Hemoglobin

**Major roles : overview**

<table>
<thead>
<tr>
<th>Requirements:</th>
</tr>
</thead>
<tbody>
<tr>
<td>Transport of O₂ from the lungs to peripheral tissue</td>
</tr>
<tr>
<td>'Just-in-time delivery' of O₂</td>
</tr>
<tr>
<td>Transport of CO₂ and H⁺ from peripheral tissue to the lungs</td>
</tr>
<tr>
<td>Plasma buffer</td>
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</tbody>
</table>

**Factoids:**

- Each red blood cell (RBC) contains about 280 million hemoglobin (Hb) molecules.
- The average adult contains about 5 billion RBCs/mL of blood.
- The average adult has about 5 liters of blood.
- Thus, the average adult has about 790 grams (or 1.74 pounds) of Hb.
- Assuming the blood re-circulation time at rest is about 1 minute, the net transport of oxygen by Hb is about 100mL/minute.
- The average life time of a RBC is about 120 days.
- ~20 million RBCs are degraded and synthetised per second (~500 trillion Hbs/sec)
- ~97% of dry mass of a RBC is Hb
- ~35% of wet mass of a RBC is Hb
- Hb enhances the solubility of O₂ in blood seventy fold compared to water.
- The degradation products are AAs (recycled), Fe[II] (recycled) and, bilirubin & CO.
- Elevated levels of plasma bilirubin can be caused by pre-, intra- and post-hepatic problems and leads to jaundice.
- Hb deficiency is called 'hypoemia', whereas low oxygen conditions is called 'hypoxia'.
- Low [Hb] can be due to decreased blood volume, poor nutrition, bone marrow, liver and kidney disfunction and chemotherapy
- High [Hb] can be due to exposure to high altitudes, smoking, dehydration, congenital heart diseases, pulmonary fibrosis, elevated erythropoetin (EPO), and some tumors.
- The [Hb] can be assessed by a 'hemocrit' value.
- A pulse oximeter is used to determine the relative the ratio of deoxy to oxy Hb using visible and far infra-red spectrometry since the spectra have significant differences at these wavelengths.
- Hb can bind CO (see later), cyanide ion, NO₂⁻, and H₂S resulting in decreased O₂ transport

**Structural features of hemoproteins**
**HEME:**
The porphyrin moiety of heme is synthetised from glycine and succinoyl CoA to initially form δ-aminolevulinate (ALA) that dimerises into porphobilinogen that contains a pyrrole ring (Fig. 1). Extensive processing of four of the latter compounds and incorporation of the iron cation leads to heme synthesis.

![Heme structure](image)

**Figure 1. Heme structure.**
Heme units in hemoglobins can bind other small chemical species such as CO, cyanide ion, NO₂⁻, and H₂S as Fe[II] and NO and N₂O but the latter two causes oxidation to Fe[III].

<table>
<thead>
<tr>
<th>Monomeric hemoproteins</th>
<th>Tetrameric hemoproteins</th>
</tr>
</thead>
<tbody>
<tr>
<td>Myoglobin (storage of O₂ in muscle) with 156 AA residues</td>
<td>HbA: two α (141AA residues) and 2 β subunits (146 AA residues)</td>
</tr>
<tr>
<td>Cytochromes (ETS in mitochondria and P₄₅₀ system in the liver)</td>
<td>HbD, HbF, HbS, etc (see latter)</td>
</tr>
<tr>
<td>Redox enzymes (peroxidase, catalase, etc)</td>
<td></td>
</tr>
<tr>
<td>Chlorophylls (with central Mg[II])</td>
<td></td>
</tr>
</tbody>
</table>
Besides Mb and Hb, several other proteins contain heme units. Each subunit contains one heme that can bind one molecule. The role of the heme in Hb and Mb is to reversibly bind oxygen i.e. 'oxygenation' or binding of the O₂ to the Fe[II]) without becoming 'oxidised' to Fe[III]. If the latter occurs, MetHb and MetMb are formed that cannot bind oxygen. In the other hemoproteins, mentioned in the left column of the table above, the iron cation is cycled repeatedly between the Fe[II] and Fe[III] oxidation states as part of their role in electron transfer.

**GLOBIN:**

The polypeptide chain provides a binding site for the heme in a hydrophobic interior pocket with the non-polar groups (methyls and vinyls, (see above, Fig. 1) on the porphyrin rings in this pocket and the two propanoate groups on the exterior (see above, Fig. 4, the red balls represent the O atoms in the -CH₂CH₂CO₂⁻ groups). In addition to these non-covalent interactions, the heme unit is bound covalently through the 'F8' histidine N-Fe bond. (see Figs. 2, 3 & 4)

Another role of the polypeptide chains is to prevent close approach of two Fe[II] ions that is required to prevent the formation of an oxygenated 'sandwich' intermediate (Fe-O=O-Fe) that is a pre-requisite for the oxidation of Fe[II] to Fe[III].

In addition, the globin affects relative affinities for hemoglobin to CO. The affinity of CO to heme (alone) is 2500 times that of the affinity to O₂ but this relative affinity is reduced to 200:1 for hemoglobin. It is thought that steric hindrance by amino acid residues near the distal histidine in the globin chains interferes with the end-on (or linear) alignment of CO molecules compared to the 120° binding by oxygen molecules (see Figs. 2 & 4).

Carboxyhemoglobin is very bright red and causes the skin of CO affected individuals to become very pink. 0.02% CO in air causes headaches and nausea, 0.1% will lead to unconsciousness. Heavy smokers may have 20% of available oxygen sites on hemoglobin blocked by CO. Interestingly, CO is
one of the degradation products in vivo of hemoglobin to bilirubin.

The subunits are mainly (75%) α helical, with no β sheets nor SS bonds. All but the two histidines (F8 and E7) interior AA residues are non-polar and all the exterior AA residues are virtually all polar and/or charged. The helices in Hb and Mb are labeled from the N terminus A through H and each AA residue in a helix given a specific number. In addition, the AA residues can also numbered by the primary sequence, again from the N terminus (Fig 5).

Figure 5. Myoglobin with labeled helices.

The sub-units in Hb are attracted to each other mainly by an extensive (but very specific) network of salt bridges and hydrogen bonds that play an important role in the cooperative nature of Hb.

HEMOGLOBIN:

The polypeptide chains of Hb and Mb fold into very close packed globular shapes (4.5 x 3.5 x 2.5 nm diameters) (Fig. 7). The UV and visible spectra of the crystalline forms of these proteins are very similar to that of the proteins dissolved in aqueous buffer @ pH 7.4, so it assumed that the 3D structures obtained by X-ray crystallographic data is very close to that found in red blood cells.

Figure 7. Cartoon drawing of tetrameric hemoglobin. Hemes are shown in red.
The chemical properties of Hb and Mb have been studied extensively since their behavior has served as a model for many proteins especially enzymes, i.e. the binding curves for the single polypeptide chain Mb resemble classic Michaelis-Menten enzyme kinetics whereas those of the tetrameric Hb are analogous to the observed kinetics of multiple sub-unit and allosteric cooperative enzymes. Hb (and related developmental and mutated iso-forms) illustrate succinctly the structure-activity-biological function relationship in that a significant change in structure (i.e. HbS and sickle cell anemia) changes the chemistry of oxygen binding and thus affects the ability of Hb to adequately reversibly bind, release and transport oxygen.

Further, many relatively common 'genetic' disease states (i.e. sickle cell anemia and the thalassemias) have been shown to be due to mutations in the primary structures of Hbs. Other diseases that are known to be caused by aberrations in hemoglobin metabolism include the porphyrias (defective enzymes in the synthesis of the porphyrin rings) and jaundice (since bilirubin is the breakdown product of heme).

**OXYGEN BINDING CURVES:**

Mb exhibits a hyperbolic relationship whereas Hb shows a sigmoidal relationship. The latter is indicative of the cooperative binding found in only Hb. The relative affinity to O₂ (as quantitated by the P₅₀ value i.e. the O₂ concentration, usually estimated as the partial pressure of oxygen or pO₂, when 50% of the Hb subunits have bound oxygens) of Mb is much higher that that of Hb. Thus given the ranges of pO₂ in resting muscle is 20Torr, working muscle of 40Torr and in the lungs as 100-120Torr. Mb is fully saturated under most conditions (except severe hypoxia as in vigorous exercise) and the most responsive part of the curve of oxygen binding is found in the normal pO₂ for muscles. See Figs. 7 & 8)

![Oxygen Binding Curves](image)

Figure 7; Hb binding curve P₅₀ ~24 Torr

![Oxygen Binding Curves](image)

Figure 8: Binding curves for Hb and Mb.

The conformation of deoxy Hb is called the "T" (or tense state; the α2β2 subunits are more tightly bound than in the oxyHb "R" (or relaxed) state. There is a change in shape and size between the T and R states (Fig. 9). The allosteric effectors bind the T form in the manner that causes:

(a) enhanced stability it and,
(b) compaction.

Hb acts like an αβ dimer so that when O₂ binds, one a αβ in the deoxy form moves away and around the other the αβ unit so the complex 'expands' and changes conformation.
The 'chemomechanical' model of the reversible binding of oxygen to Hb (*as per Max Perutz (1959) (Nobel Prize 1962 with A. Kendrew*)

In Hb, the heme ring is positioned between the proximal (F8) and the distal (E7) histidine residues. In deoxy Hb, the Fe[II] has five ligands (i.e. to the four porphyrin and one to a proximal F8 histidine nitrogen) whereas in oxyHb, the Fe[II] has six ligands; the sixth is the bound oxygen near the distal E7 His, E11 Val and CD1 Phe (see Fig. 10)

The steric requirements for these two different states of Fe[II] are different in that the Fe[II] cation in oxyHb is slightly smaller that in the deoxy form (partially due to electronic differences in d orbital hybridisation for 5 and 6 ligands).

Thus, in deoxy heme, the iron cation resides about 30pm above the plane of the ring towards the nitrogen atom in the F8 proximal histidine, and when oxygen binds, the ferrous cation can 'drop' down almost to the center of the ring (actually 10pm above). (Figs. 11 & 12)

The result of this movement, since the Fe[II] is covalently bound to the N in the proximal F8 histidine,
is that this His residue also moves towards the ring. This F8 histidine is bound the F helix and so there is motion of this F helix towards the heme plane as well. This subsequently causes changes in the relative conformation F, G and H helices of this sub-unit. The result of these steric changes is to cause realignment of the salt bridges and hydrogen bonds between this β unit and its α neighbor. The resultant conformation in this α unit can more easily bind oxygen than the first sub-unit due a change in the positions of two AA residues that are near the O2 binding site. The cycle then repeats. (Figs 11 & 12)

**To summarise:**

Mechanism of **BINDING** and cooperability **IN THE LUNGS**
1. O2 binds between Fe[II] and distal E7 His in a β unit
2. This pulls Fe[II] almost into plane of heme
3. This pulls proximal F8 His 'down' (towards heme)
4. This pulls F helix 'down' (towards heme)
5. This causes a conformational change in the β subunit in the F, G and H helices i.e. a F helix Tyr rotates from between the F & G helices and into the αβ space.
6. This causes a rearrangement of the salt bridges and H-bonds along the αβ interface (Fig 11 & 13).
7. This causes a conformational change in an adjacent α sub unit
8. This causes a movement of the Val [E11] and Phe [CD1] near the distal His i.e. the O2 binding site (see Fig. 11)
9. This makes it slightly easier for the second O2 to bind...
10.... repeats 1-9....and on and on to saturation

Figure 11: Movement of (a) Fe[II] from deoxy (upper) to oxy Hb (lower) and (b) of the Tyr HG3 from between the G & H helices to αβ interface.

Figure 12. Movement of Fe[II] and F helix from deoxy (blue) conformation to oxyHb (red)
The tetrameric hemoglobin thus exhibits 'cooperability' in that the binding of one O$_2$ to a subunit affects/increases the binding affinity of the other subunits. This is illustrated by the sigmoidal Hb binding curve (Fig. 8).

THE RELEVANT EQUILIBRIA:

1. Oxy Hb ('R') $\text{HbO}_2$ $\rightleftharpoons$ $\text{HbO}_2^-$ + $\text{H}^+$ (pKa = 6.6 so $\text{HbO}_2^-$ is dominant form @ pH 7.4)
2. Deoxy Hb ('T') $\text{Hb}$ $\rightleftharpoons$ $\text{Hb}^-$ + $\text{H}^+$ (pKa = 8.2 so $\text{Hb}$ is dominant form @ pH 7.4)
3. $\text{Hb}.\text{CO}_2.\text{BPG}.\text{Cl}.\text{NO}$ + $\text{O}_2$ $\rightleftharpoons$ $\text{HbO}_2^-$ + $\text{H}^+$ + $\text{CO}_2$ + $\text{BPG}$ + $\text{Cl}^-$ + NO
4. $\text{CO}_2$(g) $\rightleftharpoons$ $\text{CO}_2$(aq) + $\text{H}_2\text{O}$ $\rightleftharpoons$ $\text{H}_2\text{CO}_3$ $\rightleftharpoons$ $\text{H}^+$ + $\text{HCO}_3^-$ (overall pKa = 6.2)

From equations #1 & 2, the difference in structure between the oxy and deoxy Hb have the effect of altering the pKa of the two states and since the two pKa lie on either side of the physiological pH (7.4), so that the R form the conjugate base $\text{HbO}_2^-$ is the most stable form whereas in the T form the conjugate acid $\text{Hb}$ is the more stable species. This difference is the major influence of how Hb can reversibly bind and release oxygen in vivo.

Equation #3, illustrates the effect of binding and releasing the allosteric effectors. All these chemical species stabilise the T form. Thus increase in their concentrations in vivo will result in their binding to the T form, using Le Chatelier's principle, will cause a shift of the equilibrium to the right resulting in oxygen release (as to peripheral tissues). By contrast, decreasing the concentrations of the allosteric effectors, will cause oxygen binding(as in the lungs).

Equation #4, illustrates the equilibrium between gaseous CO$_2$ the dissociation of carbonic acid to give bicarbonate and a proton.

MOLECULAR MECHANISM OF OXYGEN RELEASE/BINDING: (Fig 14)

1. A respiring tissue releases CO$_2$ that causes (EQ#4) production of protons and bicarbonate in plasma.
2. These protons $\text{H}^+$ enter the RBC and bind to $\text{HbO}_2^-$ to give the unstable $\text{Hb}$. This releases molecular oxygen (EQ#1).
3. O$_2$ is transferred across the RBC and peripheral tissue membranes for use in the ETS to produce ATP.
4. Some of the released CO$_2$ can bind $\text{Hb}$ to form $\text{Hb}.\text{CO}_2$ by binding to N terminal amino

Figure 13: Changes in the non covalent interactions (salt bridges and hydrogen bonds) along the $\alpha\beta$ interface from the T to R due to oxygen binding.
groups and some Lys groups (HbNH$_3^+$ + CO$_2$ $\rightleftharpoons$ HbNHCO$_2^-$)

5. The RBCs now contain increased H.Hb, bicarbonate, HbH. CO$_2$ and some dissolved CO$_2$ (the latter represent the three ways CO$_2$ equivalents are transported to the lungs for exhalation)

6. In the alveolar cells of the lungs, the concentration of O$_2$ is high (see earlier) and causes the H.Hb to get oxygenated to HbO$_2$ that dissociates to give the more stable HbO$_2^-$ and a proton H$^+$ (EQ#1). This H$^+$ combines with the plasma bicarbonate (EQ#4) to yield CO$_2$

7. In the lungs HbH.CO$_2$ releases more CO$_2$ and the HbH gets oxygenated as above.

8. The net result of chemistry is that, in the lungs, oxyHb and CO$_2$ are formed.

![Figure 14: Exchange processes for carbon dioxide, protons and oxygen in lung and peripheral tissues.](image)

**ALLOSTERIC EFFECTORS:**

H$^+$: as described above; the Bohr effect (Christian Bohr (son of Niels) was first reported in 1930s)

![Figure 15: Bohr effect: Left shift from pH7.4 to pH7.6 and right shift to pH7.2 (blue)](image)

**BPG:**

2,3 Bisphosphoglycerate is an anion with a net charge of -5 (Fig. 17). Its binding site is in the central cavity of T form that is 'lined' with His and Lys residues (Fig. 18, blue). The [BPG] has been observed to increase in the blood of individuals who (a) suffer from chronic anoxia i.e. pulmonary emphysema and (b) during prolonged exposure to high altitudes where, due to ambient decreased oxygen concentrations, it will enhance needed oxygen release from the lowered [oxyHb] and (c) anemia (more BPG releases O$_2$ from less RBCs). This is concurrent with increased RBC production & Hb
concentration in the RBCs.

![Structure of BPG](image1)

**Figure 17.** Structure of BPG

It is a structural isomer of 1,3 bisphosphoglycerate and can be interconverted *in vivo* by a mutase. 1,3 BPG is a glycolytic intermediate (formed from glyceraldehyde-3' phosphate in a reaction requiring NAD*+*). Some of the resultant NADH can be used to reduce any Fe [III] cations in methemoglobin to Fe[II] to yield viable Hb.

**Cl**⁻: enters RBC via membrane antiport (with bicarbonate via the 'Band 3' protein). The binding site in the T form is in the interface where it stabilises this T form by salt bridges.

![BPG binding site in the central cavity of the deoxy ('T') form](image2)

**Figure 18.** BPG binding site in the central cavity of the deoxy ('T') form

![Binding site for chloride ion in deoxy(T) (b) resultant new salt bridges in oxy(R)](image3)

**Figure 19.** (a) Binding site for chloride ion in deoxy(T) (b) resultant new salt bridges in oxy(R)

**NO:** The allosteric nature of this radical was only discovered in the past 10 years. Its binding site at a Cys residue. It is also a vasodilator so its presence causes oxyhemoglobin to move faster through the capillaries carrying O₂ (a good trick!). This explains the use of 'nitro' compounds (nitroglycerin, amyl nitrite, etc) for angina sufferers and...is involved in the molecular activity of Viagra!

Increases in any of these chemical species will cause a 'right' shifts and has the effect of increasing O₂ release and *vice versa*. The chemical binding of all these allosteric effector have the
effect of stabilising the T form (and vice versa).

The amount of O₂ released can be estimated from the length of the vertical line to the binding curve from the level for 100% saturation or the value of the partial pressure of O₂ at the P₅₀.

One other parameter that can effect oxygen release to peripheral tissues is temperature. Increasing temperature (as in a fever) will decrease affinity of O₂ to Hb and will result in enhanced release assisting the accelerated metabolism concurrent with a fever. Administration of anti-pyretic drugs may be counter productive in such circumstances since lower temperatures will reduce O₂ release since neurophils require molecular O₂ for a O₂-dependent myeloperoxidase that kills bacteria.

OTHER NORMAL HEMOGLOBINS:
At different stages of development (see Fig. 20), human synthetise several different types.

- **HbA**  α2β2 Normal adult Hb (~90%)
- **HbA₂ or HbDα2δ2** Normal adult Hb (~2-5%)
- **HbF**  α2γ2 The 2 His residues in the central cavity on the β units are part of the BPG binding site (see Fig. 18) are mutated to Ser and, thus, increases O₂ affinity (relative to maternal HbA) due to lowered BPG binding. This provides a mechanism for the fetus to acquire O₂ from maternal blood. The relative binding curves for HbA and HbF are shown in Fig.21).
- **HbE**  ζ2ε2  (ζ and ε are embryonic forms of α and γ/β)
- **HbA₁c**  α2β2-glucose ; glucose binds to N termini of β units. The levels** of HbA₁c are used in diabetes diagnosis. It is considered a more reliable long term marker (30-60 days) for elevated plasma glucose levels than monitoring just serum [glucose].(3-9%)

**detected and quantitated by serum electrophoresis.

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**Figure 20. Changes in Hb composition during development.**

**Figure 21. Comparison of HbA (red) and HbF (blue) oxygen binding curves.**

**HEMOGLOBIN GENES:**
Both α (on chromosome 16) and the β (on chromosome 11) clusters of globin genes are expressed in coordination and each has three exons (Fig 22). Each cluster contains the genes for variants of the sub units that combine to form the different tetrameric hemoglobins described above (Fig. 23).
HEMOGLOMOPATHIES:
These are defined as being due to:
(1) structurally abnormal Hb (i.e. HbS) or
(2) decreased synthesis of normal Hb subunits (i.e. the thalassemias) or
(3) both. (rare)?
Over 800 are known (Fig 25); mutations cause instability** in Hb structure, increased or decreased oxygen affinity, decrease in cooperability, impair BPG binding, increased susceptibility to oxidation of the Fe(ii) to Fe(III)(the 'methemoglobinins' HbM).

**Unstable Hbs denature easily and can precipitate to form 'Heinz' bodies that damage RBCs leading to anemia, reticulocytosis, splenomegaly and urobilinuria.

Two of the most common are due to mutations at the same AA residues (β6 Glu): in HbS ('sickle cell anemia', see below) this a Val and in HbC, the β6 is a Lys. These variants can be usually detected by comparison with with HbA by PAGE.

**SICKLE CELL ANEMIA:**
Homozygotes with HbS (α2σ2) produce hemoglobin molecules that precipitates as long aggregates

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**Table 7-1 Some Hemoglobin Variants**

<table>
<thead>
<tr>
<th>Name</th>
<th>Mutation</th>
<th>Effect</th>
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</thead>
<tbody>
<tr>
<td>Hammersmith</td>
<td>Phe CD1(42)β → Ser</td>
<td>Weakens heme binding</td>
</tr>
<tr>
<td>Bristol</td>
<td>Val E11(67)β → Asp</td>
<td>Weakens heme binding</td>
</tr>
<tr>
<td>Bibba</td>
<td>Leu H19(136)α → Pro</td>
<td>Disrupts the H helix</td>
</tr>
<tr>
<td>Savannah</td>
<td>Gly B6(24)β → Val</td>
<td>Disrupts the B-E helix interface</td>
</tr>
<tr>
<td>Philly</td>
<td>Tyr C1(35)β → Phe</td>
<td>Disrupts hydrogen bonding at the α1−β1 interface</td>
</tr>
<tr>
<td>Boston</td>
<td>His E7(58)α → Tyr</td>
<td>Promotes methemoglobin formation</td>
</tr>
<tr>
<td>Milwaukee</td>
<td>Val E11(67)β → Glu</td>
<td>Promotes methemoglobin formation</td>
</tr>
<tr>
<td>Iwate</td>
<td>His F8(87)α → Tyr</td>
<td>Promotes methemoglobin formation</td>
</tr>
<tr>
<td>Yakima</td>
<td>Asp G1(99)β → His</td>
<td>Disrupts a hydrogen bond that stabilizes the T conformation</td>
</tr>
<tr>
<td>Kansas</td>
<td>Asn G4(102)β → Thr</td>
<td>Disrupts a hydrogen bond that stabilizes the R conformation</td>
</tr>
</tbody>
</table>

*Hemoglobin variants are usually named after the place where they were discovered (e.g., hemoglobin Boston).*

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Figure 25. Examples of some known Hb variants

Figure 26. Normal and sickle RBCs images.
that cause RBCs that are relatively inflexible and have the characteristic crescent shape. The solubility of deoxy HbS in plasma is very low.

Sickling is enhanced by hypoxia (i.e. high altitude, decreased serum pH, poor air quality, increased partial pressure of CO2, etc.).

On the molecular level, the surface β6 Glu residue in HbA is mutated to a Val. This causes a non-polar 'patch' on the exterior of (especially) the deoxy hemoglobins to associate (via London dispersion forces) with another non-polar 'patch', a Phe residue near the surface in a neighboring molecules (Figs. 28 & 29). These HbS molecules spontaneously form supra-molecular assemblies that eventually lead to the formation of insoluble poly-HbS fibers (Figs. 26(right), 28 & 29). The latter distort the shape of the RBS in such a way that they become (a) sickle shaped and (b) too rigid to pass through micro filters in the vascular regions of joints. This causes both decreased oxygen transportation and painful joints in homozygous individuals.

Sickle cell anemia causes chronic hemolytic anemia, painful acute 'crises' and increases the risk of stroke, organ damage, bacterial infections due to *Pneumococcus, Hemophilus Influenzae, Meningococcus* and *Salmonella* species, and complications of blood transfusions. The average life expectancy is about 50 years. A high rate of painful vaso-occlusive crises in considered an index of clinical severity that correlates with early death.

The incidence of sickle cell anemia is most common in the regions where malaria is endemic i.e. those shaded dark blue and red in Fig 30. The trait affects about 20% of African tropical regions. The disease is thought to found in 1-2% of new born babies in Africa annually. About 60,000 people in the USA suffer from the disease. HbS protects against malaria; heterozygotes are more resistant to malaria than HbA individuals. One explanation (*inter alli*) is that the *Plasmodium* malarial vector lowers the pH of the infected RBCs and so leads to sickling. These cells are degraded in the spleen at a higher rate than normal RBCs.
TREATMENT OF SICKLE CELL ANEMIA:
The most beneficial medication involves the administration of hydroxyurea (Fig. 31). This compound promotes the production of fetal hemoglobin [HbF, see earlier] but the long-term effects of hydroxyurea and safety are unknown. Transfusions are used but here the treatment could be more harmful that the disease as excess iron ('hemosiderosis') can be extremely deleterious. Further, frequent transfusions could increase the risk of immune reactions and infections, such as HIV and hepatitis B or C. 

For more see the video made by students from E08 UWSOM:WWAMI: Spokane students as part of their 'conference' report http://www.youtube.com/watch?v=DDtiL3EiHDI

Individuals with the HbS trait do not present with any manifestation of illness.

Hemoglobin C (β6 Glu->Lys) forms a different shaped aggregate from that HbS in that it forms blunt-ended crystalloids. This decreases the life time of the RBCs but exhibits a more limited pathological effects than HbS.

Both HbS and HbC are relatively common in black African populations and in such populations, individuals with HbSC are found with intermediate anemia between HbS and HbC.

THALASSEMIAS:
Whereas sickle cell anemia represents a classic example of a single point mutation and mis-folding of proteins, the thalassemias can be also caused by several other molecular mechanisms, i.e. deletions, insertions, and rearrangements in DNA. The result can be:

<table>
<thead>
<tr>
<th>(a) for particularly for the α-globin genes</th>
<th>(b) for particularly for the β-globin genes</th>
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<tr>
<td>Complete deletions of globin genes deletions. Many of the these have been observed in ethic and regional populations in that α-thalassemia is relatively common in northern Europeans, Philipinos, black Africans and people living around the Mediterranean. It is most common thalassemia or hemoglobinopathy.</td>
<td>Reduced RNA production due mutations within the transcription control regions (several involve mutations in the STOP codon AUG)</td>
</tr>
<tr>
<td>Frameshift and nonsense mutations (resulting in rapid degradation of RNA)</td>
<td>RNA processing (splicing) ;the most common.</td>
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All these conditions result in hemolytic anemia.
GENETIC MECHANISMS:

α-Thalassemia caused by reduced or non synthesis mRNA for the α units that results in either hemoglobins that are β4 or α1β3 in subunit composition.

There are 2 expressed α-globin genes; this diseases arises because of deletions of either 2, 3 or all 4 copies of the α-globin genes in the gene clusters on chromosome 16. The disease becomes obvious when 3 or more genes are missing and hemolytic anemia is observed: β4 is fatal at or before birth ('hydrops fetalis'). See Fig 32.

The possibilities are : (where α = normal gene expressed and - = deleted genes)

- normal instance of all four genes expressed => α α/α α
- 1 deletion/ transformation => α α/α α :
- 2 deletions/transformations => α α/ - or α -/α - ;
- 3 deletions/transformations => - -/α - ('HbH');
- 4 deletions/transformations => - -/ - - ('hydrops fetalis')

Another variant is Hb Bart's that has the composition γ4 (recall the γ gene is on the same chromosome as the β gene and α2γ2 is HbF )

These altered combination of 4 subunits form Hb that are non-cooperative and the binding curves look like that of Mb.

Figure 32. Genes expressed in α-thalassemia (from Champe)
**β-Thalassemia** caused by reduced or non synthesis mRNA of the β units that results in either hemoglobins that are α4, α3β1 in subunit composition. (Fig. 33)

There is only 1 expressed β-globin gene on chromosome 11

- **β/β** is normal
- **Thal β minor** => β/-
- **Thal major** (or Cooley's anemia) => -/-

The unstable α4 tetramers are insoluble and damage the RBC whilst in the bone marrow.

![Diagram showing β-globin gene expression and subunit composition](image)

**Figure 32. Genes expressed in α-thalessemia (from Champe)**

By continuing to express the HbF gene (α2γ2) turned on (HPFH) in conjunction with HbD (α2δ2) can allow people with some thalassemias to live quite long lives.

However, in thalassemia major, the treatment can be the cause of death. Transfusions are used to relieve the symptoms but iron (II) overload ('hemosiderosis') and subsequent toxicity in the liver, myocardium, adrenals, etc that results in diabetes, liver dysfunction, congestive heart failure, etc (c.f HbS)

Chelation therapy is used to remove the iron. Further, bone marrow replacement has been successfully used and stem cell therapy may be useful in the foreseeable future for affected individuals.

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