1. Hemoglobin and the Movement of Oxygen

Respirator system/Biochemistry
YOU MUST BE ABLE TO:

1. Compare structure of myoglobin and hemoglobin
2. Understand the mechanism of how oxygen binding to iron affects hemoglobin structure
3. Understand the T and R states of hemoglobin and how they are converted to each other
4. Understand the concept of cooperativity in hemoglobin and the importance of having quaternary structure
5. Differentiate between oxygen saturation curves of myoglobin and hemoglobin and know the implications of these curves on the oxygen delivery (loading and unloading)
6. Understand the effects of H+, CO2, and 2,3BPG on oxygen binding to hemoglobin and what are the important physiological aspects about these effects
7. Know how CO2 is transported by hemoglobin to lungs and exhaled
8. Understand the molecular mechanism of Bohr's effect
9. Know the difference between the structures of fetal hemoglobin (HbF) and adult hemoglobin (HbA)
10. Understand the importance of 2,3BPG binding to fetal hemoglobin and how this affects fetal life

Hemoglobin and the Movement of Oxygen

specific aims
Hemoglobin and the Movement of Oxygen

- Introduction

Animals Have Widely Varying Needs for Oxygen
Demand for Oxygen Can Change in Seconds
Basal Needs are Significant - Diffusion not Enough
Exercise, Fight/Flight Add to the Need
ATP Energy Produced Aerobically 15 Times More Efficiently Than Anaerobically

Respiration versus Fermentation
Efficient, Adaptable Oxygen Delivery is Necessary
**Quaternary Structure**
- Interaction of multiple protein subunits

- **Hemoglobin** - 4 Subunits ($\alpha_2\beta_2$), 1 Heme Each, 1 O$_2$ Each, 1 “Donut Hole”
- **Myoglobin** - 1 Subunit, 1 Heme, 1 O$_2$ Each

This is the Donut Hole
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• Structure and Function

Heme Prosthetic Group
Ferrous Iron - Methemoglobin Won’t Work
Only Fe$^{2+}$ Binds Oxygen
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- Structure and Function

Edge-on View →

Attached to Remainder of Global Subunit

Histidine’s Movement Changes →

Global Unit’s Shape
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- Cooperativity

Binding of the first O$_2$ favors binding of second, etc. - Cooperatively Important as Hemoglobin Rapidly Passes Through Lungs
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• Cooperativity

At Low $O_2$, Myoglobin Holds More than Hemoglobin

At High $O_2$, Both Hold 100%

As Curves Move to Right
Less Affinity for Oxygen
Sigmoidal Binding Curve

Hyperbolic Binding Curve

Myoglobin Better for Storing Oxygen
Hemoglobin Better at Delivering Oxygen

$P_{50} = 2$
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- Bohr Effect

Less Oxygen Bound at Same Pressure

Less Affinity

More Affinity

Protons Can Bind to Hemoglobin
Protons Change Hemoglobin’s Shape
Reshaped Hemoglobin Loses Oxygen
Rapidly Metabolizing Tissues Release Protons
Rapidly Metabolizing Tissues Get More Oxygen From Hemoglobin

More O₂ Required To Have Same Fraction Bound
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• Bohr Effect & CO

Acid Favors Release of O₂ From Hemoglobin
CO₂ Favors Release of O₂ From Hemoglobin
Acid and CO₂ are Released by Rapidly Metabolizing Tissues
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- 2,3 BPG

**Byproduct of Glycolysis**
Exercising Muscle Cells Rapidly Use Glycolysis
Exercising Muscle Cells Produce Acid, CO₂, and 2,3 BPG
Binds in Hole of Donut
Locks Hemoglobin in T-State

![2,3 Bisphosphoglycerate Structure](image)

![Hemoglobin Structure](image)

*Hole of the Donut*
Hemoglobin and the Movement of Oxygen

- 2,3 BPG and Oxygen Binding

Slow Metabolic Rate

Rapidly Metabolizing Cells Produce Acid
Rapidly Metabolizing Cells Release CO₂
Rapidly Metabolizing Cells Release 2,3 BPG
All Favor O₂ Release from Hemoglobin
So

Fast Metabolic Rate

Rapidly Metabolizing Cells Get More O₂
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- 2,3 BPG and Smoking

2,3 BPG Big Concern for Smokers
Blood of Smokers has High Levels of 2,3 BPG
Hemoglobin Gets Locked in T-state in Passage Through Lungs
Oxygen Carrying Capacity of Blood Reduced
Carbon Monoxide Levels Also Higher in Smokers

2,3 Bisphosphoglycerate
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- Movement of CO₂

Rapidly Metabolizing Tissue

Blood

Blood

H₂O + CO₂ → Lungs

Blood

O₂ + H⁺:Hb-CO₂ → Exhaled

HbO + H⁺ → Remnant

Blood

H⁺:Hb-CO₂ → Lungs
CO2 and O2 Transport are linked to Bohr effect protons

Figure 9.31. The isohydric transport of CO2 as bicarbonate.
Figure 9.32. Transport of CO₂ as carbamino-hemoglobin.
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- Carbon Monoxide and Heme

An Additional Histidine is Present at the Heme Iron Site Reduces Affinity to CO, but Does Not Eliminate it
Carbon Monoxide in Cigarette Smoke
Note That CO$_2$ Does Not Bind to Heme, nor do Protons
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- Fetal Hemoglobin

The Body Makes Different Globins Over Time
Most Variations Centered on Birth
Fetal Hemoglobin Mostly $\alpha_2\gamma_2$

High Most of Life
Highest in Fetus
At Adult Levels by 24 Weeks
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• Fetal Hemoglobin

Fetal Hemoglobin Can’t Bind to 2,3 BPG
Mostly Remains in R-state
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- Sickle Cell Anemia

Sickle Cell Anemia is a Genetic Disease Affecting Hemoglobin
Multiple Forms - Mutation of Glu to Val at Position #6 Most Common
Red Blood Cells Lose Rounded Shape and Form Sickles
Shape Change Happens in Low O$_2$ Conditions - Exercise
Change Caused by Polymerization of Hemoglobin
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• Sickle Cell Anemia

Rounded Cells Move Easily Through Capillaries
Sickled Cells Get Stuck
Sickled Cells Removed by Spleen
**Clinically Important Hemoglobinopathies**

<table>
<thead>
<tr>
<th>structural defect</th>
<th>clinical consequences</th>
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</thead>
<tbody>
<tr>
<td>abnormal solubility</td>
<td>hemolytic anemia; pain</td>
</tr>
<tr>
<td>(sickle cell disease, Hb S)</td>
<td></td>
</tr>
<tr>
<td>decreased O$_2$ affinity</td>
<td>cyanosis</td>
</tr>
<tr>
<td>increased O$_2$ affinity</td>
<td>polycythemia</td>
</tr>
<tr>
<td>ferric-heme</td>
<td>cyanosis</td>
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<tr>
<td>(methemoglobin, Hb M)</td>
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<tr>
<td>unstable hemoglobins</td>
<td>Heinz body anemia</td>
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<tr>
<td>(heme loss; dissociation)</td>
<td></td>
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<tr>
<td>abnormal globin synthesis</td>
<td>anemia</td>
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<tr>
<td>(dominant thalassemia)</td>
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</tbody>
</table>
Oxygen Affinity of Hemoglobin Variants

- Methemoglobins (acquired)
- Hemoglobin A
- Hemoglobin variants with decreased oxygen affinity
- Hemoglobin variants with increased oxygen affinity
Myoglobin and hemoglobin are related proteins involved in (respectively) storing and carrying oxygen in the body. Myoglobin is a single subunit protein with high affinity for oxygen. It holds oxygen tighter than hemoglobin and serves in a battery-like capacity in tissues to release oxygen when tissue oxygen concentration is very low. Myoglobin can take oxygen from hemoglobin.

Hemoglobin is a four-subunit protein complex (two alpha subunits and two beta subunits) that serves to carry oxygen from the lungs to the tissues where it is needed. Hemoglobin is genetically related to myoglobin and is evolutionarily derived from it.
4. Myoglobin and hemoglobin both have porphyrin rings (like in chlorophyll) to hold ferrous (Fe++) iron. The specific porphyrin in these proteins is Protoporphyrin IX. Ferrous iron is the form of iron involved in carrying oxygen. Ferric iron (Fe+++) is the oxidized form of iron that will not carry oxygen. Heme is a term used to describe the protoporphyrin IX complexed with iron.

5. The iron in hemoglobin and myoglobin is held in place by five molecules: four nitrogens of the protoporphyrin IX ring and a histidine (called the proximal histidine). Oxygen is carried between the iron and an additional histidine (called the distal histidine) not involved in holding the iron.
6. If one plots the percentage of oxygen sites bound versus partial pressure for myoglobin, a hyperbolic curve is generated, consistent with a molecule with a single binding site and a high affinity for oxygen. The P50, which is the partial pressure of oxygen necessary to fill 50% of the myoglobins with oxygen, is very low for myoglobin, consistent with high affinity. Because myoglobin has high affinity for oxygen, it doesn't release much oxygen until it is in an environment with very low oxygen pressure. For this reason, it would be a poor oxygen transport protein.
7. Hemoglobin is much better designed to meet an organism's physiological needs for carrying oxygen than myoglobin. This is due to its four-subunit organization (one heme per subunit and one oxygen carried per subunit) which behaves in a cooperative fashion in binding oxygen.

8. Binding of oxygen by the iron atom causes it to be pulled 'up' slightly. This, in turn, causes the histidine attached to it to change position slightly, which causes all the other amino acids in the subunit to change slightly. The changes in shape (different 'states') result in the protein gaining affinity for oxygen as more oxygen is bound. The phenomenon is referred to as cooperativity.

9. Hemoglobin can exist in a "tight" state, called 'T', which exhibits low oxygen binding affinity. Hemoglobin in the T state will tend to release oxygen.
10 A second state of hemoglobin is the "relaxed" or R state, which exhibits increased oxygen binding affinity. Binding of oxygen by hemoglobin helps it to "flip" from the T to the R state and release of oxygen by hemoglobin helps it to flip from R to T.

11. A molecule called 2,3-bisphosphoglycerate (2,3 BPG) is produced by actively respiring tissues. It can bind in the gap in the center of the hemoglobin molecule and in doing so, stabilize the T state and favor the release of oxygen. Thus, tissues that are actively respiring get more oxygen. 2,3 BPG is noteworthy because the blood of smokers has a higher concentration of the molecule than the blood of non-smokers.

12. The Bohr effect describes physiological and molecular responses to changes in pH with respect to oxygen and carbon dioxide in the body. The oxygen effects arise from changes in the tertiary structure of hemoglobin arising from binding of protons to histidines in the molecule when under low pH.
13. Rapidly metabolizing tissues (such as muscle) generate low pHs, due to release of carbon dioxide and the conversion of this to carbonic acid by carbonic anhydrase. Carbonic acid readily loses a proton, becoming bicarbonate.

14. Thus, rapidly metabolizing tissues generate protons, which get absorbed by hemoglobin, which releases oxygen to feed the tissues.
15. CO$_2$ can also be taken up by hemoglobin at amine residues, causing protons to be released. Note that CO$_2$ binds hemoglobin at a site other than what oxygen binds. Carbon monoxide, however, can compete with oxygen for binding to the heme.

16. In the lungs, a reversal of this process occurs. Remember that the oxygen concentration in the lungs is high, so oxygen forces off the carbon dioxide and the carried protons from the hemoglobin. Addition of a proton to bicarbonate re-creates carbonic acid, which undergoes the reversal of the earlier carbonic anhydrase reaction, causing CO$_2$ to be released out of the lungs as a gas.
Hemoglobin and the Movement of Oxygen

• Summary

Animals Have Widely Varying O\textsubscript{2} Needs
ATP Generated Much More Efficiently in Presence of O\textsubscript{2}
Hemoglobin and Myoglobin are Related, but Have Different Functions
Hemoglobin has Four Subunits and Hemes. Myoglobin has One of Each
Bind of O\textsubscript{2} by Heme’s Iron Pulls up on a Histidine and Change’s Hemoglobin’s Shape
Changing Hemoglobin’s Shape Converts Hemoglobin from T-state to R-state
R-state Binds Oxygen Better. T-state Releases O\textsubscript{2} Better
In the Bohr Effect, Binding of CO\textsubscript{2} and H\textsuperscript{+} Favors O\textsubscript{2} Release
The Bohr Effect Explains How Oxygen and CO\textsubscript{2} Exchanged in Lungs
2,3 BPG is Produced by Rapidly Metabolizing Cells. It too Favors O\textsubscript{2} Release
Fetal Hemoglobin Can’t Bind 2,3 BPG and has Greater O\textsubscript{2} Affinity Than Adult Hemoglobin
Sickle Cell Anemia (SCA) is a Genetic Disease of Hemoglobin
In Low O\textsubscript{2} Concentration, Red Blood Cells of SCA Sufferers Form Sickle Shapes
Sickled Cells Stick in Capillaries and Can be Fatal
People Heterozygous for the Mutated Gene Survive Malaria Better Than Others