

Critical Oxygen Delivery: The Crux of Bypass with a Special Look at the Microcirculation

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Abstract: The microcirculation can be defined as those vascular structures where respiratory gas flux occurs. These are generally the arterioles, venules, and capillaries. Larger vascular conduits tend to have thicker walls, are at considerable distance from cellular sites of oxygen utilization, and therefore contribute little to oxygen flux. The microcirculation is complex, not a simple straight line of parallel groups of pipes with unidirectional flow. Rather, the complex network has most vascular structures not open (held in reserve) and often has bidirectional flow. Understanding the movement of O₂, CO₂, and other gases within this network has only recently been the center of focused

research. The cardiopulmonary bypass machine is meant to keep the microcirculation normal, but research is demonstrating major changes within. This review looks at what is known today in spontaneously perfusing animals as well as early findings noting differences in cardiopulmonary bypass. We, as yet, do not understand all the mechanisms involved in the changes of the microcirculation so thoughts regarding future areas for research are discussed. **Keywords:** oxygen delivery, oxygen demand, critical oxygen delivery, microcirculation, endothelium, glycocalyx, cardiopulmonary bypass, cardioplegia, inflammation, thrombosis. JECT. 2011;43:P10–P16

Critical oxygen delivery (DO_{2crit}) is a key physiologic parameter for organ and organism survival. It defines, physiologically, the boundary of shock (1,2). The purpose of cardiopulmonary bypass is to support that physiologic variable (DO_{2crit}), keeping the patient out of shock, while the heart and lungs are removed from the normal circulation during repair. An understanding of DO_{2crit} is paramount to understanding shock and being able to treat it appropriately. Although many in cardiac surgery think they understand O₂ delivery, the process is complex and few understand the ramifications and limitations of DO_{2crit} . Cardiopulmonary bypass (CPB) has at times been compared to other shock states, but every effort is made to assure specific organ demands (brain, heart, kidney, and intestine) are met. The microcirculation has been under intense study over the last 10–15 years in highly specialized physiology laboratories. With new techniques we are learning a great deal about how O₂ fluxes through the complexity of arterioles, venules, and capillaries that together constitute the microcirculation. Although wonderful animal

models of hemorrhagic shock and hemodilution have been studied, the data we have gained is limited. Almost all of the studies to date have been performed in rodents and most often in striated muscle. We know a good deal about endothelial cells, vasoregulation, blood cell interactions with endothelial cells, and the glycocalyx and these observations are intriguing. Unfortunately we make assumptions that what we see in striated muscle might well translate to brain, heart, or kidney microcirculation. However that may not always be true. Some data and hypotheses generated from these experiments could well explain some of the phenomenon that we encounter in CPB.

DO_{2crit}

The physiologic definition of shock is a state in which there is an inadequacy of O₂ delivery, in relation to O₂ demand (1–3). O₂ delivery is maintained in a surplus state. All of us are aware of the O₂ content equation based upon hemoglobin (Hgb) level, saturation, and the amount of dissolved O₂ in plasma (Table 1).

That equation utilizes a constant (1.36) multiplied by the measured hemoglobin level. The constant is based upon a usual physiologic oxy-hemoglobin dissociation curve, however not all hemoglobin binds O₂ in accordance with the usual curve. This becomes important when a pharmaceutical

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Table 1. Oxygen content.

CaO_2 (oxygen content) = $(1.36 \times Hgb_{con} \times Hgb_{sat}) + (.0031 \times O_2, PaO_2)$
 DO_2 (oxygen delivery) = $CaO_2 \times CO$ (cardiac output or CPB flow)
 $VO_2 = DO_2 (P_{CO_2} - P_{mitoO_2})$ the gradient of O_2 from erythrocyte (Hgb) to the mitochondria is driven by partial pressures within each sub-cellular region.

company tries to design a hemoglobin based oxygen carrier as a blood substitute or when Hgb is dramatically different (stored banked blood, sickle Hgb, fetal Hgb, etc.). The O_2 content equation also has a component for dissolved O_2 ($.0031 \times PaO_2$). In many clinical situations that dissolved O_2 is disregarded as an insignificant contributor to the total O_2 content. In severe anemia, as well as in hyperbarics, the dissolved O_2 content may well be a major portion of the total O_2 content. It is dissolved O_2 that is the O_2 physiologically used for cellular metabolism.

Oxygen content is important for O_2 delivery (Table 1). Cardiac output (CPB machine flow) is multiplied by the total O_2 content for an estimate of O_2 delivery. This is the classic teaching. What we have learned from the microcirculation in the last 10 years is that such calculated numbers may not reflect the actual delivery of O_2 to tissues. Calculated whole body numbers may not be real in terms of minute to minute biology. The microcirculation will auto-regulate its own O_2 delivery and extraction is based upon instantaneous tissue utilization, acid base levels, and a number of other complex mechanisms. Hemoglobin dissociation curves are dramatically manipulated through acid base equilibrium, chloride ion concentration, and 2,3 diphosphoglycerate (2,3 DPG) concentration. The production of 2,3 DPG is highly O_2 and energy dependent.

A key, and little recognized fact, is that in striated muscle the hematocrit (Hct) of blood in the capillary network is approximately 15% (4,5). Even if the aorta and large arterioles carry a hematocrit of 40%, the pre-capillary sphincter cells along with a complex set of physics (micro-tubular rheology) allows that red cells cannot be stacked tighter in the capillaries than the 15% Hct (Figure 1). We do not know whether in other key tissues such as heart, kidney or brain this 15% Hct limit exist as well.

If cardiac output (CPB flow) drops, then total calculated O_2 delivery will drop as well. The compensatory event that occurs in the capillaries will be that O_2 extraction rises to meet tissue O_2 demands. Eventually if either systemic or local flow drops enough, or if anemia is so bad (less than 15–20% Hct), then a level of critical O_2 is encountered. For the vast majority of our lives all of our tissues exist with a luxury O_2 delivery and this is known as flow independent O_2 delivery (Figure 2).

At the point at which O_2 extraction has hit its limits or if anemia is so severe (<15% Hct) then flow dependent O_2 extrac-

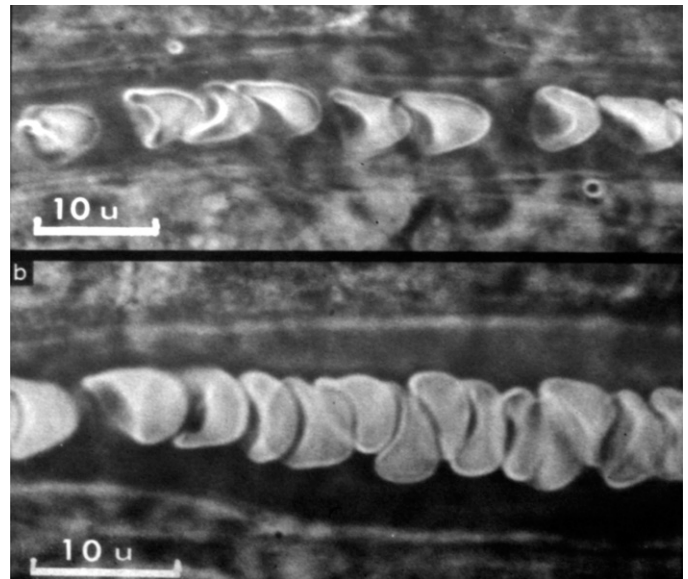


Figure 1. Erythrocytes traversing a capillary. Note that they “stack” and appear to be touching. However, the cells are all viewed in an obtuse angle and they all flex/fold to fit through microcirculation. One can see the plasma gaps and appreciate the distance from the red cell membrane outer limit to the edge of the capillary. In the lower picture, at a slightly greater magnification one should appreciate the lining of the capillary. This area is made up of the glycocalyx and represents a highly complex glycosaminoglycan surface, which holds a number of key molecules that have anti-coagulation and anti-inflammation function. The thickness of the glycocalyx can be measured using these microvascular techniques. Photomicrograph provided by Torres-Filho et al. (14).

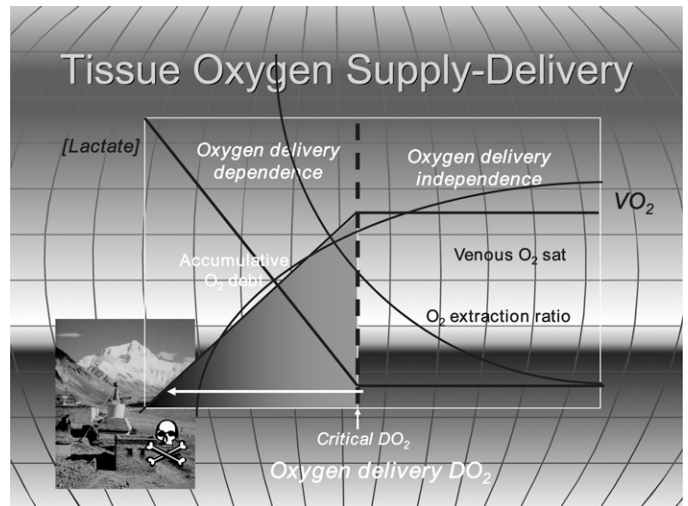


Figure 2. Supply independent and supply (flow dependent) oxygen delivery. Note that as there is a drop in oxygen delivery, there is an increase in oxygen extraction. The point of DO_{2crit} is the definition of when shock occurs. The more time spent to the left of DO_{2crit} affects tissue, organ, and whole body survival. Left of DO_{2crit} can be thought of as “the killing zone.”

tion occurs (Figure 2). As one approaches and exceeds that interesting physiologic point a number of key events happen.

This inflection point is known as the point of critical O_2 delivery or DO_{2crit} (1–3). When an animal or a tissue

is to the left, on the curve, of DO_{2crit} it is by definition in anaerobic metabolism. The length of time spent to the left of DO_{2crit} has a direct correlation to survival. With anaerobic metabolism cells do not die immediately. They begin to create metabolic acids, lactate being the most important. The mitochondria shift their adenosine tri-phosphate (ATP) production to anaerobic biochemistry but the other cellular organelles involved with DNA/RNA transcription cease to work and protein synthesis drops off dramatically. As this imbalance continues, cellular ion pumps, particularly calcium flows, change eventually leading to other side effects such as cellular, mitochondrial edema, and eventually apoptosis. But, it all begins to be dysfunctional with the point of DO_{2crit} . So it is that the study of DO_{2crit} ought to provide us great data on tissue and animal survival.

To further understand DO_{2crit} , by way of illustration, if a mountain climber ascends the Himalayas he will increase his/her Hct leading to increased O_2 carrying capacity. This is in direct response to a lowered partial pressure of O_2 increasing erythropoietin from the kidney. The cardiac output will increase also but factors such as viscosity and fluid losses will lead to a point of diminishing enhanced cardiac output. Eventually viscosity and cardiac output increases reach their maximum. Once the climber enters a level above 24,000 feet, the PaO_2 is so reduced that all human physiology will be forced to the left of DO_{2crit} . This is known in the climbing world as “killing zone.” The entire body is constantly anaerobic and in an oxygen debt. Of interest, total O_2 carrying capacity has dramatically increased but the dissolved O_2 content has dropped so low that all the increased carrying capacity cannot make up for the reduced dissolved O_2 and diffusivity of O_2 combined with viscosity overtake the compensatory mechanisms.

Oxygen Debt

Oxygen debt is a concept not often understood (nor even studied) in cardiac surgery, and is the total amount of O_2 (quantity of O_2 per mL tissue volume \times time) not delivered to an organism or tissue (Table 1) (6,7). In trauma and hemorrhagic shock it is well understood that the amount of O_2 debt again correlates with survival or death (1–3,8–10). The amount of O_2 debt is directly related to the amount of left shift beyond DO_{2crit} and the length of time spent in that physiologic (shock) state. A rough way to estimate this is to look at lactate levels and even rising potassium levels (a result of tissue ion leakage).

Those animals that are successfully resuscitated after hemorrhage will regain a normal blood pressure, cardiac output, and oxygen carrying capacity. But, if they had an existing O_2 debt it may well be that all the vital signs appear normal (blood pressure, heart rate, etc.) yet repayment of O_2 debt is not complete. Oxygen debt cannot be

measured in our usual operating rooms or Intensive Care Units. To measure O_2 debt a metabolic measurement must be precisely made of O_2 uptake and CO_2 production. When O_2 uptake does not meet the demands set by CO_2 production, O_2 debt is occurring. We as yet do not know the sub-cellular mechanisms of repair that go on with repayment of O_2 debt, but it probably is at least the restoration of ATP stores as well as the recreation/repair of dysfunctional or destroyed cellular protein machinery. In O_2 debt it may well be that a trauma victim could be 1–3 L behind in O_2 delivery (1–3). Generally, more than 3 L behind in O_2 delivery will mean certain death. To replete that amount of debt may take hours, depending upon O_2 delivery. Those patients who can repay their O_2 debt within 60–90 minutes often survive whereas those that cannot repay O_2 debt by 4 hours or more will almost certainly die. No one has ever investigated cardiac surgery patients in terms of DO_{2crit} and O_2 debt.

O_2 Flux

The microcirculation is where the “rubber meets the road” in terms of tissue O_2 delivery. The microcirculation is a complex, highly dynamic, redundant network of arterioles, capillaries, and venules. Flow is not constant through all vascular channels at all times. Erythrocyte flow stops and starts depending upon tissue demands. Many channels cannot be seen with routine trans-illumination microscopy if there are no red cells within the lumens. We do know that at some times plasma flows through channels either devoid of erythrocytes, or at different flow rates than the erythrocytes are moving. Oxygen flows from all vascular channels out to the tissues (Figure 3). That fact cannot be overemphasized. It is not just capillaries that interact in the delivery of O_2 to cells. Arterioles and venules contribute to O_2 delivery but generally there is a network dependent upon one or more feeder arterioles. Any cell cannot survive if it is further than 40–50 microns from a vascular O_2 source. If arteries and venules are in juxtaposition they actually transfer O_2 between them, and venules can be very active in the delivery of O_2 to tissues. At any given time, in most tissue, only about 30% of capillaries are open and flowing. This allows for increased O_2 demand to be supplied by a regulated mechanism of delivery. Unfortunately, most of what we know regarding the microcirculation is from striated muscle with assumptions made to other tissue. Again, we know relatively little about flow in the microcirculation during CPB.

Oxygen moves from hemoglobin in red cells into the surrounding plasma and from that plasma out to the tissues. Although such a process sounds easy the route of an O_2 molecule leaving hemoglobin and entering a mitochondria or onto myoglobin is difficult (11–13). Oxygen is poorly soluble in water. Plasma is essentially water with some proteins, hormones, and of course cellular elements. Each red

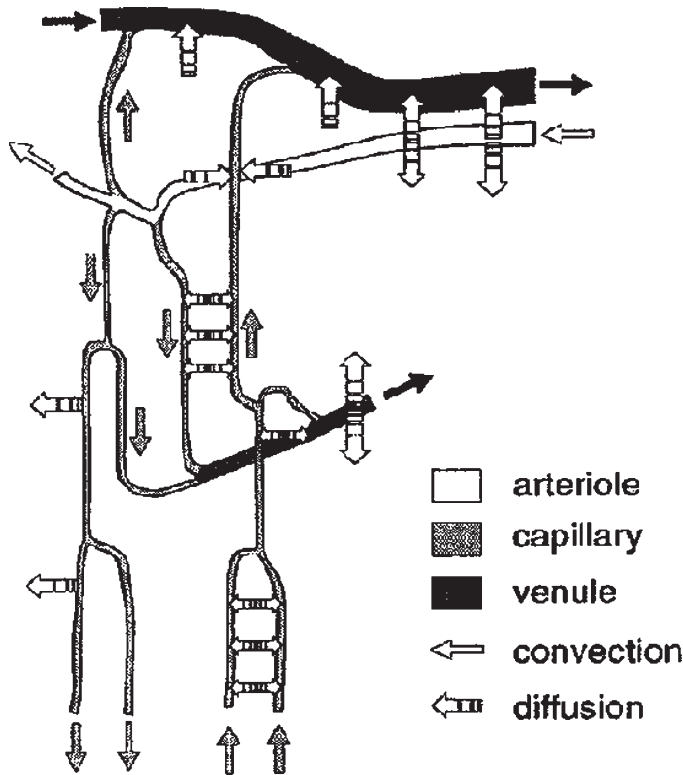


Figure 3. The flux of O_2 from arterioles, venules, and capillaries. One should realize that O_2 flows freely from levels of high partial pressure to areas of lower partial pressure. Hgb exerts a pull and push based upon relative saturation. Mitochondria pull O_2 based upon their needs but the movement of O_2 is based upon its solubility in water based (plasma) and lipids (membrane) as well as its binding and release from Hgb.

cell has approximately 300,000,000 molecules of Hgb and on each Hgb there are four O_2 molecules. Per mL of blood there are 4-5,000,000 red blood cells. It would therefore seem that the amount of available O_2 , no matter what the demand, would be massively in excess. However Hgb binds O_2 very tightly. We now understand that the movement of O_2 from Hgb to target sites is dependent upon the erythrocyte acting as a localized super charger of dissolved O_2 , and it is the dissolved O_2 that is available for metabolic function. The larger the plasma gap from the surface of an erythrocyte the larger is the resistance to movement of O_2 (Figure 4).

The erythrocyte functions with a corona of O_2 surrounding it and as one moves by angstroms away from the cell membrane, the partial pressure of O_2 drops. The way in which Hgb recharges the surrounding plasma is through the biochemistry of changed O_2 binding. The way the microcirculation auto-regulates O_2 supply to local tissue demand is by increasing red cell transit time and by increasing O_2 extraction ratio, with the system being limited by the 15% Hct and the associated physics of stacking red cells in capillaries. Of interest, there is a fascinating network interaction that leads to countercurrent movement of O_2 from venules

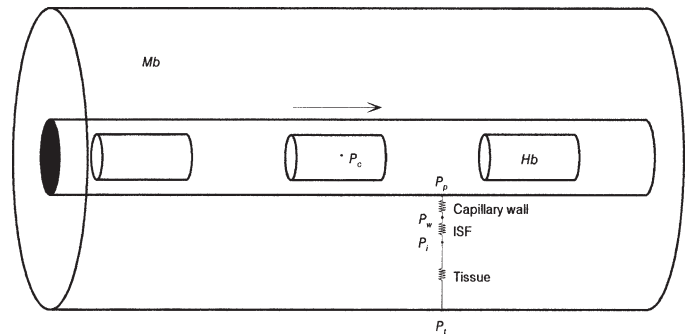


Figure 4. Representation of a tissue bore with a capillary running through it. The cylinders inside the capillary represent red blood cells separated by plasma gaps. Various resistances to O_2 movement are noted by the figure, with corresponding partial pressures of O_2 within the plasma, vessel wall, and tissue itself. Reprinted and adapted with permission from Cabrales and Intaglietta (12). Mb, myoglobin; P_c , partial pressure of O_2 in capillaries; P_p , partial pressure in plasma; Hb, Hemoglobin; P_w , Resistance to O_2 flow from vessel wall; P_i , resistance to O_2 flow due to interstitial fluid; ISF, Interstitial fluid; P_t , resistance to O_2 flow due to tissue (e.g., cell membranes, intracellular water).

to capillaries and from arterioles to both other vessel types. So O_2 is in constant flux diffusing down gradients, but convectively carried by plasma and red cell movements.

All mammalian species (we do not know about reptiles and fish) have the same level of DO_{2crit} in terms of Hct. That one observation should be contemplated for a bit, as it has profound implications. Whether you are a mouse, rat, pig, goat, chimp, or human at or near 15% Hct, flow independent oxygen delivery is maxed out, O_2 extraction ratio has hit its limit, and lactate production begins (14-16). This means that at 3.5-4 gm/dL Hgb, no matter what else is done, shock will occur. Blood pressure may be preserved (although likely it will be depressed) and cardiac output is maximized but the cells somewhere in the organism will revert to anaerobic glycolysis and metabolic acid production will begin. Irrespective of everything else, with a level below 3.5-4 gm/dL Hgb O_2 debt is occurring. Therefore 3.5-4 gm/dL becomes a floor below which we cannot electively accept going especially at normothermia. No one knows the DO_{2crit} at different levels of hypothermia, although O_2 usage drops about 4% per degree. Therefore, to fully understand DO_{2crit} , Hgb, and microcirculation O_2 fluxes during CPB, a wide range of basic physiology should be studied. Not only does temperature change O_2 demand but it also changes extraction ratio, oxy-Hgb curves, acid base, etc.

Historically, when blood transfusion was first conceived in the early years of the 20th century and blood banking was not yet viable, the trigger for transfusion was a level of Hgb between 3-5 gm/dL. This was the point at which cardiac failure and unacceptable deaths increased. Of note, in databases following outcomes both in cardiac surgery and other surgeries for Jehovah's Witnesses, it is not until the levels of Hgb drop to around 5 gm/dL or below that

death rates rise (17,18). Both of these facts seem to relate to the limit of the microcirculation to function at or near DO_{2crit} . In CPB we have long had the debate about what is the “best” Hgb or Hct to transfuse. Both measurements are surrogates for potential O_2 delivery. One day perhaps we can understand and talk in terms of DO_{2crit} and study O_2 debt in CPB rather than such gross measurements as Hgb and Hct.

MEASUREMENTS IN EXPERIMENTAL MICROCIRCULATION WORK

Today the use of microvascular/microcirculation research techniques is moving from the highly instrumented animal research laboratory to the operating room. In the research laboratory, the standard has been trans-illumination intravital and confocal microscopy. These techniques use one of several standard animal preparations to view a representative piece of tissue left intact to its native circulation. Hamster cremaster muscle, hamster cheek pouch, rat, and other animal mesentery and rat spinotrapezius muscle preparations have all been used. Recently some exciting work using an imbedded plastic “window” in the rat skull has made it possible for surface microscopy investigations of brain blood flow (19–21). Work is underway to adapt such techniques to intact spinal cord blood flow as well (22).

From these preparations, capillary density, vessel flow rates, and vessel sizes can be measured off line. Usually videos of the vessels to be interrogated are captured and then computer programs are adapted for automated or semi-automated calculations of parameters. Cell types, erythrocytes, platelets, and white cells can be distinguished. White cell rolling, sticking, and diapedesis can be followed at a site of capillary or vessel interest. Work with laser injury has been able to create distinct lesions of endothelial cells to assess platelet adhesion, clot formation, and anticoagulation pharmaceuticals. The lining of the endothelial cells with the glycosaminoglycans is available for study as well. Its size can be measured using overlaid digital subtraction photomicroscopy. Using a number of molecular markers with immune-fluorescence, the presence, clearance, and production of key endothelial cell products such as nitric oxide, hydrogen peroxide, endothelin, etc. can be directly visually assessed. Furthermore, again with immune-fluorescent techniques individual endothelial cells can be seen to be healthy or undergoing apoptosis.

Vascular O_2 content, as well as tissue O_2 content, can be directly measured in real time during microcirculation research. With the use of specific laser wavelengths of light a technique of phosphorescence quenching has been perfected. This technique uses a known amount of phosphorous attached to albumin. With the right laser light it gives

a decay curve directly and inversely related to the partial pressure of O_2 . Such techniques allow for assessments of vascular and tissue O_2 delivery in real time under any desired Hgb, Hct, or shock (low blood pressure, hemorrhage, etc.) to be investigated. Phosphorescence quenching cannot be done in humans and neither can routine intravital microscopy.

However, about 10 years ago a new technique was commercially created—orthogonal polarization video microscopy (23–25). This technique allows for using polarized light at 550 nm, which is the wavelength reflected by Hgb. By using this technique and shining the device the orthogonal (90° reflected light) forms a picture of red blood cells flowing through the microcirculation. Video images can then be made of nail beds, oral mucosa, and even rectal mucosa in humans during any number of adverse physiologic conditions. Measurements of red cell velocity, red cell concentration, vascular diameter, etc. can all be made by off line analysis. Work from our center has created a technique using Raman spectroscopy (light scattering) at the right wavelength such that microvascular oxygen content and Hgb O_2 saturation can be read without touching the organ or organism. This means that in the future we should be able to get readings of tissue or even cellular O_2 amounts in humans without using phosphorescence quenching.

STUDIES IN CARDIOPULMONARY BYPASS AND MICROCIRCULATION

The use of orthogonal polarization video analysis has led to some recent literature regarding the changes of the microcirculation during CPB (23–25). In a small study from Belgium, nine patients undergoing cardiac surgery were compared to six patients undergoing cardiac surgery without CPB and seven patients undergoing thyroidectomy (complete controls) (25). At baseline, prior to surgery the percentage of perfused vessels was the same in all groups. When anesthesia was induced the levels of vessel perfusion dropped to about 70% perfused. Of interest, during CPB the levels of perfused vessels dropped to 53% and when patients concluded their surgery (on entrance to intensive care unit) the perfusion had begun to increase. Those that underwent CPB had a lower perfusion than either thyroid patients who had normalized or nonCPB cardiac patients (64%) and still had only about 60% of vessels perfused even with normalized hemodynamics. The severity of obstructed vessels correlated with measured systemic lactate. Others have similarly confirmed that once CPB is begun there is a measurable decrease in microvascular flow index (26–28). In our research we have found that micro-air embolism is a universal event during CPB (29,30). Furthermore, air embolism causes destruction of the glycocalyx, up-regulates white cell sticking, and has effects upon

hydrogen peroxide reperfusion injury of endothelial cells. Whether these mechanisms are important in routine CPB microcirculation events or are more rare situations we simply do not know.

In animal work with transillumination video microscopy the effects of some vasoconstrictors have been examined as well as basic mechanisms of CPB. In a study of small bowel microcirculation, it was shown in rats on CPB that even if the hemodynamics were maintained in a normal range (stable and normal mean blood pressure) there was a decrease in functional capillary density, arteriolar vasoconstriction, and blood velocity reduction. Increased leukocyte accumulation occurred with more sticking and rolling of leukocytes during CPB as well as an extravasation of albumin. These observations signal that endothelial cells and the glycocalyx are dysfunctional, but they have not been directly studied to date. The use of phenylephrine, vasopressin, and other vasoconstrictors to enhance or normalize blood pressure appear to be particularly bad on the maintenance of microvascular perfusion, capillary density, and O_2 delivery. Large blood vessel flow went up whereas small vessels (where O_2 is transferred) dropped. The endothelium is responsible for vasoconstriction/dilation, local blood flow, inflammatory mediation, coagulation mediation, vascular permeability, and vascular growth/repair. Think about how many of these events we manipulate in cardiac surgery and how few of them we truly understand (31). The microcirculation is where blood and endothelium interact.

THE FUTURE

The initial foray into microcirculation biology research with CPB is disturbing. Observations that flow in the key units for O_2 flux are decreased dramatically suggest that even though we do our best to support hemodynamics, the complexity of the microcirculation and the endothelial biology lead to a dysregulation of DO_{2crit} . Mechanisms for this can easily be suggested. They include the near universal micro emboli that occur with CPB, inflammatory events, changes in hormones, nitric oxide synthesized, and perhaps many more (Figure 5).

The fact that we use CPB in an attempt to maintain homeostasis and preserve organs during repair of the heart and lungs suggests that at the best we are far from performing anything normal. This is not new news. However, the widespread efforts by anesthesiologists and perfusionists to maintain blood pressure in a normal range using infused vasoconstrictors again suggests that we are sailing in waters we know little about. The use of understanding DO_{2crit} and O_2 debt coupled with advanced physiologic measurements of the microcirculation, endothelial blood interface will surely yield exciting results in the future. With these studies will come new models for testing pharmacologic inter-

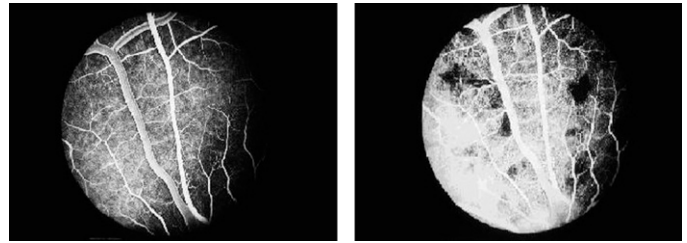


Figure 5. Before and after retinal angiograms of a human after cardiopulmonary bypass. Note the changes in the microcirculation and the loss of capillary network. These are transient and are thought to be due to micro-air emboli. The retinal microcirculation is a direct reflection of the brain microcirculation. Reprinted with permission from Arnold et al. (20).

ventions, new CPB techniques, and strategies that should make CPB safer and improve outcomes.

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