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.

Anna Paczek, Liliana Wawrzyńska, Ewa Mendek-Czajkowska, Jarosław Kober, Adam Torbicki. Acquired methemoglobinemia - case report. *Pneumonol Alergol Pol 2010;78(2):153-158*

- 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.

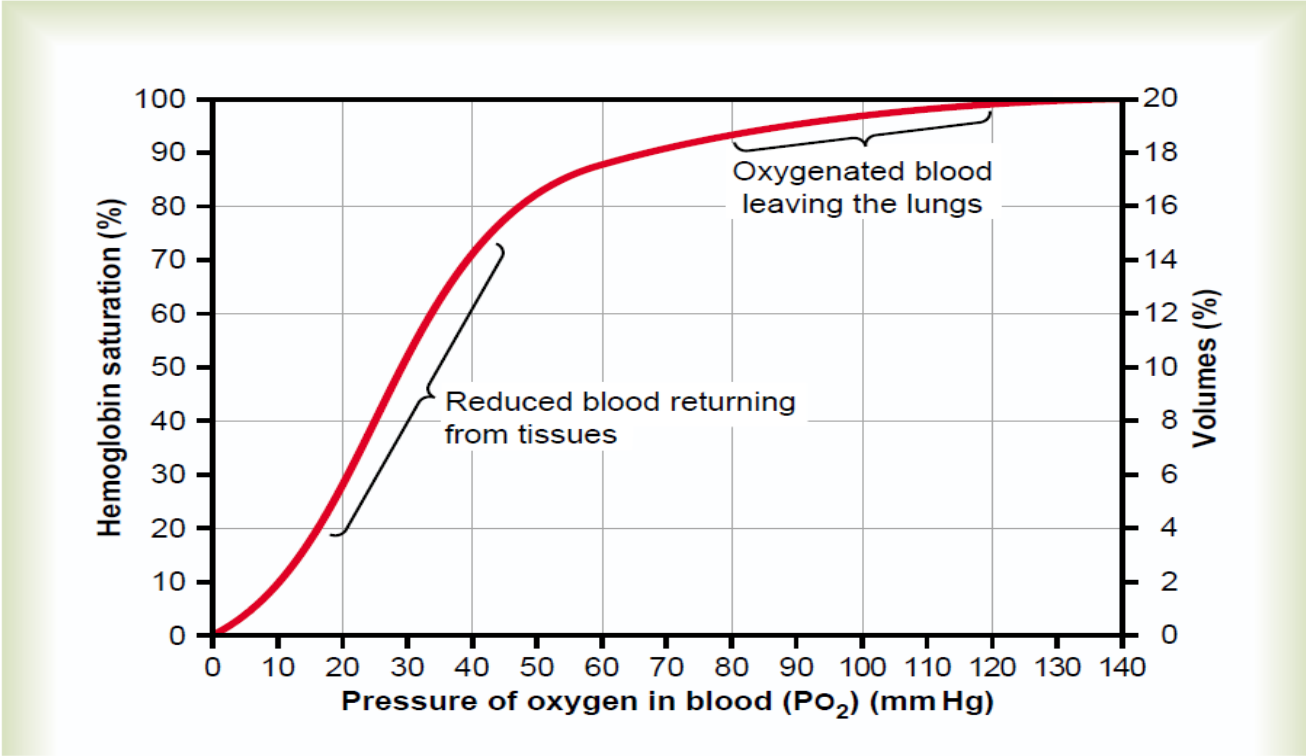
Relationship of O_2 saturation with pO_2

- 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.

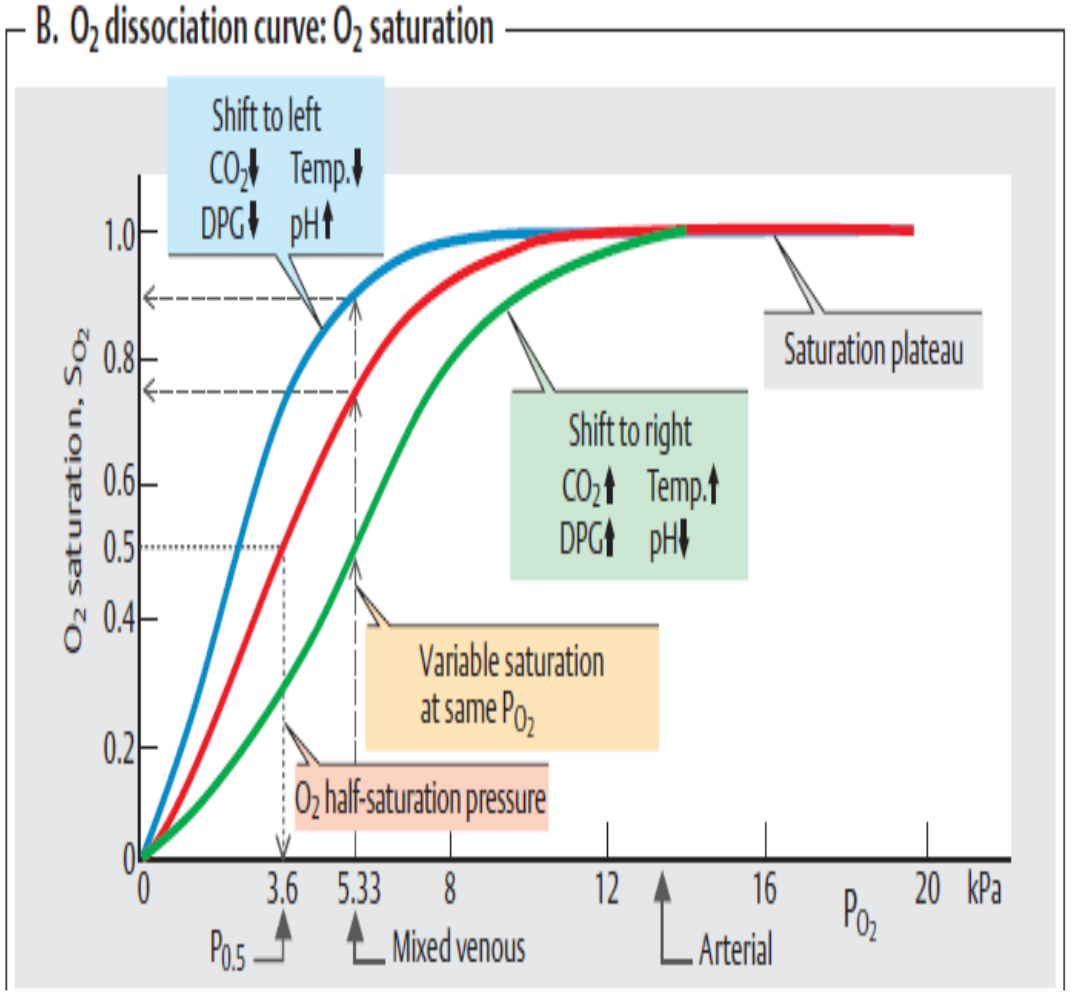
- The oxygen dissociation curve (ODC) describes this relationship graphically (sO_2 denotes Hb affinity)

Oxygen Hemoglobin Dissociation curve



Although pO_2only 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

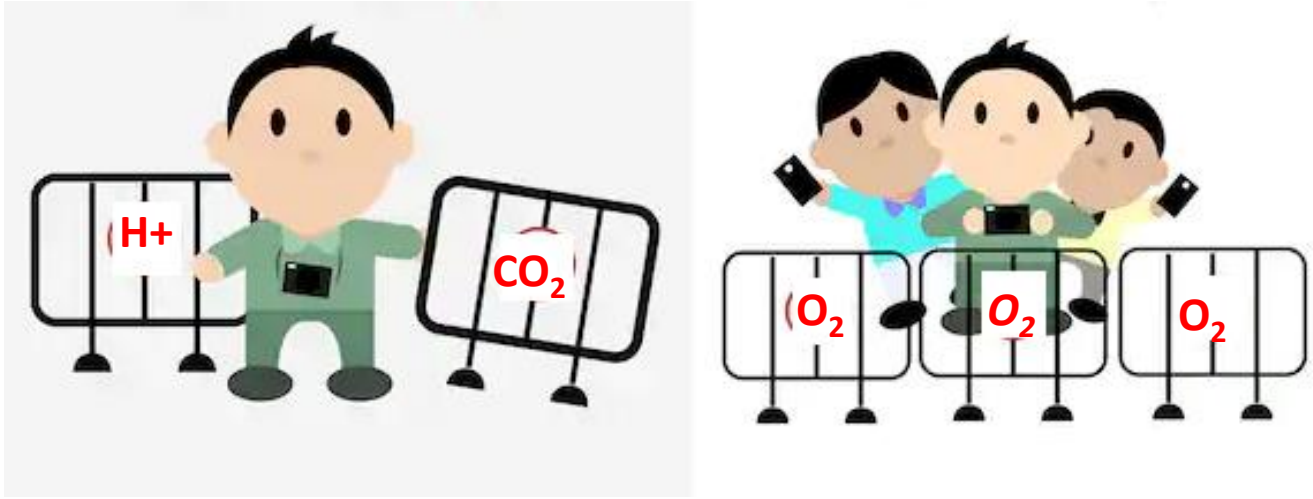


1. Carbon dioxide
2. Protons (↓pH)
3. Temperature
4. 2,3 BPG

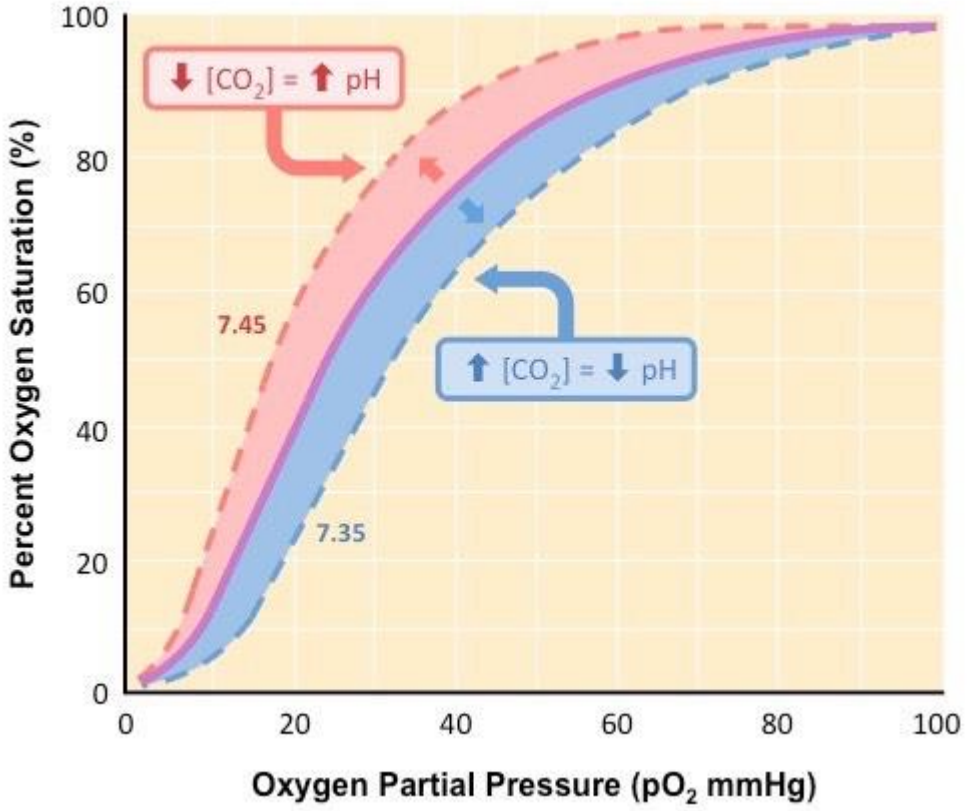
Increase in any of the above shifts curve to right and vice-versa

Bohr Effect

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

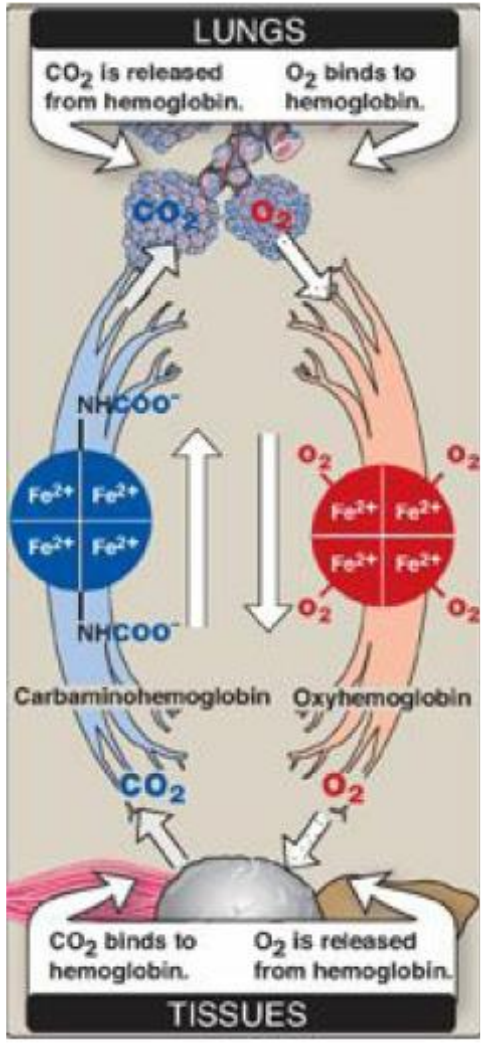


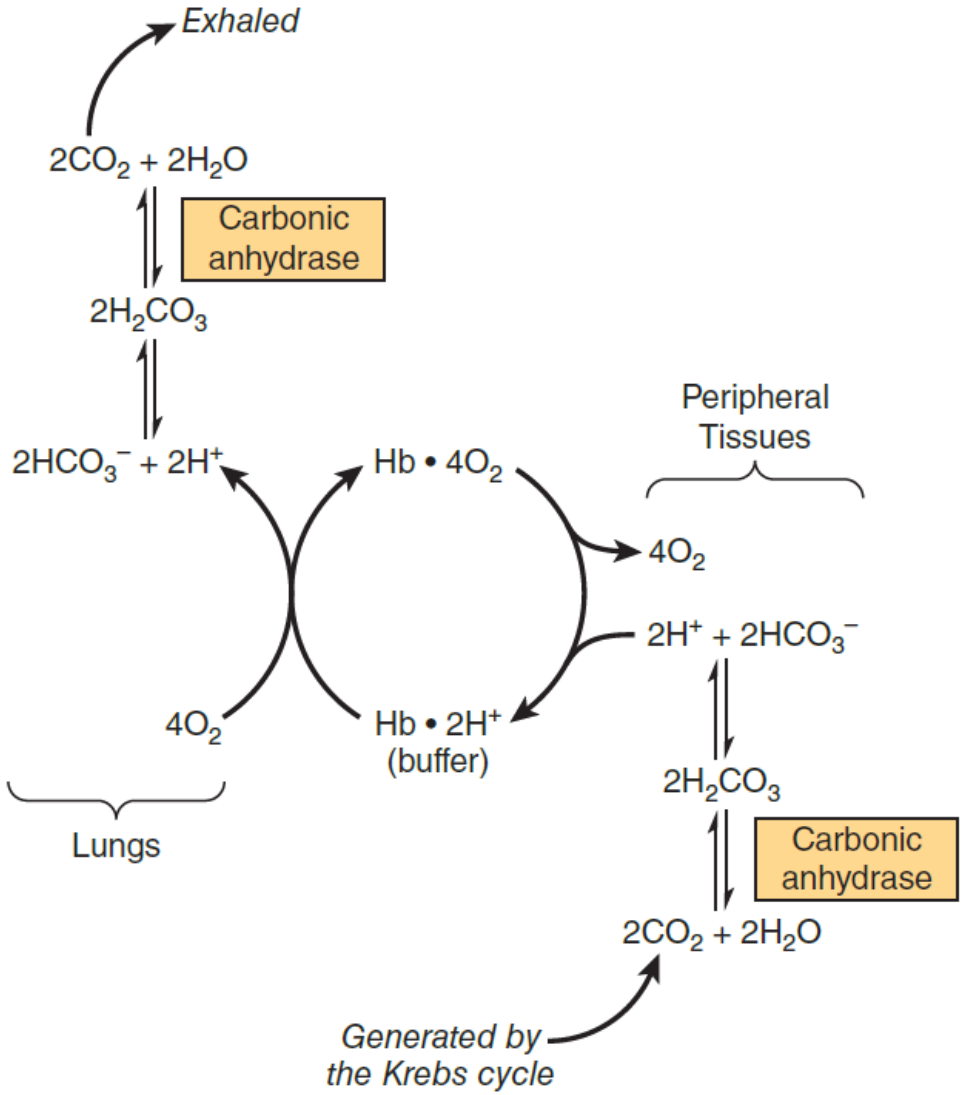
Importance of Bohr Effect



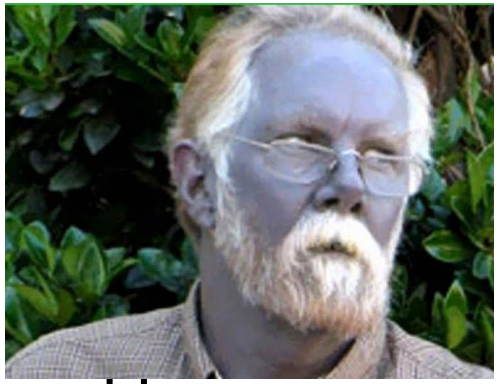
- Bohr Effect shifts ODC to right increasing oxygen delivery

Bohr Effect and Haldane Effect





Methemoglobinemia
(Discussion of case)



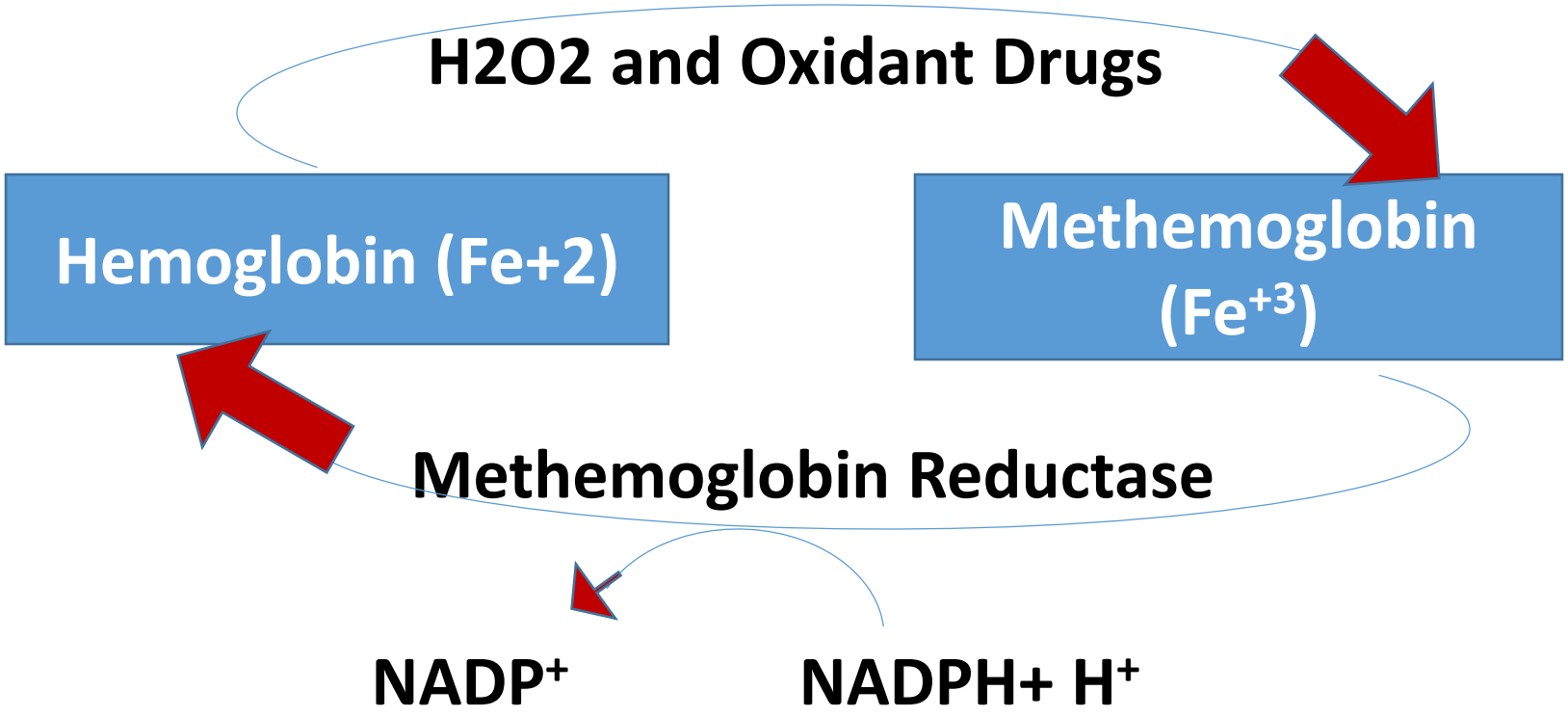
- Cyanosis(chocolate cyanosis) with structurally normal heart.
- Important D/D for an acquired or drug induced cause
- Hemoglobin can accept and transport oxygen only when the iron atom is in its ferrous form
- When haemoglobin becomes oxidized, the iron atom is converted to the ferric state (Fe3+), 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 [α^{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

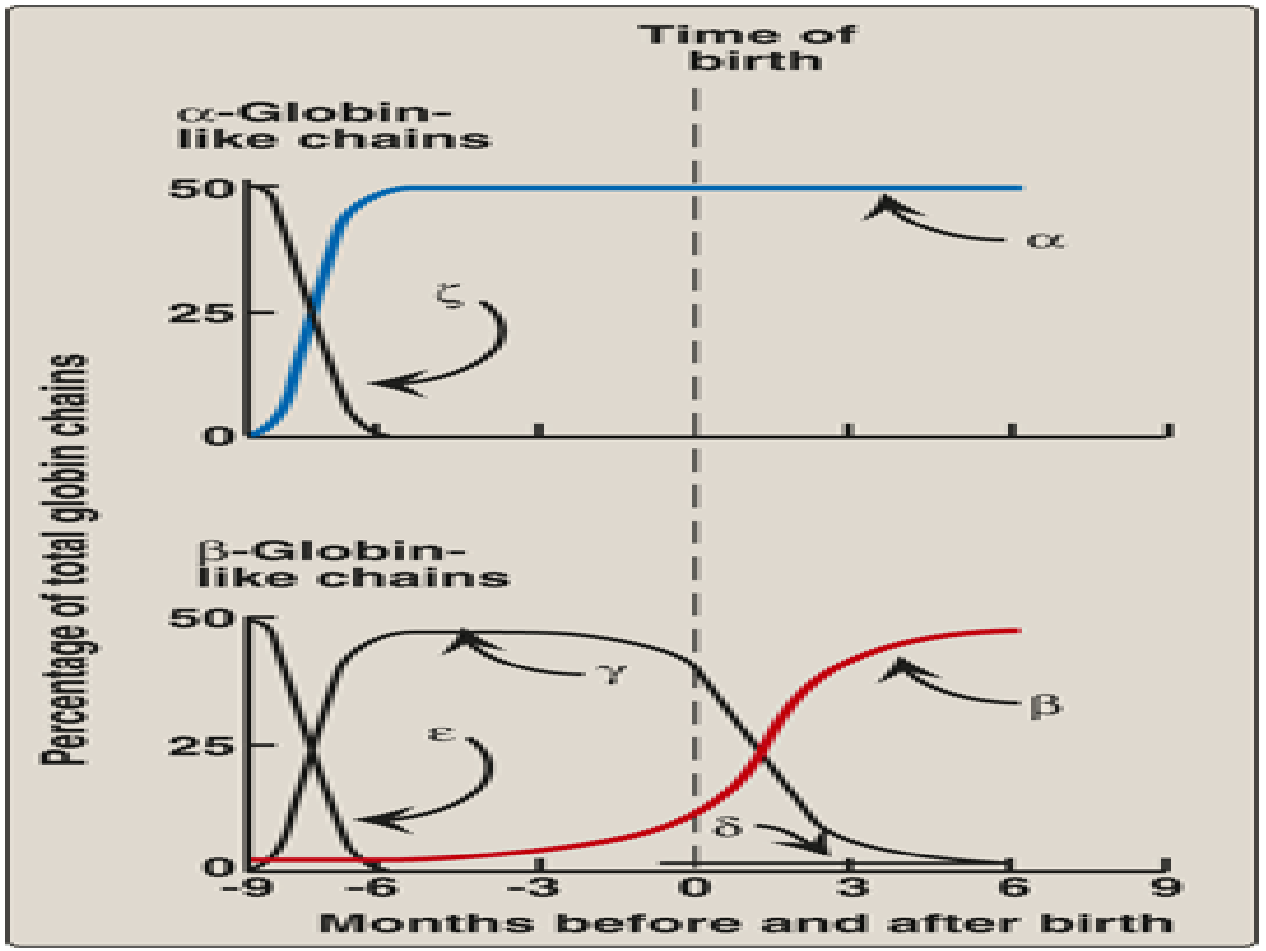
- Intravenous (IV) methylene blue is the first-line antidotal agent.
- Exchange transfusion and hyperbaric oxygen treatment are second-line options for patients with severe methemoglobinemia

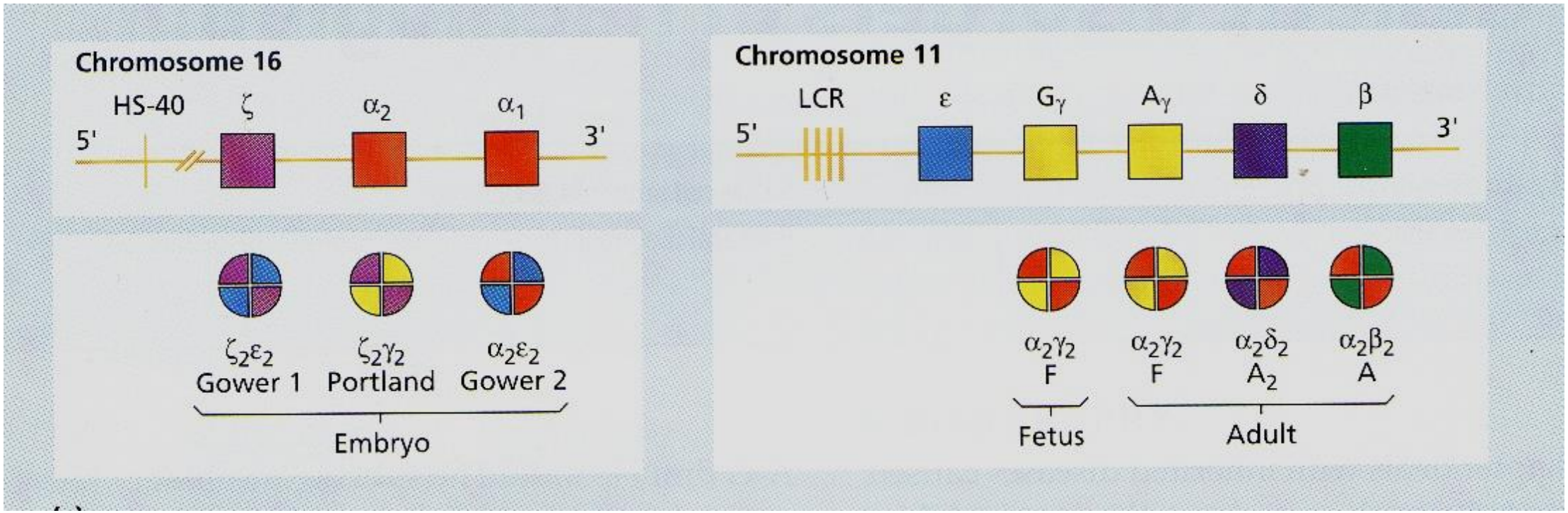
Therapeutic Induction of Methemoglobin Formation

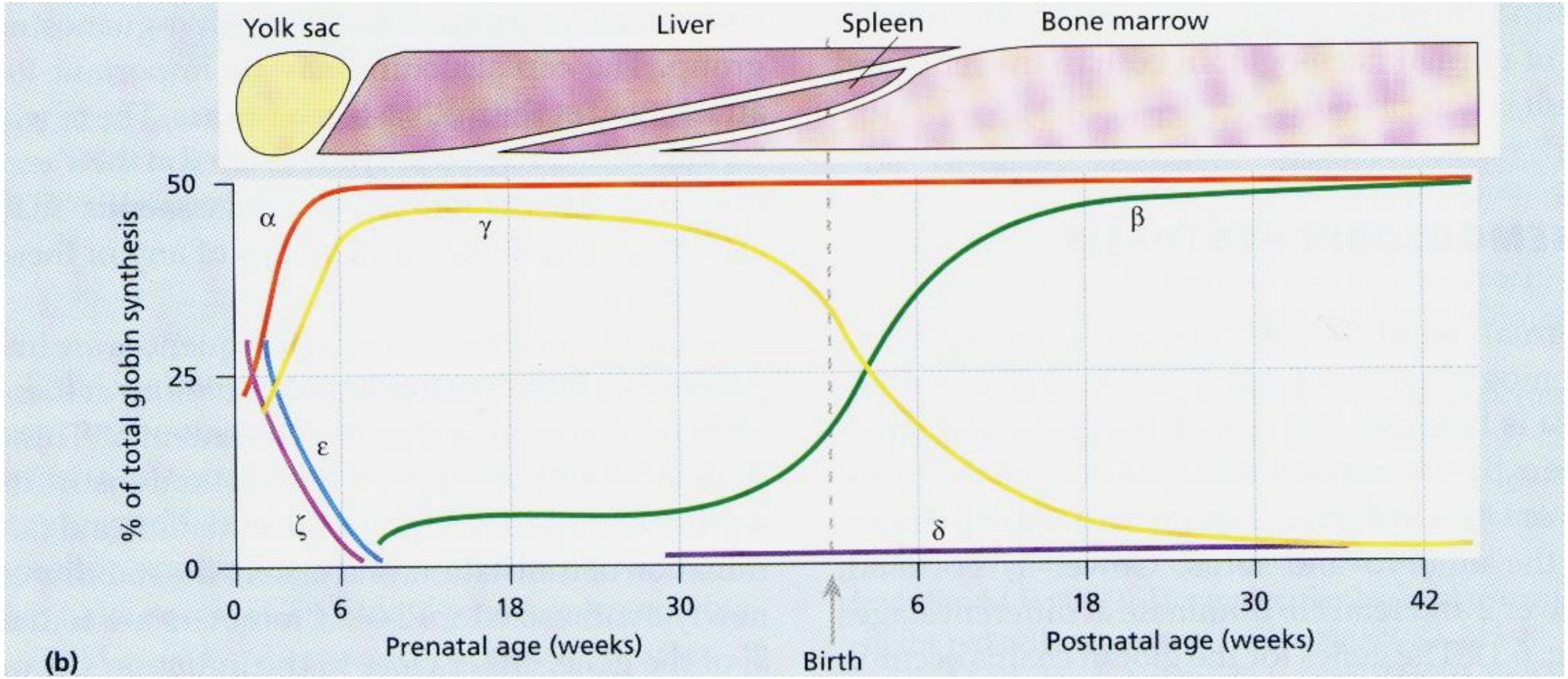
- Cyanide competes with cytochrome oxidase for Fe+++ of methemoglobin to form cyanmethemoglobin which is eliminated
- Thereby, the activity of inhibited cytochrome oxidase is restored.
- Agents used as antidote: sodium nitrite, amyl nitrite, 4-dimethylaminophenol

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%

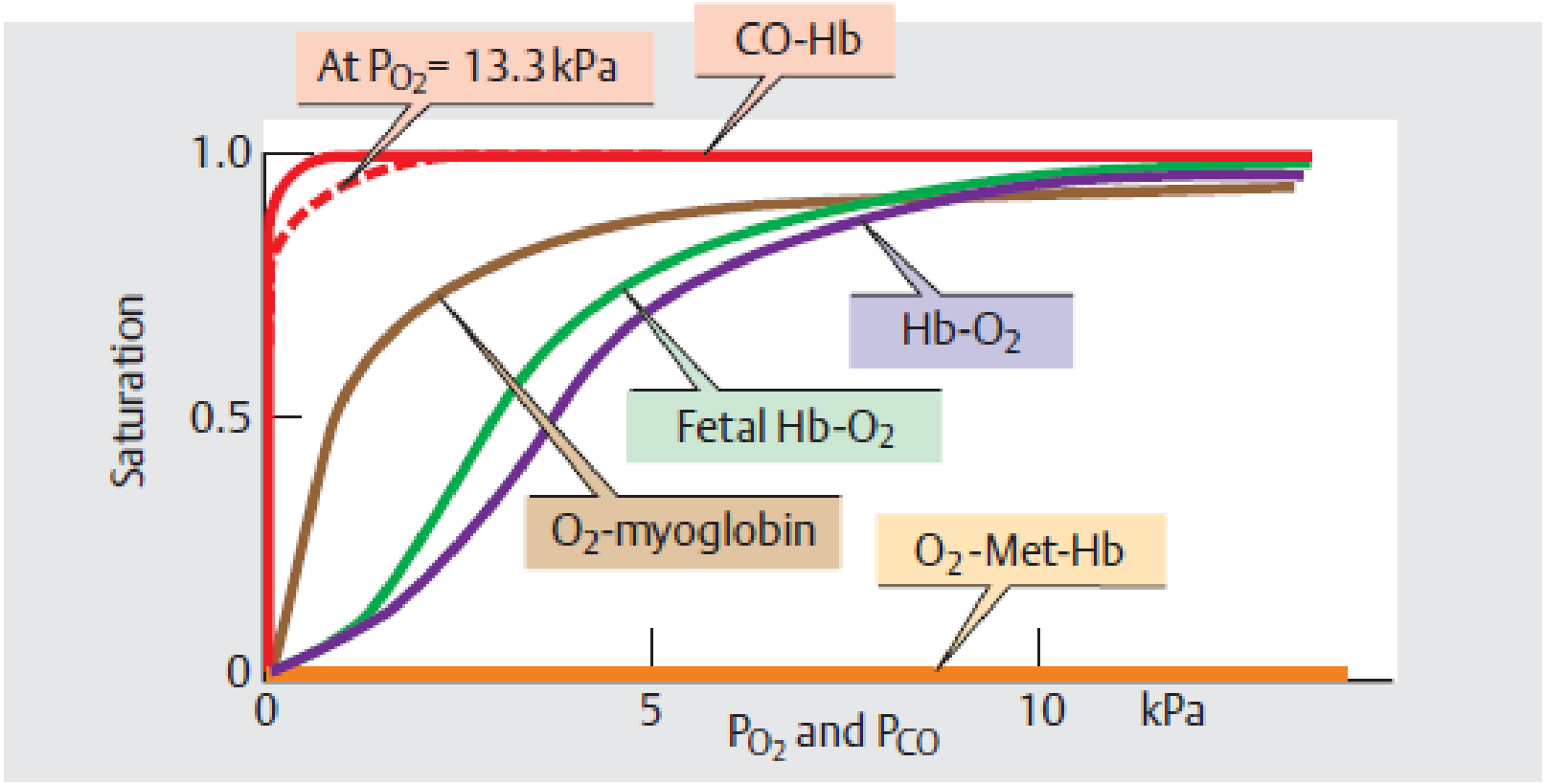



Chromosome 16
HS-40
ζ
α2
α1
5'
3'
ζ2ε2
Gower 1
ζ2γ2
Portland
α2ε2
Gower 2
Embryo
Chromosome 11
LCR
ε
Gγ
Aγ
δ
β
5'
3'
α2γ2
F
α2γ2
F
α2δ2
A2
α2β2
A
Fetus
Adult

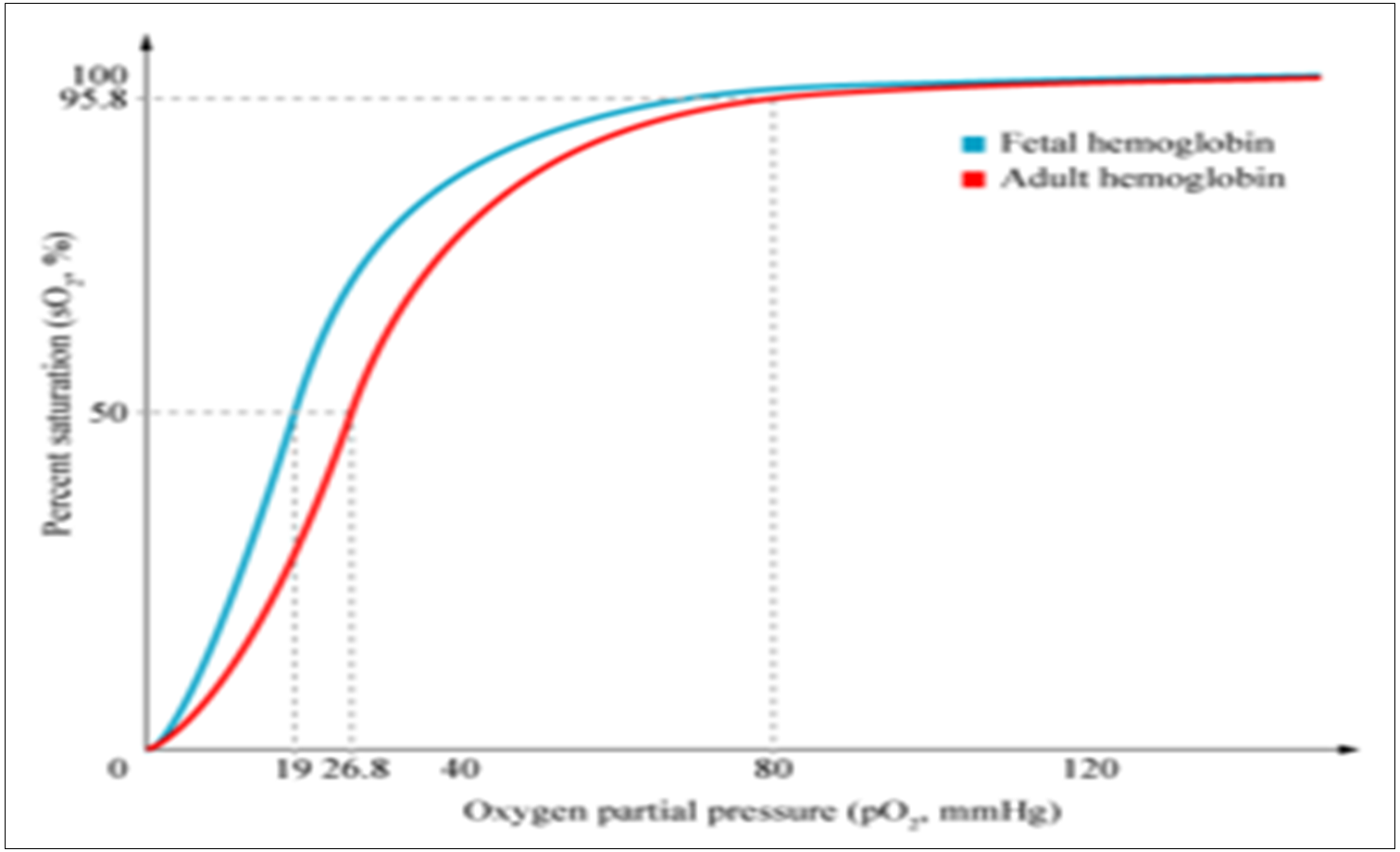

Yolk sac
Liver
Spleen
Bone marrow
50
25
0
% of total globin synthesis
α
γ
β
ε
ζ
δ
0
6
18
30
6
18
30
42
Prenatal age (weeks)
Birth
Postnatal age (weeks)
(b)

ODC with different types of Hb and Mb

C. O_2 and carbon monoxide (CO) dissociation curves

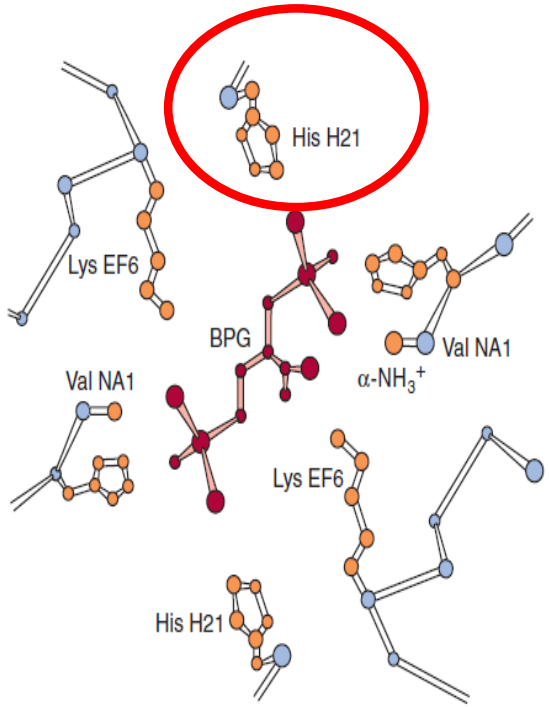


HbF(Fetal Hemoglobin)



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

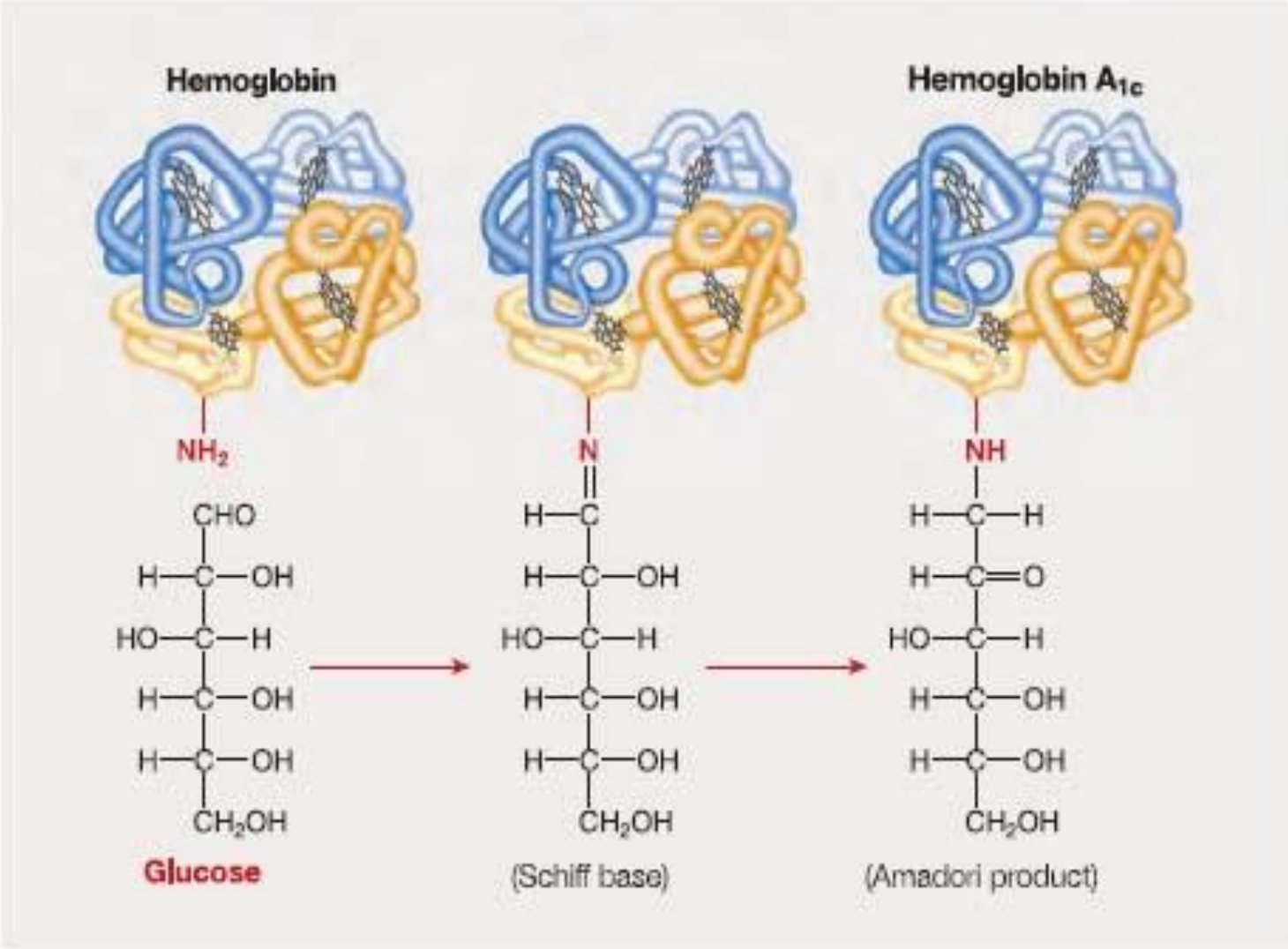
- ? Importance



- 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

Diabetes Care 2018;41(Suppl. 1):S55–S64

HbA1c

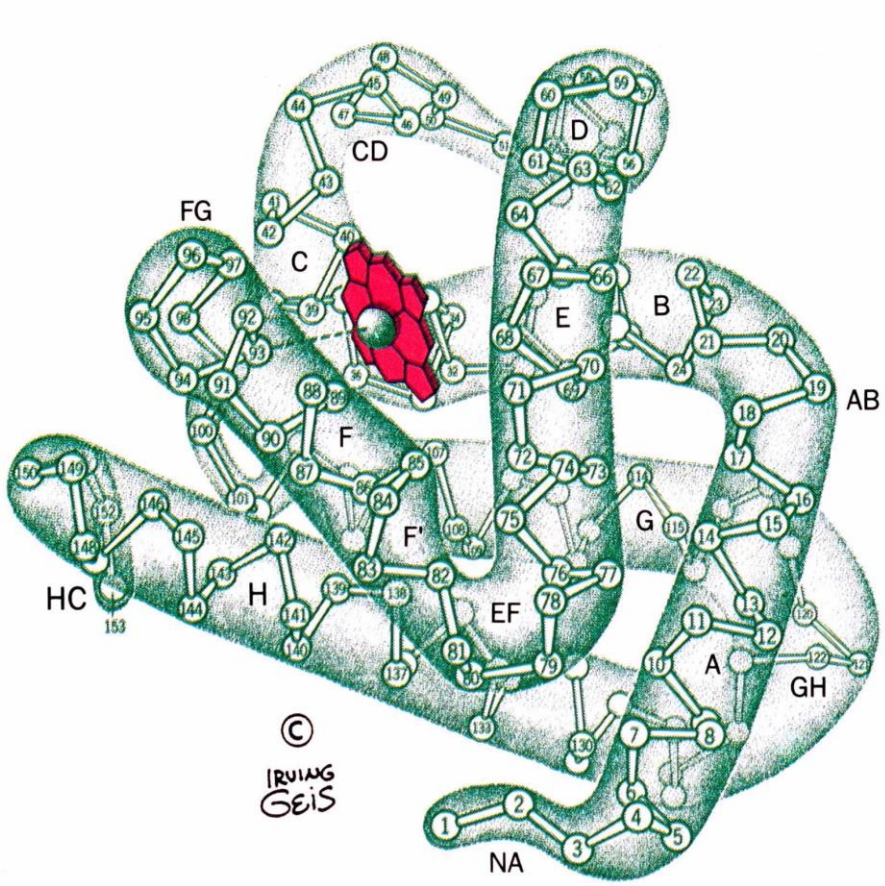
- Variations by Variable Red cell Turnover: hemolytic and other anemias, recent blood transfusion, use of drugs that stimulate erythropoesis, end-stage kidney disease, and pregnancy

Methods:

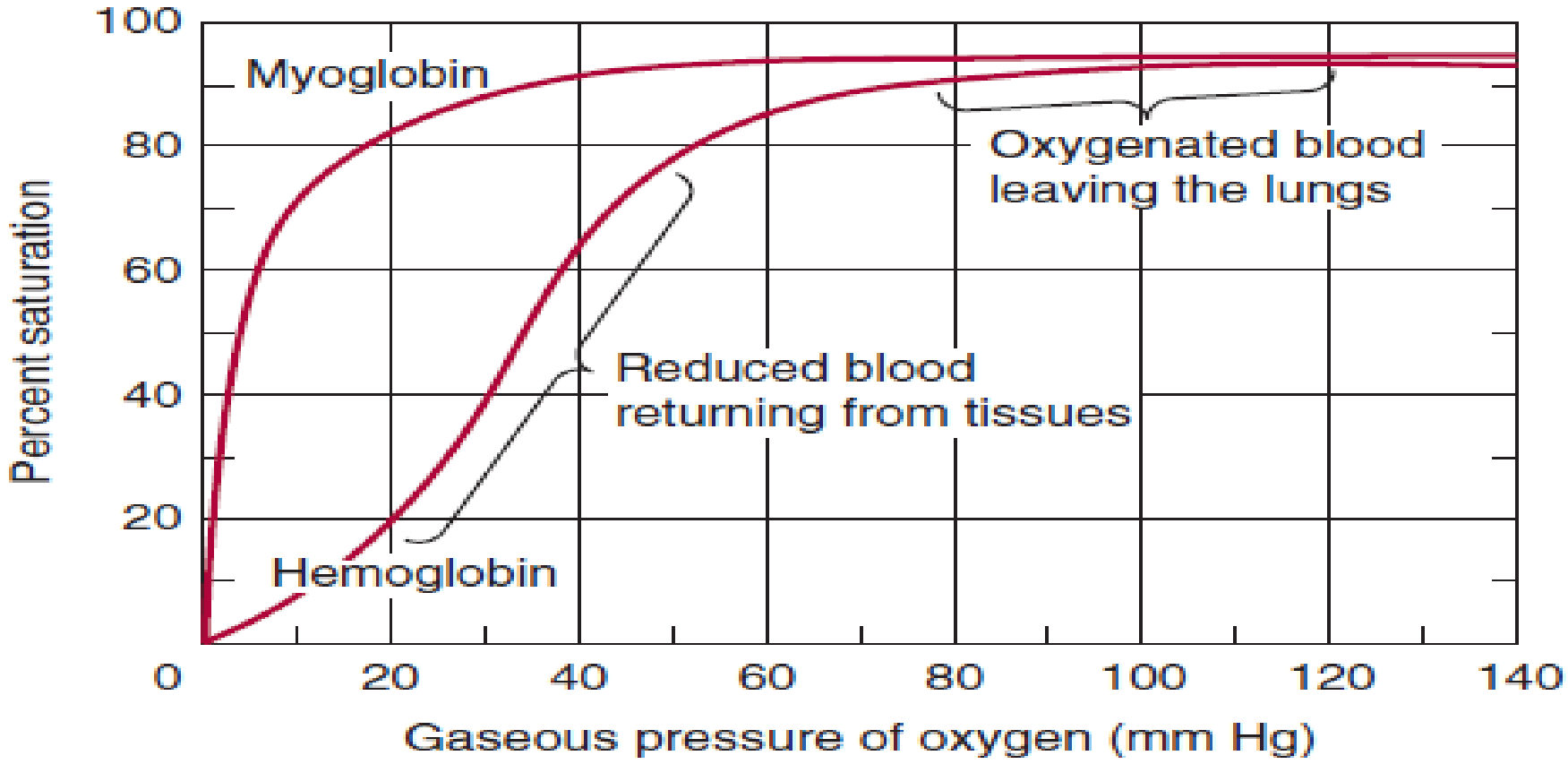
- Ion-exchange high-performance liquid chromatography (HPLC),
- Boronate affinity assay,
- Immunoagglutination √

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. **P50 for HbA1 is 27 torr.**	MbO2 unloads oxygen at cell cytosol when pO2 is at 5 mmHg. to rapidly respiring cells **P50 for Mb is 2 torr.**
5.	**ODC is sigmoid shaped**	**ODC is hyperbolic shaped.**
6.	**Hb has 574 amino acids.** **Mol .wt-67,000 Daltons.**	**Mb has 153 amino acids.** **Mol wt-17,200 Daltons.**

- Thank You!