The Bohr and Haldane Effects

Dr Kobus Bergh

UCT Dept of Anaesthesia & Perioperative Medicine

The Bohr and Haldane effects have got to do with the loading of oxygen to the haemoglobin molecule and the unloading of oxygen from the haemoglobin molecule [6]. Just in case you were wondering, both these effects got their names from the people who described them.

I will first discuss some essential theory that is needed to understand these effects, before exploring them in detail.

Essential knowledge needed to understand the Bohr and Haldane effects

Some essential facts about haemoglobin [1,2,7]

Haemoglobin is the red, O_2 carrying pigment in the red blood cells (erythrocytes) of vertebrates. There are about 200 – 300 million haemoglobin molecules in each red blood cell.

Haemoglobin is a protein and this protein is made up of 4 subunits (Fig. 1). Each subunit contains a *haem moiety* attached to a polypeptide chain. (Definition of moiety = a distinct part of a large molecule). The *haem moiety* is a complex made up of a porphyrin and a central iron atom in the ferrous state (Fe^{2+}). The polypeptides are referred to collectively as the *globin portion* of the haemoglobin molecule. Some finer detail to take note of is that the *haem moiety* is attached (at a constant distance) to a histidine group on the *globin portion*, and this forms one subunit of haemoglobin.

There are 2 pairs of polypeptides in each haemoglobin molecule. In normal adult human haemoglobin (HbA), the 2 types of polypeptides are called ***α chains***, (each α chain contains 141 amino acid residues), and ***β chains***, (each β chain contains 146 amino acid residues).

Each of the 4 iron atoms can bind reversibly to 1 molecule of oxygen, and therefore each haemoglobin molecule can bind to 4 oxygen molecules. Bear in mind that the iron stays in the ferrous state, so that the reaction is an oxygenation, not an oxidation! HbA can have its ferrous ion (Fe^{2+}) oxidized to the ferric form (Fe^{3+}) by drugs and chemicals such as prilocaine, nitrates, nitrites, sulfonamides, and acetanilid. Deficiency of the enzyme methaemoglobin reductase within the red blood cell whose job it is to convert Fe^{3+} to Fe^{2+} may also cause this. When the iron atom is in its ferric form it is known as methaemoglobin and is unable to carry oxygen. [1,7]

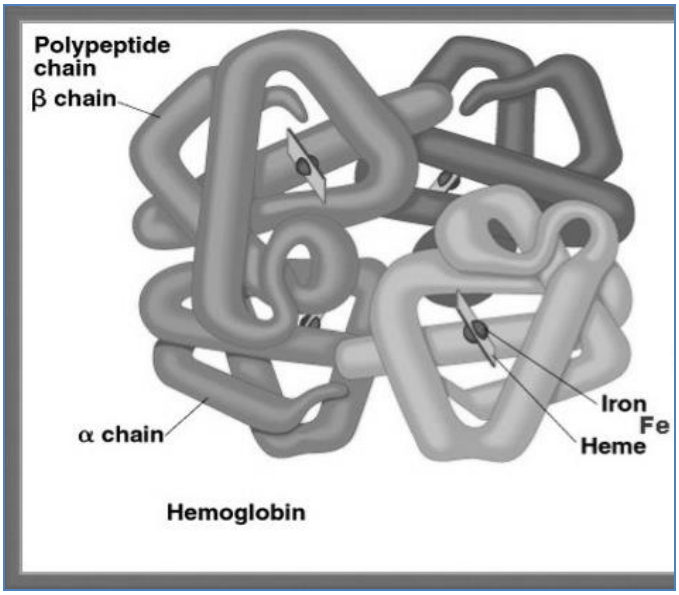

Fig.1 Haemoglobin A

Image taken from: https://bhavanajagat.com/2013/10/26/wholedude-wholedesigner-red-blood-cell

Oxygen transport in the blood [1,7]

It is important to appreciate that oxygen is carried in blood in two forms: *dissolved* and *combined with haemoglobin (Hb).*

Dissolved oxygen

- Like all other gasses, oxygen obeys Henry's Law, which states that the gas dissolved in a liquid is proportional to its partial pressure.
- For each mmHg of PO_2, there is 0,003 ml O_2 dissolved per 100 ml of blood at 37°C.
- Therefore arterial blood with a PO_2 of 100 mmHg (13,3 kPa) contains 0,3 ml oxygen per 100ml.
- It is clear that this way of transporting oxygen is inadequate and that an additional method for transporting oxygen is required…!

Combined with haemoglobin

- I have discussed most of this already under Haemoglobin.
- Taken note that oxygen bound to Hb does not contribute directly to the PO_2 of the blood, only dissolved oxygen contributes to PO_2.
- At normal atmospheric pressure, 98% of oxygen in blood is bound by Hb.

The reaction of haemoglobin and oxygen [2,3,7]

The quaternary structure of haemoglobin determines its affinity for O_2. The change in Hb from the fully oxygenated state to its deoxygenated state is accompanied by a conformational change in the molecule. The oxygenated form is the *R (relaxed) state*, while the deoxygenated form is the *T (tense) state*.

In de-oxyhaemoglobin, the globin units are tightly bound in a *tense (T) configuration*, which reduces the affinity of the molecule for O_2. When O_2 is first bound, the bonds holding the globin units are released, producing a *relaxed (R) configuration*, which exposes more O_2 binding sites. The net result is a 500-fold increase in O_2 affinity! In the tissues these reactions are reversed and O_2 is released.

The oxygen-haemoglobin dissociation curve (which will be extensively discussed in another lecture) is a curve that plots oxygen saturation of haemoglobin against PaO_2. It has its characteristic sigmoid shape due to the T-R interconversion. Combination of the first haem in the haemoglobin molecule with O_2 increases the affinity of the second haem for O_2, and oxygenation of the second, increases the affinity of the third and oxygenation of the third increases the affinity of the fourth! Therefore the affinity of haemoglobin for the fourth O_2 molecule is many times that for the first.

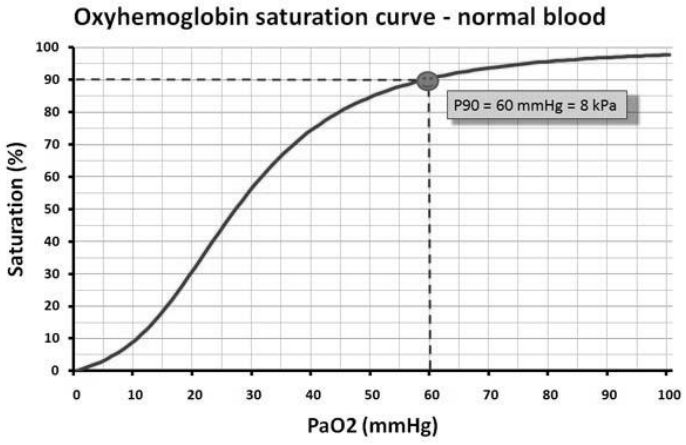

Fig. 2 The Oxygen-haemoglobin dissociation curve

- *Image taken from: http://engineering.stackexchange.com/questions/3058/how-to-generate-a-synthetic-infrared-and-red-led-signal-for-spo2-calculation*

Factors affecting the affinity of haemoglobin for oxygen [2, 7]

Five important conditions affect the oxygen-haemoglobin dissociation curve:

- the **pH**
- the **hydrogen ion** concentration
- the amount of **CO_2**
- the **temperature**
- the concentration of **2,3 diphosphoglycerate (2,3 DPG)**.

A rise in temperature or a fall in pH shifts the curve to the right (Fig 3.7). A rightward shift means more unloading of oxygen at a given PO_2 in a tissue capillary. A simple way to remember these shifts is that an exercising muscle is acidic, hypercarbic, and hot, and it benefits from increased unloading of oxygen from its capillaries. When the curve is shifted to the right, a higher PO_2 is required for haemoglobin to bind a given amount of O_2. On the other hand, a fall in temperature or a rise in pH shifts the curve to the left, and a lower PO_2 is required to bind a given amount of O_2. A convenient index of such shifts is the P_{50}. The P_{50} is the PO_2 at which haemoglobin is half saturated with oxygen. The higher the P_{50}, the lower the affinity of haemoglobin for oxygen.

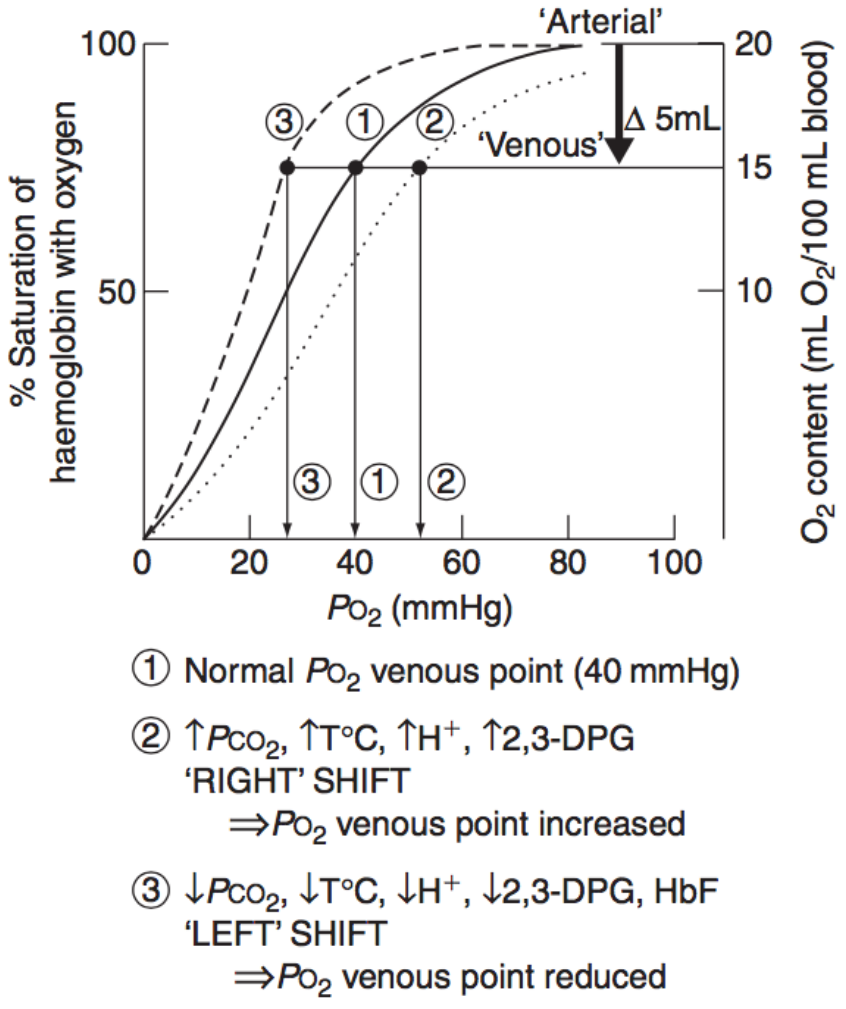

Figure 3.7 *Graphical representation of the Bohr effect. 2,3-DPG, 2,3-diphosphoglycerate.*

Taken from Power I, Kam P. Chapter 3: Respiratory physiology. Principles of Physiology for the Anaesthetist, second edition. (p. 84). Hodder Arnold

Carbon dioxide transport in the blood [2, 7]

It is important to know that CO_2 is carried in blood in the following three forms:

1. Dissolved (in plasma and RBC)
2. Bicarbonate (in plasma and RBC)
3. In combination with proteins as carbamino compounds (in plasma and RBC)

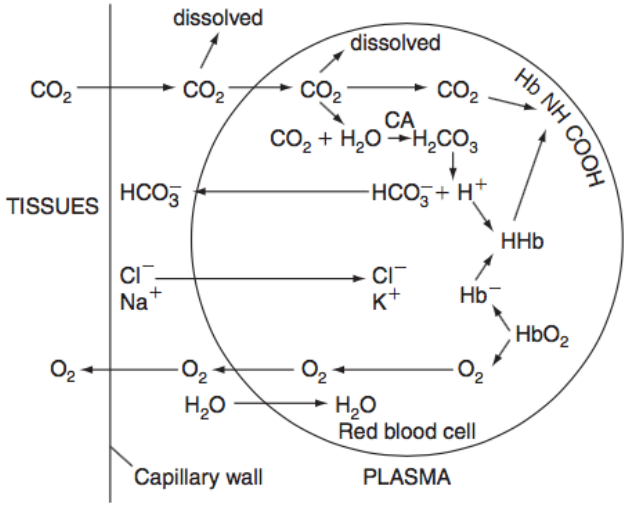

Figure 3.9 *Diagrammatic representation of the uptake of CO_2 and liberation of O_2 in systemic capillaries. CA, carbonic anhydrase. (Reproduced, with permission, from* Respiratory Physiology – the essentials, *5th Edition. John B West, 1995, Williams and Wilkins.)*

Taken from Power I, Kam P. Chapter 3: Respiratory physiology. Principles of Physiology for the Anaesthetist, second edition. (p.86). Hodder Arnold

1. Dissolved CO_2

Like O_2, CO_2 also obeys Henry's Law. CO_2 is however about 20 times more soluble than O_2 in simple solution at equal partial pressures. As a result, dissolved CO_2 plays a significant role in its carriage in blood. About 10% of the gas that is evolved into the lung from the blood is in the dissolved form.

2. Bicarbonate

Bicarbonate is formed in the blood by the following sequence:

$$CO_2 + H_2O \underset{\text{CA (carbonic anhydrase)}}{\leftrightarrows} H_2CO_3 \rightleftarrows H^+ + HCO_3^-$$

The CO_2 that diffuses into red blood cells is rapidly hydrated to H_2CO_3. The fist reaction is very slow in plasma but is fast within the red blood cell, because of the presence of the enzyme carbonic anhydrase (CA) in the red blood cell. The second reaction, which is the ionic dissociation of carbonic acid to form H^+ and HCO_3^- is fast without an enzyme!

What happens to the formed HCO_3^-?
Well, this is the ideal place to discuss the Chloride Shift…

The Chloride Shift (Hamburger phenomenon)
The rise in the HCO_3^- content of red cells is much greater than that in plasma as the blood passes through the capillaries, and about 80% of the HCO_3^- formed in the red blood cells diffuses out into the plasma. H^+ cannot easily diffuse out because the red cell's membrane is relatively impermeable to

cations. Therefore to maintain electrical neutrality, Cl^- ions move into the red blood cell from the plasma. This is the so-called chloride shift, also known as the Hamburger phenomenon, named after Hartog Jakob Hamburger.The chloride shift is responsible for the fact that the chloride content of the red cells in venous blood is significantly greater than in arterial blood. The chloride shift occurs rapidly and is essentially complete in 1 second. Note that for each CO_2 molecule added to a red cell, there is an increase of one osmotically active particle, either an HCO_3^- or a Cl^- in the red cell. Consequently the red cells take up water and increase in size! When the cells pass through the lung again they shrink a little!

What happens to the formed H^+?
Some of the H^+ ions bind to de-oxyhaemoglobin, which is illustrated below.

$$H^+ + HbO_2 \leftrightarrows H^+ - Hb + O_2$$

This occurs because de-oxyhaemoglobin is less acidic and is therefore a better proton acceptor than the oxygenated form. The presence of de-oxyhaemoglobin in the peripheral blood helps with the loading of CO_2, while the oxygenation that occurs in the pulmonary capillary assists in the unloading. The fact that deoxygenation of the blood increases its ability to carry CO_2 is known as the Haldane effect. (I will explain the Haldane effect in much more detail later!)

3. Carbamino compounds

Carbamino compounds are formed by the combination of CO_2 with terminal amine groups in blood proteins. The most important protein is the globin of haemoglobin, and is illustrated by the following reaction:

$$Hb\text{-}NH_2 + CO_2 \rightleftarrows Hb\text{-}NH\text{-}COOH \text{ (This is carbamino-haemoglobin)}$$

This reaction occurs rapidly without an enzyme, and de-oxyhaemoglobin ($Hb\text{-}NH_2$) can bind more CO_2 to form carbamino-haemoglobin (Hb-NH-COOH) than oxyhaemoglobin (HbO_2). I hope that it is clear to you that the unloading of O_2 in peripheral capillaries, facilitates the loading of CO_2, while oxygenation in the lungs has the opposite effect.

A last word on CO_2 carriage…
Note that the greatest bulk of the CO_2 is in the form of bicarbonate (80 - 90%). The amount of dissolved CO_2 is small (5 - 10%), as well as the amount of CO_2 carried as carbamino-haemoglobin (5 - 10%).

Summary of Carbon Dioxide Transport [2]

In Plasma	*In Red Blood Cells*
1. Dissolved 2. Formation of carbamino compounds with plasma proteins 3. Hydration, H^+ buffered, HCO_3^- in plasma	1. Dissolved 2. Formation of carbamino-Hb 3. Hydration, H^+ buffered, large% of HCO_3^- enters the plasma 4. Cl^- shifts into cells

Of the approximately 49 ml of CO_2 in each 100ml of arterial blood, 2,6 ml is dissolved, 2,6 ml is in carbamino compounds, and 43,8 ml is in HCO_3^-. In the tissues 3,7 ml of CO_2 per 100ml of blood is added; 0,4 ml stays in solution, 0,8 ml forms carbamino compounds, and 2,5 ml forms HCO_3^-. The pH of the blood drops from 7,40 to 7,36. In the lungs, the process is reversed, and 3,7 ml of CO_2 is discharged into the alveoli. In this fashion, 200 ml of CO_2 per minute at rest and much larger amounts during exercise are transported from the tissues to the lungs and excreted.

CO_2 Dissociation curve [1,7]

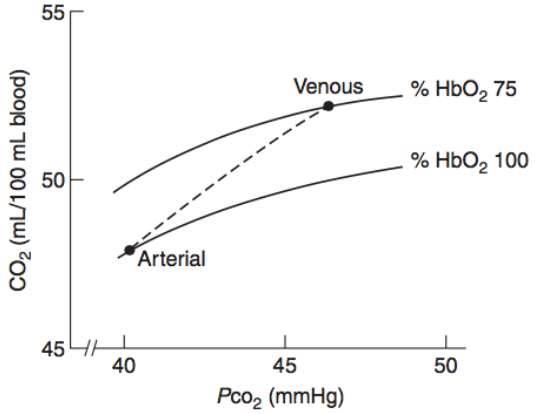

Figure 3.8 *The carbon dioxide–blood dissociation curve.*

Taken from Power I, Kam P. Chapter 3: Respiratory physiology. Principles of Physiology for the Anaesthetist, second edition. (p.86). Hodder Arnold

In contrast to the dissociation of oxygen from haemoglobin, the dissociation of CO_2 from blood is directly related to the PCO_2 and therefore the dissociation curve for CO_2 is linear. Note also that the lower the saturation of Hb with O_2, the larger the CO_2 concentration for a given PCO_2, This is the Haldane effect, and a detailed explanation will follow later.

The Bohr effect[1,2,4,6]

The decrease in oxygen affinity of haemoglobin when the pH of blood falls is called the Bohr effect and is related to the fact that de-oxygenated haemoglobin binds H^+ more actively than does oxyhaemoglobin.

The pH of blood falls as its CO_2 content increases, so that when the PCO_2 rises, the oxygen haemoglobin dissociation curve shifts to the right and the P_{50} rises. Note that most of the unsaturation of haemoglobin that occurs in the tissues is secondary to the decline in the PO_2, but an extra 1-2% of haemoglobin's unsaturation is due to the rise in PCO_2 and the consequent shift of the dissociation curve to the right (Fig. 3.7)

The Bohr effect takes place in tissues that are metabolically active, for example in the quadriceps muscles when you are walking! As I have mentioned earlier this magnificent piece of physiology helps with the unloading of oxygen from the haemoglobin molecule and it does so proportionally to the metabolic activity of the tissue. As more metabolism takes place, the carbon dioxide partial pressure increases and will cause larger reductions in local pH and in turn will allow for greater oxygen unloading. This is especially true in exercising skeletal muscles which may also release lactic acid that further reduces local blood pH and therefore enhances the Bohr Effect.

Now its time for a simplified explanation [6], some parts of this explanation have been discussed under the carriage of CO_2 already but repetition is good!
Imagine an individual quadriceps muscle cell in which CO_2 will be produced due to the Krebs cycle in the mitochondria. The produced CO_2 will diffuse out of the muscle cell down a partial pressure gradient into the interstitium, across the capillary wall, and into the plasma. Some CO_2 will dissolve in the plasma, and some will dissolve in the erythrocyte.
From our essential knowledge discussion, remember that approximately 10% of the CO_2 will directly dissolve in the plasma, and 90% will diffuse into the red blood cell (RBC). You may ask, what will happen to the 90% that diffuses into the RBC? Well 10% of the 90% of CO_2 that diffuses into the RBC will bind directly to haemoglobin to form carbamino haemoglobin and the remaining 80% will combine with H_2O to form H_2CO_3 (carbonic acid) with the help of the enzyme *carbonic anhydrase (CA)*.

$$CO_2 + H_2O \rightleftarrows H_2CO_3$$

(↑ carbonic anhydrase (present in RBC's and endothelium, not plasma))

H_2CO_3 will spontaneously dissociates into H^+ (hydrogen ion) and HCO_3^- (bicarbonate)

What happens with this formed HCO_3^- and H^+?

The HCO_3^-:
The HCO_3^- will leave the RBC and remember that 80% of the CO_2 produced by the quadriceps muscle will be transported to the lungs as bicarbonate in the plasma.
When HCO_3^- leaves the cell, Cl^- will enter the cell to maintain electrical neutrality. This is the *chloride shift*, which we have discussed earlier.

The H^+:
The H^+ will combine with the haemoglobin molecule. The H^+ ions bind to the α-amino and imidazole groups of haemoglobin and alter the allosteric conformation of the haemoglobin, which reduces the affinity of oxygen to haem [1]. Remember that this haemoglobin molecule is saturated with 4 oxygen molecules because it is coming from the lungs.
The H^+ will protonates the histidine residue in the haemoglobin molecule and this will cause stabilization of the T state. The T state of haemoglobin is not favourable for oxygen to be bound to haemoglobin and oxygen will be released for usage by the tissues.

The Haldane effect[1,2,7]

The increased capacity of deoxygenated haemoglobin to carry CO_2 is referred to as the Haldane effect.

De-oxygenated haemoglobin binds more H^+ than oxyhaemoglobin and de-oxygenated haemoglobin forms carbamino compounds more readily than oxyhaemoglobin.

Let's dissect and explore above statement:

De-oxygenated haemoglobin forms carbamino compounds more readily than oxyhaemoglobin
Explanation:
The attachment of oxygen to haem reduces the capacity of haemoglobin to carry CO_2. The reason for this is that O_2 increases the ionization of nitrogen groups, which reduces the capacity of the globin chain to carry CO_2 as carbamino compounds. De-oxyhaemoglobin can carry more CO_2 in the form of carbamino compounds, which account for about one third of the arterial venous difference of CO_2 carried in blood.

De-oxygenated haemoglobin binds more H^+ than oxyhaemoglobin
Explanation:
De-oxyhaemoglobin is more basic (due to the large number of imidazole groups present in the histidine moieties) and therefore has an increased capacity to mop up the H^+ ions produced when carbonic acid dissociates and so has an increased buffering capacity for CO_2.

Consequently, venous blood carries more CO_2 than arterial blood, and CO_2 uptake is facilitated in the tissues and CO_2 release is facilitated in the lungs.

A simplified way to explain the Haldane effect [6]

Let me set the scene:
Imagine a RBC that is returning from the hard working quadriceps muscle and is now arriving in a pulmonary capillary adjacent to an alveolus. Remember that the haemoglobin molecule in this RBC has given off oxygen to the quadriceps muscle. The P_AO_2 of the alveolus = 90 – 100 mmHg (12 – 13,3 kPa). In the plasma of this pulmonary capillary is HCO_3^-.This HCO_3^- will diffuse into the RBC, and as this happens, Cl^- will diffuse out of the cell (remember that at the quadriceps muscle, HCO_3^- diffused out of the RBC, and Cl^- diffuses into the RBC)
The haemoglobin in this RBC (de-oxyhaemoglobin) got a H^+ attached to it as well as CO_2 .
Now that the scene is set, let's see what happens:

1. Oxygen diffuses down its partial pressure gradient from the alveolus into the RBC.
2. As oxygen enters the RBC, it combines with haemoglobin.
3. When oxygen binds to haemoglobin, the H^+ and CO_2 are released from the haemoglobin molecule.
4. What happens to the released H^+?
    a. Well, H^+ will combine with HCO_3^- to form H_2CO_3 (carbonic acid)
    b. H_2CO_3 (carbonic acid) will dissociate with the help of CA (carbonic anhydrase) into $CO_2 + H_2O$
    c. The CO_2 formed from the dissociation of H_2CO_3 will diffuse out of the cell and will diffuse down a partial pressure gradient into the alveolus from where it will get exhaled!
5. What happens to the CO_2 that is released from the haemoglobin?
    a. This CO_2 will also diffuse out of the cell and will also diffuse down a partial pressure gradient into the alveolus from where it will get exhaled

The Double Bohr effect [1,8]

The double Bohr effect got to do with the exchange of oxygen and carbon dioxide between the mother and fetus. Let me set the scene to explain the double Bohr effect:

Think about the mother and fetus and think about the total amount of oxygen that goes from the mother to the fetus. Let's quickly revise some important anatomy that will help us to understand.

Umbilical cord:
Remember that there are 2 umbilical arteries and 1 umbilical vein in the umbilical cord. The umbilical arteries carry de-oxygenated blood from the fetus to the placenta and the umbilical vein carries oxygenated blood from the placenta to the fetus.

Placental structure:
The umbilical arteries branches into the chorionic plate. On the maternal side of the chorionic plate is a pool of blood. The chorionic plate got little extensions that dip into this pool of blood. In these extensions are fetal capillaries, which are tiny extensions of the umbilical vein and umbilical arteries. The mother's uterine arteries also open into the pool of blood, supplying oxygenated blood and the mother's uterine veins drain deoxygenated blood to the mother's lungs. Behind the pool of blood on the mother's side is the thick muscular uterine wall.

Let's look at the oxygen content on the mother's side and the oxygen content on the side of the fetus:

	PO_2	**HbA saturation**	**HbF saturation**
Uterine artery (mother)	100 mmHg	98%	
Uterine vein (mother)	40 mmHg	75%	
Umbilical artery (fetus)	18 mmHg		45%
Umbilical vein (fetus)	28 mmHg		70%

Have a look now at fig 14.12. Look at the differences between the 4 drawn curves.

- The **umbilical artery** has a higher carbon dioxide content and lower pH (and higher hydrogen ion concentration) than the **umbilical vein**.
- The difference in oxygen saturation between the umbilical artery and umbilical vein lines is called the Bohr effect.
- The **uterine vein** has a higher carbon dioxide content and lower pH (and higher hydrogen ion concentration) than the **uterine artery.**
- Similarly, the difference in oxygen saturation between the uterine artery and uterine vein is also called the Bohr effect.

Remember from our discussion earlier, the Bohr effect happens when carbon dioxide and hydrogen ions makes oxygen 'fall off' haemoglobin or haemoglobin doesn't bind oxygen well in the presence of carbon dioxide and hydrogen ions.

Let's discuss another obvious difference between the 2 curves of the fetus and the 2 curves of the mother.

- Appreciate that the fetal curves (umbilical artery curve and umbilical vein curve) are pushed to the left, this is because the fetus got haemoglobin F which has a higher affinity for oxygen compared to haemoglobin A.

Finally, let's look at the Bohr effects:

Bohr effect on the fetal side:

- The Bohr effect takes place when the release of carbon dioxide from the fetal blood inside the chorion enhances the uptake of oxygen. To illustrate this on the diagram, it is the vertical difference between the umbilical artery curve and the umbilical vein curve.

Bohr effect on the maternal side:

- Inside the pool of blood, the carbon dioxide levels are slowly rising, and the Bohr effect causes the release of oxygen molecules from the maternal haemoglobin, which causes a right shift of the curve. Again it can be illustrated on the diagram by the vertical distance between the uterine artery and uterine vein curve.

Because both these Bohr effects are happening in the placenta we call it the double Bohr effect. The double Bohr effect refers to the 4 lines illustrated in the diagram. All of this is happening in the placenta at the same time!

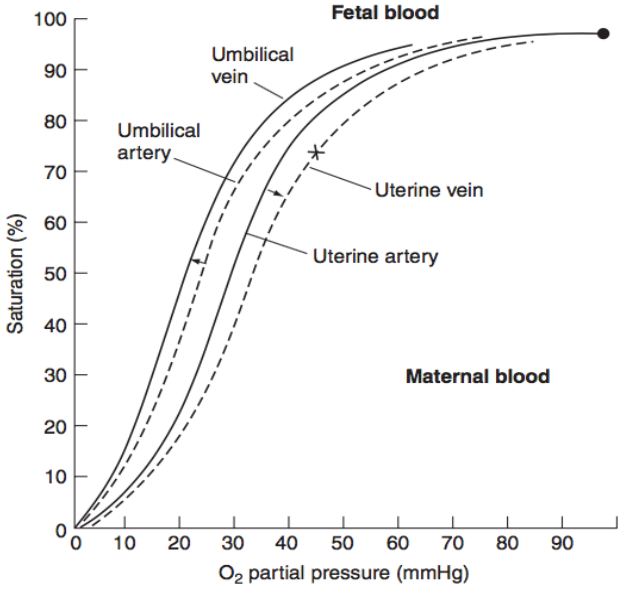

Figure 14.12 *Transport of oxygen from the mother to the fetus: the double Bohr effect.*

Taken from Power I, Kam P. Chapter 3: Respiratory physiology. Principles of Physiology for the Anaesthetist, second edition. (p.86). Hodder Arnold

References

1. Power I, Kam P. Chapter 3: Respiratory physiology. *Principles of Physiology for the Anaesthetist,* second edition. (pp. 81 – 86). Hodder Arnold.
2. Ganong WF. Chapter 35: Gas transport between the lungs and the tissues. *Review of Medical Physiology*, twenty-first edition. (pp. 669 – 674). Lange.
3. Yentis S, Hirsch N, Smith G. *Anaesthesia and Intensive Care A-Z*, fourth edition. Churchill Livingstone Elsevier.
4. Retrieved from http://www.pathwaymedicine.org/bohr-effect
5. Retrieved from https://www.khanacademy.org/science/health-and-medicine/advanced-hematologic-system/hematologic-system-introduction/v/bohr-effect-vs-haldane-effect
6. Retrieved from https://youtu.be/PM6maiHEnIM
7. JB West. Chapter 6: Gas transport by the blood. *Respiratory Physiology, The Essentials, seventh edition*. (pp. 75 – 83).
8. Retrieved from https://www.khanacademy.org/science/health-and-medicine/circulatory-system/fetal-circulation/v/double-bohr-effect