# Oxygen hemoglobin dissociation curve and its clinical importance

## Case

- 49-year-old man who was admitted to the department of chest medicine with dyspnea, weakness and cyanosis in whom differential diagnosis excluded acute and chronic pulmonary and cardiovascular disease.

- Saturation measured with a finger pulse oximeter was 89%.
- Despite administration of oxygen through a nasal cannula, saturation measured with a pulse oximeter did not change.

- Arterial blood gas analysis revealed a saturation of 97.9%, $PaO_2$ of 102 mm Hg, PaCO2 of 35 mm Hg, HCO3 of 3.4 mmol/l, pH of 7.44.

- Clinical cyanosis and low measured oxygen saturation in the presence of normal arterial oxygen tension was highly suggestive of methemoglobinemia ("**saturation gap**").

- Methemoglobin level, measured at the acute phase of disease was elevated at 16%. Episode resolved spontaneously.

# Saturation Gap

- The "oxygen saturation gap" is the difference between the calculated oxygen saturation from a standard blood gas machine and the reading from a pulse oximeter.

- If it is greater than 5%, the patient's hemoglobin may be abnormal, representing carbon monoxide poisoning, methemoglobinemia, or sulfhemoglobinemia.

- In present case(97.9%- 89%= 8.9%)

# Pulse Oximetry
# (measured oxygen saturation


- Pulse oximetry is based on measurement of a ratio of light absorption by tissues at a red wavelength (660 nm) and at an infrared wavelength (940 nm).
- OxyHb absorbs infrared and deoxyHb absorbs red light

- Uses empirically derived calibration curves that converts ratio of oxy to deoxyHb into %saturation.

# Calculated oxygen saturation(ABG Machine)

- Calculates % oxygen saturation by following formula

**sO2(%) = cHbO2/cHbo2+cHHb**

| | |
|---|---|
| pH | 7.35–7.45 |
| $pCO_2$ (mm Hg) | 35–45 |
| $HCO_3^-$ (mmol/L) | 22–26 |
| Total $CO_2$ content (mmol/L) | 23–27 |
| $pO_2$ (mmol/L) | 80–110 |
| $SO_2$ (%) | >95 |
| $O_2Hb$ (%) | >95 |

- It is important to note that the denominator in this equation is not the concentration of total hemoglobin.

- There are two species of hemoglobin present in blood that are incapable of binding oxygen. They are carboxyhemoglobin (COHb) and methemoglobin (MetHb)

- In health, COHb and MetHb together comprise less than ~5 % of total hemoglobin so that, normally, the concentration of total hemoglobin (*c*tHb) approximates to the sum of $cO_2Hb$ and *c*HHb.

- However, there are pathologies – most notably carbon monoxide poisoning and methemoglobinemia – that are associated with a marked increase in COHb or MetHb, and a resulting marked reduction in the oxygen-carrying capacity of blood, that is *not* reflected in $sO_2$.

This results in "**Saturation Gap**"

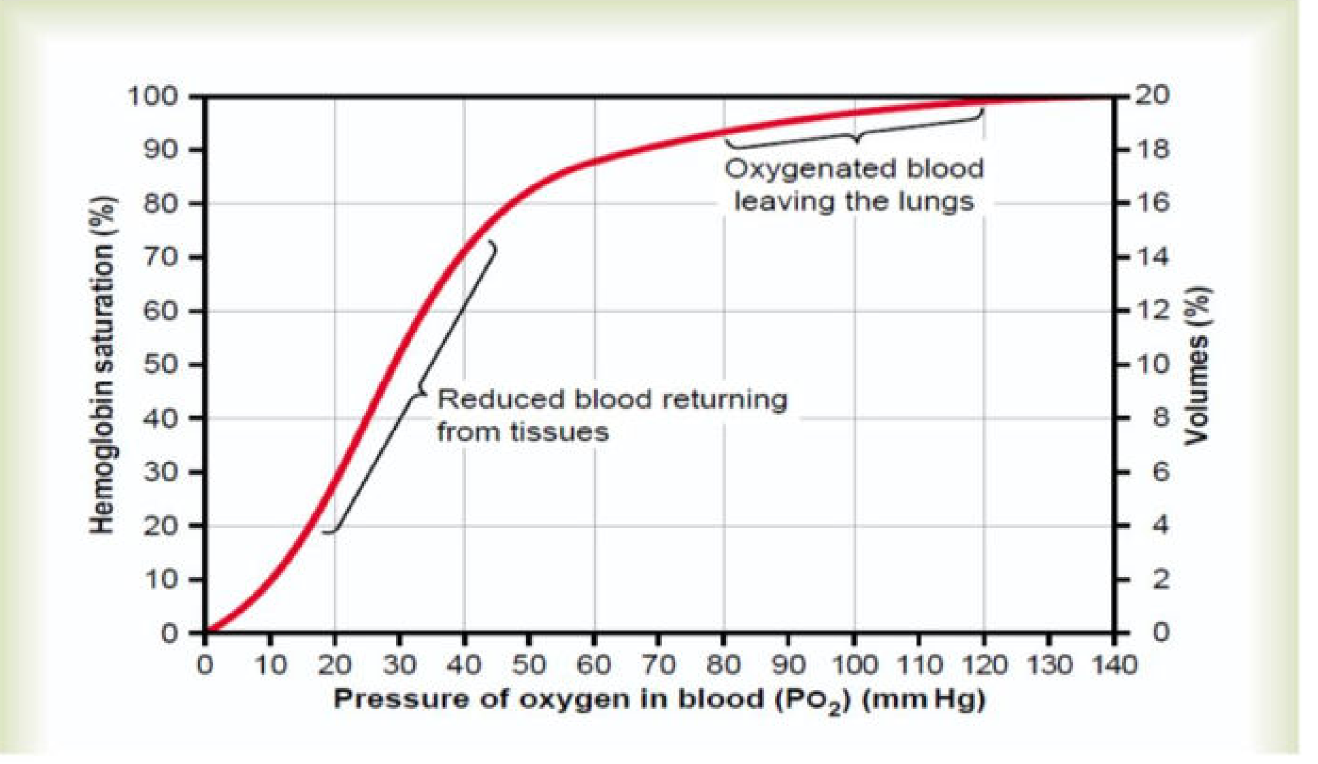
# Co-Oximeter

- Many modern blood gas analyzers have an incorporated CO-oximeter
- The measurement is based on spectrophotometric analysis of the hemoglobin released from a sample of arterial blood
- The four hemoglobin species present in blood (oxyhemoglobin, $O_2Hb$; deoxyhemoglobin, HHb; carboxyhemoglobin, COHb; and methemoglobin, MetHb) each have a characteristic light-absorption spectrum.

# Relationship of $O_2$ saturation with $pO_2$

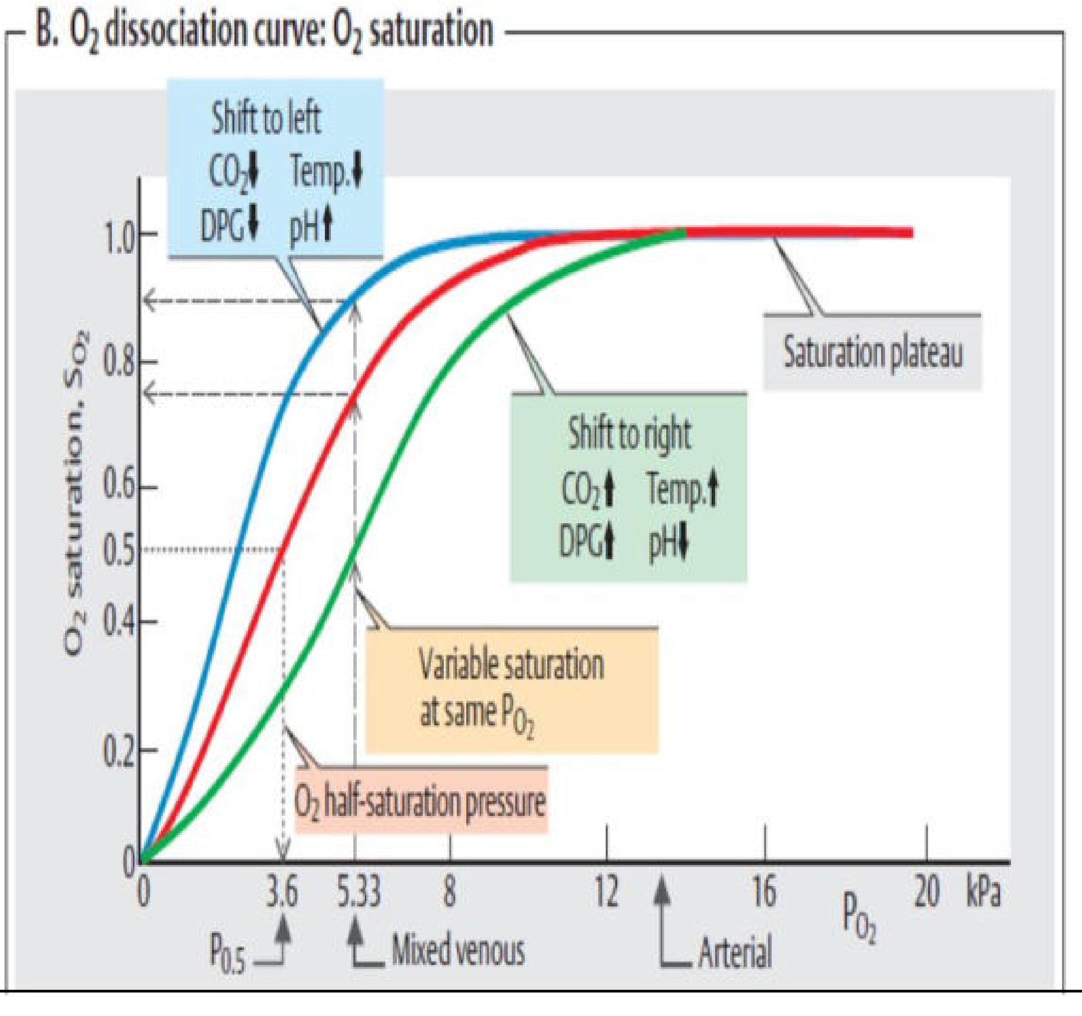
- A number of environmental factors in blood determine the relative affinity of hemoglobin for oxygen. The most significant of these is $pO_2$.
- Hemoglobin present in blood with relatively high $pO_2$ has much greater affinity for oxygen than hemoglobin present in blood with relatively low $pO_2$.
- The oxygen dissociation curve (ODC) describes this relationship graphically ($sO_2$ denotes Hb affinity)

# Oxygen Hemoglobin Dissociation curve




Although $pO_2$ only reflects a very small proportion (3 %) of the oxygen in arterial blood, it is highly significant because, as the ODC implies, it determines the $sO_2$ and therefore the total amount of oxygen that is contained in arterial blood for delivery to tissues.

# Factors affecting ODC

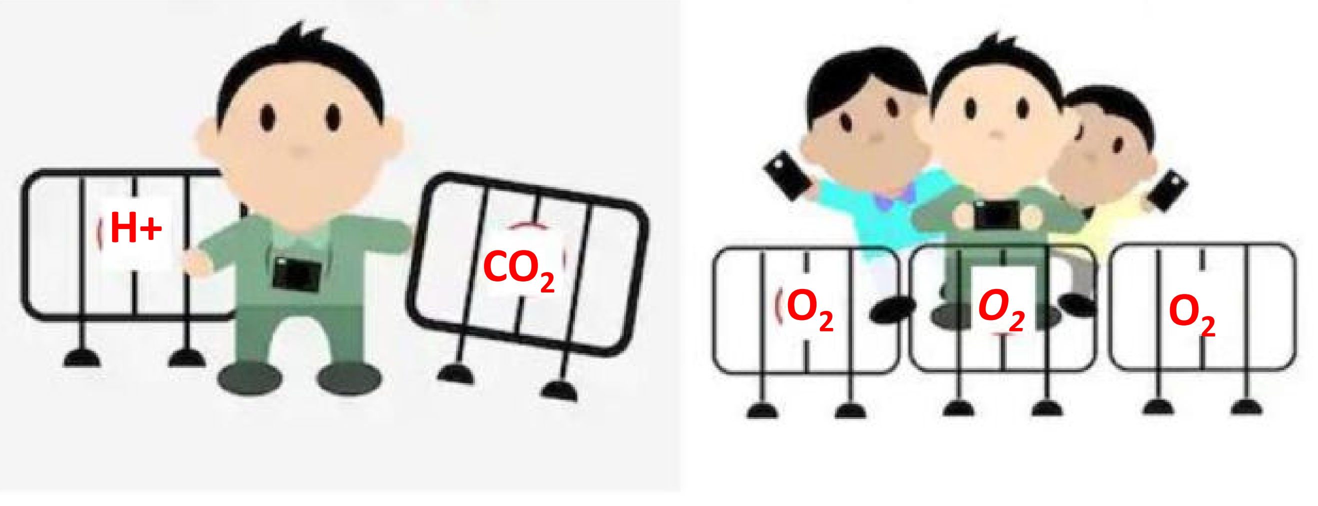



1. Carbon dioxide
2. Protons (↓pH)
3. Temperature
4. 2,3 BPG

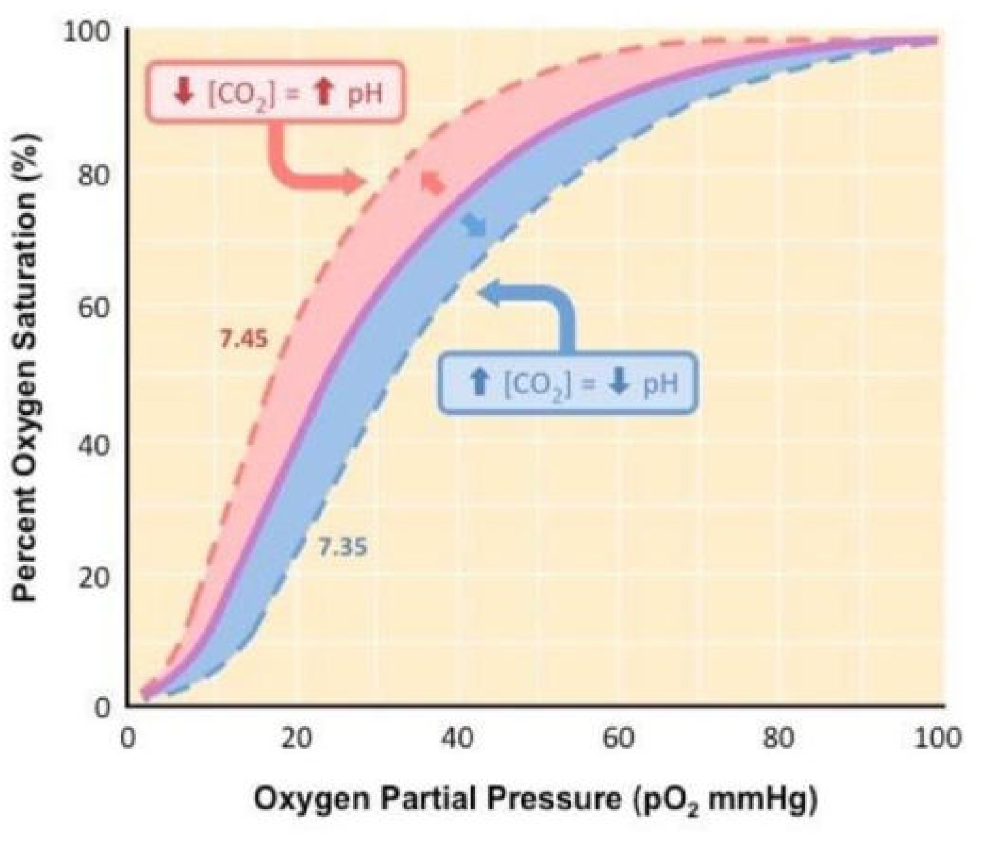
Increase in any of the above shifts curve to right and vice-versa

# Bohr Effect

- The Bohr effect is decreased affinity of hemoglobin for oxygen with increase in H+ or $CO_2$

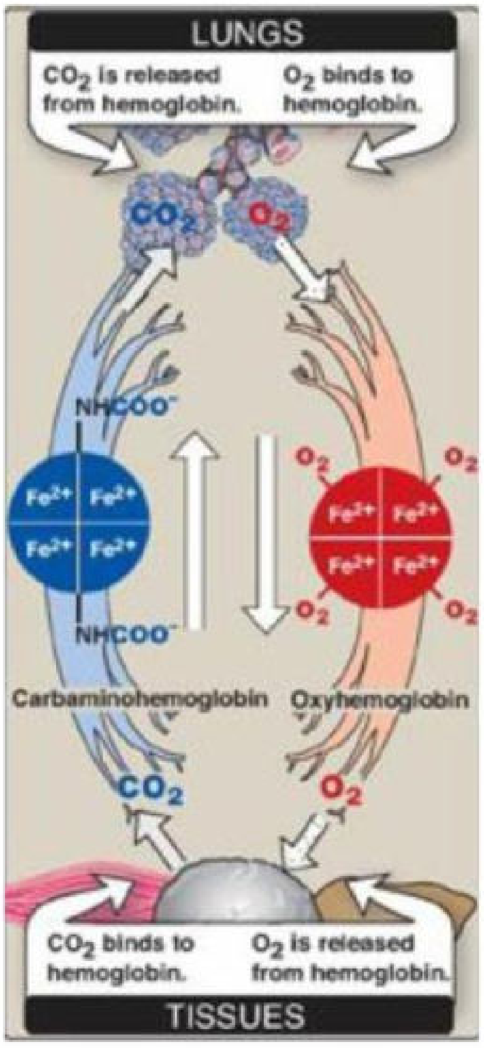


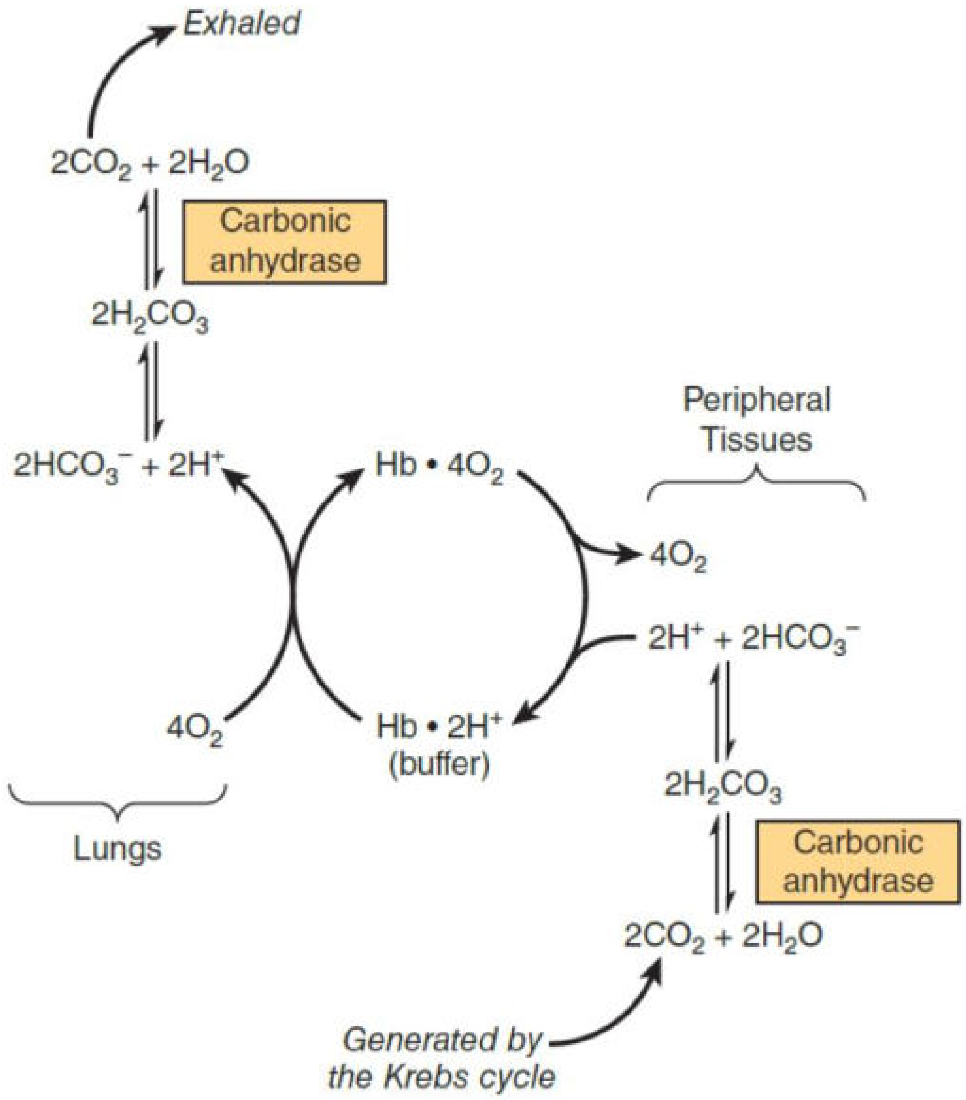

# Importance of Bohr Effect




- Bohr Effect shifts ODC to right increasing oxygen delivery

# Bohr Effect and Haldane Effect




# Methemoglobinemia
## (Discussion of case)

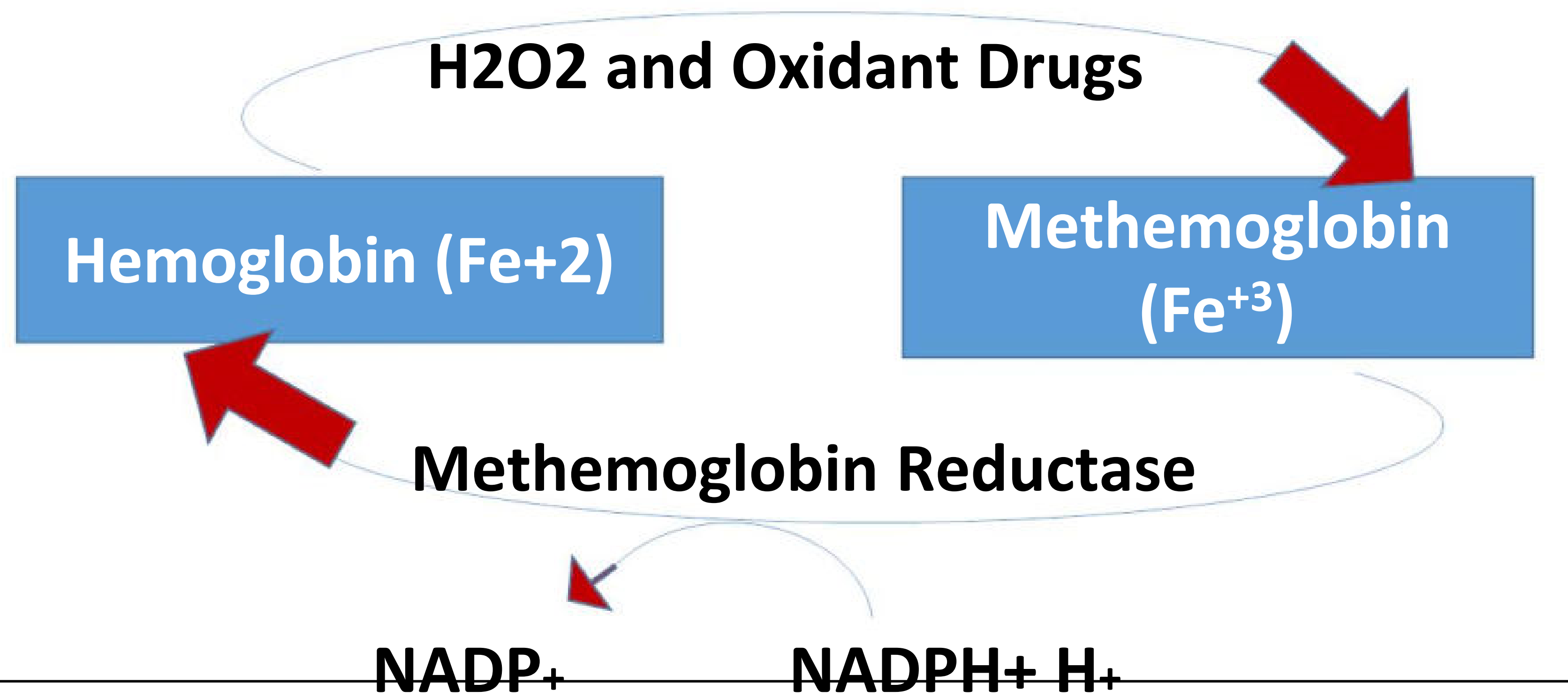
- Cyanosis(chocolate cyanosis) with structurally normal heart.
- Important D/D for an acquired or drug induced cause
- Hemoglobin can accept and transport oxygen only when the iron atom is in its ferrous form
- When haemoglobin becomes oxidized, the iron atom is converted to the ferric state (Fe3+), resulting in the formation of methemoglobin
- Methemoglobin lacks the electron that is needed to form a bond with oxygen and thus is incapable of oxygen transport.

- The low level of methemoglobin is maintained through 2 important mechanisms

1. HMP shunt pathway within the erythrocyte. Through this pathway, oxidizing agents are reduced by glutathione.
2. Enzyme cytochrome b5 reductase(Methemoglobin reductase) , requires NADH to reduce methemoglobin to its original ferrous state.

- Any drug that interferes with these mechanisms can lead to Methemoglobinemia

# Conversion Of Methemoglobin To Hemoglobin is NADPH+H+ Dependent



- **Congenital Methemoglobinemia**

1. arises from globin mutations that Stabilize iron in the ferric state (e.g. HbM Iwata [$\alpha^{87}$His↓Ty]

2. from mutations that impair the enzymes that reduce methemoglobin to hemoglobin (e.g.methemoglobin reductase, NADP diaphorase).

- **Acquired Methemoglobinemia** is caused by toxins that oxidize heme iron, notably nitrate and nitrite-containing compounds including drugs commonly used in cardiology and anesthesiology.

# Management of Methemoglobinemia

## Diagnosis

- Arterial blood with elevated methemoglobin levels has a characteristic chocolate-brown color(chocolate cyanosis)
- Saturation Gap

## Treatment

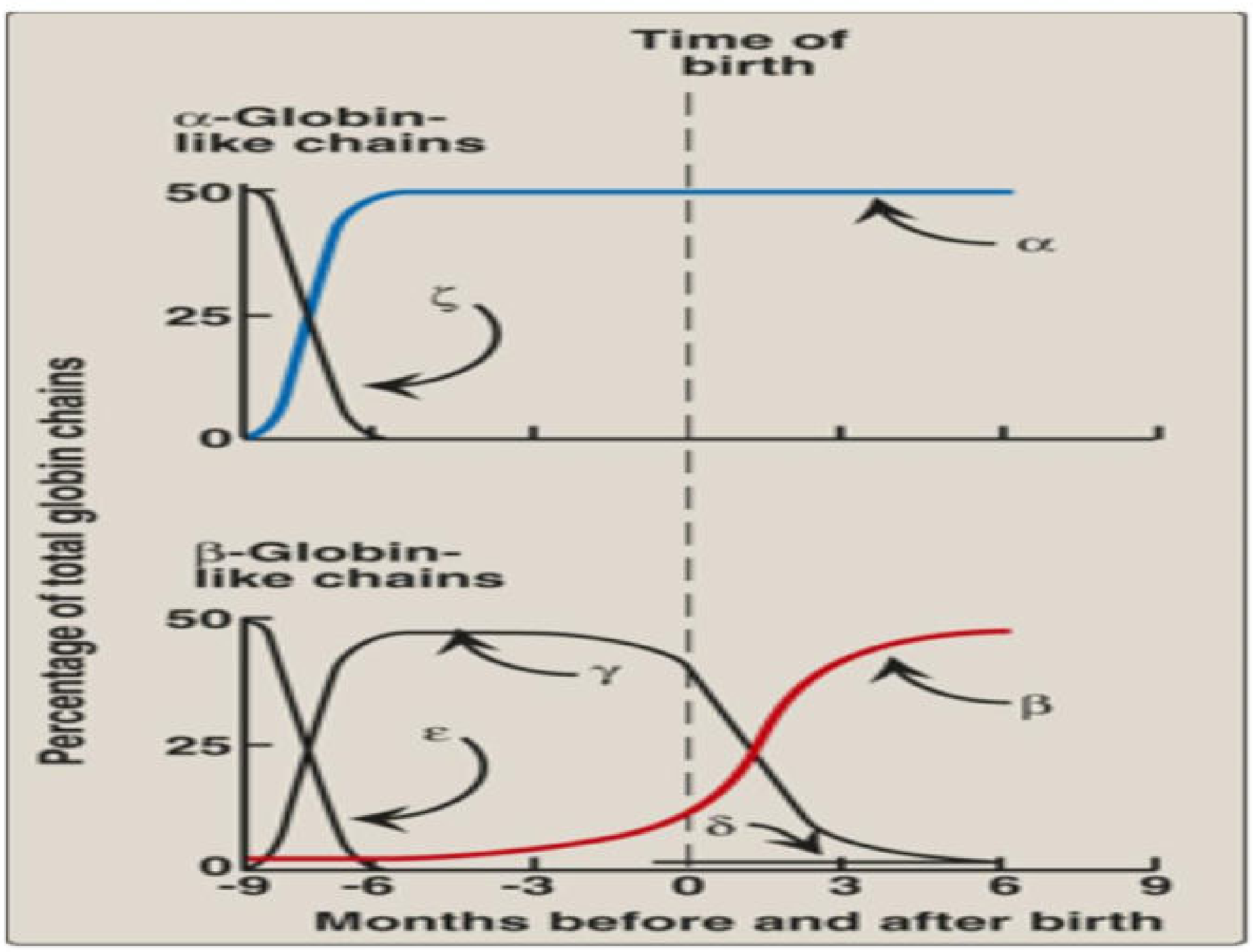
- Intravenous (IV) methylene blue is the first-line antidotal agent.
- Exchange transfusion and hyperbaric oxygen treatment are second-line options for patients with severe methemoglobinemia

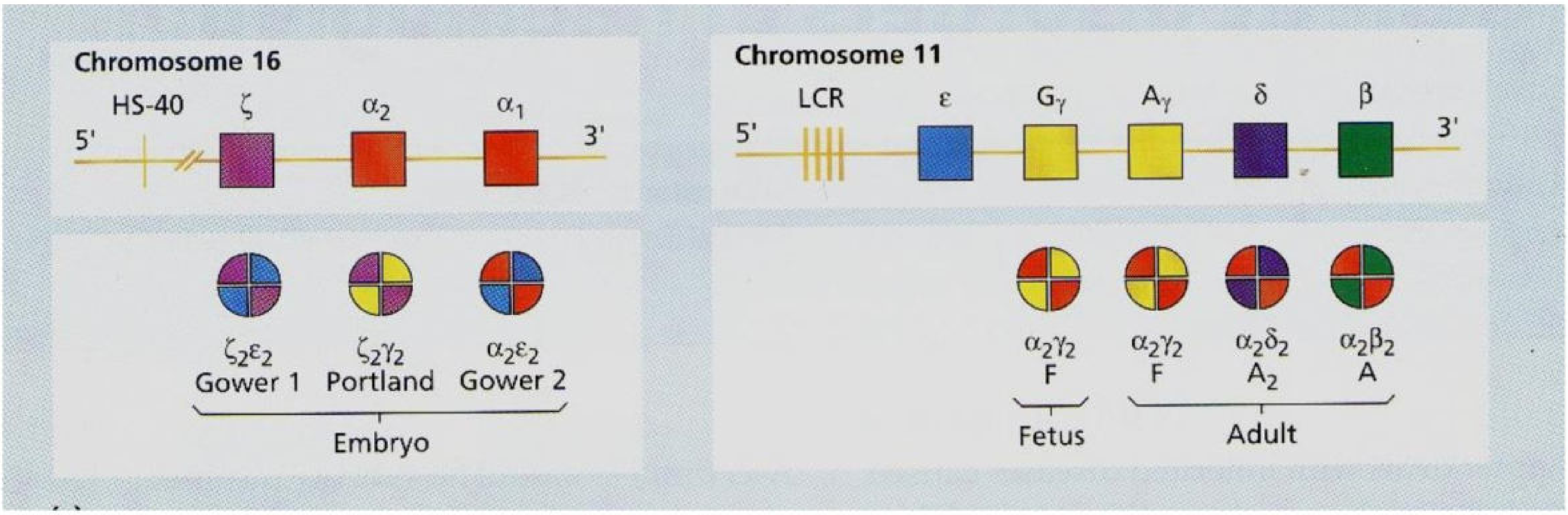
# Therapeutic Induction of Methemoglobin Formation

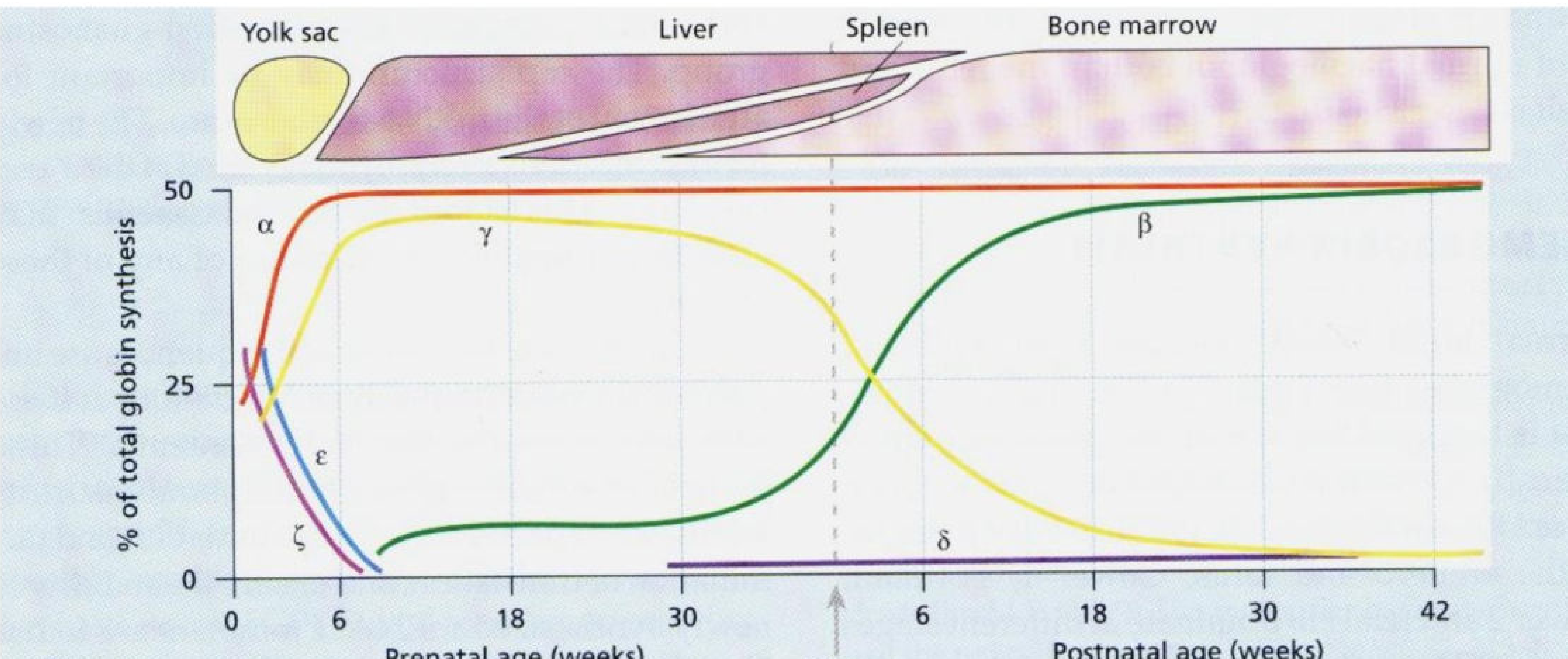

- Cyanide competes with cytochrome oxidase for Fe+++ of methemoglobin to form cyanmethemoglobin which is eliminated
- Thereby, the activity of inhibited cytochrome oxidase is restored.
- Agents used as antidote: sodium nitrite, amyl nitrite, 4-dimethylaminophenol

# Minor Hb

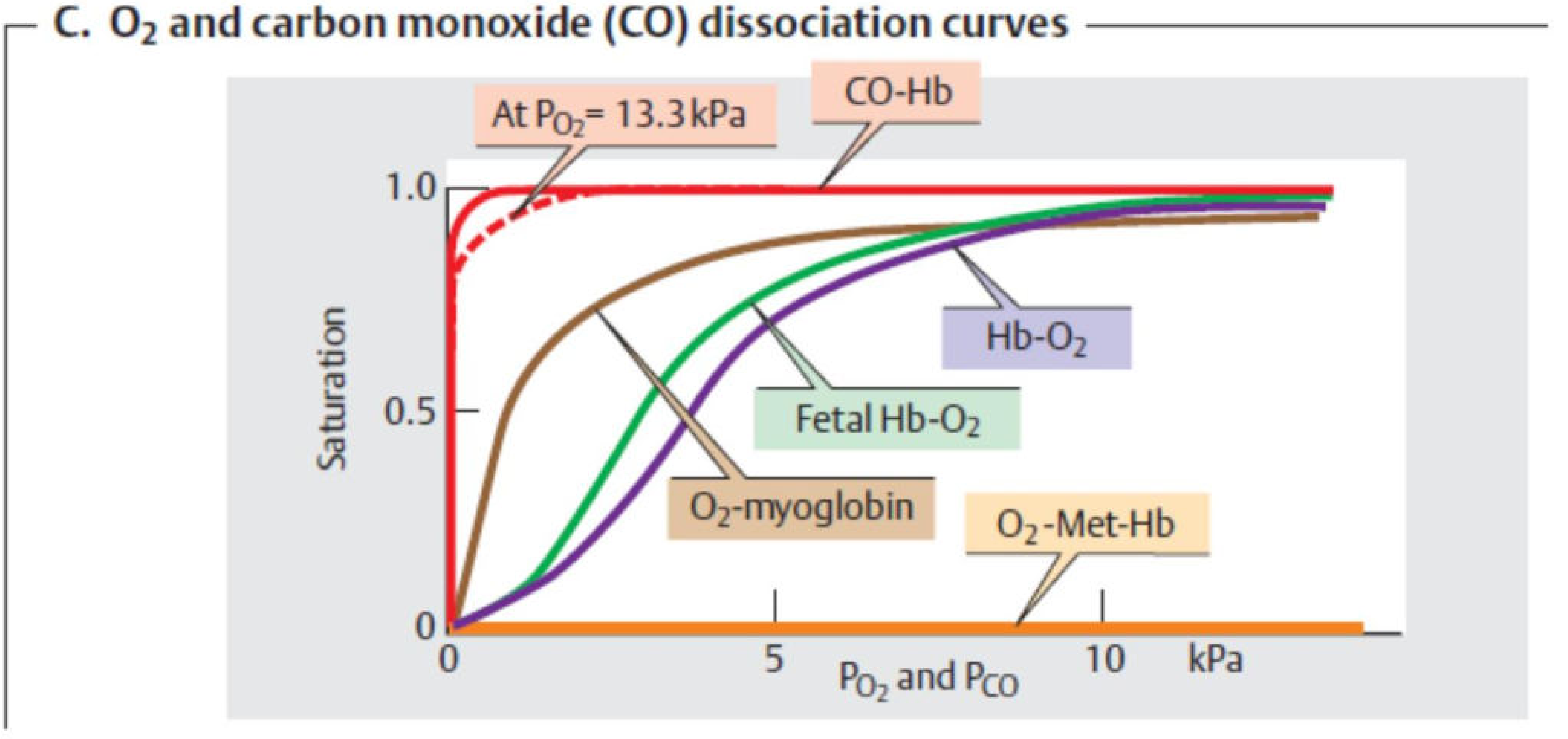
| Form | Chain composition | Fraction of total hemoglobin |
|---|---|---|
| HbA | $\alpha_2\beta_2$ | 90% |
| HbF | $\alpha_2\gamma_2$ | <2% |
| $HbA_2$ | $\alpha_2\delta_2$ | 2–5% |
| $HbA_{1c}$ | $\alpha_2\beta_2$-glucose | 3–9% |



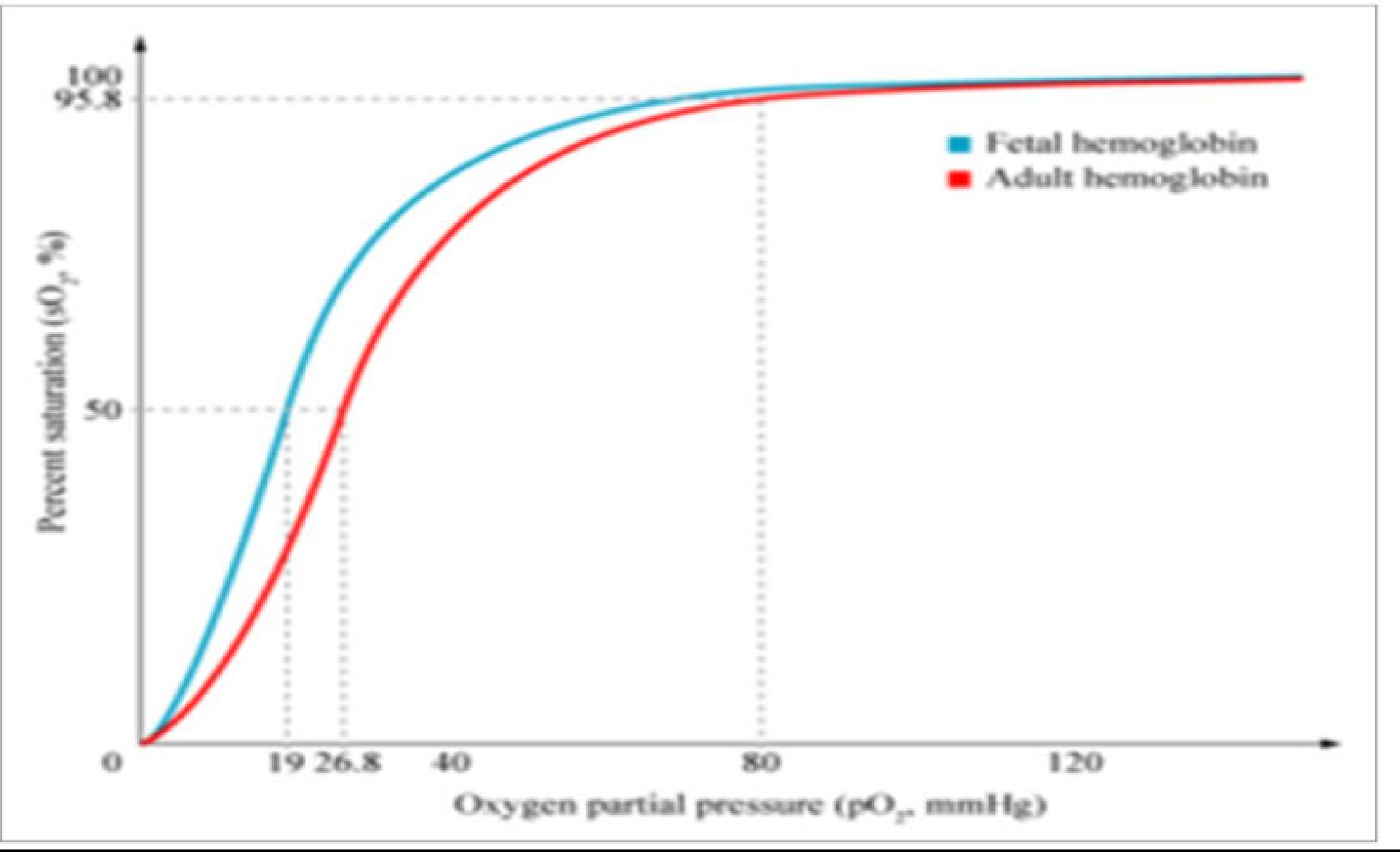

Chromosome 16
HS-40
ζ
α2
α1
5'
3'
ζ2ε2
Gower 1
ζ2γ2
Portland
α2ε2
Gower 2
Embryo
Chromosome 11
LCR
ε
Gγ
Aγ
δ
β
5'
3'
α2γ2
F
α2γ2
F
α2δ2
A2
α2β2
A
Fetus
Adult


Yolk sac
Liver
Spleen
Bone marrow
50
25
0
% of total globin synthesis
α
γ
β
ε
ζ
δ
0
6
18
30
6
18
30
42
Prenatal age (weeks)
Birth
Postnatal age (weeks)
(b)

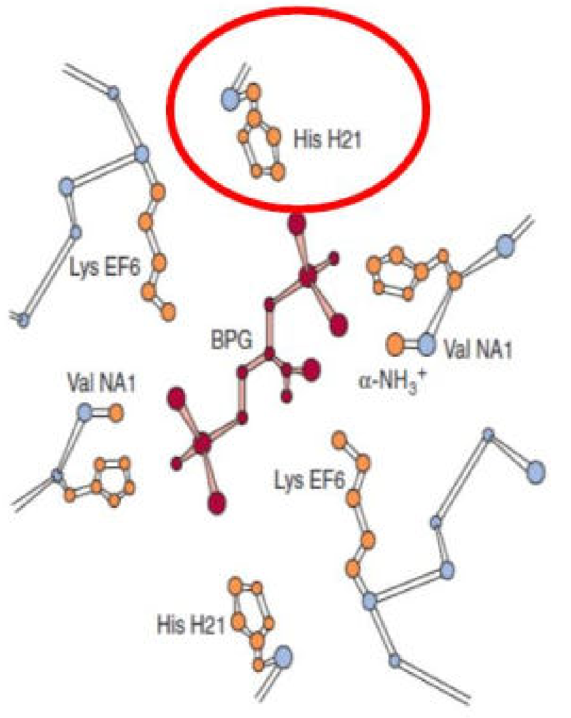
# ODC with different types of Hb and Mb




# HbF( Fetal Hemoglobin)

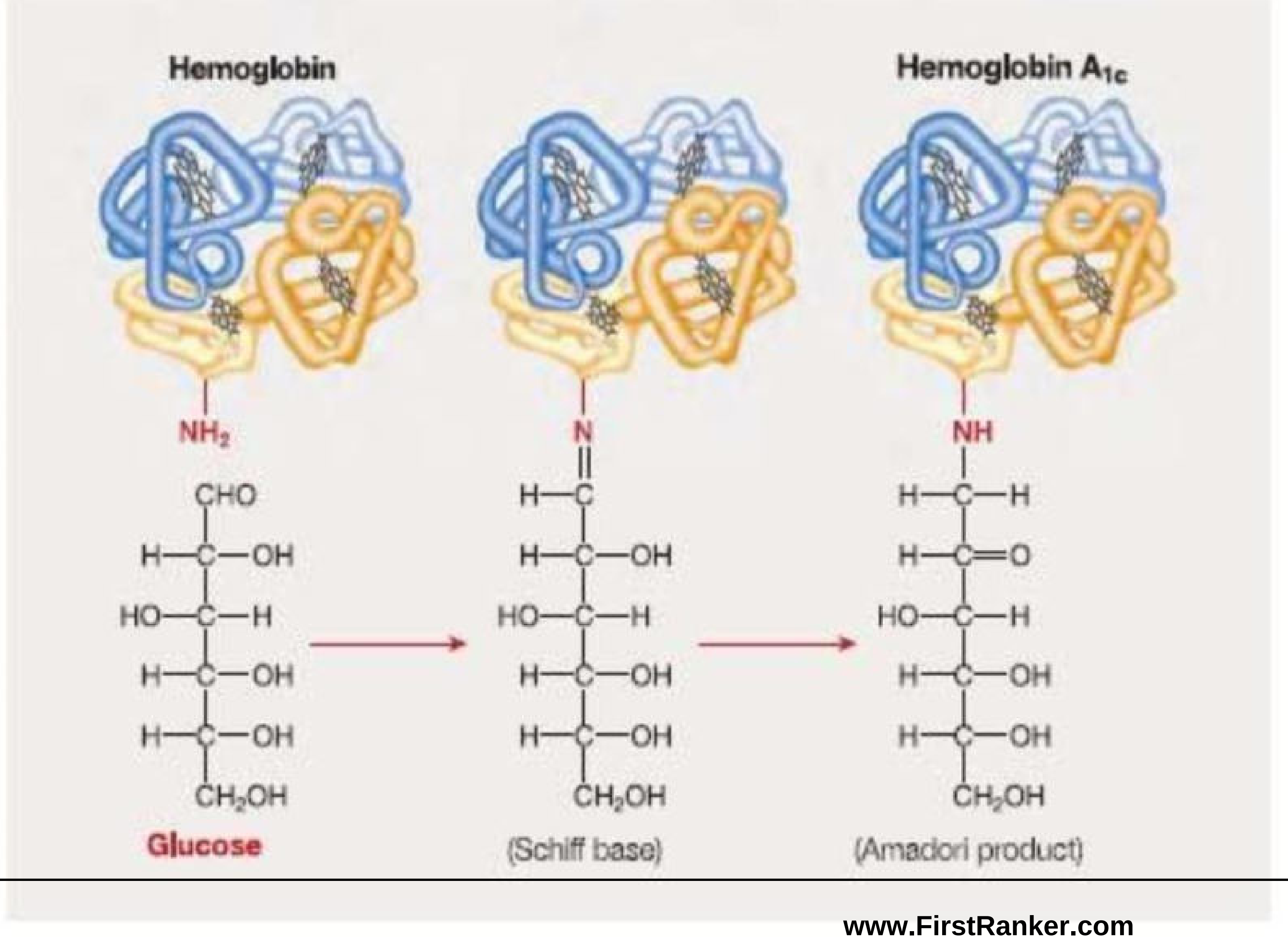


- **Binding of 2,3-BPG to HbF:** weak
- ? Importance




- Residue H21 of the γ subunit of HbF is Ser rather than His. Since Ser cannot form a salt bridge, BPG binds more weakly to HbF than to HbA.
- The higher oxygen affinity of HbF facilitates the transfer of oxygen from the maternal circulation across the placenta to the RBC of the fetus.

# HbA1c




**ADA Criteria for Diabetes Mellitus**
**HbA1c > 6.5%**

# HbA1c

- A1C reflects average glycemia over approximately 3 months and has strong predictive value for diabetes complications
- A1C testing should be performed routinely in all patients with diabetes—at initial assessment and as part of continuing care
- Factors affecting HbA1c measurement:

1. Glucose concentration
2. Red cell turnover
3. Analytical Variations

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# HbA1c

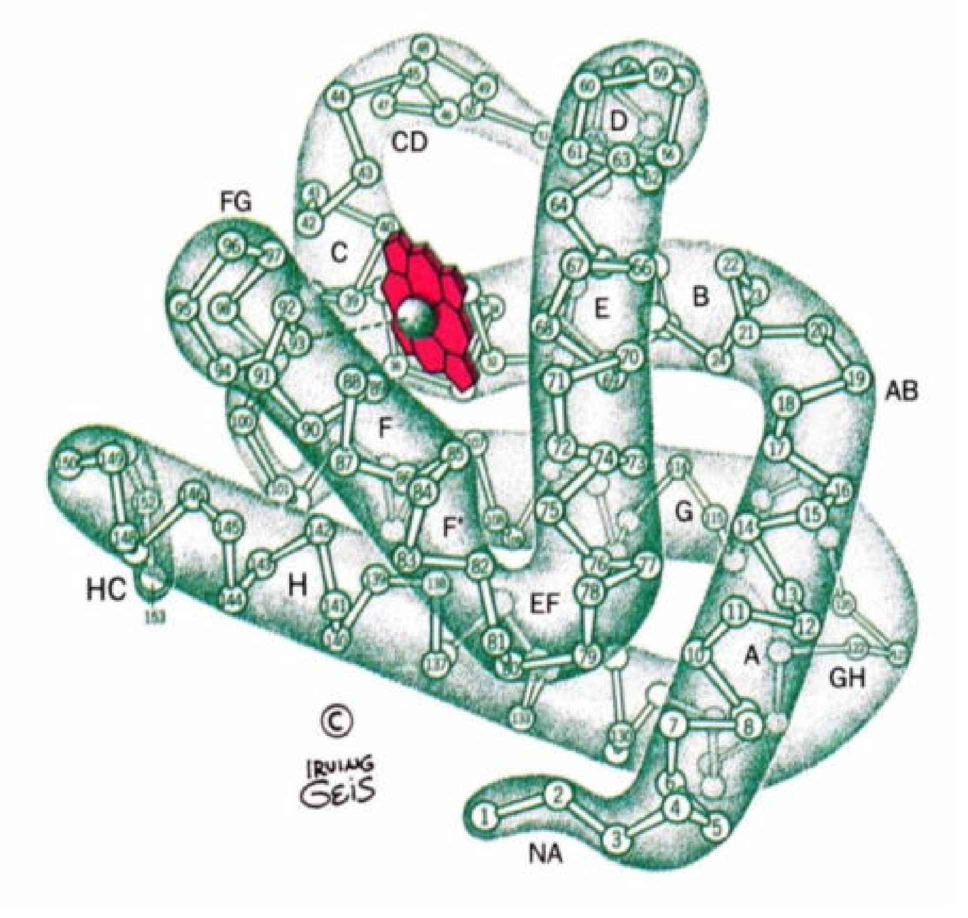
- Variations by Variable Red cell Turnover: hemolytic and other anemias, recent blood transfusion, use of drugs that stimulate erythropoesis, end-stage kidney disease, and pregnancy

Methods:

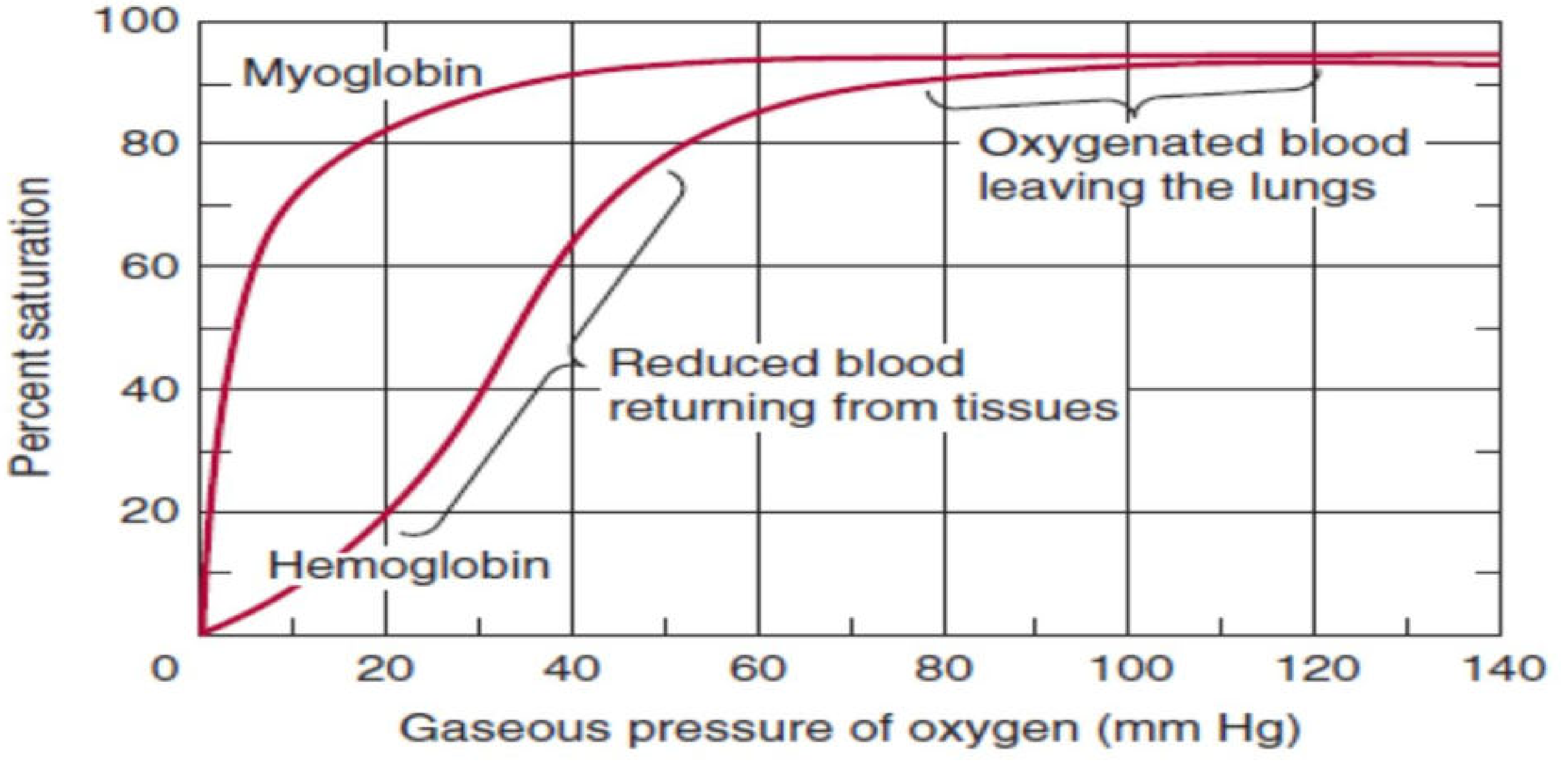
- Ion-exchange high-performance liquid chromatography (HPLC),
- Boronate affinity assay,
- Immunoagglutination √

Ref Range: 4-6.2%

# Myoglobin




# Why is myoglobin unsuitable as an $O_2$ transport protein but well suited for $O_2$ storage?



| S.No | Hemoglobin (Hb) | Myoglobin (Mb) |
|---|---|---|
| 1. | Hb is **Oxygen transport protein in RBCs of blood**. | Mb is **Oxygen storing protein in muscles**. |
| 2. | **Tetrameric** has four Heme and **binds with 4O2** | **Monomeric** has one Heme and **binds with 1 O2.** |
| 3. | **Oxygenated at Lungs** | **Oxygenated at Muscle Cell Cytosol.** |
| 4. | HbO2 unloads oxygen at tissues when pO2 is at 40 mmHg.<br><br>**P50 for HbA1 is 27 torr.** | MbO2 unloads oxygen at cell cytosol when pO2 is at 5 mmHg. to rapidly respiring cells<br><br>**P50 for Mb is 2 torr.** |
| 5. | **ODC is sigmoid shaped** | **ODC is hyperbolic shaped.** |
| 6. | **Hb has 574 amino acids.**<br>**Mol .wt-67,000 Daltons.** | **Mb has 153 amino acids.**<br>**Mol wt-17,200 Daltons.** |

- Thank You!