



Virginia Commonwealth University  
**VCU Scholars Compass**

---

Theses and Dissertations

Graduate School

---

2008

## Effects of Light Exposure on the Release of Oxygen from Hemoglobin in a Red Blood Cell Suspension

Tanikka Toler  
*Virginia Commonwealth University*

Follow this and additional works at: <https://scholarscompass.vcu.edu/etd>



Part of the [Physiology Commons](#)

© The Author

---

Downloaded from

<https://scholarscompass.vcu.edu/etd/1637>

This Thesis is brought to you for free and open access by the Graduate School at VCU Scholars Compass. It has been accepted for inclusion in Theses and Dissertations by an authorized administrator of VCU Scholars Compass. For more information, please contact [libcompass@vcu.edu](mailto:libcompass@vcu.edu).

**EFFECTS OF LIGHT EXPOSURE ON THE RELEASE OF OXYGEN FROM  
HEMOGLOBIN IN A RED BLOOD CELL SUSPENSION**

A thesis submitted in partial fulfillment of the requirements for the degree of Master of  
Science at the Medical College of Virginia Campus, Virginia Commonwealth University

by

**Tanikka Daneille Toler**  
B.S., Virginia Union University, 2006

Director:  
**Roland N. Pittman, Ph.D.**  
Professor  
Department of Physiology and Biophysics

Virginia Commonwealth University  
Richmond, Virginia  
December, 2008

## ACKNOWLEDGEMENTS

First and foremost I must express my extreme gratitude to my wonderful mentor, Dr. Roland N. Pittman. His kindness, compassion, encouragement and unwavering patience are all methods of mentorship that I will always cherish. He supported me through my undergraduate years and now has guided me through another milestone in my life. Words can not express how much respect and appreciation I have for such an honorable man as Dr. Pittman. Thank you!

Secondly, my research could not have been completed without the contributions and support of the members of this laboratory. Dr. Helena Carvalho helped in a tremendous way. She took time out of her schedule to teach me the skills needed for my research and always was available to answer the many questions that arose. I am grateful to Dr. Aleksander Golub for his amazing technical skills with all the equipment in the laboratory. To Pete Meliagros, Bjorn Song, and Michael Kim, thank you so much for providing the samples utilized in my experiments. I would also like to extend a thank you to Matt Barker, Phoebe Dacha and Drew Yannaccone for always having a smile on their faces and words of encouragement to give throughout the year. I graciously give a million thanks to this genuine group of laboratory team members that always displayed a great sense of camaraderie and made my experience in the laboratory enjoyable.

This would not have been possible without the immense love and support of my mother, grandmother, Jonas, and the remainder of my family and friends. They were always there to give much needed encouragement and motivation to accomplish this goal. Without this genuine love, completion of this project would not have been possible.

## TABLE OF CONTENTS

ACKNOWLEDGEMENTS.....	ii
TABLE OF CONTENTS.....	iii
LIST OF TABLES.....	v
LIST OF FIGURES.....	vi
LIST OF ABBREVIATIONS.....	vii
ABSTRACT.....	x
INTRODUCTION.....	12
Circulation of Blood and the Cardiovascular System.....	12
Microcirculation.....	13
Blood Composition.....	13
Structural and Functional Components of Red Blood Cells.....	14
Hemoglobin.....	15
Oxygen Dissociation Curve.....	16
Oxygen as a Cellular Necessity.....	17
Oxygen Transport.....	18
Rate of Oxygen Uptake.....	18
PO <sub>2</sub> Experimental Measurements.....	19
Previous Work by Others.....	21
Purpose of Present Research Study.....	22
MATERIALS AND METHODS.....	24
Animals.....	24
Withdrawal of Blood from <i>In Vivo</i> Experiments.....	24

PBS Solution.....	24
Red Blood Cell Suspension.....	25
PO <sub>2</sub> Electrode Calibration.....	26
PO <sub>2</sub> Measurement of RBC Suspension.....	29
Measuring Hematocrit of RBC Suspension.....	30
Measuring Hemoglobin of RBC Suspension.....	30
Data Analysis.....	30
Baseline Correction.....	31
Changes in Temperature.....	32
Statistical Analysis.....	35
RESULTS.....	36
PO <sub>2</sub> Electrode Calibration.....	36
Temperature Corrected Slope and Intercept for Sample Chamber .....	38
Baseline Correction.....	40
Final $\Delta$ PO <sub>2</sub> due to Light Exposure.....	45
DISCUSSION.....	47
Comparison of Results with Previous Experiments.....	47
Limitations.....	51
Conclusions.....	52
Future Studies.....	53
BIBLIOGRAPHY.....	55
VITA.....	59

## LIST OF TABLES

Table 1. PO <sub>2</sub> Electrode Calibration at Different Temperatures.....	34
Table 2. Electrode Calibration.....	37
Table 3. Corrected Slope and Intercept for Sample Chamber Temperature.....	39
Table 4. Baseline Correction.....	43
Table 5. Summary Table for Data.....	46
Table 6. In Vivo Data from the Literature on Oxygen Release from Arterioles .....	51

## LIST OF FIGURES

Figure 1. PO <sub>2</sub> Electrode Current Tracing for Calibration using 0% O <sub>2</sub> (100% N <sub>2</sub> ), 5.02% O <sub>2</sub> , and 10% O <sub>2</sub> .....	27
Figure 2. PO <sub>2</sub> Electrode Calibration Linear Regression for Current (I) versus PO <sub>2</sub> .....	28
Figure 3. Original Current Recording for Experiment on 4/16/08(4) .....	41
Figure 4. PO <sub>2</sub> Electrode Linear Regression for the 5 Minute Dark Period before Light Exposure .....	42
Figure 5. Experimental Data from 4/16/08(4) with Baseline Correction .....	44

## LIST OF ABBREVIATIONS

2, 3 DPG	2, 3 diphosphoglycerate
A	Amperes (current)
A'	new intercept of the calibration line
ATP	adenosine triphosphate
$\alpha$	alpha
$\beta$	beta
B'	new slope of the calibration line
$b^{\text{CORR}}$	corrected intercept for a given temperature
cm	centimeter
$^{\circ}\text{C}$	degrees Celsius
db/dT	first derivative of electrode calibration intercept with respect to temperature
dm/dT	first derivative of electrode calibration slope with respect to temperature
EATs	erythrocyte-associated transients
$\text{Fe}^{2+}$	ferrous iron
$\text{Fe}^{3+}$	ferric iron
g	gram
Hb	deoxyhemoglobin
HbCO	carboxyhemoglobin
HbO <sub>2</sub>	oxyhemoglobin
Hct	hematocrit of the RBC suspension
I	PO <sub>2</sub> electrode current in Amperes
$\Delta I$	change in current after the baseline correction was applied
JO <sub>2</sub>	oxygen flux from arterioles
KCl	potassium chloride
kg	kilogram
m	slope of PO <sub>2</sub> electrode calibration
M	molar
$m^{\text{CORR}}$	corrected slope for a given temperature



metHb	methemoglobin
ml/kg	milliliter per kilogram
ml	milliliter
mm	millimeter
mM	millimolar
mmHg	millimeter of mercury
n	Hill coefficient
N <sub>2</sub>	nitrogen gas
nA	nanoamps
NaCl	sodium chloride
NaNO <sub>2</sub>	sodium nitrite
O <sub>2</sub>	oxygen gas
ml O <sub>2</sub> /dl	ml oxygen per deciliter
ml O <sub>2</sub> /g Hb	ml oxygen per gram of hemoglobin
ODC	oxygen dissociation curve
OSM	oxygen saturation meter
PBS	phosphate buffered saline
PCO <sub>2</sub>	partial pressure of carbon dioxide
PE-50	size 50 polyethylene tubing
PO <sub>2</sub>	partial pressure of oxygen
RBC	red blood cell
RO <sub>2</sub> <sup>LIGHT</sup>	rate of oxygen release from Hb due to light exposure
RO <sub>2</sub> <sup>OBS</sup>	observed rate of oxygen release from blood in arterioles
rpm	revolutions per minute
SO <sub>2</sub>	fractional oxygen saturation of red blood cell hemoglobin
T	temperature
Δt	change in time
ΔT	change in temperature between the sample chamber and calibration
T <sup>CALIB</sup>	temperature of the calibration chamber
T <sup>SAMPLE</sup>	average temperature of the sample chamber during the 5 minute light exposure period

$\mu\text{m}$	micrometer
V	volts
$V_{\text{RBC}}$	volume of an RBC

## ABSTRACT

### **EFFECTS OF LIGHT EXPOSURE ON THE RELEASE OF OXYGEN FROM HEMOGLOBIN IN A RED BLOOD CELL SUSPENSION**

By: Tanikka D. Toler

A thesis submitted in partial fulfillment of the requirements for the degree of Master of Science at the Medical College of Virginia Campus, Virginia Commonwealth University

Virginia Commonwealth University, 2008

Advisor: Roland N. Pittman, Ph.D.  
Department of Physiology and Biophysics

The main function of the cardiovascular system is to deliver a sufficient quantity of oxygenated blood to the tissues, cells, and organs of the body in order to provide the cells with essential nutrients for metabolism and for the removal of waste products. All cells require and utilize oxygen. Oxygen is transported to various cells and tissues via red blood cells flowing through the microcirculation of an organism. Measurement of oxygen transport in the microcirculation has shown that about ten times more oxygen appears to leave the blood of arterioles than can be accounted for by diffusion. One possibility to explain the high oxygen loss is an increased release of oxygen due to exposure of blood to light. In the present *in vitro* study the release of oxygen from red blood cells was measured during exposure of the sample to light by monitoring the change in  $PO_2$  of the suspension during light exposure. A  $PO_2$  electrode was calibrated using PBS solution and utilized to monitor the change in current in the present study. Red blood cell suspensions were made using blood withdrawn from male Sprague-Dawley rats. The red blood cell suspension was placed in a closed sample chamber and exposed to light for 5 minutes. A method to correct for the drift of the  $PO_2$  electrode and

temperature change during the experiment was implemented. The calculated change in  $PO_2$  of the RBC suspension due to light exposure was small. The change of  $PO_2$  in the sample chamber during light exposure was an average of  $1.60 \pm 0.9$  mmHg (SEM). The contribution of photo-dissociation of oxygen from oxygenated hemoglobin molecules to the observed oxygen loss per RBC can account for only about 0.01% of the observed *in vivo* results. Therefore, light-associated oxygen release is negligible. These findings disprove the hypothesis of the present study, in which light exposure does not have a significant effect on oxygen release and thus rules out this possible explanation for the discrepancy between experiment and theory.

## **INTRODUCTION**

### **Circulation of Blood and the Cardiovascular System**

The main function of the cardiovascular system is to deliver a sufficient quantity of oxygenated blood to the tissues, cells, and organs of the body in order to provide the cells with essential nutrients for metabolism and for the removal of waste products. This system heavily relies on the coordinated actions of the lungs, heart, and blood vessels. Blood is circulated continuously throughout the body by the pumping action of the heart. The heart contracts thereby generating the pressure needed to drive the blood through blood vessels of the various organs. The arteries supply the tissues with blood from the heart, while the veins carry blood away from the tissues back to the heart. Capillaries are the primary vessels involved in nutrient, fluid, and waste exchange. They are found between the arteries and veins and dispersed throughout the tissues. The circulation of blood begins with blood entering the right atrium of the heart from the systemic veins and pumped into the right ventricle via contraction of the heart. The blood then enters the lungs by way of the pulmonary artery and becomes oxygenated while expelling carbon dioxide. After gas exchange in the lungs is complete the oxygenated blood enters the left atrium via the pulmonary vein and then fills the left ventricle. With a forceful contraction, the left ventricle propels the blood into the aorta and it is then distributed throughout the body by way of the meshwork of arteries, arterioles and capillaries. All the organs are replenished with oxygen when the blood is distributed throughout the body and the now deoxygenated blood returns to the heart via two major veins, the superior vena cava and inferior vena cava (Costanzo 2006).

## **Microcirculation**

The microcirculation is a more intricate aspect of the circulatory system, owing to its collection of a large number of microscopic vessels connected in complicated networks. It consists of arterioles, capillaries, and venules which are all blood vessels smaller than about 100  $\mu\text{m}$  with partially permeable thin walls that allow for transfer of mainly small molecules to and from the tissues. In this case the small diameter of the capillaries only permits the passage of RBCs in single file. It is here in the microcirculatory system that oxygen passes to the parenchymal cells by passive diffusion and the cells engage in gas exchange (Pittman 2005). One can consider that the microcirculation begins with the arterioles which were formed by the continuous branching and decrease in size of the arteries. The arterioles play a key role in blood pressure regulation, since most of the total peripheral resistance is located in these vessels. Arterioles also continually branch and decrease in diameter before forming capillaries and supplying them with oxygenated blood. Capillaries are made up of only a thin wall composed of a single layer of endothelial cells, thereby creating the greatest surface area-to-volume ratio in the microcirculation (Costanzo 1998). The capillaries feed into the venules, tiny vessels that link the capillaries to the veins, and the deoxygenated blood flows back to the heart to start the circulatory process over again.

## **Blood Composition**

On average the human body contains about 5 liters of blood in the circulatory system which makes up approximately 7% of the body weight. Blood, which on a macroscopic scale appears to be homogeneous, is actually a heterogeneous fluid that

functions as a medium for the transport of physiological necessities such as hormones, oxygen, and glucose to muscles and other tissues of the body. After centrifugation, whole blood separates into three distinct layers. The upper layer is known as plasma, a translucent yellowish, aqueous substance. Plasma makes up about 55% of the total blood volume due to the presence of mainly water, plasma proteins, and electrolytes. Its purpose is to transport insoluble substances along with organic and inorganic substances around the body by allowing them to bind to protein molecules while also functioning as a suspending solution for the other blood cells. Together, the middle and lower layers make up the remaining 45% of the blood volume and contain blood cells and platelets. The thin middle layer is known as the “buffy coat” because of its straw-like color, and it consists of white blood cells, or leukocytes. This layer comprises less than 2% of the blood volume and plays a major role in immunity by protecting the body from foreign and harmful particles. The lower, most dense layer consists of red blood cells (RBCs) or erythrocytes. RBCs are the most abundant cell type in the blood. There are about 5 million RBCs per cubic millimeter of blood which is equivalent to about 250 million RBCs in every drop of blood. RBCs have numerous roles to fulfill, but most importantly they play a major role in the process of respiration. RBCs are involved in the removal of carbon dioxide from the parenchymal cells, to be expelled from the body in the lungs, and the transport of oxygen to the cells.

### **Structural and Functional Components of Red Blood Cells**

Red blood cells, or erythrocytes, are biconcave disk-like cells with a flattened center which lacks a nucleus. They arise from pluripotent stem cells and are permanently

differentiated. On average the life span of red blood cells in humans is 120 days (Berne and Levy 1998), while in rats it has been found to be about 68 days (Berlin 1951). Red blood cells are destroyed by macrophages in the liver and spleen, which causes the release of iron that is then stored in the liver. It is inside these cells where the protein, hemoglobin, is found. Hemoglobin is the molecule that gives red blood cells their oxygen carrying capacity.

## **Hemoglobin**

Hemoglobin is a globular protein with four subunits. This tetrameric protein is made up of two alpha ( $\alpha$ ) chains and two beta ( $\beta$ ) chains. Each subunit contains a polypeptide chain ( $\alpha$  and  $\beta$  chains) and a non-protein iron-binding porphyrin (a heme group). The heme group of each subunit can bind one molecule of oxygen, so that a total of four oxygen molecules can be bound per molecule of hemoglobin (Costanzo 2006). On average a normal blood concentration of hemoglobin is about 15 g/100 ml of whole blood and each molecule of hemoglobin is able to carry 1.34 ml  $O_2$ /g Hb. Therefore, the total oxygen carrying capacity of human blood is 20 ml  $O_2$ /dl (Berne and Levy 1998). The binding of oxygen to hemoglobin is a reversible process that occurs rapidly and continuously for maintenance of adequate cellular respiration (Weibel 1984).

Hemoglobin molecules may exist as one of four main species: oxyhemoglobin ( $HbO_2$ ), deoxyhemoglobin (Hb), carboxyhemoglobin (carboxyHb or  $HbCO$ ), and methemoglobin (methHb). The majority of hemoglobin is found in the  $HbO_2$  form which is hemoglobin formed during passage of blood through the pulmonary circulation and it possesses oxygen bound to its heme group ( $Fe^{2+}$ ). Hb lacks bound oxygen. CarboxyHb



(1-2%) is found when carbon monoxide competitively replaces oxygen at the binding sites of the heme group. MetHb (1-2%) occurs when ferrous iron ( $\text{Fe}^{2+}$ ) is oxidized to ferric iron ( $\text{Fe}^{3+}$ ) and can no longer bind oxygen.

### **Oxygen Dissociation Curve**

The oxygen dissociation curve shows the percent oxygen saturation of hemoglobin as a function of the partial pressure of oxygen ( $\text{PO}_2$ ) found in the blood. It is a graphic representation of how the percent saturation increases steeply as the  $\text{PO}_2$  increases from zero to about 40 mmHg and then starts to level off between  $\text{PO}_2$  values of 50 mmHg to 100 mmHg. This relationship between percent saturation and  $\text{PO}_2$  yields the unique sigmoidal shape of the oxygen dissociation curve. When individual oxygen molecules bind to a subunit of hemoglobin, the hemoglobin molecule undergoes conformational changes (Hillman and Finch 1996). This change in shape leads to an increased binding affinity of each subsequent oxygen molecule, i.e., binding of the first oxygen molecule increases the affinity of the second heme group for oxygen, while oxygenation of the second heme group increases the affinity for the third (Ganong 1977) until all four binding sites on the heme group are occupied; this phenomenon is known as positive cooperativity. As a result of positive cooperativity, shifts in the dissociation curve are possible when influenced by factors such as temperature, pH,  $\text{PCO}_2$ , and 2, 3 diphosphoglycerate (2, 3 DPG) concentration, which ultimately affect the rate of dissociation of oxygen from hemoglobin. Overall, this process of positive cooperativity explains why oxygen is easily loaded into pulmonary capillary blood from alveolar gas and unloaded from systemic capillaries into the tissues (Costanzo 2006). The  $\text{PO}_2$  levels

are at their highest in the systemic arterial blood, entering tissues at an oxygen tension of 95 torr, which means that hemoglobin's affinity for oxygen is greatest. The lowest  $\text{PO}_2$  values are seen in the venous blood, exiting with an average oxygen tension of 40 torr, showing that this is where hemoglobin has a lower affinity for oxygen. Hence, under normal conditions, about 20% - 25% of oxygen attached to the hemoglobin in red blood cells is released while a corresponding amount of carbon dioxide is taken up and transported to the lungs (Hillman and Finch 1996).

### **Oxygen as a Cellular Necessity**

It is well known that all cells in the body rely on an adequate supply of oxygen. This continuous oxygen supply provides energy to the cells to enable them to perform routine functions. Oxygen is used as the common oxidizing agent for the breakdown of energy-producing molecules such as glucose, amino acids and fatty acids. The energy released during respiration is used to make adenosine triphosphate (ATP). ATP is a form of stored energy that is used and made in both aerobic and anaerobic processes. Anaerobic pathways do not require oxygen and, as a result, much less energy is produced compared to the aerobic pathways, which require oxygen. Aerobic metabolism of substrate molecules is 19 times more efficient than anaerobic metabolism in producing energy for cells. Therefore, this explains why there is a cellular necessity for oxygen during cellular respiration (Taylor, Green, and Stout, 1997).

## **Oxygen Transport**

Oxygen carriage by blood can exist in two possible forms: as free or dissolved molecules in the plasma and RBC cytoplasm or bound to hemoglobin molecules in the RBCs. Because dissolved oxygen constitutes only 2% of the total oxygen content of blood, it plays only a minor role in oxygen transport and is not sufficient to supply the cells with adequate amounts of oxygen. In contrast, the bound form of oxygen makes up the remaining 98% of the oxygen content of blood, so oxygen content is primarily determined by the oxygen binding capacity and the concentration of hemoglobin in the RBCs. Although dissolved oxygen only counts for 2% of the total oxygen content, it is the only form of oxygen that produces a partial pressure which is what drives oxygen diffusion (Costanzo 2006; Hillman and Finch 1996).  $PO_2$  is a key factor because it is what is measured during microcirculatory experiments.

## **Rate of Oxygen Uptake**

Studies have shown that the rate of oxygen uptake in human RBCs is about 40 times slower than the corresponding rate of oxygen combined with free hemoglobin. One limitation that may explain the difference in rates of oxygen uptake is the ability of oxygen molecules to diffuse through red blood cell cytoplasm. It has been hypothesized that the membrane of the red blood cell presents a barrier to oxygen diffusion and is the major rate limiting step to oxygen uptake (Hartridge and Roughton 1923; Coin and Olson 1979). Another possible limitation of oxygen uptake is due to the presence of unstirred layers of solvent around the red blood cell surface. It was shown that, when mixed, these layers form and rapidly become depleted of oxygen that is taken up by the cells. Oxygen

molecules are better taken up when they diffuse over short distances, 1.0-5.0  $\mu\text{m}$ , before they are able to penetrate RBCs (Coin and Olson 1979). Based on Middleman's analysis, Coin and Olson believed that diffusion through alveolar and capillary endothelial cell layers of the lung is the actual rate limiting step of oxygen uptake into RBCs (Middleman 1972; Coin and Olson 1972).

Due to extensive research in the microcirculatory field, a mathematical model was developed to predict oxygen transport by RBCs and acellular hemoglobin solution mixtures flowing through vessels equivalent to arteriolar size. Studies have shown comparative data between the rate of oxygen uptake and delivery of hemoglobin solutions, RBC/hemoglobin solution mixtures and RBC suspensions. The work of Boland, Nair, and Page suggested that the oxygen uptake and delivery by hemoglobin solutions and RBC/hemoglobin solution mixtures were more efficient compared to RBC suspensions of the same overall hemoglobin content in an artificial capillary (Boland *et al* 1987; Nair *et al* 1989). The model was created to estimate the possible effects that altering the oxygen affinity of extracellular hemoglobin would have on oxygen transport. In the end it showed that the rate of oxygen uptake is fairly insensitive to the oxygen binding affinity of hemoglobin, yet the rate of oxygen release relies heavily on hemoglobin oxygen affinity (Vandegriff and Olson 1984; Lemon *et al* 1987).

## **PO<sub>2</sub> Experimental Measurements**

Blood appears as a homogeneous fluid when flowing through large vessels in which the various components such as blood cells and plasma are impossible to differentiate. This is seen until the blood reaches the capillaries where red blood cells

must flow through in single file, thereby making it easy to distinguish the individual RBCs and the plasma spaces surrounding them. Hellums named the phenomenon of  $PO_2$  fluctuations between RBCs and plasma in capillaries erythrocyte-associated transients (EATs) (Hellums 1977). EATs refer to the fluctuations of  $PO_2$  seen in capillaries caused by the alternate passage of individual RBCs and plasma gaps. These EATs depict the  $PO_2$  gradients between plasma and RBCs with amplitudes that vary depending on cell shape, spacing, and position. The diffusion of oxygen found outside of capillaries creates a gradient that ultimately influences the overall  $PO_2$  gradient. Numerous mathematical models have shown that intracapillary resistance makes a considerable contribution to the overall resistance to oxygen transport. This finding is possibly a result of oxygen's low solubility in the plasma, which impedes the diffusion of oxygen down its gradient from the RBCs through the surrounding plasma sheath and plasma gaps and on into the tissue (Golub and Pittman 2005; Pittman 2005).

Measuring the  $PO_2$  levels in the microcirculation has been approached by various methods. The use of phosphorescence of excited phosphors is a non-invasive *in vivo* method that has been used to determine oxygen levels in blood vessels and tissues. It is a method with some technical limitations and large non-uniformities of oxygen concentrations inside the microvessels require special data analysis for the application of this technique (Zheng, Golub, and Pittman 1996). Using  $PO_2$  sensing microelectrodes is another *in vivo* method to measure  $PO_2$  levels. The  $PO_2$  electrode senses and measures the dissolved form of oxygen only. Oxygen bound to hemoglobin molecules requires other techniques that directly measure the oxygen bound to hemoglobin.

## Previous Work by Others

August Krogh, the Father of Oxygen Transport, developed a mathematical model of oxygen transport derived from measurements of the diffusion coefficient of oxygen through tissues. This model became known as the Krogh Cylinder Model and explains how Krogh concluded that a very high  $PO_2$  in the blood was not needed to adequately supply the surrounding oxygen-consuming tissues; rather, only a small radial gradient in oxygen tension ( $PO_2$ ) was sufficient to supply an adequate amount of oxygen to the tissue cylinder (Krogh 1959; Pittman 2005). Years later, Duling and Berne successfully measured oxygen in the microcirculation. Duling, using the technical developments of Whalen with a recessed tip oxygen-sensitive microelectrode, discovered that there was a significant and progressive decline in  $PO_2$  in the arterioles, which is an observation that contradicts Krogh's expectation that the main site of oxygen diffusion from the circulation was in the capillaries (Duling and Berne 1970; Pittman 2005). This new finding for oxygen transport made it important to determine if a corresponding longitudinal gradient in hemoglobin oxygen saturation existed in the arterioles. The rationale behind this concept was that if oxygen were released from hemoglobin fast enough and could get out of the RBCs fast enough, then there should be an equilibrium established between oxygen inside and outside the RBCs. In the end, it was proven that a longitudinal gradient of both  $PO_2$  and  $SO_2$  is present (Pittman and Duling 1977).

This advancement in measurements of oxygen transport was remarkable, but after several experimental and theoretical tests, a problem emerged showing that there was an order of magnitude discrepancy between the experimental results and the theoretical predictions (Popel *et al* 1989). The amount of oxygen that is released from the blood

appears to be about 10 times higher than predicted. Due to this dramatic discrepancy, questions have been asked such as: what is the destination for the “lost” oxygen? Some possible reasons have been proposed that could account for the discrepancy between measured and predicted oxygen levels. The main three reasons that have been considered are that (1) some of the oxygen could be consumed by nearby parenchymal cells after diffusion from arterioles, (2) some is consumed by the microvessel wall on its route from the blood to the perivascular space, and (3) some oxygen is “lost” by its diffusion to other nearby microvessels where it is picked up by the flow of blood through those vessels (Pittman 2005). A few assumptions are still under investigation while some were quickly dismissed, such as the idea that the diffused oxygen was consumed by cells located in microvessel walls (Swain and Pittman 1989). One possible reason for the unaccounted “lost” oxygen that has not received attention previously is the possible effect of light exposure on oxygen release from the RBCs as they pass through the microcirculation.

### **Purpose of Present Research Study**

The purpose of the present study was to investigate the effect, if any, of light exposure on oxygen release from hemoglobin in red blood cells. All microcirculatory experiments past and present are performed under microscopes in which the tissue and microvessels were illuminated with high intensity light. It is possible that this direct light exposure may facilitate the release of oxygen from hemoglobin molecules. We hypothesized that light exposure will indeed affect the release of oxygen and can be manifested by an increase in  $PO_2$  measurements recorded during *in vitro* experiments using red blood cell suspensions. If light exposure does cause release of some of the

oxygen bound to hemoglobin in the red blood cells, the question is posed as to how much would the  $PO_2$  of the red blood cell suspension rise? The findings of the present study will investigate this possible cause of the unexpectedly large amount of “lost” oxygen in the microcirculation or disprove that light has a significant effect on oxygen release and thus rule out this possible explanation for the discrepancy between experiment and theory.



## **MATERIALS AND METHODS**

### **Animals**

Male Sprague-Dawley rats (~250 g) were housed, two per cage, in plastic containers and under a 12/12 hour light/dark cycle in a climate-controlled room (temperature 20-23 °C and humidity 40-60 %). The animal protocol was approved by the Institutional Animal Care and Use Committee (IACUC) of Virginia Commonwealth University, and was consistent with the National Institutes of Health (NIH) Guidelines for the Humane Treatment of Laboratory Animals.

### **Withdrawal of Blood from *In Vivo* Experiments**

Initially, a mixture of ketamine and acepromazine (75 mg/kg of ketamine and 2.5 mg/kg of acepromazine, Henry Schein, Melville, NY) was injected intraperitoneally to sedate the animal. Following sedation, the carotid artery was cannulated with PE-50 tubing (Clay Adams, Parsippany, NJ) for withdrawal of blood. A 10 ml syringe pre-treated with heparin (Baxter, Deerfield, IL) was utilized to collect the blood. Following blood collection, the rat was euthanized with 0.5 ml of Euthasol (pentobarbital 390 mg/ml and phenytoin 50 mg/ml, Delmarva Laboratories, Inc., Midlothian, VA).

### **PBS Solution**

Phosphate-buffered saline (PBS) was prepared for use in the calibration of the PO<sub>2</sub> electrode and to make the red blood cell suspension, by dissolving one packet of dry powdered phosphate buffered saline (Sigma) in 1 liter of distilled, deionized water. This yielded 0.01M phosphate buffered saline (NaCl, 138 mM; KCl, 2.7 mM; phosphate, 10

mM) with a pH 7.4 at 25°C. PBS solution was stored in the refrigerator (temperature 10 °C) until needed.

### **Red Blood Cell Suspension**

Following withdrawal of blood from the Sprague-Dawley rats, it was divided equally into two 16x150 mm glass test tubes. The tubes were weighed, sealed with Parafilm (American Can Company, Greenwich, CT) and centrifuged for 5 minutes at 3000 rpm (Fisher Scientific Marathon 6K Centrifuge). After centrifugation, the red blood cells (RBCs) were separated from other cell types in the whole blood. The plasma layer and white blood cells layer were removed using glass pipettes. The packed RBCs were removed from the tubes and resuspended in clean glass tubes with PBS solution at room temperature. The tubes containing RBCs and PBS (~ 2 ml) were balanced, sealed with Parafilm and gently inverted ~ 4-5 times to mix the RBCs and PBS. The suspensions were then centrifuged for 5 minutes at 3000 rpm. After removal from the centrifuge, the PBS layer was removed from the packed RBCs and fresh PBS solution was added. This washing process was repeated 3 times. Before use in experiments the packed RBCs were resuspended in a 2:1 ratio, 2 parts PBS and 1 part packed RBCs, producing a hematocrit of approximately 33 %. Immediately before use, the tubes were sealed with Parafilm and gently inverted ~ 3-4 times to produce an approximately homogeneous distribution of RBCs.

## **PO<sub>2</sub> Electrode Calibration**

For calibration of the PO<sub>2</sub> electrode room temperature PBS solution was added to a glass aeration chamber. The PO<sub>2</sub> electrode was placed in the PBS solution and allowed to polarize at -0.7 V for about one hour. The glass chamber was connected to a water circulating pump which circulated room temperature water through the outer jacket of the chamber. First, 0% oxygen (100% nitrogen gas) was bubbled into the bottom of the PBS-filled chamber. The current through the PO<sub>2</sub> electrode was measured by a picoammeter (6485 Picoammeter, Keithley Instruments, Cleveland, OH) and recorded every second using a laptop PC and saved to a Microsoft Excel spreadsheet. Next, 5 % and then 10 % oxygen (balance nitrogen) gas was bubbled through the chamber and the current was measured as above. Recording of the current for each oxygen gas mixture was monitored until the PO<sub>2</sub> electrode reached a steady plateau at each oxygen level before switching to the next calibration gas, seen in Figure 1. A pictorial of the linear regression of the PO<sub>2</sub> electrode calibration can be seen in Figure 2.

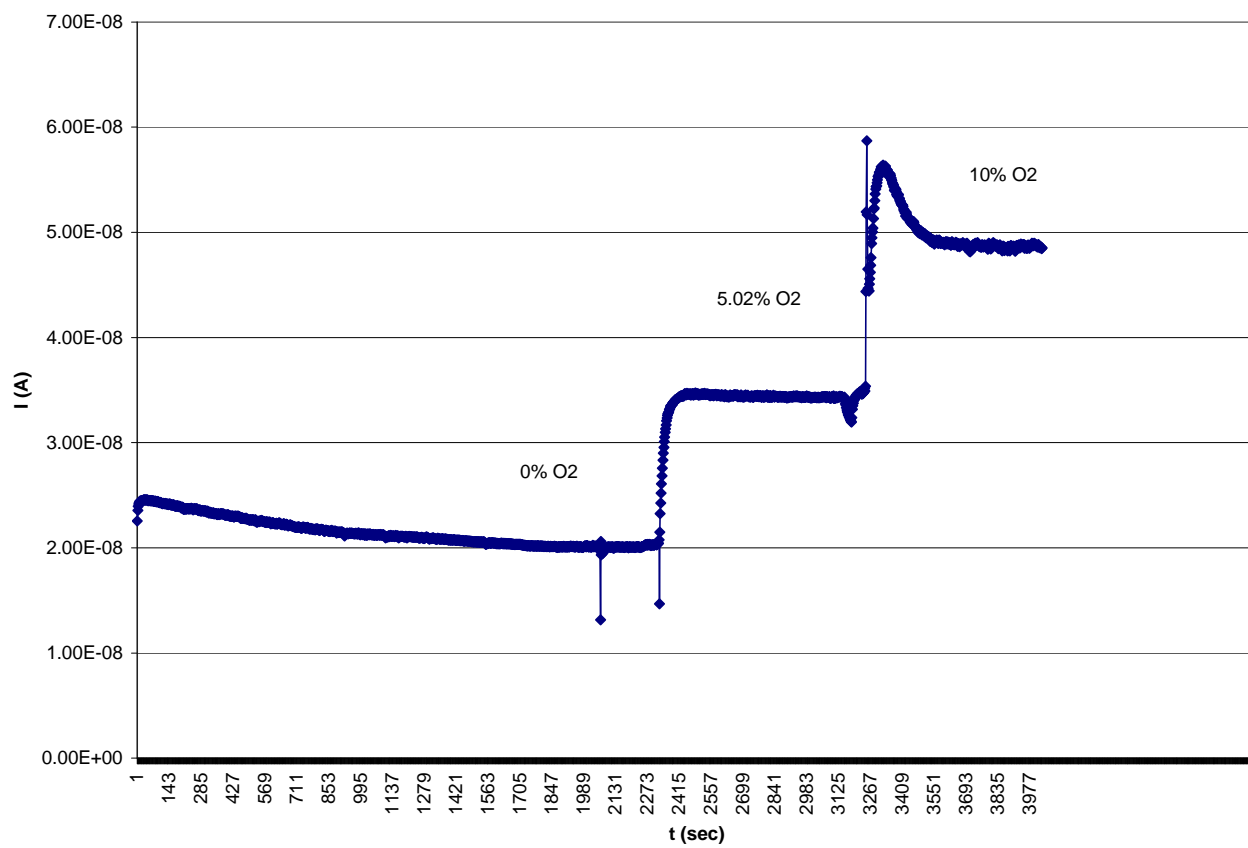


Figure 1: PO<sub>2</sub> Electrode Current Tracing for Calibration using 0% O<sub>2</sub> (100% N<sub>2</sub>), 5.02% O<sub>2</sub>, and 10% O<sub>2</sub>.

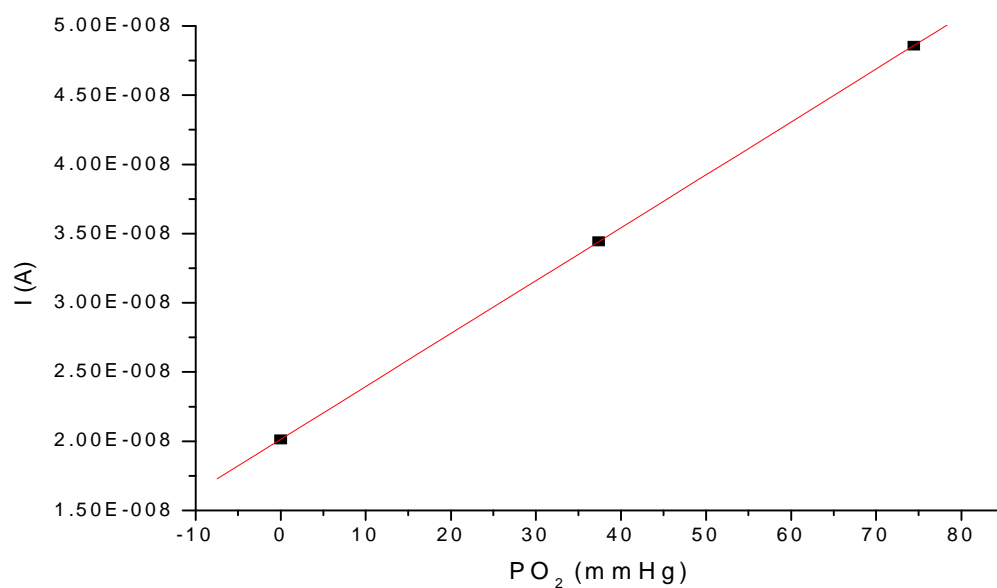


Figure 2:  $PO_2$  Electrode Calibration Linear Regression for current ( $I$ ) versus  $PO_2$  is  $I = m PO_2 + b$ , where  $m = 3.82 \times 10^{-10} \text{ A/mmHg}$  and  $b = 2.01 \times 10^{-8} \text{ A}$ . Temperature =  $22.5^\circ\text{C}$

## **PO<sub>2</sub> Measurement of RBC Suspension**

A multi-port measurement chamber (NOCHM-4 World Precision Instruments, Sarasota, FL) was placed on a stirring plate (Corning Hot Plate Stirrer PC-351). A magnetic stirring bar was placed inside the multi-port chamber and the stirring plate was set to a low setting. Water at room temperature was circulated through the outer jacket of the multi-port chamber. The PO<sub>2</sub> electrode was placed securely into one of the ports of the chamber. 1.5 ml of the RBC suspension was pipetted into the multi-port chamber and continuously stirred by the stirring bar. A thermometer was placed inside the chamber to measure the temperature of the RBC suspension. The chamber was capped and the PO<sub>2</sub> electrode current was measured while it was recorded by the picoammeter. The whole apparatus was enclosed in a darkened box to shield it from ambient light and the room was darkened before and during measurements. The PO<sub>2</sub> electrode was allowed to stabilize for about 20-30 minutes before measurements were begun. Once the PO<sub>2</sub> electrode reached a steady baseline current, the RBC suspension in the chamber was exposed to light from a xenon arc lamp (Zeiss, 75 W) through an opening in the sealed, darkened cardboard box (30 x 25 x 11 cm). The box was made dark by lining it with thick black construction paper and black electrical tape. The RBC suspension was exposed to light for 5 minutes and then the light beam was interrupted to end the light exposure period. The PO<sub>2</sub> electrode was monitored for 10 minutes after the light was removed to observe the response to the light on/light off transient.

### **Measuring Hematocrit of RBC Suspension**

A sample of the RBC suspension was used to measure the hematocrit. Heparinized micro-hematocrit capillary tubes (Fisher Scientific, Pittsburgh, PA) were filled with a sample of the RBC suspension being tested and one end was sealed with Critoseal. The capillary tubes were centrifuged for 30 seconds and the hematocrit was calculated as the length of the RBC column divided by the length of the RBC plus PBS column. The hematocrit for every sample was measured before and after each experiment.

### **Measuring Hemoglobin of RBC Suspension**

Some of the RBC suspension sample was used to measure the hemoglobin concentration of the sample. Heparinized micro-hematocrit capillary tubes were half filled with a sample of the RBC suspension being tested. The sample was run through the Oxygen Saturation Meter 3 Hemoximeter (OSM 3 Hemoximeter, Radiometer Copenhagen). The read-out of the amounts of different types of hemoglobin was observed and recorded.

### **Data Analysis**

While conducting the *in vitro* experiments using the PO<sub>2</sub> electrode, it was observed that in all cases, the PO<sub>2</sub> electrode needed time to stabilize once placed into the sample chamber containing the RBC suspension. This stabilization process lasted on average 20-30 minutes. Once the electrode stabilized, or came to a steady baseline, the light-induced experiments were conducted. An overview of the compiled data showed

that the PO<sub>2</sub> electrode did not stabilize at the same current reading for any given experiment. Ideally, we would like for the electrode to come to a steady baseline at the same current reading before every experiment was conducted, but this was not the case. Therefore, a “baseline correction” procedure was created to unify the data and to create a baseline that would be consistent for all the experiments.

### **Baseline Correction**

To perform the baseline correction for each experiment, it was assumed that the baseline of the PO<sub>2</sub> electrode changes linearly with time. The 5 minute period just before the light was turned on was used to estimate how the current (I) changed with time. All the current readings for this 5 minute period were fit to a straight line and from this linear fit values for the slope and intercept of the line were calculated. This equation was expressed as:  $y = A + B \cdot X$ , where  $y$  = current,  $A$  = intercept,  $B$  = slope, and  $X$  = time. This “baseline correction” was then subtracted from the actual data recorded for the beginning of the 5 minutes before the light was on period to the end of the 10 minutes after the light exposure was removed. By subtracting the “corrected baseline” from the 5-minute period before the “light on” period, the PO<sub>2</sub> electrode currents all began at about 0 nA as the steady baseline. The purpose of this baseline correction procedure was to correct the PO<sub>2</sub> electrode current versus time, due to drift of the electrode, so that the current change during the “light on” period of the experiment was more accurate. An example of the outcome of this procedure can be seen in Figure 3.



## Changes in Temperature

Before each experiment was conducted, the PO<sub>2</sub> electrode was calibrated using the calibration procedure described above. The temperature was monitored during each calibration. When the experiments were conducted using the sample RBC suspension, the temperature was also monitored. Unfortunately, the temperature of the calibration chamber for the PO<sub>2</sub> electrode and the sample chamber for the RBC suspension experiments usually were not the same, but differed by a small amount. The calibration relation between PO<sub>2</sub> and electrode current depends on temperature. As the temperature increases, the PO<sub>2</sub> electrode current increases. Because of this temperature difference, a method to correct for the temperature difference was formulated. This temperature difference was corrected by considering how the calibration line changes with temperature. To correct for the difference in temperature, the PO<sub>2</sub> electrode was calibrated at several temperatures: 22.5 °C, 30.7 °C, and 37.1 °C. A linear relationship exists between the current (I) and PO<sub>2</sub>. For each calibration, the measured current (I) was plotted versus the PO<sub>2</sub> which yielded a slope and intercept for the calibration line at that particular temperature. The slope and intercept of each temperature range can be seen in Table 1. The slope and intercept for each calibration line was plotted versus temperature to observe the temperature dependence. This was computed using the following equations:

$$m = A + B T$$

$$b = A' + B' \cdot T$$

where m is the slope of the calibration line, A and A' are the intercepts of the slope and intercept of the calibration line, respectively, B and B' are the slopes of the slope and

intercept of the calibration line, respectively, and  $T$  is the temperature at which the calibration was conducted. The temperature difference between the calibration chamber and the sample chamber was calculated using the following formula:

$$\Delta T = T^{\text{SAMPLE}} - T^{\text{CALIB}}$$

where  $\Delta T$  is the difference in temperature between the sample chamber and calibration chamber,  $T^{\text{SAMPLE}}$  is the average temperature of the sample chamber during the 5 minute light exposure period, and  $T^{\text{CALIB}}$  is the temperature of the calibration chamber in which there was no light exposure period. The corrected slope and intercept for the change in temperature of the experiments were calculated using the following formulas:

$$m^{\text{CORR}} = m(T^{\text{CALIB}}) + [dm/dT] \Delta T$$

$$b^{\text{CORR}} = b(T^{\text{CALIB}}) + [db/dT] \Delta T$$

where  $m^{\text{CORR}}$  and  $b^{\text{CORR}}$  are the new corrected slope and intercept for the given temperature and  $dm/dT$  and  $db/dT$  are the first derivatives of the slope and intercept with respect to temperature derived from plotting the slope and intercept of the calibration line versus the temperature at which the calibration was conducted. The corrected slope and intercepts represents the slope and intercept of the sample chamber.

**Table 1. PO<sub>2</sub> Electrode Calibration at Different Temperatures**

Temperature (°C)	Slope, m (A/mmHg)	Intercept, b (A)
22.5°C	3.85E-19	6.30E-8
30.7°C	5.90E-10	3.61E-8
37.1°C	6.33E-10	4.79E-8

The slope and intercept of the calibration line are defined as  $I = m \text{ PO}_2 + b$ , where  $m$  is the slope and  $b$  is the intercept of the calibration line. The temperature of the PBS solution in the calibration chamber was measured during the calibration of the PO<sub>2</sub> electrode at various temperatures.

**Statistical Analysis**

The statistical analysis of all data was done using Origin Scientific Graphing and Analysis Software (Version 7.0). Values in tables are expressed as mean  $\pm$  SEM.

## RESULTS

### **PO<sub>2</sub> Electrode Calibration**

The PO<sub>2</sub> electrode was calibrated before each experiment using the procedure explained in the Materials and Methods section to confirm the stability of the PO<sub>2</sub> electrode. See Materials and Methods for an example of the calibration line (Figure 2). The slope and intercept of the calibration line were derived by fitting the calibration points to a straight line ( $I = m \cdot \text{PO}_2 + b$ ). During each experiment the temperature of the calibration chamber was monitored and recorded. The calculated slope, intercept and measured temperatures are displayed in Table 2. It was observed that in many cases the PO<sub>2</sub> electrode did not stabilize at the same range of currents among the different experiments.

**Table 2. Electrode Calibration**

Date of Experiment	Slope, m (A/mmHg)	Intercept, b (A)	Calibration Chamber Temperature (°C)
4/2/08	3.38E-10	1.81E-08	21.6
4/9/08	3.82E-10	2.01E-08	22.5
4/16/08(1)	3.04E-10	8.40E-09	23.2
4/16/08(2)	3.04E-10	8.40E-09	23.2
4/16/08(4)	3.04E-10	8.40E-09	23.2
4/24/08(1)	4.08E-09	3.36E-08	22.4
4/24/08(2)	4.08E-09	3.36E-08	22.4

The slope of the calibration line is defined as  $I = m \text{ PO}_2 + b$ , where  $m$  is the slope and  $b$  is the intercept of the calibration line. The temperature of the PBS solution in the calibration chamber was measured during the calibration of the  $\text{PO}_2$  electrode.

**Temperature Corrected Slope and Intercept for Sample Chamber**

The experimental data were collected in a plastic sample chamber with an inner volume of 1ml. The experimental protocol used 1ml of RBC suspension for each experiment. PO<sub>2</sub> measurements were made and the temperature of the sample was recorded while in the sample chamber. The temperature was recorded during the period before the light exposure, in thirty second intervals during the light exposure period, and during the period after the light source was removed from the sample. The temperature of the RBC suspension sample often was slightly different from the temperature at which the corresponding PO<sub>2</sub> electrode calibration was conducted. Because of this difference in temperature, the electrode calibration was corrected to account for the temperature change. This procedure was used to calculate the corrected slope and intercept. See Materials and Methods for a description of the temperature correction procedure. The results of the corrected slope, intercept, and sample chamber temperature are displayed in Table 3.

**Table 3. Corrected Slope and Intercept for Sample Chamber Temperature**

Date of Experiment	Corrected Slope (A/mmHg)	Corrected Intercept (A)	Sample Chamber Temperature (°C)
4/2/08	3.75E-10	1.66E-08	24.4
4/9/08	4.00E-10	1.94E-08	23.6
4/16/08(1)	3.21E-10	7.99E-09	26.8
4/16/08(2)	3.23E-10	7.94E-09	24.4
4/16/08(4)	3.31E-10	7.76E-09	24.9
4/24/08(1)	3.65E-10	1.47E-08	22.8
4/24/08(2)	3.65E-10	1.47E-08	23.6

The listed sample chamber temperature is the average temperature measured during the light exposure period.



### **Baseline Correction**

During the collection of data for each experiment, the PO<sub>2</sub> electrode did not stabilize in the same range of current. This drift in the electrode's current recordings made interpretation of the data problematic in some cases. The original current recording that is uncorrected can be seen in Figure 3. Due to this inconsistency in the stability of the PO<sub>2</sub> electrode, a baseline correction for each experiment was derived in order to view all experimental data on the same footing. The procedure of this baseline correction has been previously discussed in the Materials and Methods section, using it allowed the processing of the current change during the light exposure period to be more accurate. Figure 4 depicts the pre-light on tracing used to obtain the new slope and intercept for the baseline correction. The slope and intercept of the baseline correction for each experiment is listed in Table 4. The baseline correction allowed the data from the experiments to all begin at the same baseline, near zero A. The original recording from an experiment is presented in Figure 3 and the outcome of the application of the baseline correction is shown in Figure 5 (compare Figure 3 to Figure 5).

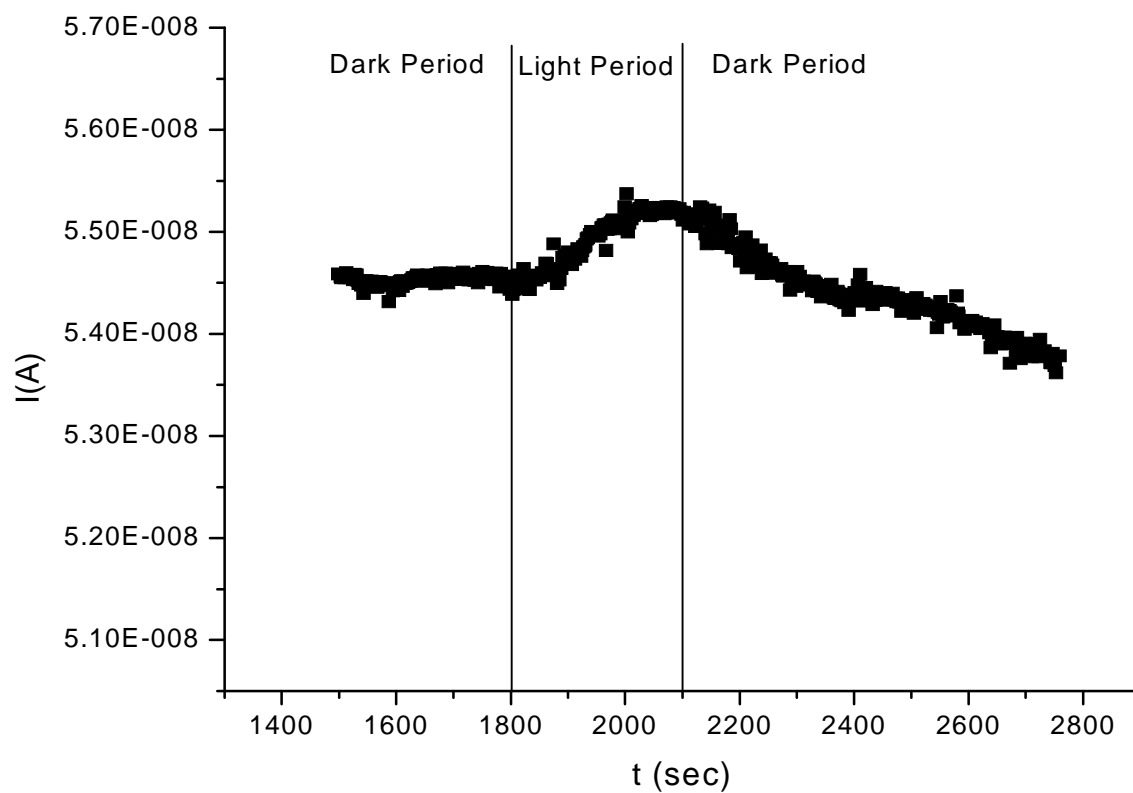


Figure 3. Original current recording for experiment on 4/16/08(4). This figure shows current ( $I$ ) vs time ( $t$ ).

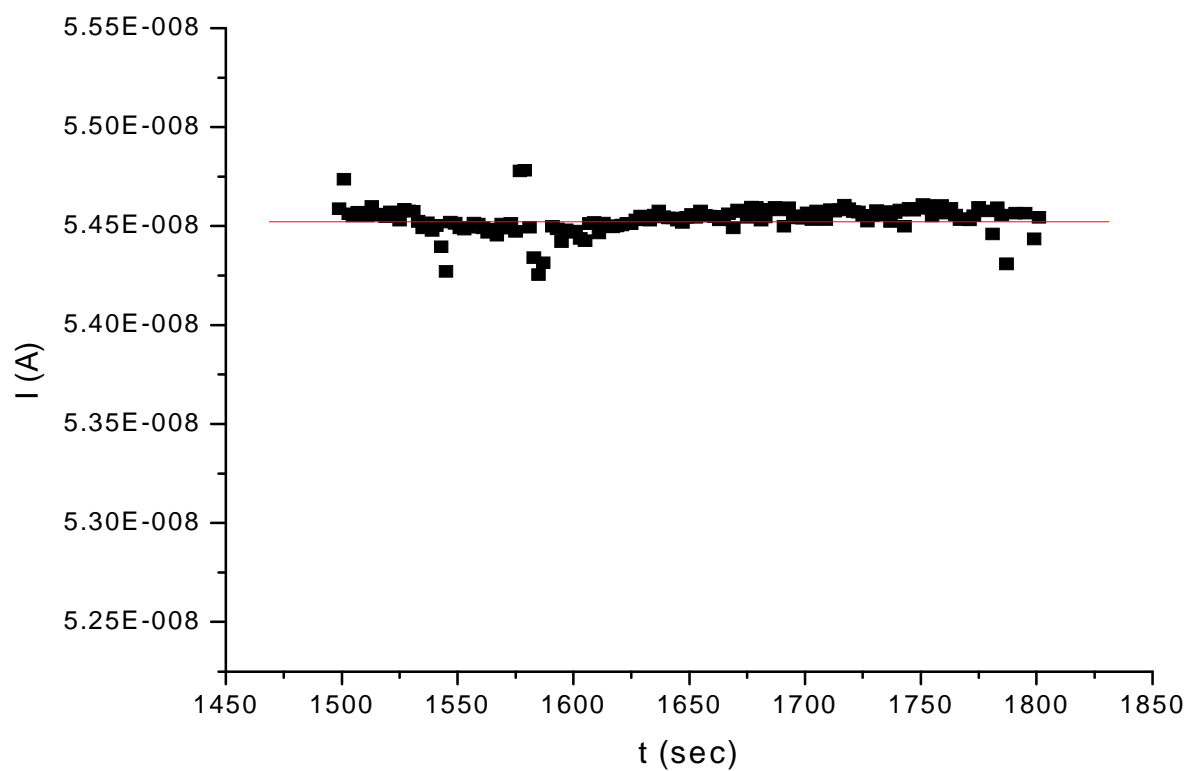


Figure 4.  $\text{PO}_2$  electrode linear regression for the 5 minute “light off” period right before the light exposure period. It shows the current ( $I$ ) versus time ( $t$ ) as  $I = A + B \cdot X$ , where  $A = 5.45 \times 10^{-8}$  A and  $B = -1.88 \times 10^{-15}$  A/mmHg.

**Table 4. Baseline Correction**

Date of Experiment	Slope, B (A/s)	Intercept, A (A)
4/2/08	-2.49E-13	4.91E-8
4/9/08	1.04E-12	4.91E-8
4/16/08(1)	1.89E-12	4.18E-8
4/16/08(2)	8.66E-12	3.26E-8
4/16/08(4)	-1.88E-15	5.45E-8
4/24/08(1)	-1.20E-11	6.42E-8
4/24/08(2)	4.57E-12	5.05E-8

The 5 minute period just before the light exposure period was used to estimate current (I) changes with time. All the current readings for this 5 minute period were fit to a straight line and from this linear fit, values for the slope and intercept of the line were obtained. This equation was expressed as:  $y = A + B \cdot X$ , where  $y$  = current,  $A$  = intercept,  $B$  = slope, and  $X$  = time.

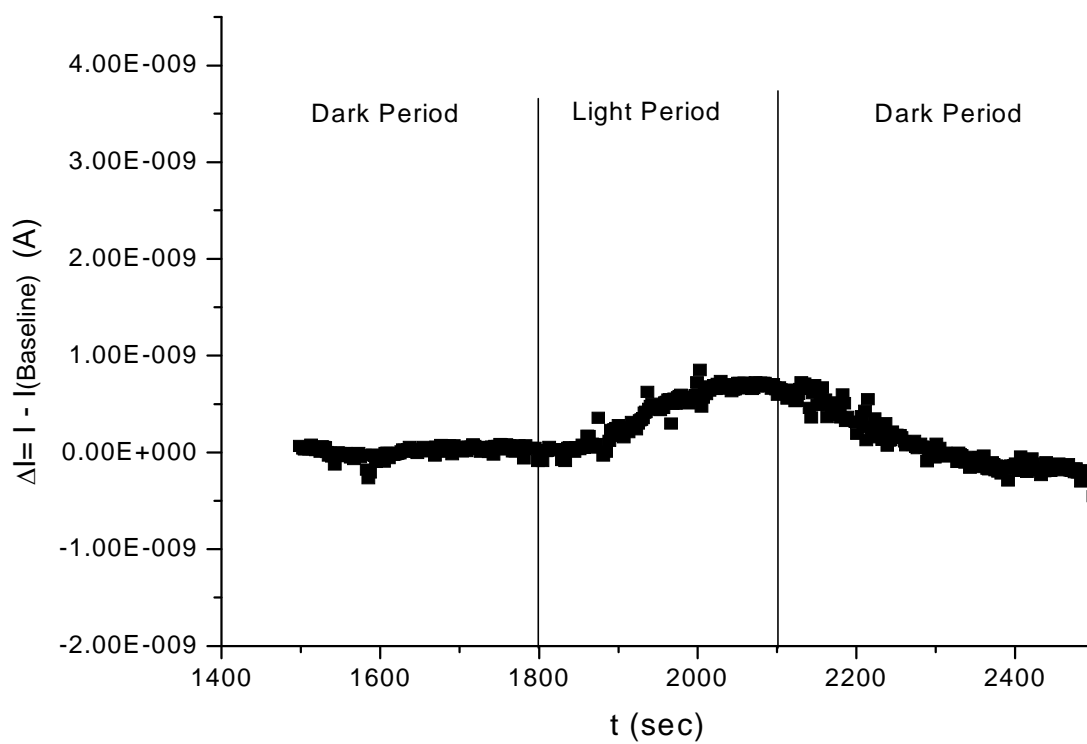


Figure 5. PO<sub>2</sub> electrode data from 4/16/08(4) with baseline correction applied.

### **Final $\Delta PO_2$ due to Light Exposure**

For the data from the present study collectively, the calculated change in  $PO_2$  of the RBC suspension due to light exposure was small. The change in  $PO_2$  was an average of  $1.60 \pm 2.4$  mmHg. The change in  $PO_2$  was computed using the following equation:

$$\Delta PO_2 = \Delta I / m^{\text{CORR}}$$

where  $\Delta I$  is the change in current after the baseline correction was applied and  $m^{\text{CORR}}$  is the corrected slope. In previous studies, the release of oxygen from hemoglobin in RBCs was measured and calculated as the observed rate of oxygen release,  $RO_2^{\text{OBS}}$ , measured in the units  $\text{mlO}_2/\text{s} \cdot \text{per RBC}$ . Therefore, the rate of oxygen release due to light exposure in the present studies must be converted to a comparable unit of measure. An equation was derived to transform the data into the rate of oxygen release per RBC due to light exposure,  $RO_2^{\text{LIGHT}}$ . The  $RO_2^{\text{LIGHT}}$  was calculated using the following equation:

$$RO_2^{\text{LIGHT}} = \alpha \cdot \Delta PO_2 V_{\text{RBC}} / (\text{Hct } \Delta t)$$

where  $\alpha$  is the oxygen solubility coefficient,  $\Delta PO_2$  is the change in  $PO_2$ ,  $V_{\text{RBC}}$  is the volume of an RBC, Hct is the hematocrit of the RBC suspension, and  $\Delta t$  is the time period corresponding to the  $PO_2$  change. The results of the calculated rate of oxygen release due to light exposure are shown in Table 5. The average ( $\pm \text{SEM}$ ) for  $RO_2^{\text{LIGHT}}$  is  $3.45 \times 10^{-17} \pm 1.09 \times 10^{-17} \text{ mlO}_2/\text{s} \cdot \text{per RBC}$ .

**Table 5. Summary Table for Data**

Date of Experiment	$\Delta I$ (A)	Corrected Slope (A/mmHg)	$\Delta PO_2$ (mmHg)	Hct	$\Delta T$ (°C)	$\alpha$ (mlO <sub>2</sub> /mmHg·cm <sup>3</sup> )	RO <sub>2</sub> <sup>LIGHT</sup> (mlO <sub>2</sub> /s·per RBC)
4/2/08	5.13E-10	3.75E-10	1.37	0.38	0.8	0.00347	2.54E-17
4/9/08	3.26E-10	4.00E-10	0.82	0.55	0.6	0.00347	1.06E-17
4/16/08(1)	7.52E-11	3.21E-10	0.23	0.30	1.6	0.00346	5.49E-18
4/16/08(2)	4.81E-10	3.23E-10	1.49	0.30	0.2	0.00347	3.56E-17
4/16/08(4)	9.07E-10	3.31E-10	2.74	0.31	0.5	0.00348	6.27E-17
4/24/08(1)	2.44E-09	3.65E-10	6.67	0.57	0.2	0.00352	8.37E-17
4/24/08(2)	5.13E-10	3.65E-10	1.41	0.55	0.1	0.00347	1.80E-17

The change in temperature,  $\Delta T$ , is the difference between the end and the beginning temperature of the light exposure period.

## **DISCUSSION**

In current microcirculation studies there has been an ongoing unsolved mystery regarding oxygen transport. The predicted rate at which oxygen is released from hemoglobin does not match the measured rate of oxygen release. The rate at which oxygen is released from hemoglobin molecules in red blood cells is observed to be about ten times higher than can be explained by passive diffusion from arterioles and subsequent consumption by the parenchymal cells. It is known that exposure of oxyhemoglobin to light causes oxygen to dissociate from the hemoglobin. This discrepancy has led to the current study to determine how much of the observed rate of loss of oxygen can be explained by the photo-dissociation of oxygen from hemoglobin.

### **Comparison of Results with Previous Experiments**

Previous studies have been done to learn more about oxygen transport in the field of microcirculation. Various researchers have investigated the diffusion of oxygen across the endothelial surface in precapillary vessels during microcirculation experiments. Duling and Berne demonstrated oxygen diffusion from arterioles and many other researchers contributed to this topic by quantifying the oxygen diffusion in different species and tissues. From the numerous experiments done on oxygen transport, it has been seen that there is a large discrepancy in the amount of oxygen released from red blood cells in arterioles. Researchers found that the observed rate of oxygen transfer from the lumen into the wall of a vessel is an order of magnitude higher than what was hypothesized from a theoretical model (Vadapalli et al). The compilation of data from the literature done by Vadapalli et al in 2000 includes results on oxygen diffusion from arterioles in experiments on the rat pancreas, rat liver, and rat mesentery. The results of



the experimental findings were expressed in terms of the oxygen flux from arterioles, expressed as  $JO_2$  ( $RO_2^{OBS}$ ) with units of  $mlO_2/(cm^2 s)$  (see Table 6). In order to compare the findings of the present study to previous work, the results of the light-induced release of oxygen from hemoglobin was expressed in terms of oxygen release rate per RBC,  $RO_2^{LIGHT}$ .

$RO_2^{LIGHT}$ , the amount of oxygen released from RBCs in the sample chamber during the light exposure, was calculated by considering the amount by which dissolved oxygen changed in the closed chamber and was expressed as follows:

$$AO_2^{LIGHT} = \alpha \Delta PO_2 V_{Chamber}$$

where  $AO_2^{LIGHT}$  is the amount of oxygen released from RBCs in the sample chamber during the light exposure,  $\alpha$  is the oxygen solubility of the medium in the chamber,  $\Delta PO_2$  is the change in  $PO_2$  during the light exposure period, and  $V_{Chamber}$  is the volume of the sample chamber. The amount of oxygen released per unit time is defined as  $AO_2^{LIGHT}/\Delta t$  where  $\Delta t$  (= 300 seconds) is the time period over which the release occurred. It is assumed that the amount of oxygen released should be proportional to the number of RBCs in the sample chamber. Therefore, the number of RBCs in the chamber can be estimated by dividing the total volume of RBCs ( $V_{RBCs} = Hct \times V_{Chamber}$ ) by the volume of a single RBC ( $V_{RBC}$ ) shown in the following equation:

$$\# \text{ of RBCs in chamber} = Hct \times V_{Chamber} / V_{RBC}$$

Therefore, the amount of oxygen released per RBC per unit time is expressed as:

$$RO_2^{LIGHT} = \alpha \Delta PO_2 V_{RBC} / (Hct \Delta t)$$

It is of interest to make a comparison of the  $RO_2^{LIGHT}$  values computed for the experiments conducted in the present study to those from the calculated oxygen flux from

arterioles (Vadapalli et al 2000), since the in vivo values are an order of magnitude higher than can be explained by conventional models of oxygen diffusion and consumption.

Thus, how much of the in vivo value can be explained by the light-induced release of oxygen from RBCs? The release of oxygen from arterioles is expressed as oxygen flux,  $JO_2$ , which is the amount of oxygen that diffuses from an arteriole per unit time per luminal surface area of arteriole. In order to make a proper comparison of results from the present in vitro study to those from published in vivo studies, it is necessary to estimate the number of RBCs responsible for the oxygen release. The number of RBCs in a vessel segment of radius,  $r$ , length,  $l$ , and filled with blood of hematocrit,  $Hct$ , is calculated as the volume of RBCs in the segment ( $\pi r^2 l Hct$ ) divided by the volume of a single RBC ( $V_{RBC}$ ):

$$\# \text{ RBCs in segment} = \pi r^2 l Hct / V_{RBC}$$

Thus, for a given diameter arteriole, how many RBCs are contained within the vessel needs to be determined and this will yield the “per RBC” value. The rate of change of the amount of oxygen in the vessel segment,  $dAO_2/dt$ , is:

$$dAO_2/dt = JO_2 2\pi r l$$

where  $dAO_2/dt$  is measured in units of ml  $O_2$ / sec. Thus, the amount of oxygen released from a vascular segment per RBC is as follows:

$$RO_2^{OBS} = 2JO_2 V_{RBC} / (r Hct)$$

Using the values presented in Table 4 of Vadapalli et al (2000), these calculations were made for arterioles in rats and the results are presented in Table 6

**Table 6. Experimental Data on the Rat from Vadapalli et al Compilation**

	d ( $\mu\text{m}$ ) (r, cm)	Hct (%)	$\text{JO}_2$ (ml $\text{O}_2$ / $\text{cm}^2$ s)	$\text{RO}_2^{\text{OBS}}$ (ml $\text{O}_2$ / s) per RBC
Rat pancreas	8.5 ( $r = 4.25 \times 10^{-4}$ )	59.2	$6.3 \times 10^{-5}$	$3.06 \times 10^{-11}$
Rat liver	9.5 ( $r = 4.75 \times 10^{-4}$ )	42.0	$1.7 \times 10^{-5}$	$1.04 \times 10^{-11}$
		34.3	$3.2 \times 10^{-5}$	$2.40 \times 10^{-11}$
		27.4	$2.0 \times 10^{-5}$	$1.88 \times 10^{-11}$
		22.5	$1.1 \times 10^{-5}$	$1.26 \times 10^{-11}$
Rat mesentery	23.2 ( $r = 11.6 \times 10^{-4}$ )	30.0	$2.7 \times 10^{-5}$	$0.95 \times 10^{-11}$

Data collected on rats found in Table 4 of Vadapalli et al (2000). Calculated values of intravascular oxygen flux from in vivo hemodynamic data compiled by Vadapalli et al 2000.  $V_{\text{RBC}} = 61 \mu\text{m}^3 = 61 \times 10^{-12} \text{ cm}^3$  for rats is used to calculate  $\text{RO}_2^{\text{OBS}}$  (Altman and Dittmer, 1971).

The  $RO_2^{\text{LIGHT}}$  value was about  $10^6$  times smaller than the  $RO_2^{\text{OBS}}$  values calculated from those listed for rats in Vadapalli et al in the *in vivo* studies. It is thought that  $RO_2^{\text{OBS}}$  is 10-100 times higher than it should be, but the explanation for these results is not yet known. Thus, the contribution of photo-dissociation of oxygen from oxygenated hemoglobin molecules to the observed oxygen loss per RBC can account for only about 0.01% of the results from the *in vivo* reports. Therefore, light-associated oxygen release appears to be negligible.

### **Limitations**

One of the limitations of the present study was controlling the temperature in the sample chamber. Because variations in temperature can have a significant influence on the oxygen dissociation curve, it is important to maintain the temperature of the sample chamber throughout an experiment. In the present studies, exposure of the red blood cell suspension to light had a tendency to raise the temperature of the suspension in the chamber. By controlling the temperature of the solution during the experimental measurements, one can ensure that any changes in  $PO_2$  are due solely to light exposure and not a change in temperature.

Another limitation of the present study was dealing with drift of the  $PO_2$  electrode. In almost all the  $PO_2$  measurements, the electrode did not reach a stable baseline. This made it difficult to accurately quantify changes in  $PO_2$ . A procedure to correct the baseline for drift in each experiment was applied to allow uniform treatment of the data.

## **Conclusions**

Results from the present study show that light exposure to hemoglobin molecules in red blood cells is not a viable explanation for the larger than expected oxygen release in arterioles and subsequent consumption by the parenchymal cells. The results showed that the observed change in  $PO_2$  was very small and can not account for the unexpectedly large amount of “lost” oxygen in the microcirculation. These findings disprove the hypothesis of the present study, that light exposure has a significant effect on oxygen release, and thus rules out this possible explanation for the discrepancy between experiment and theory.

## **Future Studies**

To further investigate the effects of light exposure on the release of oxygen from red blood cells, more in depth studies are in order. One important aspect that should be further studied is the change in  $PO_2$  levels at various temperatures. In the present study some preliminary experiments were done to observe by how much the current (I) reading of the  $PO_2$  electrode would be affected at temperatures around 23 °C, 30 °C, and 37 °C. When it comes to temperature influence on  $PO_2$  measurements, 37 °C is an important temperature to observe, since this is the normal body temperature of most mammals and the temperature at which most in vivo measurements have been conducted. It is well known that oxygen dissociation from hemoglobin molecules is greatly influenced by an increase or decrease in temperature. If the temperature is increased, a right shift in the oxygen dissociation curve is observed and the  $PO_2$  electrode current recording should increase. As a result, the  $PO_2$  measurements of a solution would be inaccurate unless corrected for the difference in temperature.

Another area of interest for the present study is to perform more control studies to validate the data. An example of a control experiment is to expose the hemoglobin in the red blood cells to sodium nitrite ( $\text{NaNO}_2$ ) to convert the hemoglobin in the red blood cells to metHb. With all the hemoglobin converted to metHb, the heme group of the hemoglobin molecule would no longer be able to bind oxygen. Therefore, there would be no oxygen to be released from the hemoglobin molecules. We would expect that the  $\text{PO}_2$  of the red blood cell suspension would remain constant even during light exposure because of the hindered oxygen binding ability to the heme group of the hemoglobin, which is now all converted to metHb.

It is recommended that future experiments could also be conducted using various hematocrit ranges. Using a wider hematocrit range to perform the experiments would test the effect of different red blood cell concentrations. Conducting the experiments using different hematocrit levels would also enable one to observe whether  $\text{PO}_2$  measurements would change due to an increase or decrease in viscosity of the red blood cell suspension.

The present study could also be conducted in different media other than red blood cell suspensions. A suggestion is to use a hemoglobin solution in place of the red blood cell suspension to observe the effects of light exposure on oxygen release from hemoglobin molecules. The process of making the hemoglobin solution includes all the same steps of making a red blood cell suspension. Once the red blood cell suspension is washed and centrifuged, a sample of the packed RBCs is mixed and diluted with distilled water. The distilled water, in turn, lyses the red blood cells due to the osmotic inflow of water. This process allows for the existence of only hemoglobin molecules in the sample solution and  $\text{PO}_2$  can be measured.

The present study was done using light from a microscope lamp, commonly used during microcirculation experiments. A suggestion for future studies is to conduct the present study using different wavelengths of light. The red blood cell suspension, or other choice of medium, could be exposed to various wavelengths of light by using optical filters in the light beam to select specific narrow wavelength ranges. This alteration to the light exposure method allows for wavelengths such as blue, green, or red regions of the visible spectrum of light to be utilized in the experiments.

## BIBLIOGRAPHY

Altman, P.L. and Dittmer, D.S., Respiration and Circulation, Bethesda, MD: Fed. Amer. Soc. Exp. Biol., 1971.

Berlin, N. and Lotz, C., Life span of the red blood cell of the rat following acute hemorrhage. Proc. Soc. Exp. Bioi. Med. 78, 788, 1951.

Berne, R.M. and M.M. Levy (1998). Physiology. St. Louis, Mosby.

Boland, E.J., D.D. Lemon, P.K. Nair, J.S. Olson, J.D. Hellums, Physiological factors affecting O<sub>2</sub> transport by hemoglobin in an *in vitro* capillary system. J Appl Physiol. 19 pp. 798-806, 1987.

Coin, J. T., and J. S. Olson. The rate of oxygen uptake by human red cells. J. Biol. Chem. 254: 1178-1190, 1979.

Costanzo, L. S. Physiology. Philadelphia, W.B. Saunders Company, 2006.

Duling BR and Berne RM. Longitudinal gradients in periarteriolar oxygen tension. A possible mechanism for the participation of oxygen in local regulation of blood flow. Circ Res 27:669-678, 1970.

Ganong, W.F., Review of medical physiology. Los Altos , California, Lange Medical Publications, 1977.



Golub, A.S., and R.N.Pittman, Erythrocyte-associated transients in PO<sub>2</sub> revealed in capillaries of rat mesentery. Am J Physiol Heart Circ Physiol, 2005.

Hartridge H and Roughton FJW. The velocity with which carbon monoxide displaces oxygen from combination with hemoglobin. Proc R Soc Lond B Biol Sci, 1923.

Hellums J.D. The resistance to oxygen transport in the capillaries relative to that in the surrounding tissue. Microvasc Res 13: 131-136, 1977.

Hillman, R. and C. Finch, Red Cell Manual. Philadelphia,PA, FA Davis Company, 1996.

Krogh A. The Anatomy and Physiology of Capillaries. New York: Hafner Publishing, 1959.

Lemon, D.D., E.J. Boland, P.K. Nair, J.S. Olson, J.D. Hellums, An in vitro capillary system for studies on microcirculatory O<sub>2</sub> transport. J. Appl. Physiol, 1987.

Middleman, S. Transport phenomena in the cardiovascular system. Wiley-Interscience New York, 1972.

Nair, P.K., J. D. Hellums, J.S. Olson. Prediction of oxygen transport rates in blood flowing in large capillaries. Ann Biomed Eng 1989.

Pittman, R.N. Oxygen supply to contracting skeletal muscle at the microcirculatory level: diffusion vs. convection. Acta Physiol Scand 168(4): 593-602, 2005.

Pittman RN and Duling BR. Effects of altered carbon dioxide tension on hemoglobin oxygenation in hamster cheek pouch microvessels. Microvasc Res 13: 211-224, 1977.

Popel AS, Pittman RN, and Ellsworth ML. Rate of oxygen loss from arterioles is an order of magnitude higher than expected. Am J Physiol 256: H921-924, 1989.

Swain, D.P. and R.N. Pittman, Oxygen exchange in the microcirculation of hamster retractor muscle., 1989.

Taylor, D.J., N.P.O. Green, and G.W. Stout, Biological science. 2. Systems, maintenance and change. Cambridge University Press, 1997.

Vadapalli A, Pittman RN, and Popel AS. Estimating oxygen transport resistance of the microvascular wall. Am J Physiol Heart Circ Physiol 279: H657-671, 2000.

Vandegriff, K.D. and J.S. Olson, Morphological and physiological factors affecting oxygen uptake and release by red blood cells. J Biol Chem, 1984.

Weibel, E.R., The pathway for oxygen: structure and function in the mammalian respiratory system. Cambridge, Mass., Harvard University Press, 1984.

Zheng L, Golub AS, and Pittman RN. Determination of  $PO_2$  and its heterogeneity in single capillaries. Am J Physiol 271: H365-372, 1996.

## **VITA**

Tanikka Daneille Toler was born on August 26, 1984 in the District of Columbia. She graduated from Frederick Douglass High School in Upper Marlboro, Maryland in 2002. She received a Bachelor of Science in Biology from Virginia Union University (Richmond, Virginia) in May 2006. Tanikka enrolled in graduate studies at Virginia Commonwealth University in 2006 and went on to receive a Master of Science in Physiology and Biophysics from Virginia Commonwealth University in December 2008.