratio. The present apparatus is capable of recording a visible spectrum of flowing erythrocytes from a spot 5 μm in diameter on single microvessels (7–25 μm in diameter). For the reproducibility of recording and the stability of light intensity, a highly precise stabilizer was used for the current source of a quartz-iodine lamp in the microscope.

To get a good absorption spectrum of erythrocytes flowing in single microvessels, noises due to the temporal instability of erythrocyte flow were minimized by prolonging the gating time for photon counting and/or by adopting the moving average of data from seven consecutive points.

For discriminating the light scattering from the absorption spectrum, five isosbestic points in the wavelength range of 500–600 nm were used. If noisy, six additional wavelengths were used for the determination of the degree of oxygenation of flowing erythrocytes by applying the moving average of seven successive points. These techniques made possible the determination of hemoglobin concentration and the oxygenation of erythrocytes flowing in microvessels of diameter <15 μm. Pittman and Duling and Duling et al. have used three wavelengths for the separation of absorption and light scatter and for the determination of oxygenation of flowing erythrocytes, but their techniques could not be adopted for such small microvessels.

To distinguish between the absorbance and the light scattering, it may be worthwhile to compare the present results with the theory for OD of erythrocyte suspension. According to Loewinger et al., the apparent OD of the erythrocyte suspension can be expressed as follows:

\[
\frac{I_0}{I} = 10^{-qdHt} + g_2dHt + q[1 - 10^{-g_2dHt} - 10^{-g_2'dHt}]
\]

where \( q \) and \( g_2 \) are coefficients of light scattering and \( g_2' \) and \( g_2'' \) are coefficients of Loewinger. The logarithmic equation is as follows:

\[
OD = \log_{10}\left(\frac{I_0}{I}\right) = g_2dHt - B
\]

The first term

\[
[c \cdot d] = (g_2/e)dHt
\]

is the light absorbance, and the second term

\[
B = -\log_{10}[1 - (g_2/e)dHt] + q[1 - 10^{-g_2dHt} - 10^{-g_2'dHt}]
\]

is empirically independent of the wavelength in the present apparatus (i.e., \( g_2, q, g_2' \) and \( g_2'' \) are independent of wavelength). The path length \( d \) and \( Ht \) can be expressed as a function of \([c \cdot d]\) and \( B \) with four parameters \((g_2, q, g_2'/e, g_2''/e)\). For the relations between \( Ht \) and \( B \) and between \( Ht \) and \([c \cdot d]\), good agreements were obtained between experimental results and those computed on the basis of the above theory by setting appropriate values for four parameters \((g_2 = 238, q = 0.121, g_2'/e = 21.6, g_2''/e = 27)\) for various path lengths (d=50–400 μm, as in Figure 3a). The values for these parameters agreed well with those reported by Anderson and Sekelj.

As a whole, using the present optical system, the following simple relation is ascertained:

\[
OD = [c \cdot d] + B
\]

where \( B \) relates to the light scattering but is almost independent of wavelength in the range of 400–650 nm. Pittman and Duling and Fenton and Gayeski have also shown that the light scattering is independent of wavelength in erythrocyte suspension.

Measurement of Flow Velocity of Erythrocytes

The dual-spot cross-correlation method for the measurement of flow velocity of erythrocytes in microvessels has been already established. In the present sys-
Oxygen Release From Single Microvessels in Rat Mesentery

It is generally recognized that oxygen transfer from flowing erythrocytes to tissues is the greatest in capillaries. However, it has been observed that a significant fraction of oxygen transport to tissues also occurs in arterioles and venules.

Under the present experimental conditions (such as rat mesentery exposed to air), erythrocytes flowing in microvessels (arterioles, capillaries, and venules) were almost fully oxygenated. However, when a part of the mesentery was covered with an acrylate plate, erythrocytes in some microvessels were partially deoxygenated, probably because of oxygen consumption by the intestine and mesentery. Such special conditions made it possible to measure the rate of oxygen release from erythrocytes flowing in the microvessels at a similar state of hemoglobin oxygenation (~80% oxygenation; below 80% oxygenation, the velocity coefficient during deoxygenation of oxyhemoglobin in the presence of dithionite is approximately constant under the same experimental conditions). In short, the rate of oxygen release was the greatest in capillaries, and the rate in arterioles was somewhat larger than that in venules, when the rate was compared in microvessels of the same diameter. These phenomena provide evidence that the rate of oxygen release in arterioles is fairly large, as observed by Popel et al.

The rate of oxygen release (i.e., the deoxygenation rate of erythrocytes) in the present experiment was estimated by measuring the difference of the oxygenation states of erythrocytes between two points of a microvessel in a steady state. The measurement was carried out when the steady state of oxygen tension in mesenteric tissue, including the microvessel, was attained at a steady flow of erythrocytes in the microvessel after superfusing deoxygenated solution (Figure 5); in this state, a gradient of oxygen tension between the surface of the mesentery and the inside of the microvessel must be formed, though the profile of the gradient is unknown. In this connection, the time required to reach the steady state was 2–3 minutes, as shown in Figure 5. Therefore, the rate of oxygen release is affected by various factors: flow velocity, oxygen diffusion, and oxygen affinity of hemoglobin.

Flow velocity. In arterial microvessels, the higher the velocity of erythrocyte flow, the smaller was the rate of oxygen release. However, the phenomenon was not necessarily true in venous microvessels. Differences in vessel diameter, thickness of vessel wall, and constituents of vessel wall should be considered, because of the difference in oxygen diffusion (see below).

Oxygen diffusion. The rate of gas diffusion is different in various tissues. Oxygen diffusion through the vessel wall and mesenteric tissue might be one of the factors affecting the rate of oxygen release. After exposure to a solution containing dithionite, the first-order velocity coefficient of deoxygenation of oxyhemoglobin, given by $\Delta n(\%$ oxygenation of hemoglobin)/$\Delta t$, where time $t$ is in seconds, is ~100 for human hemoglobin solution (at pH 7.35 and 30°C) and ~5 for human erythrocyte suspension (at pH 7.4 and 25°C), whereas the roughly estimated value is 0.01 (at pH 7.35 and 30°C) for rat erythrocytes flowing in the microvessel through mesenteric tissue in the present experiment (Figure 5). Such a low value for the first-order velocity coefficient of the deoxygenation shows that the rate of oxygen diffusion is greatly affected by the vessel wall and mesenteric tissue. In the steady state for measuring the rate of oxygen release, such diffusion barriers make gradients of oxygen tension between the inside of the microvessel and the surface of the mesentery under a constant flow of erythrocytes. Differences in the rate of oxygen release among microvessels with the same diameter (Figure 7) may be due to differences of oxygen diffusion, and characteristic differences in the rate between arterioles and venules may be due to the properties of the vessel wall.

The rate of oxygen release decreased ~27% as temperature decreased from 30°C to 15°C. This decrease in the rate must be largely due to the decreased rate of gas diffusion. The temperature coefficient of Krogh’s diffusion constant is nearly 1%/°C in most tissues. If simply estimated, half of the decreased rate is due to the temperature effect on gas diffusion, and the rest comes from the flow effect and the increased oxygen affinity of hemoglobin (see below). The observed rate of oxygen release was much less than the estimated value by the well-established temperature coefficient of oxygen dissociation from erythrocytes in vitro. This may suggest that the temperature in the microvessel was still high because of the continuous blood flow (the rectal temperature was maintained at 35°C); a temperature gradient is probably formed between the inside of the microvessels and the surface of the mesentery.

Oxygen affinity of hemoglobin. The rate of oxygen release from erythrocytes is dependent on the oxygen affinity of hemoglobin. The effect of pH on the rate of oxygen release was of interest. The rate was maximum at pH 7.0–7.2, and it decreased at more alkaline and acidic pH values. The change above pH 7.0–7.2 may be partly explained by the pH effect on the rate of oxygen dissociation from oxyhemoglobin. For the degree of pH dependency on the rate, it should be taken into consideration that rat erythrocytes have low affinity toward oxygen because of high 2,3-diphosphoglycerate content (the oxygen tension at half oxygenation of hemoglobin in erythrocytes is 38.7±1.8 mm Hg) and have a high Bohr effect (the Bohr factor, $\Delta \log P_O_2/\Delta \text{pH}$, is ~0.603±0.066). Furthermore, a pH gradient may be formed between the inside of microvessels (possibly near pH 7.4) and the surface of the mesentery. Such a
gradient of pH may modify the magnitude and the profile of the pH-dependent changes in the rate of oxygen release and may explain the changes in the rate for acidic pH. The temperature effect on the rate of oxygen release is as follows: with a decrease in temperature, the rate also decreases because of the increased oxygen affinity of hemoglobin (see above).

In physiological conditions, oxygen transport from blood to tissues is essentially performed in the region of the microvessels, and the rate of oxygen release is determined by the changes of physicochemical conditions around erythrocytes; these changes are induced by changes in the metabolism of tissues (e.g., pH, PCO2, and temperature) and in the environment around tissues (e.g., temperature). In these conditions, some gradients of pH and temperature must be formed between the inside of microvessels and the tissues. This situation is very similar to that in the present experiment. Therefore, the technique used in this study will be important for future studies of the physiological significance of oxygen transport from blood to tissues.

References

1. Krogh A: The number and distribution of capillaries in muscles with calculations of the oxygen pressure head necessary for supplying the tissue. J Physiol (Lond) 1919;52:409–415
23. Ivanov KP, Derry AN, Vovenko EP, Samoilov MO, Seminov DG: Direct measurements of oxygen tension at the surface of arterioles, capillaries and venules of the cerebral cortex. Pflugers Arch 1982;393:118–120
42. Krogh A: The rate of diffusion of gases through animal tissues, with some remarks on the coefficient of invasion. J Physiol (Lond) 1909;34:239–248
A method for measuring the rate of oxygen release from single microvessels.
N Tateishi, N Maeda and T Shiga

Circ Res. 1992;70:812-819
doi: 10.1161/01.RES.70.4.812

Circulation Research is published by the American Heart Association, 7272 Greenville Avenue, Dallas, TX 75231
Copyright © 1992 American Heart Association, Inc. All rights reserved.
Print ISSN: 0009-7330. Online ISSN: 1524-4571

The online version of this article, along with updated information and services, is located on the World Wide Web at:
http://circres.ahajournals.org/content/70/4/812