Physiology of haemoglobin

Caroline Thomas MB ChB FRCA
Andrew B Lumb MB BS FRCA

Revalidation FOR ANAESTHETISTS
RCA Revalidation matrix
Matrix reference
1A01, 2A03

Key points

Haemoglobin comprises four globin chains, each containing a haem molecule which reversibly binds to oxygen.

Binding of oxygen to haem alters oxygen affinity by inducing structural changes in the adjacent globin chains.

This molecular 'co-operativity' within haemoglobin is responsible for a sigmoidal-shaped oxygen dissociation curve and is influenced by pH, carbon dioxide, and 2,3-diphosphoglycerate.

Haemoglobin forms carbamino compounds with carbon dioxide and buffers hydrogen ions within the erythrocyte, so facilitating the carriage of carbon dioxide in blood.

Abnormal haemoglobins arise from changes in either the globin chains, the iron atom, or from binding of ligands other than oxygen.

Caroline Thomas MB ChB FRCA
Specialty Registrar in Anaesthesia
Department of Anaesthesia
St James's University Hospital
Leeds
UK

Andrew B Lumb MB BS FRCA
Consultant Anaesthetist
Department of Anaesthesia
St James's University Hospital
Beckett Street
Leeds LS9 7TF
UK
Tel: +44 113 2065789
Fax: +44 113 2065630
E-mail: andrew.lumb@leedsth.nhs.uk
(for correspondence)

This article describes the structure and physiological functions of haemoglobin, including abnormal forms of haemoglobin and their significance. The mechanism of oxygen binding and the factors affecting oxygen affinity will be discussed.

Synthesis and destruction of haemoglobin

Haemoglobin is present in blood at concentrations of 13.5–18.0 g dl^{-1} in men and 11.5–16.0 g dl^{-1} in women. Each erythrocyte contains around 200–300 million molecules of haemoglobin.

Synthesis

A haemoglobin molecule is composed of four polypeptide globin chains (Fig. 1). Each contains a haem moiety which has an organic part (a protoporphyrin ring made up of four pyrrole rings) and a central iron ion in the ferrous state (Fe^{2+}). Normal adult haemoglobin molecules (HbA) have a molecular mass of 64 458 Da with a complex quaternary structure, the function of which has been extensively studied and is described below. Erythrocytes containing haemoglobin are produced in the bone marrow of the long bones, such as femur and humerus, and flat bones, such as sternum and ribs. Erythropoiesis is mainly under the control of erythropoietin, which is released from the kidney in response to cellular hypoxia mediated by hypoxia-inducible transcription factors.

Haem

Haem synthesis occurs both in cytosol and in mitochondria of erythrocytes. Protoporphyrin is synthesized from the condensation of glycine and succinyl coenzyme A, eight molecules of each being required to form a linear tetrapyrrole molecule, which finally cyclizes into the protoporphyrin ring. The protoporphyrin then binds to a Fe^{2+} ion to form haem.

Iron

The Fe^{2+} ion forms six bonds within the haem moiety. Five of these bind the Fe^{2+} firmly: four with nitrogen atoms in the centre of the protoporphyrin ring, and one to a 'proximal' histidine residue at position 87 on an α-globin chain. The final bond is made with an oxygen molecule as required. Close to the oxygen-binding site on the haem group, there is another crucial histidine residue, the 'distal histidine'. This occupies position 89 on the α-globin chain. It has two important functions: through steric hindrance, it prevents haem groups on other globin molecules oxidizing the iron in the Fe^{2+} state to the Fe^{3+} state and it prevents carbon monoxide binding irreversibly to the Fe^{2+} ion.

Globin chains

More than 95% of an adult's haemoglobin is in the form of HbA with two α- and two β-globin chains. Each α-chain has 141 amino acids, and each β-chain has 146. Genes for the α-chain are found on chromosome 16 and those for the β-chain on chromosome 11. Globin chains are synthesized in the cytosol of erythrocytes.

Of an adult's haemoglobin, 2.2–3.5% is HbA_2, composed of two α- and two δ-chains. This form of haemoglobin is poor at oxygen carriage. Fetal haemoglobin (HbF) comprises two α-chains and two γ-chains. At birth, 50–95% of a baby's haemoglobin is HbF, but these levels decline after 6 months as more HbA is produced. In a healthy adult, <1% of haemoglobin is HbF. The oxygen affinity of HbF is substantially greater than HbA to facilitate the transfer of oxygen between the maternal and fetal circulations in the placenta.

Haemoglobin destruction

Erythrocytes are removed by the reticulo-endothelial system. Globin chains are broken down to amino acids which then return to the amino acid pool. Iron is re-used by the bone marrow to synthesize haem. Protoporphyrin degradation begins with the cleavage of the

doi:10.1093/bjaceaccp/mks025 Advance Access publication 15 May, 2012

ring to form a linear tetrapyrrole molecule, biliverdin, which is then reduced to bilirubin. Bilirubin is bound to albumin for transport to the liver, where it is conjugated with glucuronic acid. This is excreted in the bile and then into the small bowel. In the gastrointestinal tract, bilirubin is converted into stercobilin, some of which is reabsorbed into the plasma and excreted by the kidney as urobilinogen in urine. Small amounts of free haemoglobin may be released into the plasma.[1]

Functions of haemoglobin

Haemoglobin has multiple functions:

- Transport of oxygen from the lungs to the tissues, mostly to facilitate oxidative phosphorylation in the mitochondria.
- Carriage of carbon dioxide from tissues to the lungs as carbaminohaemoglobin.
- Buffering of hydrogen ions formed in the erythrocyte from the conversion of carbon dioxide into bicarbonate.
- Nitric oxide metabolism. This is discussed below.

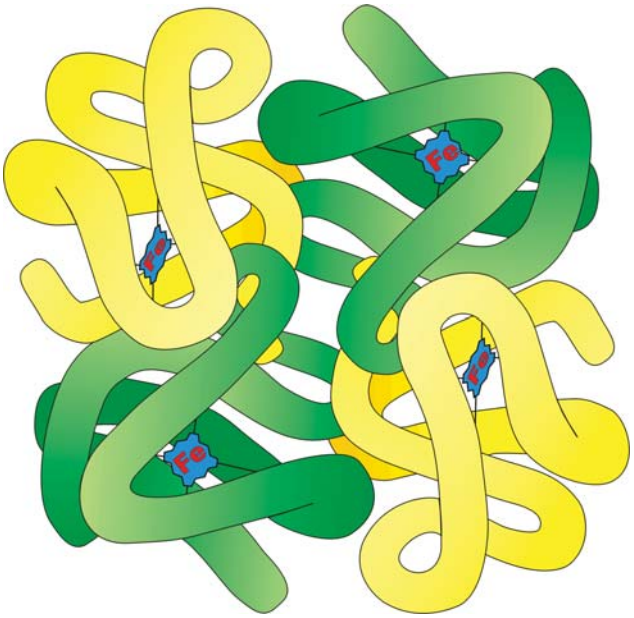

Fig 1 Schematic diagram showing the basic structure of a single haemoglobin A molecule, including two α-globin chains (green), two β-globin chains (yellow), each containing a haem–iron complex (blue).

Oxygen transport

The molecular mechanism of oxygen binding

Oxygen binds reversibly to haem, so each haemoglobin molecule can carry up to four oxygen molecules. Haemoglobin is an allosteric protein; the binding of oxygen to one haem group increases the oxygen affinity within the remaining haem groups. This 'co-operativity' between the component parts of haemoglobin means that oxyhaemoglobin has a substantially different quaternary structure to deoxyhaemoglobin. As a molecule of oxygen binds to haem, it pulls the Fe^{2+} ion closer towards the plane of the protoporphyrin ring, slightly flattening the ring and so changing its shape. This small movement within the centre of the globin chain is transmitted to the surface of the molecule. Ionic interactions holding the four globin chains together are distracted and, as they re-form in a different position, the quaternary structure is altered, which increases the oxygen binding affinity of the other globin chains.[2]

In each globin chain, the haem molecule is located in a deep crevice on the side of the globin molecule (Fig. 2). The shape of the crevice determines how easily an oxygen molecule can access its binding site. In fully deoxygenated haemoglobin, the molecule's quaternary structure is described as the 'T' or 'tense' form in which the crevices are small, making it difficult for oxygen to gain access to the haem. As each successive oxygen binds to the molecule, the structural changes described above result in the molecule relaxing, enlarging the crevice on adjacent globin chains and increasing their oxygen affinity. When fully oxygenated with four oxygen molecules, the haemoglobin achieves its 'R' or 'relaxed' quaternary structure.

The Hüfner constant

The oxygen binding capacity of haemoglobin (Bo_2) is the amount of oxygen in millilitres carried by each gram of haemoglobin, and is commonly referred to as Hüfner's constant. Over many years, different values have been obtained for this constant, with those found experimentally consistently lower than the theoretical value.

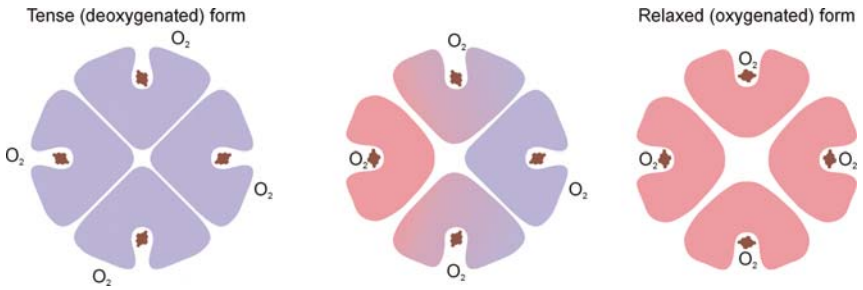

Fig 2 The transition from 'tense' to 'relaxed' haemoglobin. In its deoxygenated 'tense' form, the crevice containing the haem molecule is narrow, restricting the access of oxygen to its binding site. As each oxygen molecule binds, the position of the haem molecule changes which affects the interaction between adjacent globin chains, relaxing the molecule and so allowing easier access of subsequent oxygen molecules to their binding site.

Based on the molecular weight of haemoglobin, and the fact that one mole of haemoglobin binds four moles of oxygen, a theoretical value for Bo_2 of 1.39 ml g^{-1} is easily obtained. However, *in vivo* experiments produce values anywhere between 1.31 and 1.37 ml g^{-1}. This variation is now believed to result from the presence of small amounts of other forms of haemoglobin which are relatively poor carriers of oxygen, for example, HbA_2, methaemoglobin, and carboxyhaemoglobin. If the proportion of these forms of haemoglobin are determined using a co-oximeter and excluded from the calculations, the theoretical value of 1.39 ml g^{-1} is reliably obtained.[3]

The oxyhaemoglobin dissociation curve

The sigmoid shape of the oxyhaemoglobin dissociation curve (Fig. 3) is due to co-operativity between the component globin chains as described above. This means that the affinity of haemoglobin for oxygen is the lowest when the first oxygen molecule binds to the tense, deoxyhaemoglobin molecule, so at a very low partial pressure of oxygen (Po_2), the gradient of the curve is almost flat. Each subsequent oxygen molecule binds to haemoglobin more easily, so the curve gradient increases. As Po_2 increases further, almost all the oxygen-binding sites become occupied, so the curve levels off again. Under normal physiological circumstances, when venous oxygen saturation is 75% or above, only the final molecule of oxygen is binding and unbinding from the haemoglobin, making this a highly efficient system.

The 'P_{50}' is an important concept in the oxyhaemoglobin dissociation curve. It is a measure of oxygen affinity and is used to compare changes in the position of the curve. P_{50} is the partial pressure of oxygen in blood in which haemoglobin is 50% saturated, and a change in its value therefore describes whether the curve has shifted to the left or the right. Different forms of haemoglobin have different P_{50} values, for example, P_{50} for HbA is 3.5 kPa compared with 2.5 kPa for HbF, reflecting the higher affinity that HbF has for oxygen (Fig. 3).

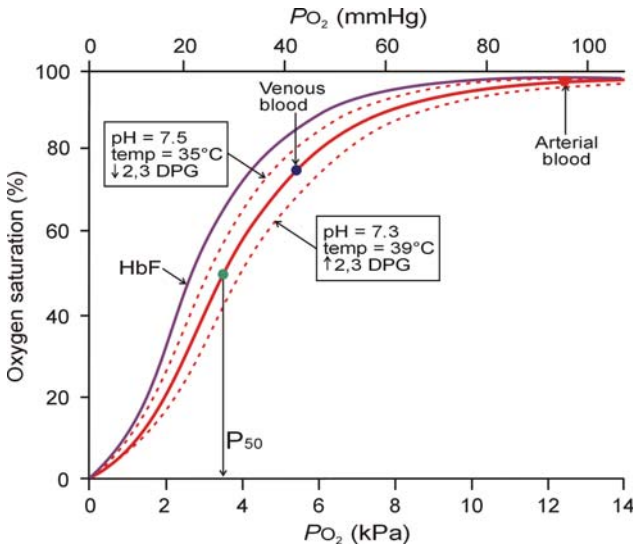

Fig 3 The oxyhaemoglobin dissociation curve. The normal curve for adult haemoglobin is shown in red, with dots showing the normal values in arterial and venous blood. P_{50}, the Po_2 at which haemoglobin is 50% saturated, is indicated by the arrow showing a normal value of 3.5 kPa. The curve can be shifted to the left or right by the factors listed in the boxes, but these physiological changes in adults are small compared with the increased oxygen binding achieved by fetal haemoglobin (purple line).

The P_{50} for any single form of haemoglobin is also variable. The hydrogen bonds and ionic interactions within the haemoglobin molecule that result in the variations in oxygen affinity described above are also affected by physical and chemical factors. Increases in hydrogen ion concentration, partial pressure of carbon dioxide (Pco_2), and 2,3-diphosphoglycerate (2,3-DPG) concentration are all allosteric effectors which favour the T-conformation of deoxyhaemoglobin. The *in vivo* effects are shown in Box 1. Each of these factors influence the structural conformation of different regions of haemoglobin and their effects are often additive.

Box 1 Factors that decrease the oxygen affinity of haemoglobin and therefore increase the P_{50}

- Increased temperature
- Increased hydrogen ion concentration (lower pH)
- Increased carbon dioxide partial pressure
- Increased 2,3-DPG concentration

The Bohr effect describes the reduction in oxygen affinity of haemoglobin when pH is low and the increase in affinity when pH is high. An illustration of the importance of the Bohr effect is seen in exercising muscle where anaerobic metabolism results in a lower pH. The Bohr effect helps with the offloading of oxygen from haemoglobin to provide a vital oxygen supply where it is needed. Similarly, the acidosis that accompanies tissue hypoxia from whatever cause improves oxygen release in metabolically challenged areas. The 'double Bohr' effect helps to increase fetal oxygenation. The transfer of carbon dioxide from fetal to maternal blood shifts the maternal oxyhaemoglobin curve to the right and the fetal curve to the left, facilitating the transfer of oxygen across the placenta from mother to fetus.

2,3-Diphosphoglycerate

2,3-DPG is a highly anionic organic phosphate which promotes the release of oxygen from haemoglobin. It is produced by a side-shunt reaction of glycolysis and is present in large quantities in the erythrocyte. One 2,3-DPG molecule binds between the β-globin chains of deoxyhaemoglobin, altering the protein structure and so reducing oxygen affinity. The production of 2,3-DPG is increased in anaemia and as a result, the relative tissue hypoxia is partially corrected by the increased P_{50}, promoting more oxygen release to the tissues. The hypobaric hypoxia occurring at altitude also causes an increase in the 2,3-DPG concentration and a subsequent right shift of the oxyhaemoglobin dissociation curve. At higher altitude, this beneficial effect may be opposed by a left shift of

the oxyhaemoglobin dissociation curve as a result of the respiratory alkalosis caused by hyperventilation.[4]

Blood that is stored for transfusion undergoes a number of changes with time. 2,3-DPG is metabolized and the ability of blood to deliver oxygen is reduced. In the storage solution, SAG–M (saline, adenine, glucose, and mannitol), 2,3-DPG levels are very low after 14 days. Oxygen is not delivered to the tissues efficiently as the oxyhaemoglobin dissociation curve of stored blood is shifted far to the left. This blood is still a better oxygen carrier than no blood at all, but the transfused red cells require more than 24 h in the recipient before normal 2,3-DPG levels are re-established.[5]

The role of haemoglobin in carbon dioxide transport

Carbon dioxide is transported in the blood in three forms: as bicarbonate, as carbamino compounds, and in solution.

(1) Approximately 89% of the carbon dioxide in blood is in the form of bicarbonate ions. As it passes through a systemic capillary, the P_{CO_2} within an erythrocyte progressively increases and bicarbonate is formed, a reaction that is facilitated by the enzyme carbonic anhydrase. Some of the hydrogen ions formed by this reaction are buffered by the haemoglobin, while the remainder are actively transported from the cell by a membrane-bound transporter protein called Band 3. This ion exchange protein simultaneously imports a chloride ion to maintain electrochemical neutrality. This exchange is known as the Hamburger effect or the chloride shift.

(2) Carbamino compounds can be formed by a chemical reaction between carbon dioxide and haemoglobin. Carbon dioxide combines with any available amino groups in the globin chains to form carbamates, but available groups are only found on the N-terminal amino group of each globin chain and on the side chains of valine residues. The bound carbamates stabilize the T-form of haemoglobin and the binding of carbon dioxide therefore lowers its oxygen affinity. Carbamino carriage of carbon dioxide is greatly influenced by the concentrations of hydrogen ions and 2,3-DPG. Both of these compete with carbon dioxide for some of the same binding sites. Carbamino compounds account for ~6% of the carbon dioxide carried in blood.

(3) Only 5% of the total carbon dioxide in blood is dissolved in solution, despite its high solubility relative to oxygen.

The Haldane effect describes the ability of deoxyhaemoglobin to carry more carbon dioxide than oxyhaemoglobin (Fig. 4). Increased formation of carbamino compounds accounts for around two-thirds of this effect, with the remainder being a result of the greater buffering capacity of deoxyhaemoglobin. Although the absolute amount of carbon dioxide carried as carbamino compounds is small, the difference in the amount carried between arterial and venous blood is large (Fig. 4), and this therefore accounts for about one-third of the difference in carbon dioxide carriage between arterial and venous blood *in vivo*.

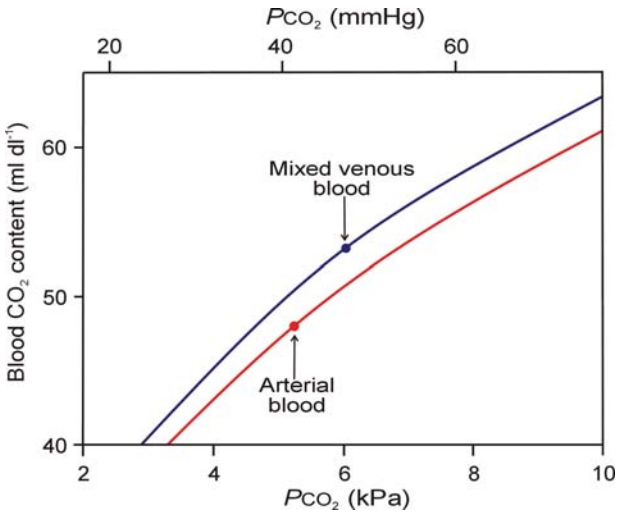

Fig 4 The carbon dioxide–blood dissociation curve for arterial (red) and venous (purple) blood. Venous blood can carry more carbon dioxide than arterial blood (the Haldane effect); so for any given P_{CO_2}, the content is greater in venous blood.

Gas exchange along capillaries

The Bohr and Haldane effects are fundamental to gas transfer in capillaries. As an erythrocyte moves along a systemic capillary, P_{O_2} declines, haemoglobin desaturates, and the molecule reshapes itself towards its T-form, and as a result the carbon dioxide carrying capacity of the blood increases due to improved carbamino carriage and hydrogen ion buffering (the Haldane effect). Simultaneously, the increasing P_{CO_2} and hydrogen ion concentration reduces the affinity of the molecule for oxygen (the Bohr effect) accelerating the release of oxygen from the haemoglobin. Both effects occur in reverse in a pulmonary capillary.

Abnormal forms of haemoglobin

A range of abnormal forms of haemoglobin exist, and these are conveniently classified according to which component is defective.

Abnormal globin chains

Genetic defects in haemoglobin are the most common of all genetic disorders. Many genetic abnormalities of globin chain synthesis exist, which either result in the impaired production of globin chains (the thalassaemias) or abnormalities in the structure of the globin chain (the haemoglobinopathies).

Thalassaemia

In health, equal quantities of α- and β-globin chains are produced. Abnormalities in the transcription of either α- or β-globin genes lead to the excessive production of the other chain, and these chains may precipitate, causing haemolysis and anaemia. The gene for the α-globin chain is duplicated on each chromosome 16, so in health, four α-globin genes exist. α-Thalassaemia results from the deletion

of between one and all four genes, with an associated variation in clinical severity. The deletion of all four genes is incompatible with life. β-Thalassaemia is usually due to a single-gene mutation and results in the reduced production of β-globin chains. It normally becomes clinically apparent at between 3 and 6 months of age, when fetal haemoglobin begins to be replaced by HbA. The excess α-globin chains combine with the available β, δ, or γ chains, forming abnormal amounts of HbA_2 (δ-chains) and HbF (γ-chains).

Haemoglobinopathies

The best-known example of haemoglobinopathy is sickle-cell disease, in which a valine is substituted for a glutamate at position 6 of the β-globin chain due to a single-base mutation in the β-globin gene. In the homozygous state, both β-globin genes are abnormal which results in sickle-cell anaemia. The P_{50} is lower than that for HbA, so the oxyhaemoglobin dissociation curve is shifted to the left. In the heterozygous state, sickle-cell trait occurs which is clinically less severe. Many other haemoglobinopathies exist, for example, HbC, but these clinical conditions are outside the scope of this article and have been reviewed recently.[6]

Other ligands

Carbon monoxide

Other ligands can combine with the haem molecule. Carbon monoxide is the most common and sources include physiological generation, air pollution, and tobacco smoke. Carbon monoxide has an affinity for haemoglobin that is around 300 times greater than that of oxygen. The presence of carboxyhaemoglobin shifts the oxyhaemoglobin dissociation curve to the left, reduces the availability of binding sites for oxygen, increases the affinity for oxygen of the remaining binding sites, and these effects lead to tissue hypoxia. The rate at which carbon monoxide dissociates from haemoglobin depends on the number of oxygen molecules present, that is, the blood P_{O_2}. The half-life of carboxyhaemoglobin therefore depends on P_{O_2} and is ~4 h when breathing air, 40 min if breathing 100% oxygen, and 20 min with hyperbaric oxygen therapy.[7]

Nitric oxide

It is well known that haemoglobin binds to nitric oxide in order to inactivate this highly vasoactive physiological molecule. There are two quite different reactions between nitric oxide and haemoglobin.

First, nitric oxide binds extremely rapidly to the haem group, but the ensuing reaction depends on the state of oxygenation of the haemoglobin. In the T-conformation (deoxyhaemoglobin), a fairly stable haemoglobin–nitric oxide complex forms which has little vasodilator activity, while in the R-conformation (oxyhaemoglobin), the oxygen is displaced by nitric oxide, a consequence of which is the oxidization of the Fe^{2+} ion to form methaemoglobin (see below) and a nitrate ion.

Secondly, nitric oxide may also react with the sulphydryl groups of cysteine residues in the β-globin chains forming stable compounds termed nitrosothiols. Nitrosothiols on the haemoglobin molecule have activity as vasodilators and survive for longer than free nitric oxide. The reaction to form nitrosothiols is faster when haemoglobin is in the R conformation (oxyhaemoglobin).

As a result of these reactions being dependent on the state of oxygenation of the haemoglobin, nitric oxide in arterial blood is mostly in the form of nitrosohaemoglobin, while in venous blood, nitric oxide is mostly bound to haem. It is believed that as haemoglobin passes through the pulmonary or systemic capillaries, the altered conformation of the haemoglobin molecule driven by changing P_{O_2}, P_{CO_2}, and pH also causes the intramolecular transfer of nitric oxide from the haem to the cysteine-bound position. This system may allow the erythrocyte to exert some control over the capillary diameter, depending on the local oxygen supply. As the haemoglobin saturation decreases along a systemic capillary, the nitric oxide molecule is released from its cysteine-bound position, from where it may either bind to the haem group or be released from the erythrocyte. Export of nitric oxide activity from the erythrocyte in this way probably involves the transmembrane Band 3 protein already described above, which may directly transfer the nitric oxide via a series of nitrosothiol reactions within the protein to the outside of the cell membrane where it can exert its vasodilator effect on the capillary. Our understanding of the role of nitrosohaemoglobin for the *in vivo* control of tissue oxygenation is at an early stage.[8]

Abnormal haem–iron complex

The iron ion may also be oxidized from the ferrous (Fe^{2+}) to the ferric (Fe^{3+}) state forming methaemoglobin, which is unable to bind with oxygen. Its presence therefore reduces the oxygen-carrying capacity of the blood, causes a leftward shift and changes the shape of the haemoglobin dissociation curve. These changes become more pronounced as the proportion of methaemoglobin increases relative to the remaining HbA. Oxidation may occur either physiologically as a result of haemoglobin's interaction with nitric oxide or as a result of exposure to drugs, for example, prilocaine, benzocaine, dapsone, or exogenous nitric oxide.[9] In health, <1% of haemoglobin is methaemoglobin, due to multiple metabolic systems for the reduction of methaemoglobin back to normal HbA:[5]

- NADH-methaemoglobin reductase is an enzyme contained within erythrocytes which uses NADH from glycolysis to reduce methaemoglobin. This system accounts for around two-thirds of methaemoglobin-reducing activity and is deficient in familial methaemoglobinaemia.
- Ascorbic acid may reduce methaemoglobin by a direct chemical effect, but the reaction is slow.
- NADPH-dehydrogenase enzyme present in erythrocytes can also reduce methaemoglobin. This is an ineffective system under physiological conditions, but the enzyme is stimulated by methylthioninium chloride (methylene blue) which is the mainstay of drug treatment for methaemoglobinaemia.

Conclusions

Haemoglobin has a complex quaternary structure, and the binding of oxygen occurs via a number of molecular interactions. The 'P_{50}' is important in understanding changes in the position of the oxyhaemoglobin dissociation curve when a single form of haemoglobin is considered and it can also be used to compare different forms. Understanding the concepts of the Bohr and Haldane effects is essential in the understanding of gas transfer along the capillaries. Haemoglobin has other functions besides the transport of oxygen; carbon dioxide is carried as carbaminohaemoglobin and hydrogen ions formed in bicarbonate production are buffered by haemoglobin. Haemoglobin has an additional role in the metabolism of nitric oxide. Many abnormalities in haemoglobin exist and can be due to altered structure and production of globin chains, the binding of other ligands, or an abnormal haem–iron complex.

Declaration of interest

None declared.

References

1. Power I, Kam P. *Principles of Physiology for the Anaesthetist*, 2nd Edn. London: Arnold, 2008
2. Hsia CCW. Respiratory function of hemoglobin. *N Engl J Med* 1998; **338**: 239–47
3. Toffaletti J, Zijlstra WG. Misconceptions in reporting oxygen saturation. *Anesth Analg* 2007; **105**: S5–9
4. West JB. *Respiratory Physiology: The Essentials*, 8th Edn. Philadelphia: Lippincott, Williams and Wilkins, 2008
5. Lumb AB. *Nunn's Applied Respiratory Physiology*, 7th Edn. Edinburgh, Churchill Livingstone Elsevier, 2010
6. Wilson M, Forsyth P, Whiteside J. Haemoglobin and sickle cell disease. *Contin Educ Anaesth Crit Care Pain* 2010; **10**: 24–8
7. Stoller KP. Hyperbaric oxygen and carbon monoxide poisoning: a critical review. *Neurol Res* 2007; **29**: 146–55
8. Gaston B, Singel D, Doctor A, Stamler JS. *S*-nitrosothiol signalling in respiratory biology. *Am J Respir Crit Care Med* 2006; **173**: 1186–93
9. Guay J. Methemoglobinemia related to local anaesthetics: a summary of 242 episodes. *Anesth Analg* 2009; **108**: 837–45

Please see multiple choice questions 17–20.