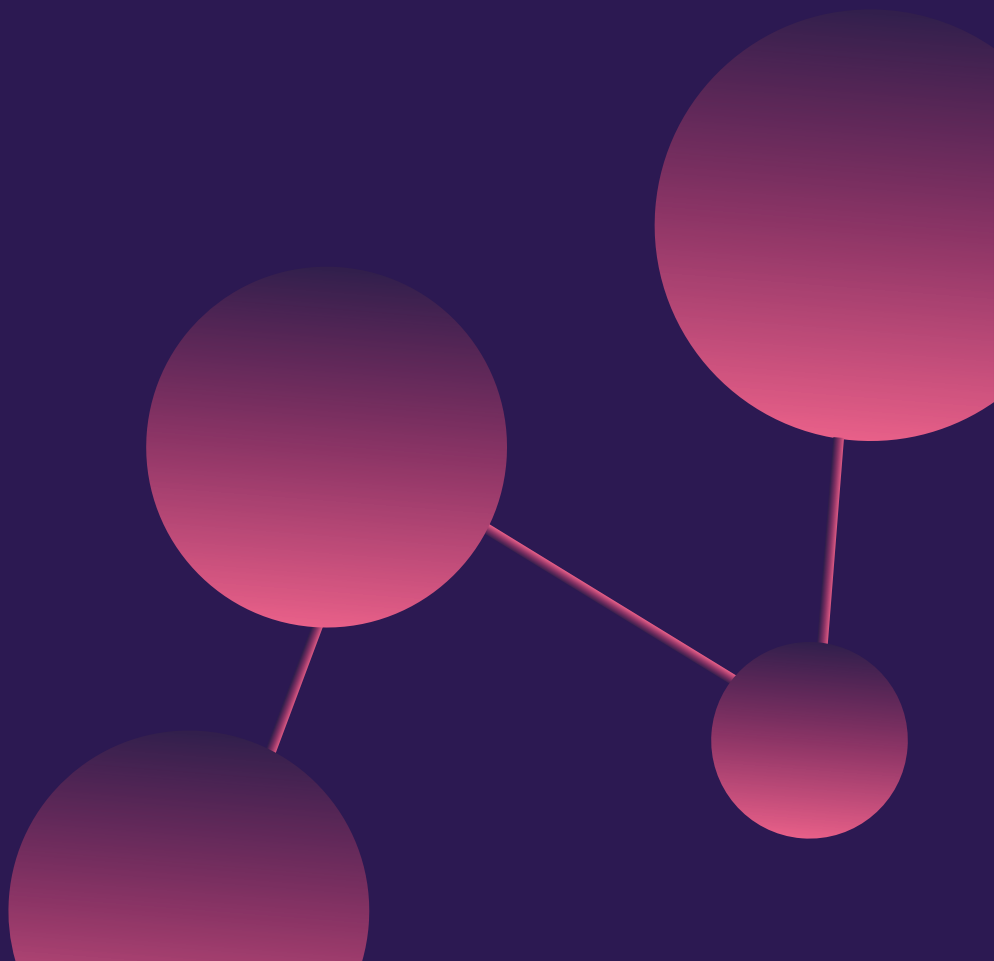
Royal College of Anaesthetists

e-LA
e-Learning Anaesthesia

e-LA Revision Guide:
Physiology

Version 1.00 July 2020



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These revision notes have been compiled from sessions in the Physiology module of e-Learning Anaesthesia to support your preparation for the Primary Exam.

The notes are presented as an interactive pdf document which can be downloaded to your smart phone, tablet or desktop computer for offline access

The Table of Contents contains active hyperlinks which allow you to jump straight to a section or topic.

Each topic also contains links to the relevant e-Learning sessions which you can access by clicking on the session id below the title of the topic e.g 07b_03_01. This will take you to the session information page on the e-LfH Hub from which you can log in and access the session.

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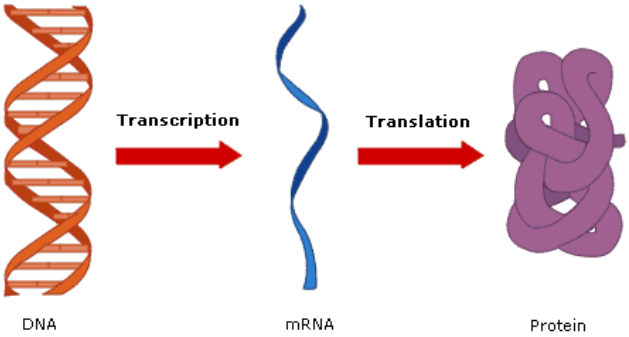
GENERAL PHYSIOLOGY

Gene Transcription and Translation and Protein Formation

(07b_01_01)

Transcription is the process of which DNA (deoxyribonucleic acid) → mRNA (messenger ribonucleic acid)

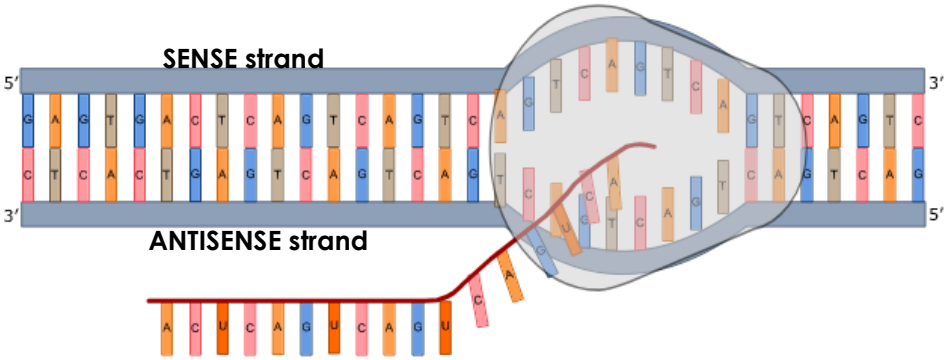
Translation is the process by which mRNA → amino acid sequence via tRNA within a ribosome.



Transcription

DNA is transcribed into mRNA. Occurs in the **nucleus**. The process is as follows:

RNA polymerase runs along the ***template strand*** of the DNA. As the hydrogen bonds are weak between the DNA strands, they can be easily pulled apart and it **'unzips'**.



As it unzips, there are **2 strands: anti-sense** and **sense strand** of DNA. The **complementary RNA nucleotides attach to the antisense strand** (3'→5') to create the mRNA through **complementary base pairing** (5'→3'). The pairings of the nucleotides (ant-sense→RNA) are as follows:

A→U **T→A** **C→G** **G→C**

The **DNA rezips** into the double helix structure once the single RNA is formed. The initially formed mRNA is then **processed into mature mRNA.**

Transcription Factors

These are **proteins** that **bind to specific DNA sequences** which affects the transcription of genetic information from DNA → RNA by being either:

1. **Promotors** → encourages transcription of specific genes
2. **Repressors** → blocks RNA polymerase activity at the gene

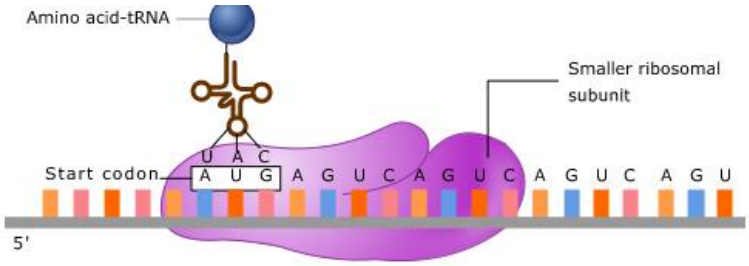
Some transcription factors **differentially regulates transcription** by interacting with the **enhancer regions of DNA** which sits next to the regulated genes.

RNA polymerase activity can also be affected by **interactions**, such as acetylation and methylation, **with histones**, which are the structural proteins of the DNA complex.

Translation

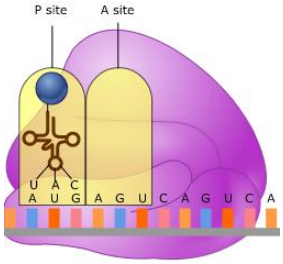
Translation of mRNA to an **amino acid sequence**. This occurs at the **ribosome** by the following steps

mRNA is bound by the **smaller 40S ribosomal unit** to form an **initiation complex**. This then allows attachment of the **specific tRNA-amino acid complex** to bind to the mRNA again through **complementary base pairing** at the **START codon** (**AUG**).

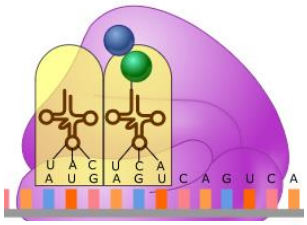


The **larger 60S ribosomal subunit** then joins the complex to form the **80S ribosome**. This has 2 sites to which tRNA can bind:

1. **Peptide (P) site** – this is the site at which the initiating tRNA binds onto
2. **Acceptor (A) site** – a tRNA that recognises the adjacent codon attaches to this site.

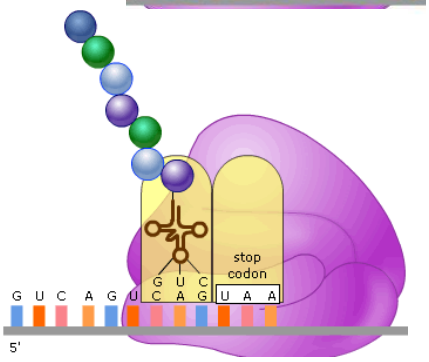


Adjacent binding of tRNA allows a covalent bond to form between the adjacent amino acids.



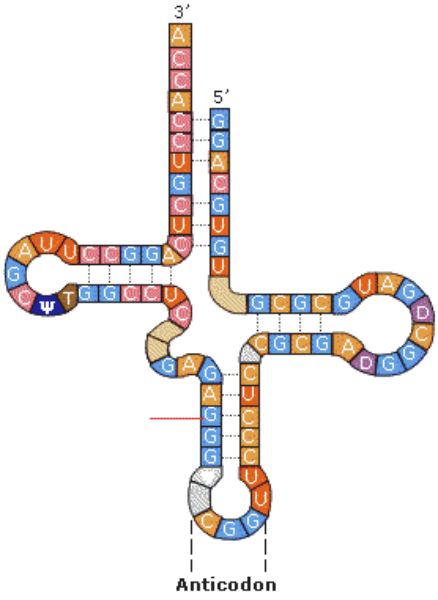
The **ribosome advances 1 codon** and the A site tRNA attaches to the P site and another tRNA attaches to the A site to form the next amino acid bond.

This continues until the **ribosome reaches a STOP codon** (**UAA**). This is a codon that doesn't code for an amino acid. The ribosome then **dissociates into its smaller subunits** and the **mRNA and protein** are **released**.



tRNA

Each tRNA molecule holds the **amino-acid through a covalent bond at the 3' end** and is loaded onto it through the action of **aminoacyl tRNA synthetases** which are highly specific enzymes.



Codon

Defined as the **sequence of 3 nucleotide bases of mRNA** which codes for a **specific amino acid** by attaching to the **complementary anti-codon of tRNA**. The sequence of codons therefore determines the amino acid sequence in the resulting protein.

- **START CODON: AUG** of mRNA. This also codes for **methionine** but if it is the 1st codon on the mRNA, it will act as a start codon.
- **STOP CODON:** May be either **UAA, UAG** or **UGA**.

Codon combinations

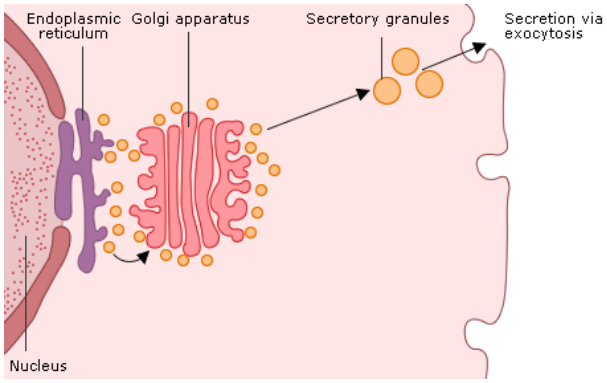
The codon-anticodon combinations are shown right. There is a total of 4^3 = 64 different codon combinations since there are 4 nucleotide bases that can have any combinations of 3. There are **20 amino acids.** If the codons were quoted using the DNA *sense strand*, then uracil will be substituted for thymine:

Second letter

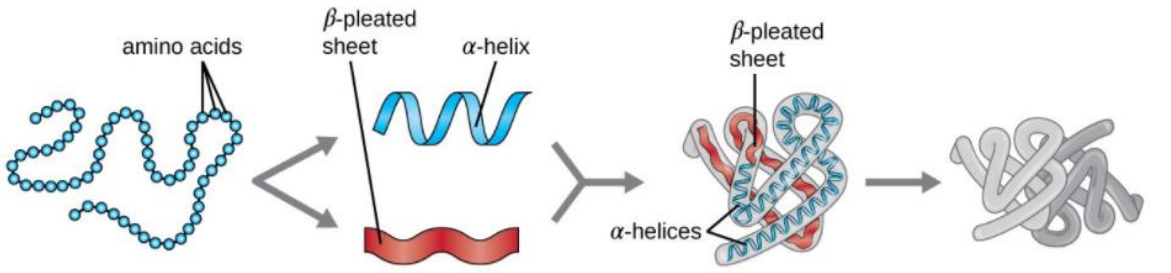
First letter	U	C	A	G	
U	UUU UUC Phenyl-alanine UUA UUG Leucine	UCU UCC UCA UCG Serine	UAU UAC Tyrosine UAA Stop codon UAG Stop codon	UGU UGC Cysteine UGA Stop codon UGG Tryptophan	U C A G
C	CUU CUC CUA CUG Leucine	CCU CCC CCA CCG Proline	CAU CAC Histidine CAA CAG Glutamine	CGU CGC CGA CGG Arginine	U C A G
A	AUU AUC AUA Isoleucine AUG Methionine; start codon	ACU ACC ACA ACG Threonine	AAU AAC Asparagine AAA AAG Lysine	AGU AGC Serine AGA AGG Arginine	U C A G
G	GUU GUC GUA GUG Valine	GCU GCC GCA GCG Alanine	GAU GAC Aspartic acid GAA GAG Glutamic acid	GGU GGC GGA GGG Glycine	U C A G

Protein Formation

The long chain of amino acids is known as a **polypeptide** or a **peptide chain**. The polypeptide leaves the ribosome and travels to the **golgi apparatus** to be further **processed and refined**. It is then 'packaged' into **secretory granules** that leave the cell through **exocytosis**.



Protein Structure



Primary Protein Structure
Sequence of a chain of amino acids

Secondary Protein Structure
Local folding of the polypeptide chain into helices or sheets

Tertiary Protein Structure
three-dimensional folding pattern of a protein due to side chain interactions

Quaternary Protein Structure
protein consisting of more than one amino acid chain

Primary structure: Describes where amino acids are linked via **covalent peptide bonds** in a **condensation reaction**.

Secondary structure: is where the polypeptide chain acquires a **specific geometric shape** due to the specific patterns of **hydrogen bonds.** The main structures are:

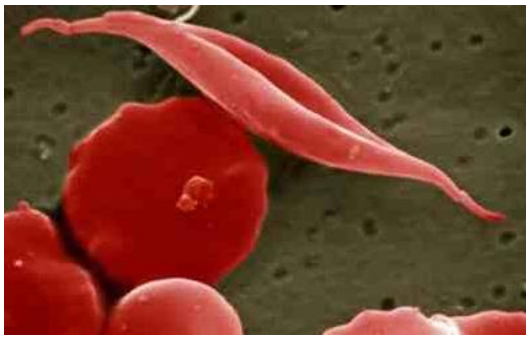
- **α helices**
- **β pleated sheets**

Tertiary structure: When the helices or sheets form a **unique 3D structure** causing various bonding arrangements.

Quaternary structure: This is when **>1 polypeptide/protein chain join together**. Fibrous or globular proteins result; haemoglobin is an example of the latter.

Genetic Mutations

These cause abnormalities in the protein structure. For example, in Sickle Cell Disease, there an **abnormal β-chain** of haemoglobin (due to **substitution mutation** glutamic acid →valine) which protrudes and hooks into the neighbouring β-chain and causes the Hb to lock together and be precipitated out of solution (sickling of the RBC).



Genetic mutations may include:

- **Substitution**
- **Insertions**
- **Deletions**

More disastrous as it changes the whole sequence of triplet codons from the mutation till the stop codon

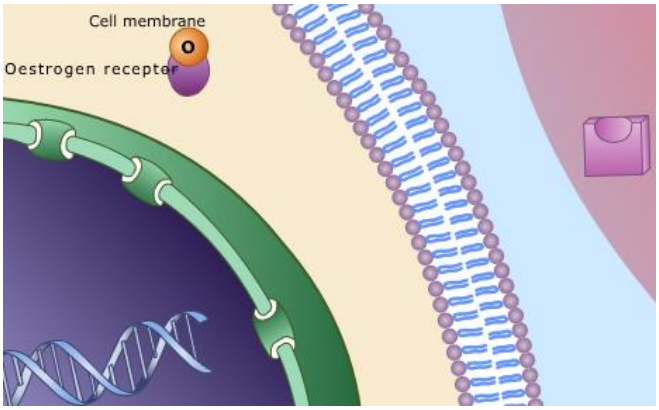
Cell Signalling with Intracellular Receptors

This is where cells communicate with each other by releasing molecules to trigger intracellular signalling in a target cell through intracellular/extracellular receptors. This may lead to **up/downregulation** of **target genes**.

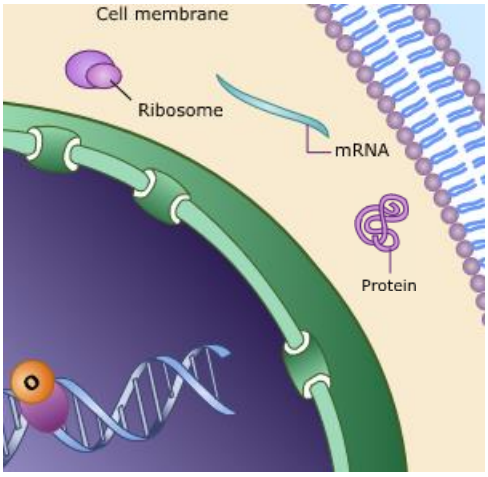
The creation of a **hormone/receptor complex** will attach to the specific region of the DNA molecule to affect gene expression. GENE TRANSCRIPTION FACTOR!

Oestrogen

Secreted by the ovaries and placenta and travels to the target cell by means of **attachment to carrier proteins** (as they are not water soluble) and readily crosses the target cell membrane via **diffusion** where it **binds to cytoplasmic oestrogen receptor** forming a complex:

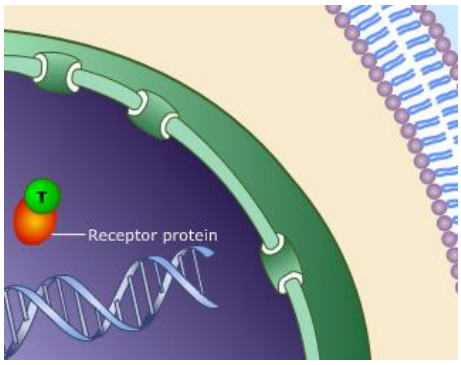


The complex enters the nucleus and binds to the specific DNA sequence known as the **'Hormone response element'** and subsequently **upregulates transcription** to form mRNA to direct protein synthesis:



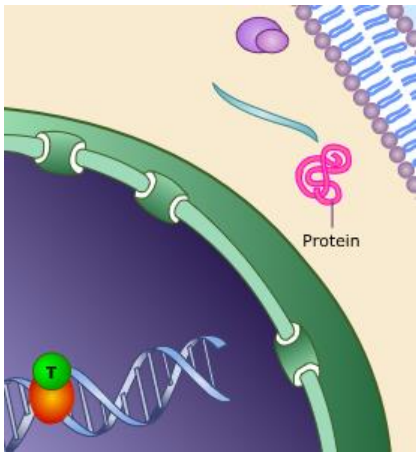
Thyroid Hormone

Thyroid hormone also travels in blood bound to carrier proteins as are not water soluble. It **enters the target cell via membrane transporter proteins** and enters the nucleus to **bind to its Intranuclear receptor**, leading to a **conformational change in the receptor**.



Hormone/receptor complex **promotes transcription** by binding to the **promoter region** of the thyroid responsive sequences of the DNA.

*NB the intranuclear receptor without thyroid hormone still normally interacts with DNA but leads to **repression** of transcription.*



Cell Membrane Characteristics and Receptors

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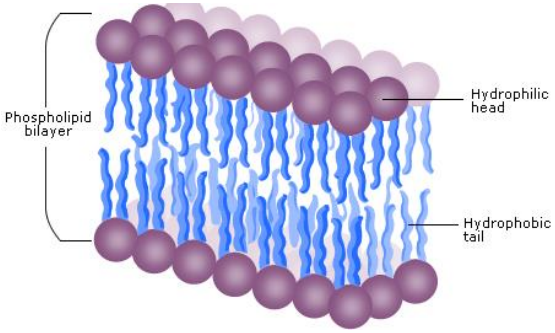
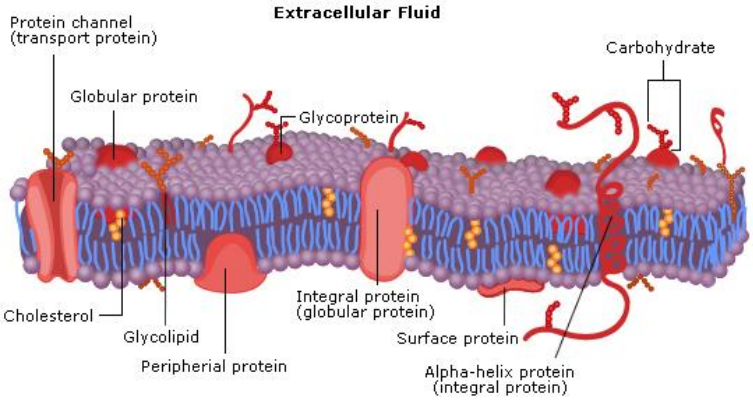
Structure



Phospholipid Bilayer: With the **hydrophilic polar water-soluble phosphate head** pointing outwards and the **hydrophobic non-polar insoluble tail** inwards. This therefore has a low permeability to ions and polarised molecules. Held together by Van der Waals forces, hydrogen bonds and non-covalent interactions.

Proteins

Accounts for 50% of the cell membrane mass and have multiple different functions (structural, enzymatic, receptor, transport). Multiple membrane proteins have **carbohydrate moieties** (functional group) which form **glycoproteins**. **Surface proteins** are anchored by glycosylphosphatidylinositol and form the basis for antigens and cell adhesion molecules.

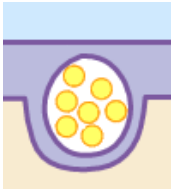


Additional functions other than structure include:

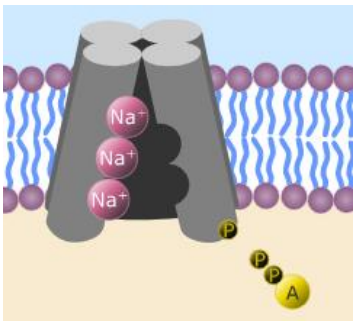
- **Pumps** for **active transport**
- **Carriers** for transport through **diffusion**
- Ion channels that open when activated
- Receptors to facilitate change within the cell
- Enzymes to catalyse reactions on the cell's surface.

Transport

1. **Diffusion** – the net movement of molecules down a concentration gradient
    a. **Simple diffusion** – across the cell membrane phospholipid bilayer
    b. **Facilitated Diffusion –** Through the action of carrier proteins
2. **Osmosis** – movement of a solvent from an area of low→ high concentration through a **semi-permeable membrane**.
3. **Active Transport** – movement of particles against their concentration gradient using energy
    a. **Primary AT –** chemical energy used from ATP see below example of Na^+/K^+ pump
    b. **Secondary AT –** energy used from an electrochemical gradient that is maintained by an active process.
4. **Endocytosis** – cell absorbs molecules through **engulfing them**. These molecules are often large or polar so are unable to cross the membrane any other way.
5. **Exocytosis –** Excretory vesicles are directed out of the cell membrane from within the cell to the EC compartment.

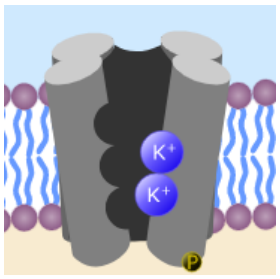


Na⁺/K⁺ Pump



3 sodium ions from within the cell bind to the cytoplasmic side of the protein **changing its conformational** shape allowing it to be **phosphorylated** by ATP.

Phosphorylation induces a **further conformational change** which **opens to the ECM** and additionally gains **lower affinity for Na⁺** which allows dissociation and diffusion of sodium to the extracellular fluid. The new conformation has **high affinity for K⁺** and **2 bind** to the extracellular side of the protein.



Phosphate dissociates and the protein reverts to its normal conformation exposing the K⁺ ions to within the cell and has a low affinity to K⁺ which then dissociate into the cell

Receptors

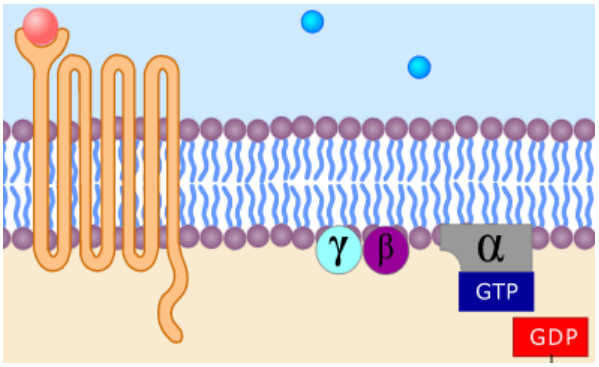
Receptors receive specific chemical signals from the environment via **ligands** to induce a cellular response through promotion or repression of an activity.

1. G-PROTEIN RECEPTORS

Metabotropic receptors are those that achieve activity via internal secondary messengers. G-Protein receptors have **7 transmembrane domains** and have an effect on GTP proteins. Examples include Muscarinic acetylcholine receptors, Adrenoreceptors and Histamine receptors and 30% of modern medicines act via G-proteins. Subsequent signal transduction is through:

- Adenylate cyclase (G_S/G_i)→ cAMP
- Phospholipase C (G_q) → Phosphatidylinositol (IP_3)

Mechanism of Action with Calcium Channels

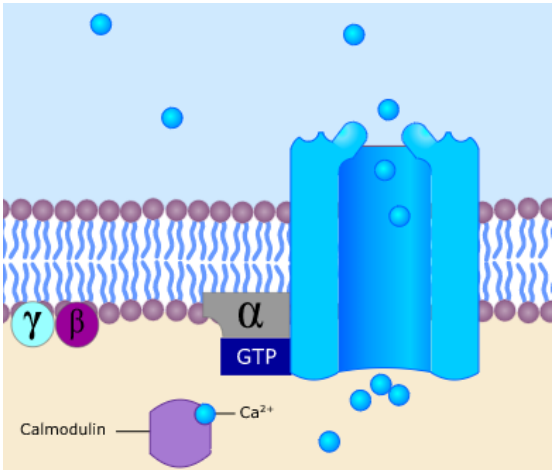


The resting position of G-protein is when the alpha subunit is bound to GDP rendering it inactive (see above image). **Ligand binds** to a receptor site which allows a **change in conformation** of the protein and the **GDP** bound to the **alpha subunit** is **replaced by GTP:**

GTP-a binds to calcium channel ion causing it to open and leads to a **diffusion of calcium into the cell** and **binds to calmodulin** which modulates the cell response.

Phosphorylase (**GTPase**) **removes a phosphate from GTP** leaving GDP bound to the alpha subunit which **detaches from the calcium ion channel** closing it and recombines with the beta & gamma subunits which recombine with the receptor

This describes **signal amplification** – a modest stimuli leads to a greater intracellular response.

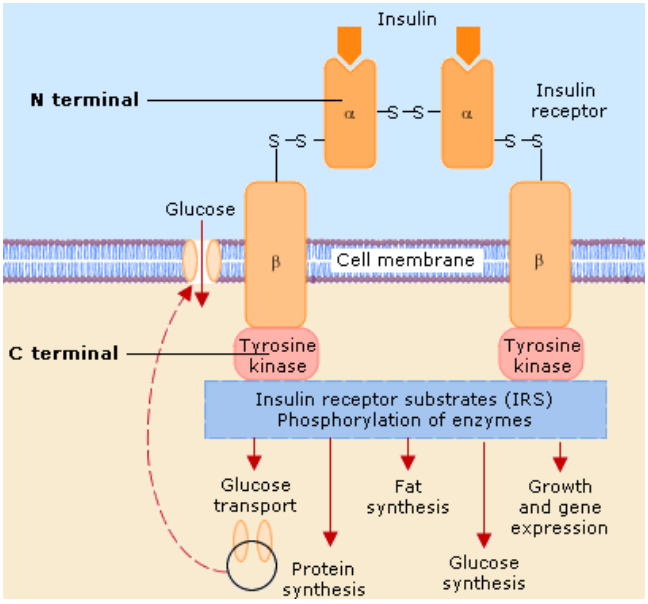


NB GTP-a complex can act on effector organs or directly on ion channels

2. TYROSINE KINASE RECEPTORS

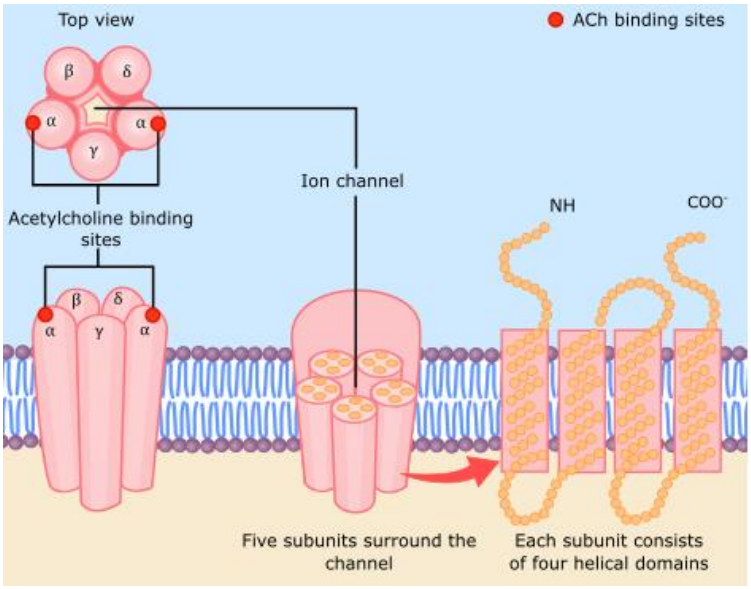
Example ligands include insulin and erythropoietin. They all have an **extracellular N-terminal** which contains the **ligand binding site** and an **intracellular C-terminal** responsible for the **kinase activity.** Kinase enzymes are responsible for **phosphorylation of tyrosine residues → signal transduction pathway activation**.

Insulin activates TK receptors through binding of insulin to alpha subunits which in turn causes phosphorylation through TK domains on the beta subunits. Through signal transduction, they induce vesicles containing **Glut-4 transporters** to insert into the membrane allowing **increased uptake of glucose into the cell.**



3. INOTROPIC RECEPTORS

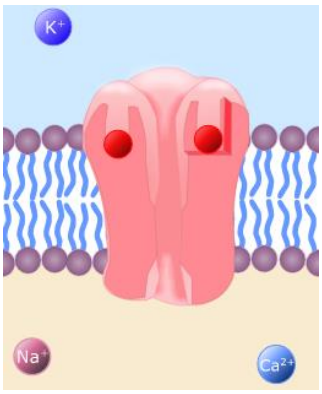
Ligand gated ion channels that open and close in response to ligand binding. Examples include nicotinic ACh receptors, NMDA glutamate receptors and $GABA_A$ receptors. They all have the same function. The **binding site is usually located on a different part of the protein** to the ion channel, this is known as **allosteric binding**.



Nicotinic Receptor

Made up of **5 subunits** arranged around a central pore. The adult form has **2 alpha, 1 beta, delta and epsilon unit**. Foetal has a gamma subunit instead of an epsilon.

2 ACh must bind to the **2 alpha subunits** which induces a conformational change to open the pore. This allows cations, mainly Na^+ in and K^+ out of the cell down the **concentration gradient**.



4. INTRACELLULAR RECEPTORS

Located within the cell and examples include thyroid (previous lecture), sex hormones, Vitamin D receptors and the IP_3 receptor found on the endoplasmic reticulum.

Acid Base Balance and Buffers

(07b_01_03)

Strong Acid is one that fully dissociates in solution as a proton donor cf weak acid

Strong base fully dissociates in solution cf weak base

Acid-base buffer - weak acid and its conjugate base. A conjugate base is the dissociated anionic product of an acid

Acid	Conjugate Base
Carbonic acid H_2CO_3	**Bicarbonate ion** HCO_3^-
Lactic acid $C_3H_6O_3$	**Lactate ion** $C_3H_5O_3^-$
Ammonium ion NH_4^+	**Ammonia** NH_3
Dihydrogen phosphate e.g. H_2PO_4	**Monohydrogen phosphate** HPO_4^{2-}
Acetoacetic acid $CH_3COCH_2CO_2H$	**Acetoacetate** $CH_3COCH_2CO_2^-$

At 25°C, a neutral pH = 7.0

At 37°C, a neutral pH = 6.8

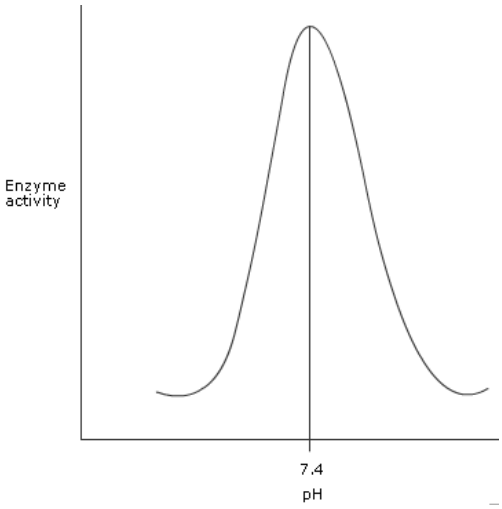
pH must be kept in physiological range in order to allow normal functioning of the proton 'pump' within the mitochondria – essential for '***oxidative phosphorylation***'

Acid Production and maintenance

Volatile acids are produced in the form of **carbon dioxide** in the citric acid (Krebs) cycle (13 000-15 000 mmol/day) and from **metabolism of amino acids** to produce **non-volatile** or **fixed acids** (50-80mmol/day).

It is important to maintain a normal $[H^+]$ as is important for:

1. **Homeostasis** – see above point for oxidative phosphorylation
2. **Metabolism –** may be the product of metabolism
3. **Ionic flux –** influences ionic gradients
4. **Other functions** including enzymes, proteins, ions and overall organ function

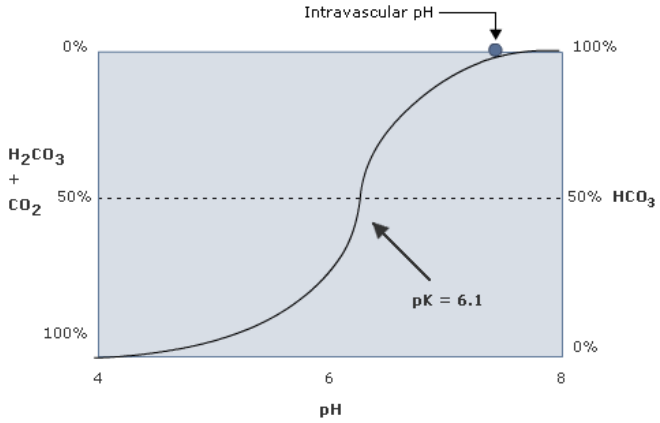


Defence against Acidaemia

Defence mechanism	Time frame
Buffer systems	Seconds
Respiration	Minutes
Renal	Hours
Hepatic	Hours

1. BUFFER SYSTEMS

There are several buffers in the body. They are measured by the **pK** – this is the value describing the pH at which the buffer exists as 50% ionised (and hence most efficient pH to act as a buffer (on the steepest part of the curve))



Henderson-Hasselbach Equation and Bicarbonate Buffer System

Allows us to explain the dynamics of a buffer system. Using bicarbonate as an example, through **mass effect, $H_2CO_3 \leftrightarrow H^+ + HCO_3^-$** pH will be as follows where **k=equilibrium constant**:

$$\frac{[H^+] \times [HCO_3^-]}{[H_2CO_3]} = K \Rightarrow [H^+] = K\frac{[H_2CO_3]}{[HCO_3^-]} \Rightarrow [H^+] = K\frac{\alpha PCO_2}{[HCO_3^-]}$$

H_2CO_3 cannot be measured so the term is replaced by "proportional to PCO_2"so all can be measured.

NB, the bicarbonate can be replaced with any other buffer in this equation and its pK.

$$pH = pK + \log\frac{[HCO_3^-]}{\alpha PCO_2}$$ **Henderson-Hasselbalch equation** where negative logarithms of each reciprocal is taken. pK is 6.1 but varies with temperature and pH.

In the body, although the pK of bicarbonate is weak (pK=6.1) and its concentrations are small, its constituents can be easily maintained with lungs and kidneys making it a **truly dynamic buffer**.

Other Buffer Systems

PHOSPHATE: $H_2PO_4^-$ and HPO_4^-, extracellular concentration is 1/12 that of bicarbonate and its pK = 6.8. It primarily acts in the tubular fluid of the kidney where phosphate is concentrated near its pK.

PROTEIN: Cell and plasma proteins have carboxyl and amino groups that can dissociate. Haemoglobin has **histidine residues** which is the most powerful buffer providing 75% of all intracellular buffering.

CARBONATE: Located in bone. Also allows ion exchange between strong ions in bone in prolonged metabolic acidosis states.

Proportions of buffering as blood pH falls from 7.4 → 7.0 are as follows:

	Haemoglobin	Bicarbonate	Plasma Protein	Phosphate
Amount of buffer per litre of blood	150 g	25 mmol	40 g	1.25 mmol
Amount of H^+ ion absorbed	8 mmol	18 mmol	1.7 mmol	0.3 mmol

'Whole Body' Buffering

Dynamic process where each buffer acts in balance to each other. The mathematical relationship between buffer systems is known as the **isohydric principle**. This describes that all buffer pairs (HA and A^-) in the system are in equilibrium with the same proton concentration $[H^+]$: Only those buffers with a pK within 1 pH unit of that in the solution participate effectively in the buffering of the solution pH.

$$H^+ = \frac{K_1 \times HA_1}{A_1^-} = \frac{K_2 \times HA_2}{A_2^-} = \frac{K_3 \times HA_3}{A_3^-}$$

2. RESPIRATORY RESPONSE

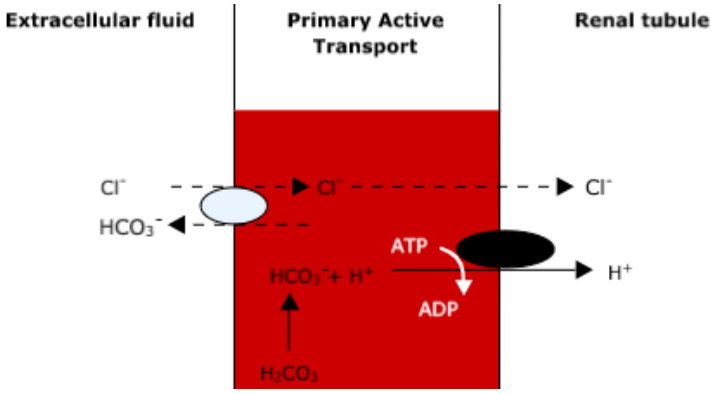
This is largely dealt with in respiratory physiology notes. It has a **control effectiveness of 50-75%** which describes the ability of a homeostatic mechanism to change the parameter it controls i.e. the lungs will only deal with 5-7.5 nmol of a 10 nmol change in hydrogen ion concentration.

3. KIDNEYS

This is largely dealt with in renal physiology notes. Allows H^+ secretion, HCO_3^- filtration and HCO_3^- production. H^+ is secreted through active transport from the epithelial cells and bicarbonate interaction within the collecting system.

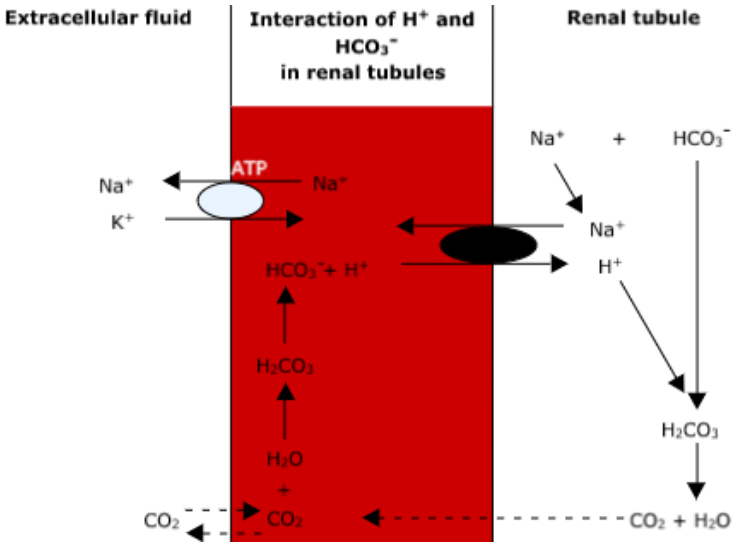
***Secondary Active Transport*:** Occurs in exchange with sodium as sodium is transported into the luminal cell by diffusion. This does not concentrate hydrogen ions as much as:

***Primary Active Transport*:** mechanism is able to concentrate hydrogen 900-fold. Specific hydrogen transporting ATPase enzyme and transport protein (hydrogen transporting ATP) allow transport at the **distal collecting duct** whilst the excess bicarbonate is reabsorbed into the extracellular fluid in exchange for chloride:



***Bicarbonate Interaction*:** Filtered by the glomerulus and combines with the hydrogen ions. Following dissociation to water and CO_2, CO_2 diffuses back in extracellular or into the cell. This allows titration within the tubules and the kidney corrects imbalance by **incomplete titration:** The difference is accounted for by non-volatile acid production

H^+ secretion = 3.5 nmol/min,
HCO_3^- filtration = 3.46 nmol/min



Other buffers exist in the tubules in case H^+ is secreted in excess. This includes **phosphate** and **ammonia**.

4. HEPATIC

The liver is highly metabolically active and is responsible for the following:

- CO_2 production from oxidation
- Metabolism of organic acid anions
- Production of plasma proteins
- Metabolism of ammonia

Ureagenesis which is the following reaction. The degree of ureagenesis is controlled by the liver and responds according to the amount of bicarbonate needing to be conserved or consumed.

M	Methanol
U	Uraemia
D	DKA
P	Paraldehyde
I	Isonicotinic Acid Hydrazide (ISH); Isoniazid
L	Lactic acidosis
E	Ethanol; Ethylene Glycol

Amino acid metabolism → HCO_3^- and NH_4^+

$2NH_4^+ + 2HCO_3^- \rightarrow NH_2.CO.NH_2 + CO_2 + 3H_2O$

Hyperlactataemia

Defined as lactate >2mmol/L and pH <7.35. Lactate is produced as a product of anaerobic metabolism along with water and ATP → H^+ is generated from the breakdown of ATP.

- *Type A hyperlactataemia* – impairs oxygen delivery
- *Type B hyperlactataemia* – doesn't impair normal oxygen delivery

It may be caused by reduced oxygen uptake/utilisation, increased cellular production and reduced lactate clearance. The **lactate:pyruvate ratio** helps determine whether there is any tissue hypoxia as pyruvate is oxidised in the presence of oxygen in the Kreb's cycle but forms lactic acid in anaerobic conditions:

- Lactate:Pyruvate > 10:1 indicates tissue hypoxia
- Normal ratio = 10:1

Anion Gap

$$(Na^+ + K^+) - (Cl^- + HCO_3^-)$$

Normal range = 8-16

Gives an indication of the severity and possible source of metabolic acidosis. The unmeasured anions are from normal proteins i.e. albumin. Paraproteins in multiple myeloma also increases the anion gap. In many situations, this measurement may be changed due to i.e. haemolysis, alterations in renal function.

High Anion Gap acidosis

High anion gap conditions result because **bicarbonate concentrations decrease without increase in Cl^-** in response to the need to buffer the increased presence of acids and is therefore replaced by the unmeasured ion to maintain electroneutrality.

M	Methanol
U	Uraemia
D	DKA
P	Paraldehyde
I	Isonicotinic Acid Hydrazide (ISH); Isoniazid
L	Lactic acidosis
E	Ethanol; Ethylene Glycol
S	Salicylates

Causes are remembered by the mnemonic **MUDPILES**

Normal Anion Gap Acidosis

Aka **hyperchloraemic acidosis** because the drop in **HCO_3^- is compensated for almost completely by an increase in Cl^-.** Multiple causes include:

Cause	Examples
Gastrointestinal loss of HCO_3^-	Diarrhoea Note: Vomiting causes hypochloraemic alkalosis
Renal loss of HCO_3^-	Proximal renal tubular acidosis
Renal dysfunction	Renal failure Hypoaldosteronism Distal renal tubular acidosis
Ingestions	Ammonium chloride Acetazolamide Hyperalimentation fluids (i.e. total parenteral nutrition)
Some cases of ketoacidosis	Particularly during rehydration with Na^+ containing IV solutions
Alcohol (such as ethanol)	Can cause a high anion gap acidosis in some patients, but a mixed picture in others due to concurrent metabolic alkalosis

Low Anion Gap

Relatively rare and most commonly due to **low albumin** (80% of unmeasured ions). Also may be caused by abnormal increases in cations. Causes of a hypoalbuminaemia include:

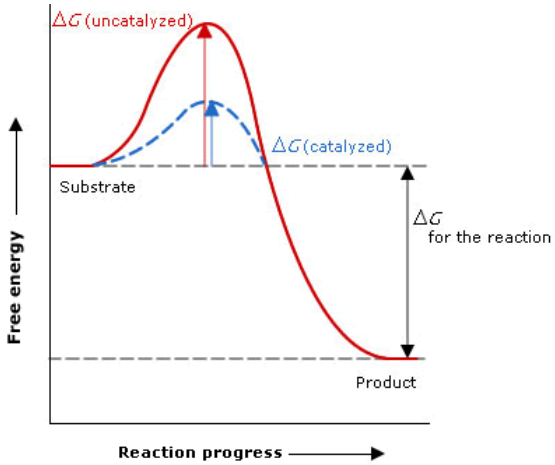
- **Decreased synthesis**
- **Increased Catabolism**
- **Increased loss**
  - Nephrotic syndrome
  - Loss through exudate in burns
  - Haemorrhage
  - Gut loss
- **Redistribution**
  - Haemodilution
  - Increased capillary permeability
  - Decreased lymph clearance
- **Capillary Leak syndrome:** occurs in SIRS associated with sepsis and leaks albumin to the extravascular space.

Cellular Metabolism and Enzymes

(07b_01_04)

Enzymes

Thermodynamic Principle: Enzymes function to **lower the activation energy** for a particular reaction usually by promoting a transition state in the substrate molecules. Uncatalyzed reactions depend on kinetic energy of molecules to bring out a reaction which at body temperature is too low. The end **free energy of the reaction is unchanged** (ΔG) with or without catalysis.



Lock and Key Model: All enzymes have an **active site** which contains multiple amino acid residues which promote a reaction i.e. oxidation/reduction or hydrolysis in order to allow other molecules to react with the substrate. They have **high specificity** to a specific reaction and few substrates.

Induced Fit Hypothesis: Enzymes do not have a rigid configuration and a **conformational change** occurs in the enzyme. The change in configuration promotes the lower energy transition states or allows other molecules to interact. This has been confirmed with **hexokinase** in Glucose + ATP → glucose-6-phosphate + ADP.

Enzymes are very efficient i.e. carbonic anhydrase speeds up conversion to bicarbonate 10^5 faster and urease speeds up hydrolysis of urea to carbon dioxide and ammonia by a factor of 10^{14}.

Co-Factors and Co-Enzymes

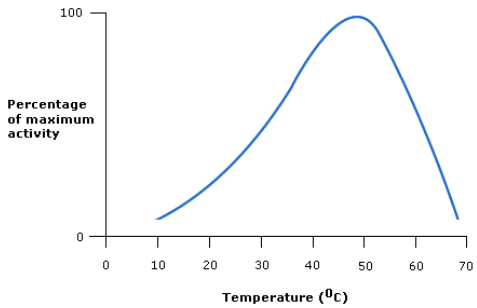
Enzymes sometimes require the presence of other factors or molecules. Broadly this is divided into 2 different groups:

1. **Metal ions or 'Co-Factors' i.e.**
    a. Zn^{2+} in carbonic anhydrase;
    b. Mg^{2+} in hexokinase,
    c. Fe^{2+} in cytochrome oxidase
2. **Organic molecules or 'Co-enzymes' i.e.**
    a. Co-enzyme A involved in acyl group reactions
    b. Co-enzyme B_{12} involved in alkyl group reactions

Effect of pH and Temperature on Enzymes

Primarily the **rate of reaction is determined by the substrate concentration** and the **intrinsic ability of the enzyme to catalyse the reaction.** Temperature and pH also affects enzyme kinetics.

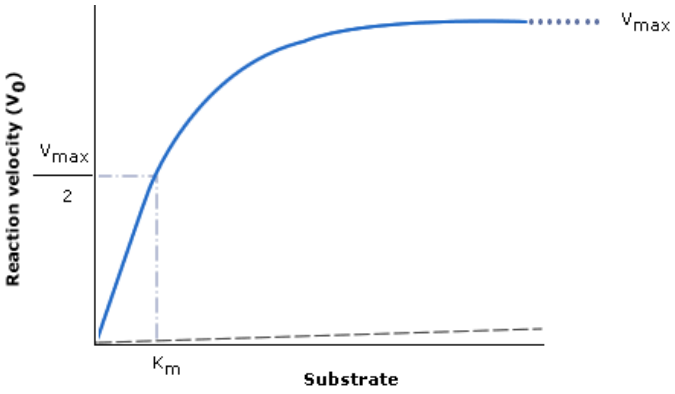
- **Temperature** – increasing kinetic energy → increases rate of reaction. However, above a certain temperature, enzymes may denature:
- **pH** – Hydrogen ions can affect the degree of ionisation at the active site and also may alter the general structure of the protein. Each enzyme has its optimal pH i.e. stomach vs intestinal.



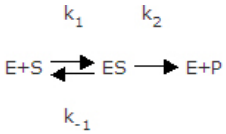
Enzyme Kinetics

Most metabolic reactions proceed too slowly at body temperature without enzymatic processes to speed them up. The following graph plotted as substrate concentration vs reaction velocity is seen in most enzymes – **hyperbolic function**.

K_M is the substrate dose at which the reaction velocity is half its maximal value. The dotted line represents the uncatalyzed version of the reaction



***Michaelis Menton*: This describes the rate of enzyme activity as substrate concentration rises until it reaches enzyme saturation**



S = Substrate, E = Enzyme, P = Product, ES = Enzyme substrate complex and k's are rate constants.

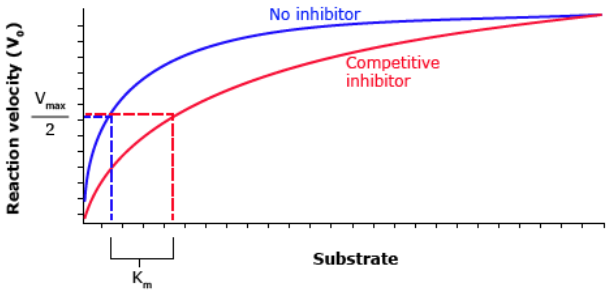
From this relationship, the equation is as follows: $$V_0 = \frac{V_{max}[S]}{K_m + [S]}$$

K_m is the Michaelis constant where $(K_{-1} + K_2)/K_1 = K_m$.

K_M changes according to the affinity of the substrate for an enzyme i.e. a higher affinity = lower K_M. For all enzymes, the kinetics are initially **first order at low substrate concentrations** whereas at **high concentrations**, the reaction velocity is constant and independent of substrate concentration(**zero order kinetics**)

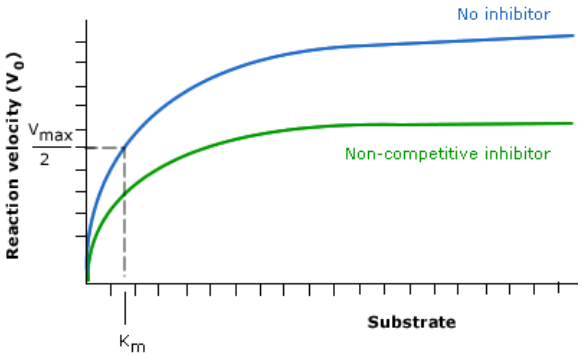
Enzyme Inhibition

Competitive Inhibition occurs when an inhibitor binds to the active site to block the normal binding with the substrate. It can also bind to a site distant from the active site to induce a conformational change at the active site. Both mechanisms can be **overcome by increasing the substrate concentration**.



Therefore, in the presence of a competitive inhibitor, **K_M is increased** and **V_{MAX} is unaffected** as can be overcome through gross increases in substrate concentration.

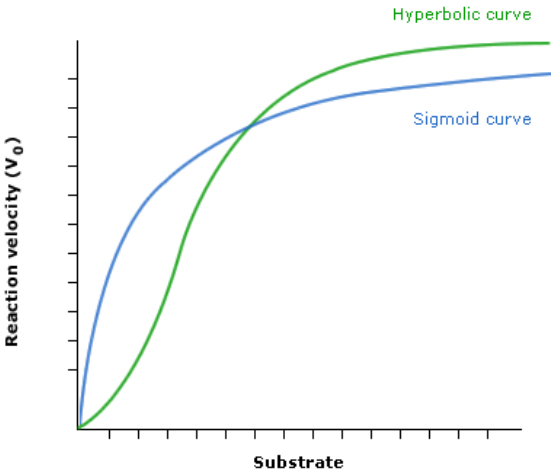
Non-Competitive Inhibition occurs when the inhibitor **binds covalently** to the enzyme causing a **permanent conformational change** in the enzyme. The catalytic activity of the enzyme is reduced by this process and the net result is similar to a total reduction of enzyme present.



Therefore, in the presence of a non-competitive inhibitor, **K_M is unchanged** and the **V_{MAX} is reduced**.

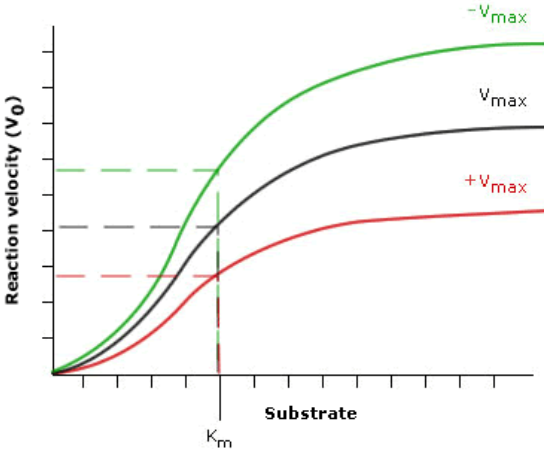
Allosteric Enzymes

Some enzymes undergo a conformational change when bound to an secondary effector substance, this may alter the affinity for and rate of conversion of the primary substrate, they are termed Allosteric Enzymes. These enzymes have a sigmoid shaped velocity/substrate profile. An example is haemoglobin with 2,3 DPG.



Regulatory Enzymes

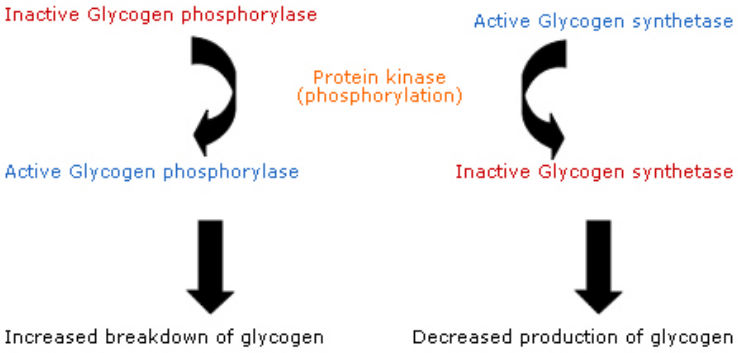
Allosteric effectors can affect V_{MAX} as well as K_M. The **K_M is unaffected** but the **V_{MAX} of the enzyme has been altered**. The graph is similar to the non-competitive inhibitor.



Some allosteric effectors affect the K_M of the allosteric enzyme but the V_{MAX} is unchanged. This graph is similar to the competitive inhibitor hyperbolic effects.

The end product of a series of catalysed reactions can inhibit the initial enzymatic process providing a **negative feedback system**.

Regulatory enzymes commonly involve **phosphorylation. Protein kinase** activation causes the **phosphorylation and inactivation of glycogen synthetase** and **phosphorylation and activation of glycogen phosphorylase**. This causes a decreased glycogen production and increased glycogen breakdown respectively. This is a SINGLE enzyme system.



Pro-Enzymes

These are created as deactivated enzymes known as **zymogens** or **pro-enzymes** and following its secretion out of the cell, subsequent cleavage activates the enzyme. This is important with **proteases** as they can break down proteins within the cell.

Body Water and Compartments

(07b_01_05)

There is approximately 60% water (42L in a 70kg man) which varies with age. Grouping tissues into compartments allow a more manageable concept of water distribution. The 2 main compartments are the **intracellular** (**ICF**) and **extracellular** (**ECF**) fluid compartments separated by the cell membrane.

- **INTRACELLULAR FLUID COMPARTMENT** ~ 2/3rd total body water (28L)
    - Consists of fluid within the cells which all have similar properties regarding ionic compositions.
- **EXTRACELLULAR FLUID COMPARTMENT** ~ 1/3rd total body water (14L)
    - Not as clearly defined as the ICF but grouped into different areas:

Fluid	Volume (L)	Total body water (%)	Body weight (%)
Intravascular fluid	**4.0**	**9.5**	**7.0**
Interstitial fluid	**9.0**	**21.0**	**13.0**
Transcellular fluid	**1.0**	**2.0**	**1.5**
Bone and connective tissue	**1.0**	**2.0**	**1.5**

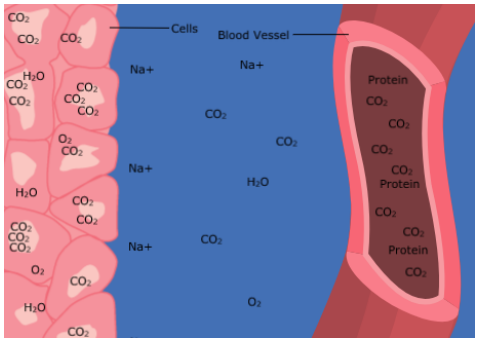
Intravascular Fluid Compartment

~1/3rd of ECF, (4L). Consists of **plasma** (55%) and blood cells (RBC, WBC and platelets and classified as ICF). The plasma consists of:

1. **Water (90%)** – the content of which depends on the relative osmotic pressure in differing compartments and retention intravascularly depends on the oncotic pressure provided by:
2. **Proteins (7%) –** Albumins (60%), Globulins (35%) and Fibrinogen (4%). Under normal conditions, they are unable to pass through fenestrations in vascular walls.
3. **Ions**

Interstitial Fluid

Largest ECF fluid compartment. Surrounds the cells to **act as a transport medium** between the **intravascular** and **intracellular fluid** compartments and includes the **lymphatic system**. It has a **similar constitution to plasma but without the protein content**. Therefore, with the same ionic concentrations as plasma, they share the same osmolality.

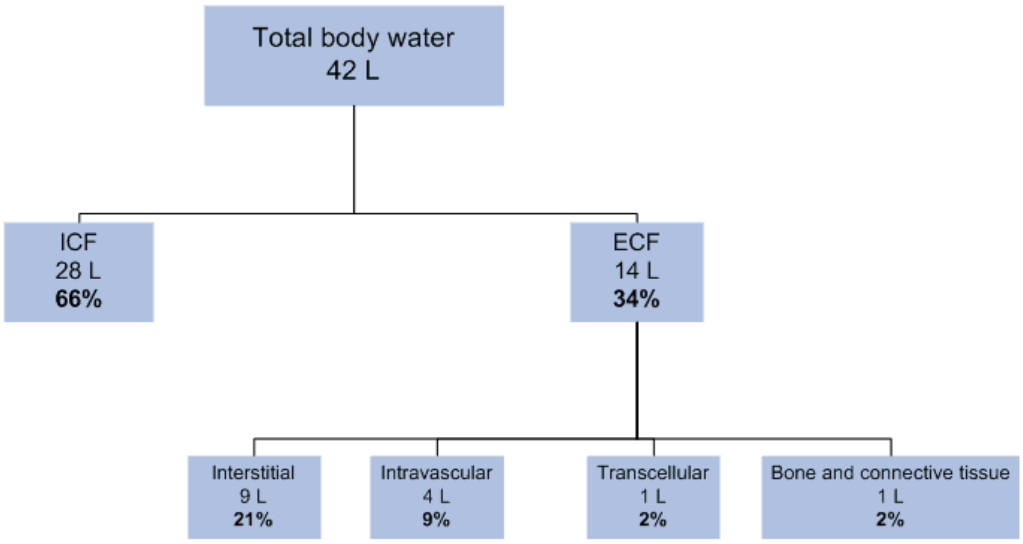


Transcellular Fluid

Consists of fluid secreted but **separated from plasma by an epithelial layer** and includes:

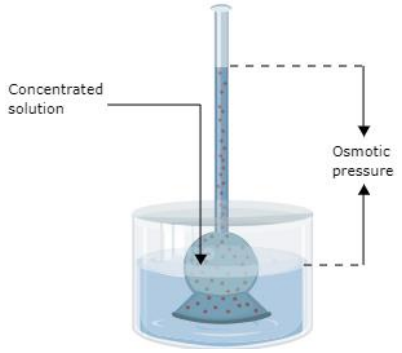
1. Gastrointestinal
2. CSF
3. Bladder urine
4. Aqueous Humor
5. Bile
6. Sweat

Summary



Movement of Fluid Between Compartments

Osmosis describes the movement of water across a semi-permeable membrane from an area of relative low to high solute concentration to achieve equal osmolality.



Osmotic Pressure: This is the hydrostatic pressure required to prevent movement of water down a concentration gradient. The greater number of osmoles, the greater the pressure required to prevent its movement:

Osmolality

This is the number of **osmoles per kg of water**. (Osmolarity is the number of osmoles per litre but this is dependent on temperature so not commonly used).

Plasma osmolality = urea + glucose + (2 x Na^+) and is approximately **280-305 mosmol/kg** (and hence the same in plasma/ISF/ICF) and is kept relatively constant. The osmolality determines the size of the compartment in question:

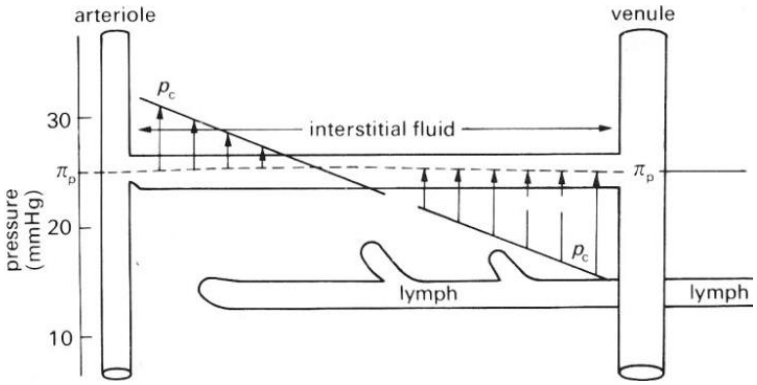
Distribution between the ICF and ECF

ICF osmolality remains constant so the **principle influence on distribution of water is the osmolality of the ECF** which is largely dependent on **sodium ions.**

Distribution within the ECF

As it allows free passage of most substances, the distribution of water therefore, occurs secondary to the **relative volumes of different compartments** and therefore determined by the actual **number of solute particles rather than osmolality** (which is equal throughout the ECF).

Water moves **out of vessels** through a **balance between hydrostatic** and **oncotic pressures** and the net force determines its movement. **Oncotic pressure remains relatively constant** along a capillary such that the main factor affecting water movement is the **change in the hydrostatic pressure** along the vessel



Regulation of Water Intake

During the un-anaesthetised patient, there is a complex regulatory process of 3 components determining the water intake:

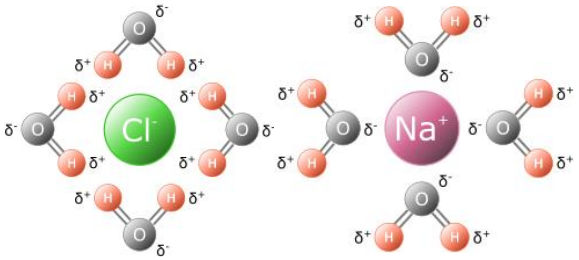
1. **Sensors**
   a. **Osmoreceptors** located in the hypothalamus respond to changes of 1-2% in ECF tonicity and largely reflect the ECF sodium concentration.
2. **Controllers** – occurs in the **hypothalamus** via complex system of interconnecting pathways.
3. **Effector**
   a. **Thirst** – conscious intake of more water
   b. **ADH secretion** – this alters the renal reabsorption of water in the collecting ducts according to the initial sensed tonicity.

Osmolarity: Partition of Fluids Across Membranes

(07b_01_07)

Water is an excellent solvent that provides a means of carriage and distribution, as well as a medium for the reactions of ionic substances in the body. It has a **high dielectric constant** which means it acts as an insulator and reduces electrostatic forces between molecules 80x more than air.

Water is also attracted to dissolved ions and charged surfaces providing an **insulating layer** to allow ions to exist in solution without reacting with other nearby ions.



Solute Exchange

The majority of capillaries pass within 5-10μm of the cell. The barriers of capillary endothelium, interstitial fluid and plasma membrane of the cell needs to be overcome for exchange to occur.

Diffusion

Molecules have thermal energy resulting in random movement and over time, they distribute evenly. The net movement is therefore **down a concentration gradient**. The rate is proportional to the gradient according to **Fick's law of diffusion**. The equation to calculate rate of diffusion is:

$$\text{Rate of diffusion} = DA\frac{\Delta c}{\Delta x}$$

Where D = diffusion coefficient, A = area of diffusion. Δc = concentration gradient and Δx = diffusion distance.

It is also **inversely proportional to √molecular weight** (Graham's law) and **inversely proportional to the distance through which the solute needs to diffuse.** This table shows diffusion time for a solute in water according to distance:

Distance to diffuse	Time taken to reach equilibrium
0.1 μm	4.56×10^{-6} s
1 μm	4.56×10^{-4} s
10 μm	0.0456 s
100 μm	456 s
1 mm	76 min

Non-ionic diffusion occurs in undissociated weak acids/bases which passively transfers across the membrane to dissociate in the cell. An example is ammonia passes into the tubules to react with hydrogen ion to produce ammonium.

Osmosis

Osmotic Pressure

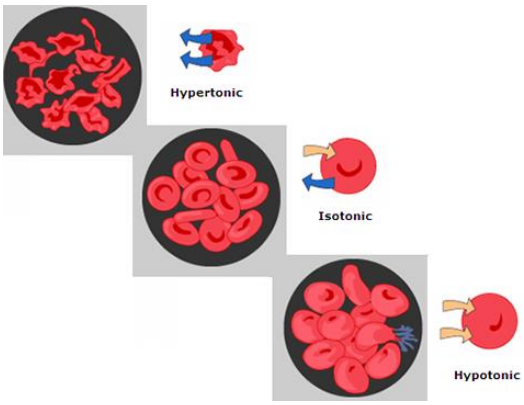
This is the hydrostatic pressure required to prevent movement of water down a concentration gradient. The greater number of osmoles, the greater the pressure required to prevent its movement: One mole of any solute in a volume of 22.4 L at 0˚C generates a pressure of 101.325 kPa, i.e. 1 atmosphere, across a semipermeable membrane.

Osmolarity

Dependent on the **NUMBER of** particles (colligative property). The particles may change its physical properties in the solvent. I.e. 1 mole placed in water may react with other ions and the actual osmolarity will therefore be less than expected. It can be calculated by PV = nRT (ideal gas equation).

Tonicity

Describe the behaviour of cells when bathed in a solution and is dependent on the semi-permeable membrane. Hyper/hypo/isotonic solutions. Cells mirror the electrolyte content extracellularly for normal metabolism to occur. If an isotonic dextrose/saline solution is given, the solution would become hypotonic from the metabolism of glucose.



Carrier Mechanisms

Facilitated Diffusion – Through carrier proteins via channels or through conformational changes. Does not require energy as down a concentration gradient.

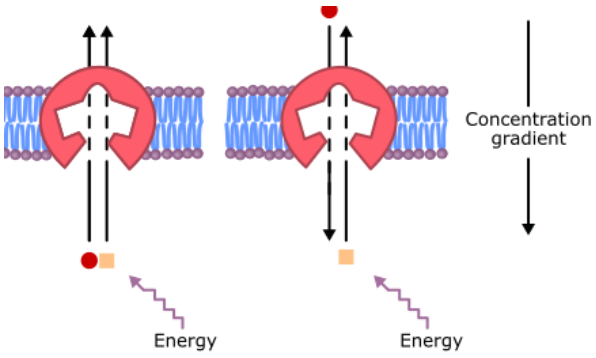
Active Transport – movement of particles against their concentration gradient using energy

Primary Active Transport – chemical energy used from ATP is directly linked to movement across the membrane. 3 main ATPase systems:

1. Calcium
2. H^+/K^+ ATPases
3. Na^+/K^+ ATPases

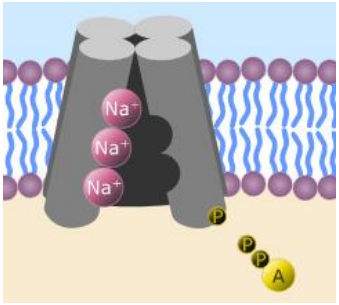
Secondary Active Transport – energy used from passive diffusion down an electrochemical gradient of a certain ion will provide energy for another solute against the gradient. This may be:

- **Co-transport** (left) i.e. amino-acids or glucose in villi of the intestine with sodium
- **Counter-transport** (right) i.e. antiports such as Ca^{2+}/Na^+, H^+/Na^+ and K^+/H^+.

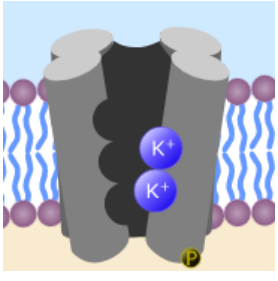


Na^+/K^+ Pump

3 sodium ions from within the cell bind to the cytoplasmic side of the protein **changing its conformational** shape allowing it to be **phosphorylated** by ATP.



Phosphorylation induces a **further conformational change** which **opens to the ECM** and additionally gains **lower affinity for Na^+** which allows dissociation and diffusion of sodium to the extracellular fluid. The new conformation has **high affinity for K^+** and **2 bind** to the extracellular side of the protein.

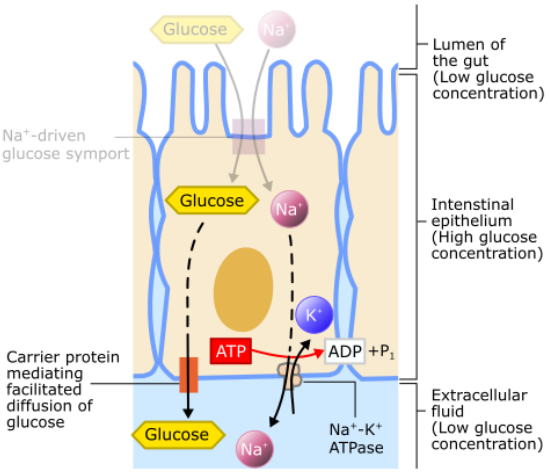


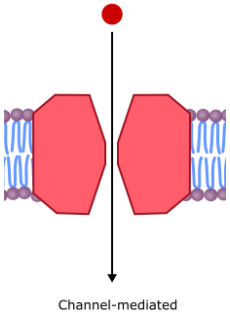
Phosphate dissociates and the protein reverts to its normal conformation exposing the K^+ to within the cell and has a low affinity to K^+ which then dissociate into the cell

This pump may be activated by thyroid hormones, aldosterone and insulin and inhibited by dopamine and cardiac glycosides i.e. digoxin. Magnesium is required for its normal function.

Glucose transport

The TK receptor activity in response to insulin is covered in prior notes. In the small intestine, uptake from the lumen to the cell is through active transport (secondary) and into the extracellular fluid through facilitated diffusion:





Membrane Channels

When open, they allow rapid transport through the membrane – between 10^6-10^9 ions per second which approaches the rate of diffusion of ions in a free solution. An example is G-protein related Ca^{2+} channel

Aquaporins

Consist of a family of 5 water channels stored in the endosomes of cells and are translocated to the luminal surface when vasopressin activates V2 receptors. Different aquaporins are as follows:

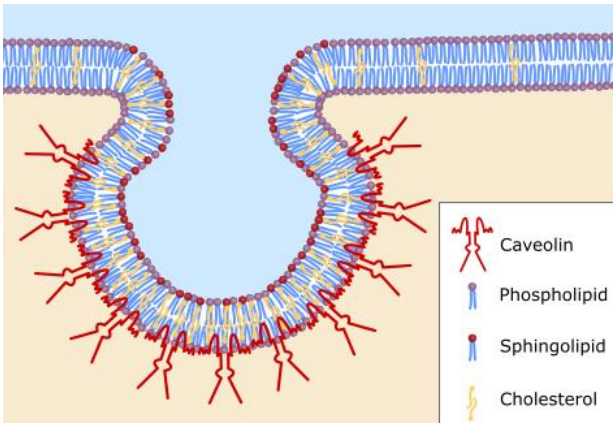
Aquaporin	Site
1-3	Found in the kidney, the most important being aquaporin 2 in the collecting duct
4	Found in the brain
5	Found in salivary glands, lacrimal glands and the respiratory tract

Gated Channels

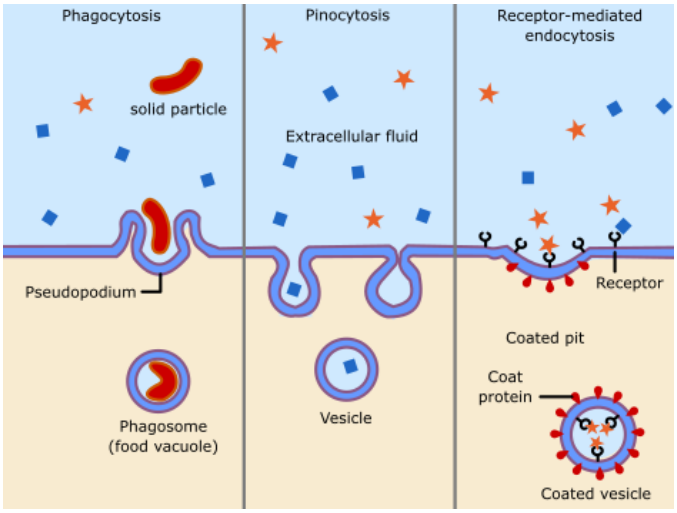
- **Voltage-gated channels** open or close in response to electrical signals i.e. sodium in action potential generation
- **Chemically-gated channels** which open through ligand binding and split into:
  - **Direct** – ligand receptor is part of the ion channel i.e. nAChR
  - **Indirect** – ligand acts to release an intracellular messenger (commonly Ca^{2+}) and the increased intracellular concentration opens the channel
- **Mechanically-gated channels –** opened by mechanical deformations of the membrane. For example, stretch activated channels on the sensory nerve terminals.

Transcytosis

Caveoli are flask-shaped depressions in the cell membrane coated by the protein **caveolin** present in the cytoplasm of endothelial cells. It allows small quantities of protein, which could not otherwise cross the membrane, to be transported across the endothelial cell into the interstitium by transcytosis (exo-/endocytosis)



Endocytosis: There are 3 types of endocytosis: phagocytosis, pinocytosis and receptor mediated endocytosis. All involve the **invagination** of the plasma membrane, **enclosure** of the EC material, **sealing** and **budding off** from the membrane without leaving a gap.

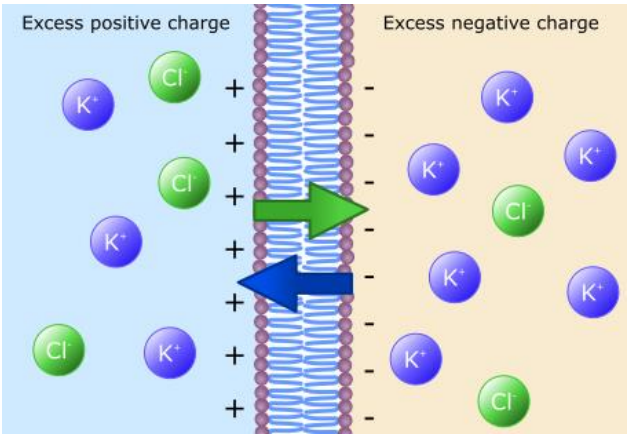


Clathrin is a triskelion shaped protein that forms around the vesicle to stabilise it and **dynamin** pinches the vesicle off from the cell membrane and forms a sphere from the flask-shaped caveoli. Clathrin therefore is responsible for internalization of low-density lipoproteins, reuptake of neurotransmitters at synapses and the internalization of nerve growth factor and caveolin is involved in protein transport.

Exocytosis: This process requires energy and is triggered by an **increasing intracellular calcium concentration.** Used to transport proteins across the capillary wall, release of peptide hormones from endocrine glands and pro-enzyme release.

Membrane Potential

When one ion cannot diffuse through the membrane it affects the distribution of the diffusible ions. Negatively charged, non-diffusible anions, hinder the diffusion of diffusible cations causing:



1. Electrical difference across the membrane from unequal distribution
2. Higher protein content inside cells trap ions attracting water via osmosis → cell swelling and a need for transport of ions outside the cell
3. Higher protein content in vessels results in a **Gibbs-Donnan effect** with excess cations in the cytoplasm:

I.E. **Albumin** binds to Na^+ and to a lesser extent Cl^- causing it to be trapped in the capillary adding 9 mmHg to the colloid osmotic pressure of albumin which is 16mmHg → total of 25mmHg.

Gradients

With uncharged solutes, driving force from one area to another is through the **chemical potential.** When the solute is **charged** i.e. an ion, there is **additionally an electrical potential** and the equilibrium potential across a membrane can be calculated by the **Nernst equation**.

Nernst Equation:

The **equilibrium potential** (electrochemical gradient) of the cell state is **calculated using the Nernst equation**. For example, there are more chloride ions outside of the cell but as the inside of the cell is negatively charged, the force is exactly opposed and no net movement occurs as mentioned above. At equilibrium, there is no electrochemical force across the membrane i.e. E = 0.

$$E_{cl-} = \frac{RT}{FZ_{cl}} \ln \frac{[Cl^-_O]}{[Cl^-_I]}$$

R = Gas constant
T = Absolute temperature
F = Faraday constant
Cl^-_O = Cl^- concentration outside
Cl^-I = Cl^- concentration inside
Z_{cl-} = Valency of chloride ion

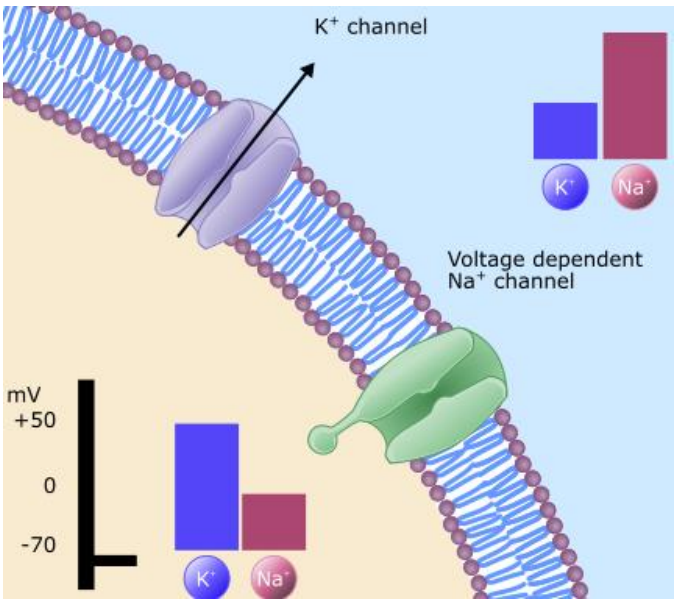
The equation on the right is applied with reference to chloride ions.

The equation can be simplified into:

$$E_{ion} = 61.5 \log \frac{[ion_2]}{[ion_1]} \quad \text{at } 37^\circ C$$

Resting Membrane Potential

This is a diffusion potential predominantly due to the distribution of potassium (-90mV) with little movement of sodium increasing it to -70mV. Neither are at their equilibrium potential so it is predicted that sodium would leak into and potassium out of the cell. This does not occur as it is maintained by the **Na^+/K^+ ATPase pump**.



The **Goldman equation** considers the impact of all other diffusible ions on the transmembrane potential

Physiology of Obesity and Effects on Organ Systems

(07b_01_08)

BMI is used to define obesity but **waist circumference** is more predictive of morbidity. There are multiple health risks associated with obesity:

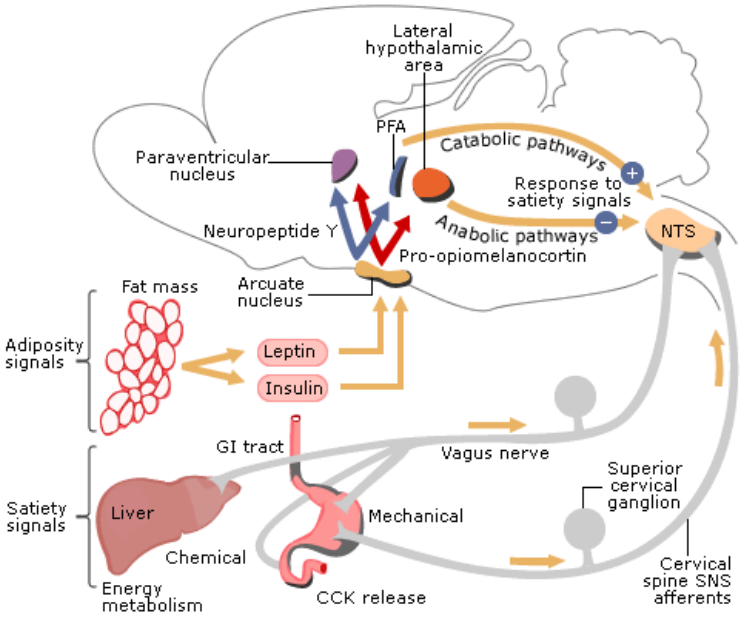
1. **Neuropsychiatric**
    a. Depression
    b. Poor self-esteem
2. **Cardiovascular**
    a. HTN
    b. Coronary artery disease
    c. Cerebrovascular
    d. Heart failure
    e. Cardiac arrhythmias
3. **Gastrointestinal**
    a. Gallstones
    b. Hiatus hernia
    c. Steatohepatitis – cirrhosis
4. **Respiratory**
    a. Obstructive sleep apnoea
    b. Hypoventilation
5. **Metabolic**
    a. Diabetes
    b. Dyslipidaemia
6. **Genitourinary**
    a. PCOS
    b. Infertility
    c. Irregular periods
    d. Erectile dysfunction
7. **Others**
    a. Headache
    b. Poor sleep
    c. Locomotor dysfunction

Pathways of Energy Balance

This includes:

- **Hyperinsulinaemia** → increased deposition of fat
- **Insulin Resistance** → T2DM
- **Suppressed leptin signalling** → reduced satiety
- **Insulin mediated reduction in dopamine clearance** → Increased food reward

The hypothalamus integrates afferent signals from the CNS, gut and adipose tissue and efferent appetite increasing/reducing signals via PNS/SNS



Leptin and Insulin

Leptin is released from adipose tissue and signals **adipose tissue mass** and therefore adequacy of energy supply to the CNS.

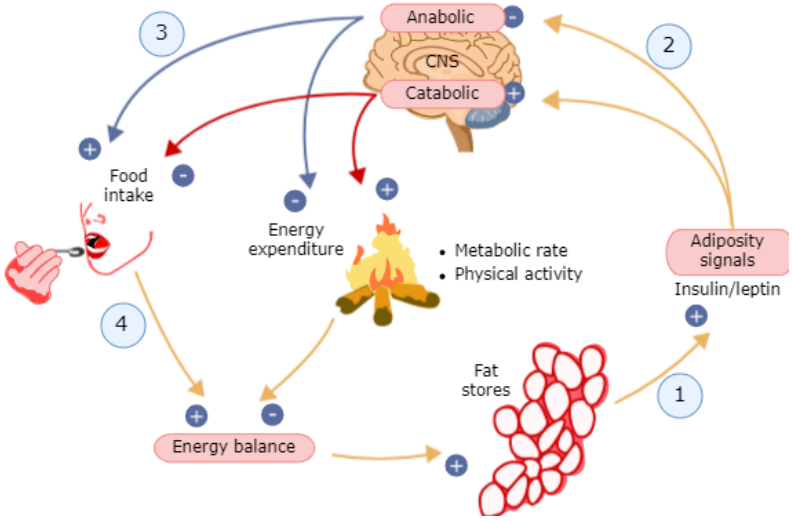
- **Stimulates melanocyte-stimulating hormone (MSH)**
- **Inhibits Neuropeptide Y.**

Overall – **reduces food intake** and **increases energy expenditure** through SNS activation

Insulin's role in the afferent CNS pathway is similar to those of leptin. It **reduces feeding** and **causes satiety**. The SNS is activated. It is thought that insulin and leptin share a common second messenger system.

During weight loss, the anabolic pathway is stimulated from low insulin/leptin levels resulting in stimulation of eating and reduced energy expenditure.

Whilst eating, the afferent pathways signal negative feedback to food intake via the hindbrain and regulates meal size to optimise energy balance.



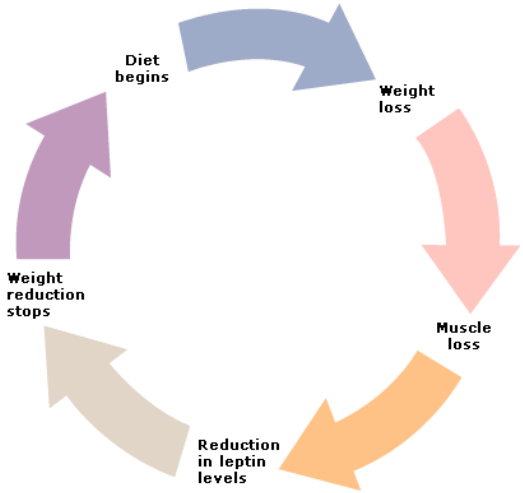
Autonomic Nervous System

SNS stimulation occurs in response to insulin/leptin and **increases energy expenditure** through glycogenolysis and through breakdown of glucose and fatty acids in skeletal muscle and lipolysis.

PNS stimulation particularly the vagus nerve oppose the SNS and modulates beta cell pancreatic function to **increase insulin secretion** to allow fatty tissue build up.

Starvation and Leptin Set Point

Appetite and obesity is regulated in different individuals according to **leptin set point**. Studies have shown that **reduced leptin levels** result in a **starvation response**: This is where there is a **20% reduction in resting energy expenditure** which balances the reduced caloric intake and therefore **weight loss is not sustained**. Even if the dietary intake is maintained at low level, the weight reduction does not continue beyond about four months.

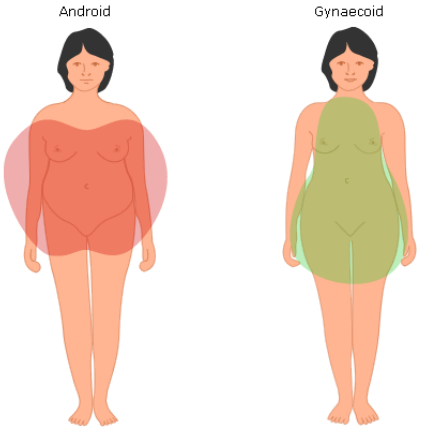


Food Reward Pathways

Insulin and Leptin also **increase dopamine release** via **D2 receptors**. It is thought that insulin resistance in the ventral segmental area causes dopamine accumulation in the nucleus accumbens hence enhancing the pleasure response to food as a possible cause of obesity.

Fat Distribution

There are 2 main categories: **Android** and **Gynaecoid:** The latter fat accumulates in the arms, legs and buttocks whilst in the former, there is fat distribution centrally and peripherally sparing so is associated with higher CV disease incidence and other complications including metabolic consequences. There is significant overlap however.

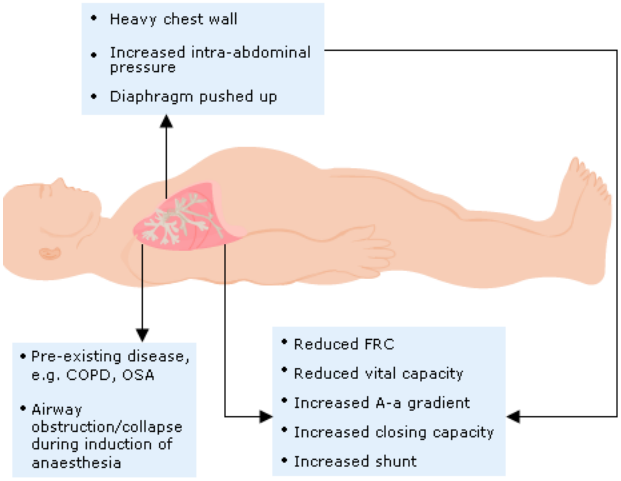


Respiratory System

Split into systemic changes affecting respiration and pulmonary changes that affect gas exchange. BMI >30kg/m^2 together with laparotomy reduces vital capacity by 40% (cf 10% in lean patients).

***Systemic Changes*:** Basal metabolic rate is increased compared to lean patients and therefore have **greater oxygen requirements** and **increased CO_2 production**. Therefore, in anaesthesia, oxygen delivery is of increasing requirements with importance when low-flow or closed-circuit anaesthetic is used. Increased minute ventilation may also be required for CO_2 clearance.

***Pulmonary Changes*:** There is a **decline in FRC and VC** as BMI increases as well as **increasing A-a O_2 tension gradient.** During anaesthesia, there is **increased shunt** when the diaphragm is pushed up into the lung bases and compression from a heavy chest wall.



Combined, the above further reduces FRC with an **overlap between closing volume** and **tidal volume.** The severity of which is due to fat distribution and intrabdominal pressure. Airway closure and shunt is further exacerbated with pre-existing airway collapse and atelectasis especially in OSA patients.

Obesity Hypoventilation Syndrome

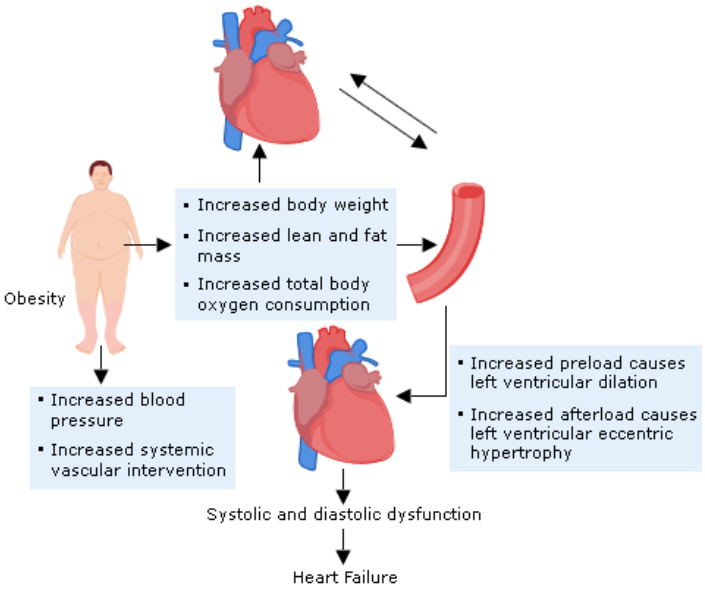
Defined as **elevated arterial carbon dioxide tension** while awake, in association with a body mass index above 30 kg/m^2. There must be an absence of any other causes of elevated CO_2 > 6kPa and may have concomitant OSA. These patients will be **difficult to wean off the ventilator** as have reduced sensitivity to CO_2 and therefore, will relatively reduce their minute volume and if ventilated to normocapnia will have a respiratory alkalosis. **Post-op respiratory depression** is common.

In laparoscopic surgery, ventilation may be of particular problem as there is an already increased airway pressure associated with obesity and a pneumoperitoneum may impede on this. **Recruitment manoeuvres** can help airway opening. Also avoiding an FiO_2 of 1 pre-intubation may protect against atelectasis.

Cardiovascular Changes

Common features of CV changes include:

- Increased blood volume and cardiac output
- Increased splanchnic blood flow up to 20% than lean individuals
- Associated hypertension
- Increased myocardial fat content which reduces contractility and increases LV work from acute reductions in contractility and peak intracellular calcium.
- Endothelial dysfunction --: increased vascular resistance. Likely secondary to leptin-mediated expression of myocardial endothelin 1.



***Right heart*:** Increased pulmonary vascular resistance and pulmonary vascular pressures from OSA and obesity hypoventilation syndrome. This will eventually cause R ventricular failure and overload which will in turn rapidly cause reduced CO and multiorgan failure.

In severely affected individuals, exercise will increase SNS → rise in end ventricular diastolic pressure, myocardial ischaemia and elevated L atrial and pulmonary artery pressures and R heart failure.

Gastrointestinal Changes

High prevalence of T2DM associated with insulin resistance and the metabolic syndrome. Increased intraperitoneal fat → **increased intrabdominal pressure** with consequent Cardiorespiratory effects and reduced organ perfusion → renal dysfunction in the bariatric surgery population.

Fatty liver occurs up to 90% which can also develop to steatohepatitis or cirrhosis.

Hiatus hernia common in morbid obesity with increased risk of acid aspiration.

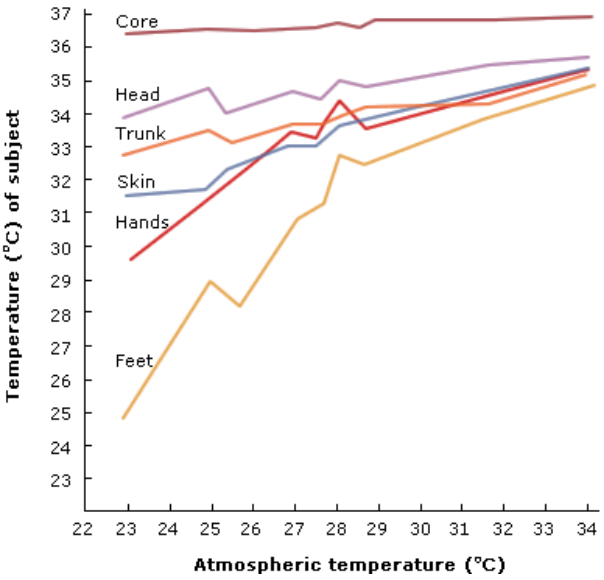
Body Temperature and its Regulation

(07b_01_09)

Core body temperature is maintained between 36.5-37.5°C to ensure optimal enzyme function. However, temperature of other elements can vary with the temperature of the environment:

There is a **circadian fluctuation** with the lowest at 06:00 and highest in the evening (varying by 0.5-0.7°C) and also during ovulation it rises 0.5°C). Some individuals have a chronically elevated basal temperature known as **constitutional hyperthermia.**

Rectally is higher 0.5°C than orally. Scrotal temperature is maintained at 32°C.

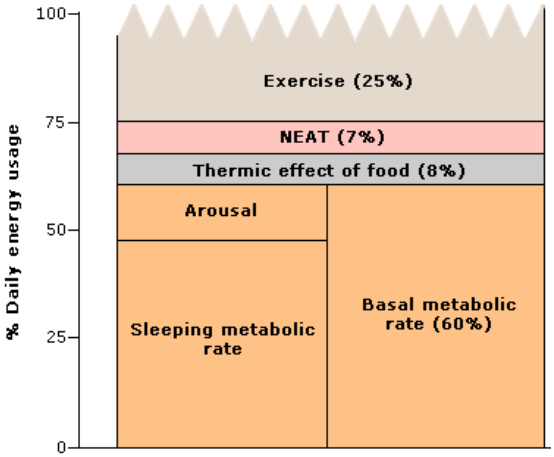


Maintenance of Normal Body Temperature

Metabolic Rate

This is the rate of heat production in the body. This changes according to level of activity and the **heat production** is due to:

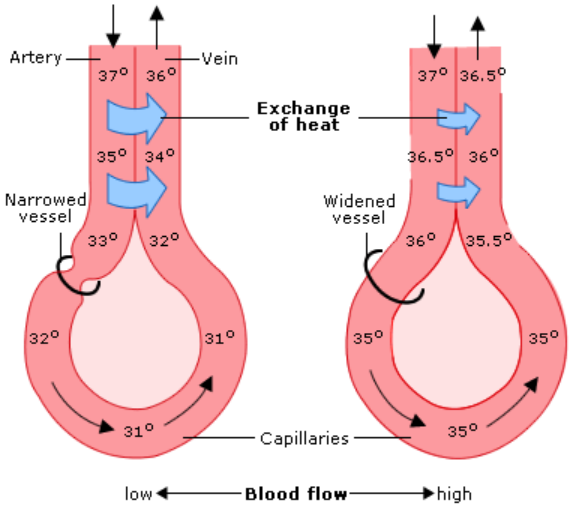
- Basal metabolic rate of cells
- Additional metabolism from digestion, absorption and storage of food (**thermogenic effect of food**)
- Activity for daily living known as **non-exercise activity thermogenesis (NEAT)**.
- Exercise states can raise heat production by ¼.



Heat loss occurs as a result of subcutaneous venous plexi receiving blood from capillaries that lose heat to the skin. Some tissues have direct AV anastomoses

Countercurrent Heat Exchange

Arteries and veins run parallel to each other so warm blood from arteries can supply heat to cooler veins returning to the core. This is enhanced in low environmental temperatures through vasoconstriction of skin capillaries (changes heat conductance to environment by up to 8-fold.

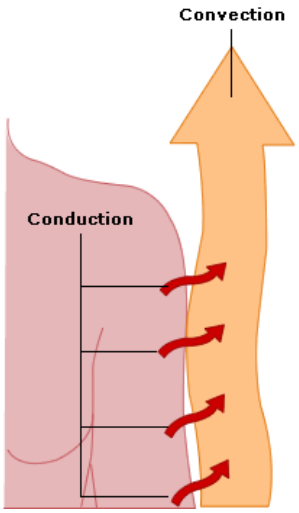


Physics of Heat Loss

Body heat is lost by 3 main mechanisms:

1. Radiation

This accounts for 60% of heat loss. Heat is lost through **infrared heat rays**. This occurs when the body temperature > environmental temperature. Gained if the opposite.

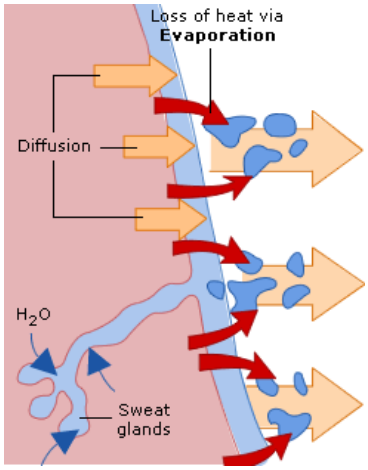


2. Conduction and Convection

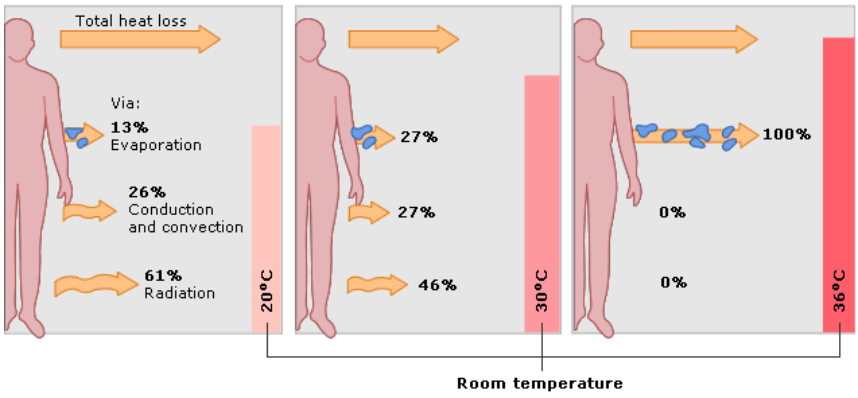
15% of heat loss where heat is lost to air in contact with skin (or with solid objects) through transfer of **kinetic energy** known as **conduction**. This occurs until a **temperature equilibrium is reached**. **Convection** is when removal of this layer is caused by rising of heater air/air currents. Liquid is more effective at heat absorption due to an increased specific heat capacity x1000 that of air.

3. Evaporation

22% of heat loss where water evaporates insensibly from skin and lungs. Evaporation is the most effective way of losing heat when the ambient temperature is higher than body temperature and can remove heat at a rate of more than 10 times the basal rate of heat production. This uses the principle of latent heat of vaporisation.

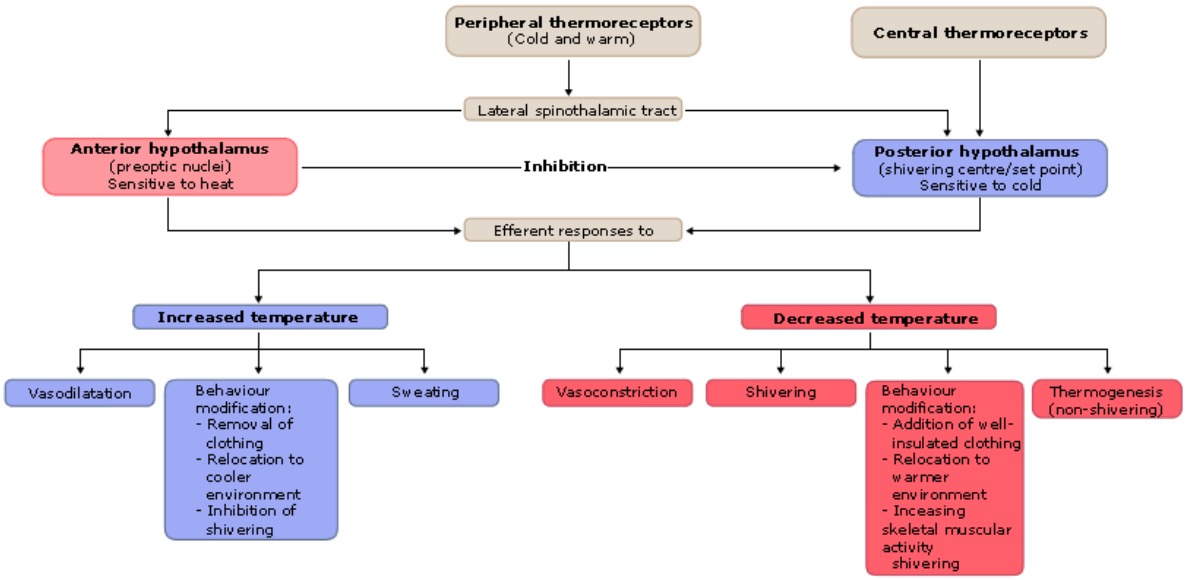


These proportions change according to the temperature of the surrounding environment:



Thermoregulation

Complex control mechanism which involves **inputs** from both **central and peripheral thermoreceptors** and integrated via **hypothalamus** to provide an **effector response**.



***Central Thermoreceptors*:** uncertain anatomical origin and less significant in temperature control. They have input into the posterior hypothalamus.

***Peripheral Thermoreceptors*:** There are 2 kinds:

- **Skin thermoreceptors** via lateral spinothalamic tracts and are either:
    - **WARM**: Send impulses via type C nerve fibres with max discharge rate at 45-50°C
    - **COLD**: Present to a greater extent with regular discharge rate which increases below 25°C and transmit impulses via type Aδ nerve fibres.
- **Deep tissue thermoreceptors** mainly in the spinal cord, abdominal viscera and great veins of upper abdomen and thorax. These can induce shivering when core temperature falls.

Hypothalamus

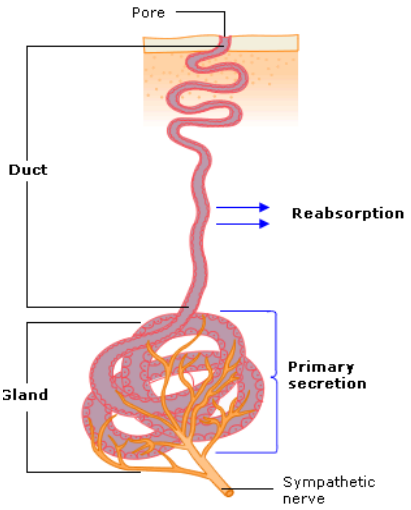
It has a determined **set-point** for temperature (see below). The **anterior pre-optic hypothalamus** is sensitive to **rises in temperature** and forms effector responses of sweating and vasodilation and is inhibitory to the posterior hypothalamus. The **posterior hypothalamus** integrates cold afferent impulses mainly from the periphery and has effector responses. The primary area for shivering is in the medial portion of the posterior hypothalamus near the wall of the third ventricle

Chemical Thermogenesis (non-shivering thermogenesis) is **SNS excitation** of heat production when **adrenaline and noradrenaline uncouple oxidative phosphorylation**. As a result, metabolic processes are able to **produce heat independently of ATP production**. Thyroxine has a similar effect but of slower action than noradrenaline/adrenaline. In infants, this occurs mainly in brown adipose tissue which is of insignificant quantities in adults.

Sweat Glands

Innervated by **cholinergic fibres** and consists of 2 distinct structures:

1. **Deep subdermal coiled gland** that secretes sweat
2. **Duct portion** that passes sweat out through the dermis to the epidermis.



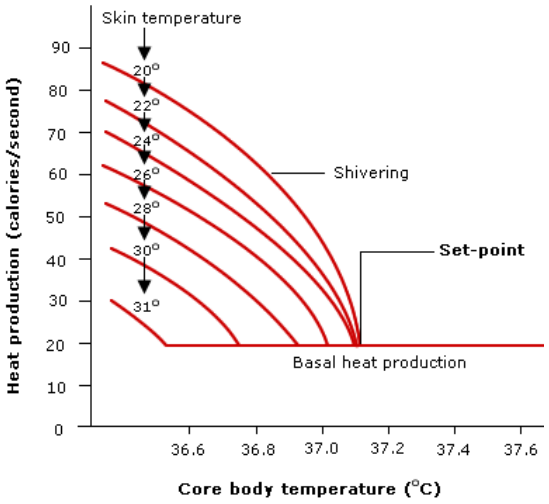
The secretion is similar to that of plasma which is modified as fluid flows via the duct and is more concentrated. Reabsorption of water, sodium and chloride is almost total at low levels of sweating.

When sweat glands are strongly stimulated, significant losses of water and sodium can occur. An unacclimatized individual can lose up to 15-30 g of sodium through sweat each day.

Acclimatisation takes 4-6 weeks exposure to hot weather and is associated with a decrease in plasma concentration of sodium and increased sweat production. Aldosterone is released to limit sodium loss to 3-5g/day.

Set Point for Temperature Control

Defined as the body temperature at which hypothalamic control centres are set and at which there is no effector response and is determined by the **posterior hypothalamus** and can be **modified by skin temperature**. The lower it is, the higher the set point will be to prevent excessive loss of body heat through sweating and to anticipate a fall in internal temperature and initiate shivering.



Abnormalities in Temperature Regulation

Pyrexia may occur from pyrogens released from toxic bacteria and can **increase the set point.**

Nervous System Abnormalities: Through **compression of the hypothalamic area** by tumour/surgery may cause **hyperthermia** (rarely hypothermia). Also, **cervical spinal cord trauma** causes **severance of SNS outflow** effecting vessels and sweat glands causing **hypothermia** so temperatures are controlled solely on behavioural responses to hot and cod sensations in the head region.

Labour epidural analgesia increases the basal temperature of pregnant women thought to be an imbalance in heat production and heat loss, and a disturbance in central thermoregulation.

CARDIOVASCULAR PHYSIOLOGY

Capillary Dynamics and Interstitial Fluid

(07b_02_01)

Capillary Anatomy

They are the only vessel that allows exchange with the ISF. They have 3 features to optimise this:

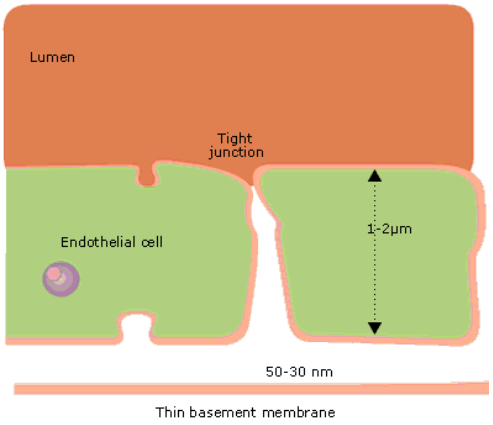
1. Blood traverses the capillary at **low velocity** 0.5-0.1 cm/s.
2. **Large area for diffusion** 6300 m^2 with cross sectional area of 4500 cm^2.
3. **Thin walls** to minimise diffusion distance according to Fick's law.

The size allows only one RBC to fit and travel in single file. The capillary is ~ 0.5-1mm in length taking 1-2s for RBC to transit (dependent on the metabolic rate).

Types of Capillaries

There are 3 types of capillaries identified microscopically:

- Continuous
- Fenestrated
- Sinusoidal

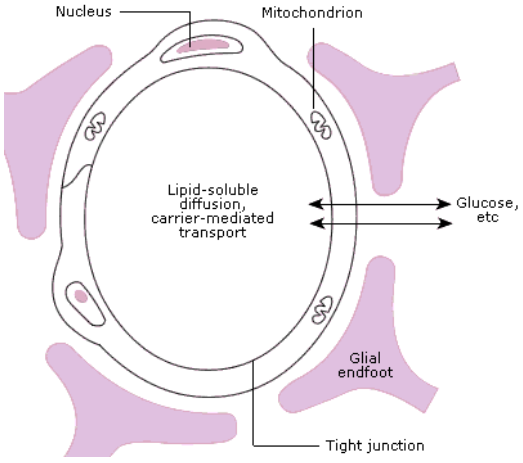


1. Continuous Capillaries

Capillary endothelial cells contain tight junctions ensuring exchange of solutes and water occur through transport proteins in the cell membrane and macromolecules through exo-/endocytosis. Lipid soluble molecules can diffuse passively.

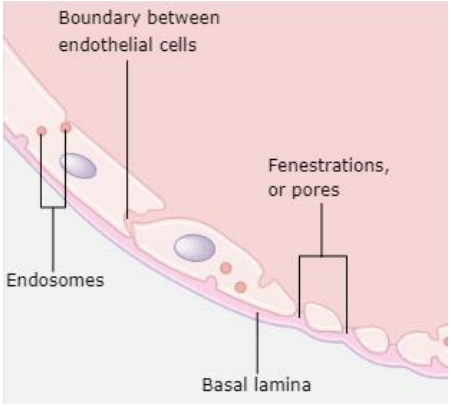
Cerebral Capillaries

A type of specialised continuous capillary with **extensive interconnected tight junctions** found in the CNS and thymus. Gasps between cells are 2nm wide and there are **few cytoplasmic vesicles for vesicular transport** but **many transport mechanisms** i.e. GLUT1 to carry water soluble solutes. Only small lipid soluble substances may pass through the membrane.



Astrocyte foot processes surround the capillaries and secrete substances to **maintain integrity of the BBB.** The degree of damage therefore, will be proportional to the permeability of the BBB.

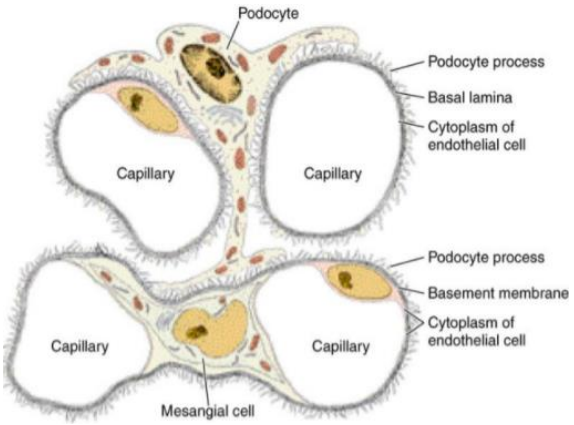
2. Fenestrated capillaries



These have pores in the endothelial surface to allow filtration of water and solutes, including peptides and hormones, to pass between plasma and interstitial fluid (20-100nm diameter). There are also areas within endothelial cells with little or no cytoplasm separating the 2 surfaces of the plasma membranes. There is still a basement membrane encircling the periluminal surface, providing additional support to the endothelial cells. Located in the following:

- **Endocrine Organs**
- **Choroid plexus** – fenestrated capillaries but also with choroid cells that form tight junctions.
- **Glomerular Capillaries** – for filtration (also located in GIT for peptides and carbohydrates)

The **hydraulic conductivity** describes the permeability factor of capillaries measured in $cm^3/s/dyne$. Brain = 3 whilst glomerular = 15,000.



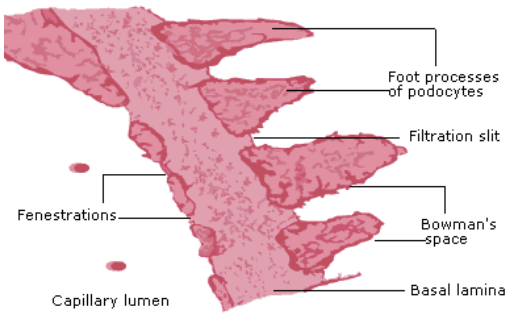
Glomerular capillaries

Pores are 70-90 nm in diameter. **Separation** of blood from glomerular filtrate is by **capillary endothelium**, specialised **Bowman's capsule epithelium** (made from **podocytes**) and **basement membrane**.

The **pseudopodia** interdigitate to form filtration slits 25nm wide which are closed by a thin basement membrane. As a result, molecules:

- <**5nm** diameter or **<7kDa** are freely filtered
- >**8nm** are excluded

Mesangial cells are located in the basal lamina endothelium regulating GFR through contractile function. They also have a role in secretion of renin and uptake of immune complexes.

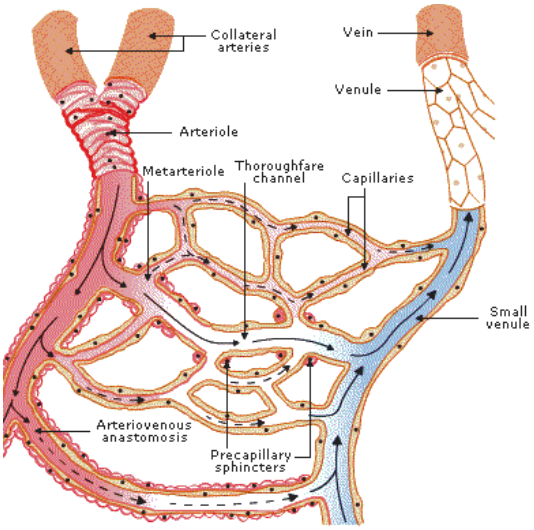


3. Sinusoidal Capillaries

Examples are spleen and liver capillaries. **Sinusoidal capillaries** are flattened irregularly shaped capillaries. They have **gaps between adjacent cells** and the **basal laminae is thinner or absent**, so there is free exchange of molecules synthesized in the liver. Molecules up to the size of plasma proteins can pass freely. Kupffer cells attach to the endothelium projecting into the lumen to engulf damaged RBC, pathogens and debris and play a central role in immune response.

Capillary Beds

Collective of capillaries that function together. They arise from one arteriole and empty into a post-capillary venule. **Venules have pericytes** in their walls (like capillaries), but no smooth muscle. They can still act as **exchange vessels**, by virtue of their **thin walls** up to a size of 30 µm.

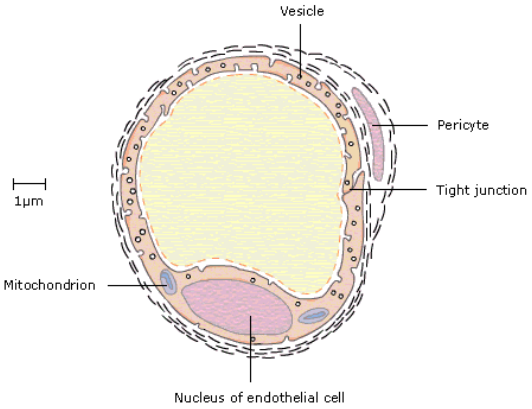


Each capillary has a pre-capillary sphincter at its afferent end and in resting state, most capillaries are closed and blood flows via the **thoroughfare channel** which starts at a metarteriole. Capillaries open up with increased metabolic demand and reduces the diffusion distance to cells.

Pericytes

Contractile cells that have long processes which wrap around the capillary and venules and have several functions:

1. Alters luminal diameter and contractile nature of the vessel
2. Synthesis and release of molecules for BM and ECM
3. Release of vasoactive agents
4. They appear to regulate flow through the junction between endothelial cells, particularly in the presence of inflammation



Vasomotion

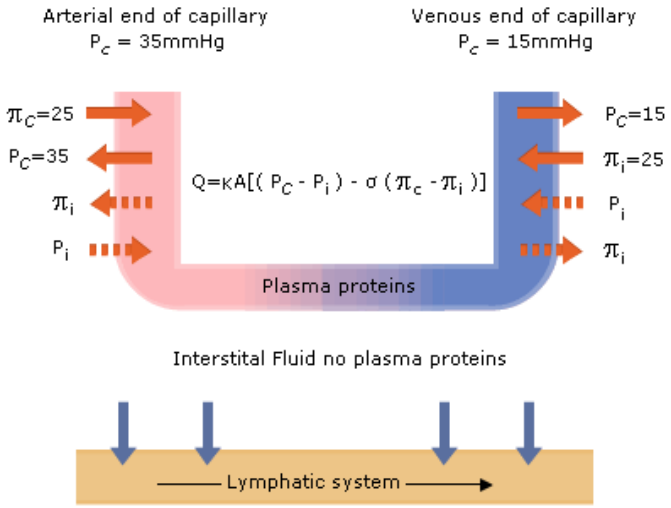
Defined as the **cycling of contraction and relaxation** controlled by a **myogenic response** to local metabolites. I.e. mechanical stimulus to the skin causes closure of the pericapillary sphincters which is why the skin goes white. Substance P and bradykinin → relaxation of precapillary sphincter

The precapillary sphincters contract approximately 12 times a minute allowing pulsatile flow in the capillary in addition to the arteriole diameter. The flow varies from zero to 0.2 cm/s. The **pulsatile nature allows different flow patterns** in the capillary bed to **alter the perfusion path**.

Starling Forces

Describes 2 major forces affecting fluid shift across the capillary: **hydrostatic** and **oncotic pressures**.

- **Hydrostatic pressure**: the pressure measured in a fluid at that point and affected by gravity. The capillary hydrostatic pressure at the foot increases from 30 mm Hg to 97 mm Hg when the person is ambulant
- **Oncotic Pressure**: pressure generated across the capillary wall due to non-diffusible molecules – principally albumin and globulins in the plasma compared to the interstitial space. Colloid osmotic pressure is the correct term to distinguish the pressure from albumin rather than the diffusible ions – pressure exerted = 26 mmHg (1.2 mosmol/kg).



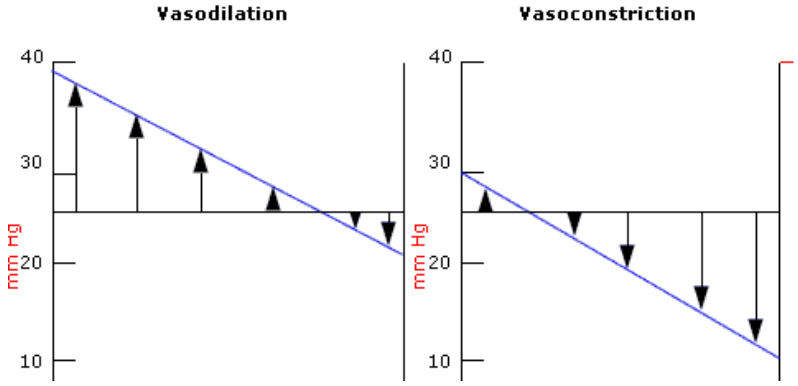
Starling's equilibrium is the balance of the forces on the capillary. **Q = fluid movement** determined by the **equation** on the diagram:

- P_c = capillary hydrostatic pressure
- P_i = interstitial hydrostatic pressure
- π_I = interstitial oncotic pressure
- π_c = capillary oncotic pressure

- σ = reflection coefficient for albumin
- A = area of membrane
- k = permeability constant

There is always slightly more filtration than reabsorption and the excess fluid is drained into the lymphatics – 2-4L/day on average. Once the capacity is exceeded however, there will be tissue oedema and may occur due to:

- Lack of plasma proteins reducing oncotic pressure
- Increased flow from vasoactive control increasing filtration from raised hydrostatic pressure (see graph).

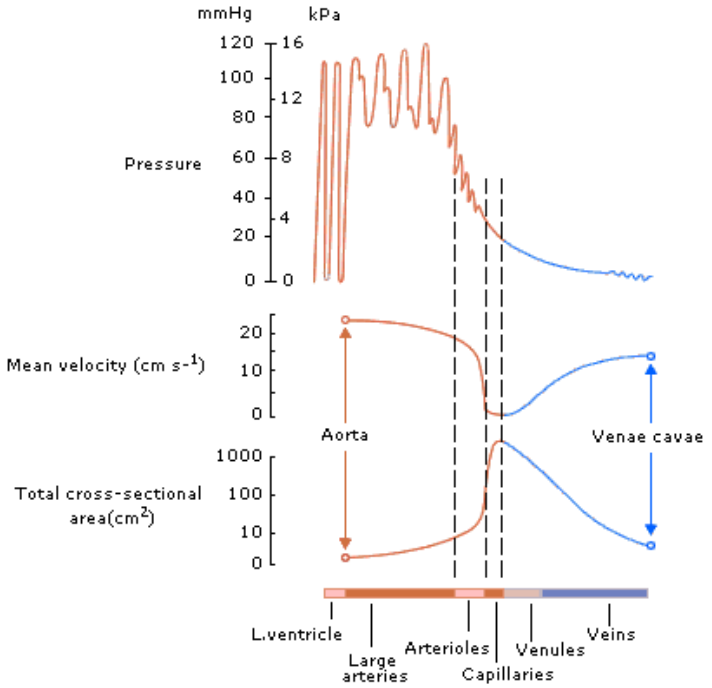


In the lung, hydrostatic pressure is < colloid osmotic pressure -osmosis prevents the alveolus filling with water

Flow Characteristics in Vasculature

Capillaries have a cross sectional area of 4500cm^3 (vs 4.5cm^3 in aorta) and therefore flow resistance is reduced. Vasomotion alters velocity, pressure and flow to different capillary beds – density is increased in brain/myocardium vs skeletal muscle. Majority of blood is in the venous circulation (54%) and capillaries have less blood than large arteries.

Typical values are 32 mm Hg at the arteriolar end and 15 mm Hg at the venous end with a pulse pressure of 5mmHg falling to 0 at the venular end.



Lymphatic System and Other Circulatory Fluids

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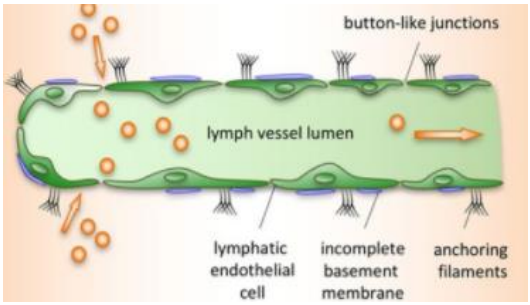
The main physiological functions of the lymphatic system are:

1. To **return excess fluid** in the ISF unable to be reabsorbed via oncotic pressure to the circulation to **maintain intravascular volume** (~2L/day)
2. **Carriage of chylomicrons** from intestines to periphery
3. **Carriage of proteins** from ISF → circulation and liver → circulation
4. **Lymphocyte carriage** from bone marrow → blood and ISF →blood

Initial Lymphatic Vessels



Histology

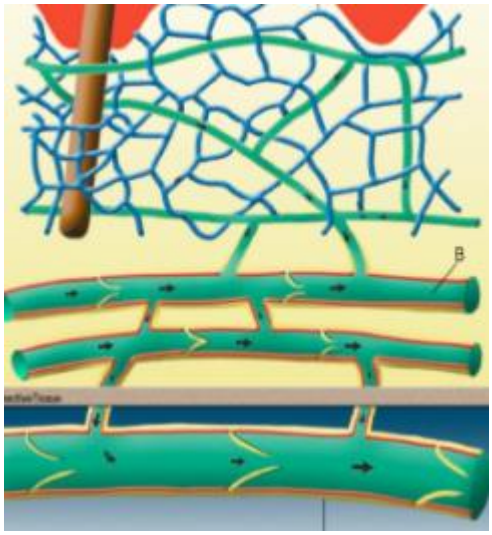
Lymphatics begin as **blind-ended vessels** and are **endothelial-lined tubes** which are **thinner** than capillary endothelium and have an incomplete or absent BM → **more permeable**. There are no valves or smooth muscle and are **intertwined within the capillary bed**.

Collagenous anchoring fibrils exert radial traction to keep the lumen of the vessels open.

Located in abundance in intestines and skeletal muscle but not located in the cartilage, CNS or eyes.

Function

They **collect interstitial fluid** via the loose button like junctions via free passage. This is done through the reliance on **negative intraluminal hydrostatic pressure** created by the holding open of the lumen through the anchoring fibrils.



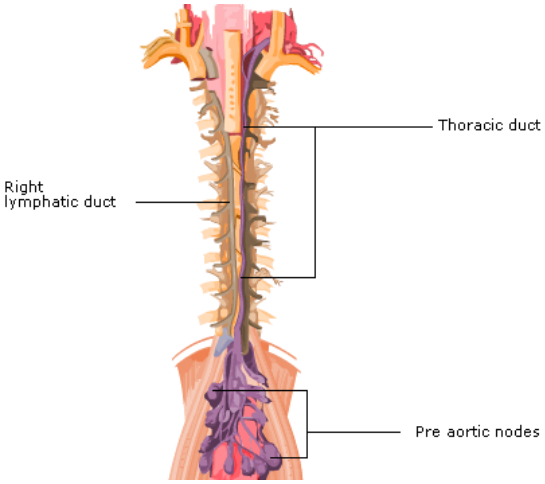
Flow of Lymph is aided by contraction of the organs, arterioles and venules associated with the lymphatics **until larger lymphatics** appear where there are **valves** and **smooth muscles in the wall** which contract in a **peristaltic** fashion ensuring unidirectional flow. Also aided by *negative intrathoracic pressure, transmitted pulsation of major arteries and suction effect of high velocity flow in brachiocephalic veins where the lymphatics terminate.*

Final conduits = thoracic and right lymphatic ducts.

Collecting Lymphatic System

Thoracic Duct

Is about 45cm and originates from the **cisterna chyli** in the abdomen and enters the thorax through the **aortic opening of the diaphragm (T12)**. It the ascends **behind the oesophagus** until C7 where it **arches posterior to carotid sheath, anterior to subclavian artery** and **enters into brachiocephalic vein**. Its function is to drain all the body below the diaphragm and above the L side of the diaphragm.

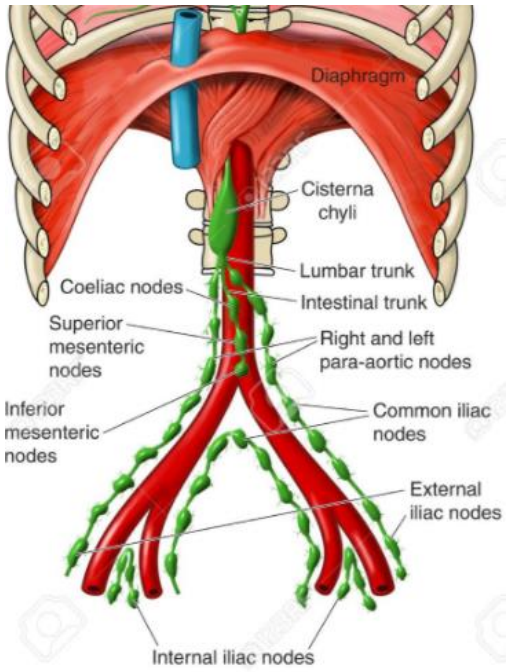


The *right lymphatic duct* may be absent but if present, drains the right body above the diaphragm from the R jugular, subclavian and bronchomediastinal lymph trunks.

Abdominal drainage

- ***Abdominal wall*** → axillary, anterior mediastinal and superficial inguinal nodes
- ***Intestinal drainage*** → preaortic nodes and kidneys
- ***Adrenal glands, posterior abdominal wall*** and ***gonads*** → paraaortic nodes
- ***Pelvic viscera, perineum*** and ***lower limbs*** → common iliac nodes → paraaortic nodes
- ***Liver***
  - → superficial plexi → anterior mediastinal nodes
  - → deep plexi → porta hepatis or posterior mediastinal nodes via the hepatic vein and IVC

Overall, the abdominal viscera drain into the para or preaortic nodes. They are arranged around the origin of the arterial supply to the relevant viscera. In the GIT, this is the coeliac, sup and inf mesenteric a.



Preaortic → cisterna chyli via intestinal lymph trunk

Paraaortic → cisterna chyli via R & L lumbar trunks.

Cisterna Chyli

Thin walled sac 5-7 cm long and slender in profile. It lies between the aorta and right crus of the diaphragm, in front of the bodies of L1 and L2 vertebrae. It drains into the thoracic duct, and receives the right and left lumbar ducts, intestinal lymph trunk, *as well* as the drainage from the posterior intercostal spaces.

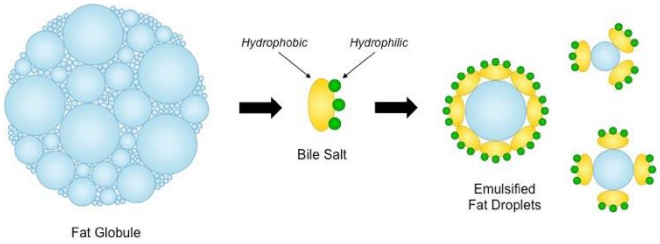
Content of Lymph

Higher concentration of **lymphocytes** than in plasma as they enter the circulation primarily via lymphatics including T, B and phagocytic cells. Lymph contains **proteins** that have traversed capillary walls but is less than the plasma concentration of 7g/dl. The protein content is dependent on the organ being drained with liver being highest at 6.2g/dl. **Water insoluble fats** are absorbed and the high concentration of **chylomicrons** give the lymph a milky appearance.

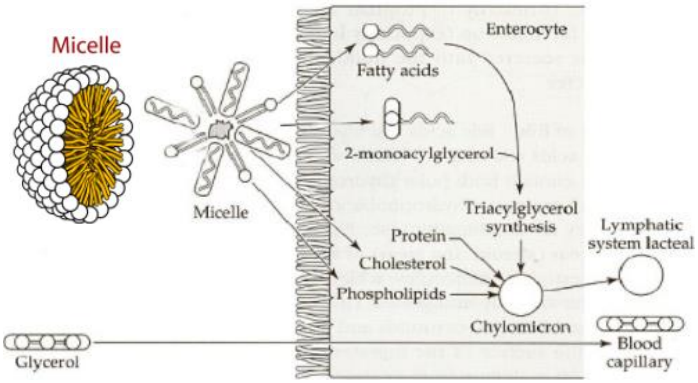
Absorption of Fat in the Intestines

Lipids are usually large and not water soluble so cannot pass readily across the basement membrane of the intestinal villi. Short to medium fatty acids (<12 carbons) are absorbed without micelle formation and are carried preferentially in the portal circulation of the liver

***Emulsification*:** The process when large lipid lipids of > 12 carbon chain are broken down and made water soluble and achieved with **bile salts** and **lecithin** to form stabilised droplets of 0.5-1µm



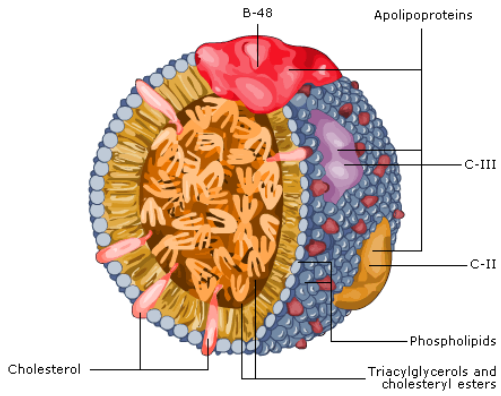
***Micelle Formation*:** Emulsified fats are acted upon by **pancreatic lipase** to convert TG into FFA and Monoglycerides. Together with lethicin, cholesterol and fat-soluble vitamins – micelles are formed with a polar hydrophilic shell on the outside of a 4-6 µm sphere composed of ~20 fat molecules.



Micelles then move into the luminal membrane of the villi to allow passive absorption of the monoglycerides and FFAs. FFA may be absorbed via FATP4 protein.

***Chylomicron Formation*:** They are approximately 100µm in diameter composed of:

- 87 % triglycerides
- 3 % cholesterol esters
- 1 % fat soluble vitamins enveloped in a hydrophobic coat of specific apolipoproteins
    - APO B-48 (transport of lipids) and APO C-II, C-III
- 9 % phospholipids and cholesterol

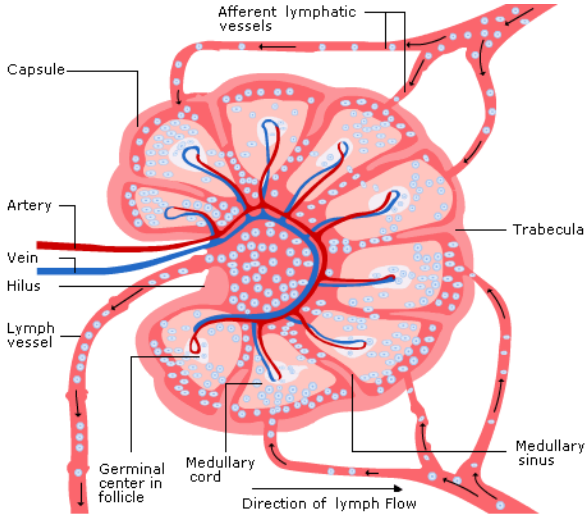


They are manufactured from long chain fatty acids in the golgi apparatus of the cells. This process occurs after the fatty acids are absorbed and re-esterified to triglycerides in the smooth endoplasmic reticulum of the epithelial cells (bypassed by smaller fats not requiring micelle formation).

***Chylomicron Absorption*:** They enter the **lymphatic lacteals** in the villi and once entered into the blood from the lymphatics, bind to sites on the capillaries of adipose tissue and muscle. Apolipoprotein CII within the chylomicron activates lipoprotein lipase present on the capillary endothelium → cleavage of the chylomicron for release of FA and MG to enter into the cell. Hepatocytes take up the rest of the chylomicron remnants (cholesterol).

Immune Response

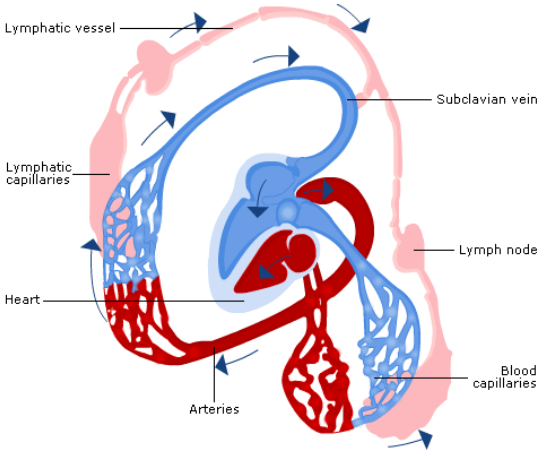
Lymphocytes (B and T-cells) are found in abundance in the spleen, **lymph nodes** and mucosal lymphoid aggregations. At one time, only 2% of the total body lymphocytes are in the blood. The entire lymphocyte population of the body **circulates 8-10x/day** which ensures antigens are picked up quickly by macrophages/dendritic cells to be transported and presented to T and B lymphocytes at the lymph nodes.



Maintaining Intravascular Fluid Volume

Daily ultra-filtration of water is 20 L/day, of which 16-18 L will be reabsorbed. This leaves 2-4 L/day to be returned via the lymphatics.

Lymph flow is slow (2-4L/day). As mentioned above driven mainly by -ve intraluminal pressure. The -ve intrathoracic pressure and suction effect of blood flow produces a constant flow of 100-125 mls/h back into the circulation. Flow in lymphatics can increase between 10-15x during exercise from skeletal muscle contractions when need increases. Following a meal there is increased smooth muscle contraction and rhythmic alterations in transmural pressures which result from the respiratory and cardiac cycles.



Lymph Drainage abnormalities

Primary Lymphoedema: Inherited conditions causing **hypoplastic/aplastic valves** leading to oedema in the affected limb. It occurs in Milroys disease and is autosomal dominant presenting within 20y of birth including SN hearing loss, extradural cysts, vertebral abnormalities and yellow nails.

Secondary Lymphoedema: May be due to:

- Surgical damage to the lymphatics
- Malignant diseases i.e. lymphoma or secondary metastases or radiation damage
- Trauma
- Infections – filariasis or TB.

Cardiac Muscle Contraction

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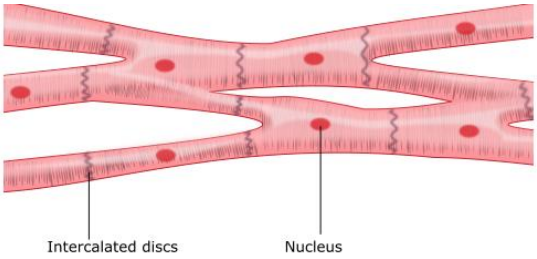
Cardiomyocytes

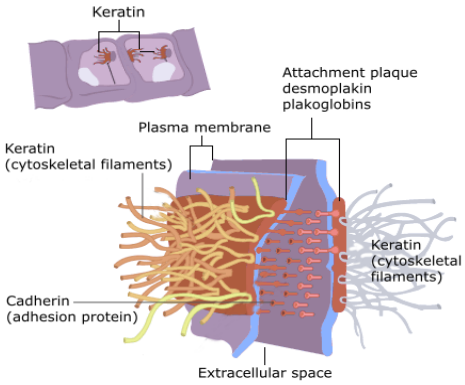
These develop from the splanchnic mesoderm which migrate to envelop the primitive heart tube. Each has a **central nucleus** and are **entirely dependent on aerobic metabolism**. **Numerous mitochondria**: up to 25% by volume, compared to 2% in skeletal muscle cells. 99% are specialised for mechanical work. Some are specialised for electrical conduction. They also have **high capillary density**.



Intercalated discs connect *groups* of cardiomyocytes laterally and end-end. There is also an **electrochemical & metabolic continuity between cardiomyocytes** creating a **functional syncytium**.

Cell Junction types

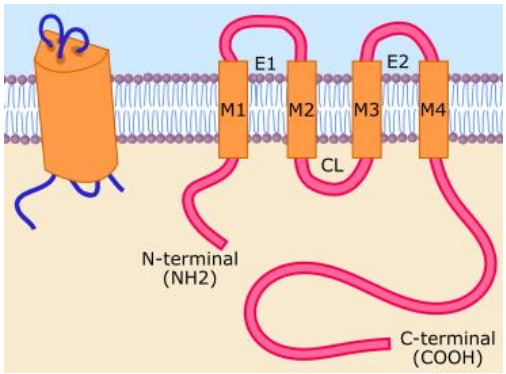
Desmosome junctional complex aka *macula adherens* - provides both structural integrity and tensile strength. The desmosomes serve as an anchor between the cytoskeleton of adjacent myocytes. Consist of an intracellular adhesion plaque and cadherins, which are transmembrane glycoproteins that span the gap between myocytes, typically 25 nm

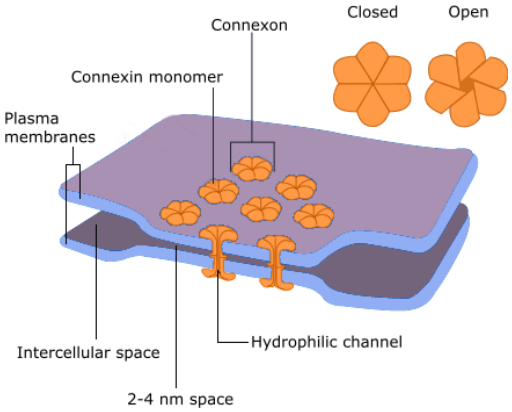


Gap Junctions: Consist of numerous, paired, hexameric ~26 kD proteins (**connexons**) that span the sarcolemma. **Six connexins**, each with:

- 4 transmembrane domains (M1-M4)
- 2 extracellular loops (E1, E2)
- Cytoplasmic loop (CL), cytoplasmic C-terminals and N-terminals

Unite to form a **connexon**. These unite in adjacent myocyte cells to allow the passage of small molecules and ions such as Ca^{2+}



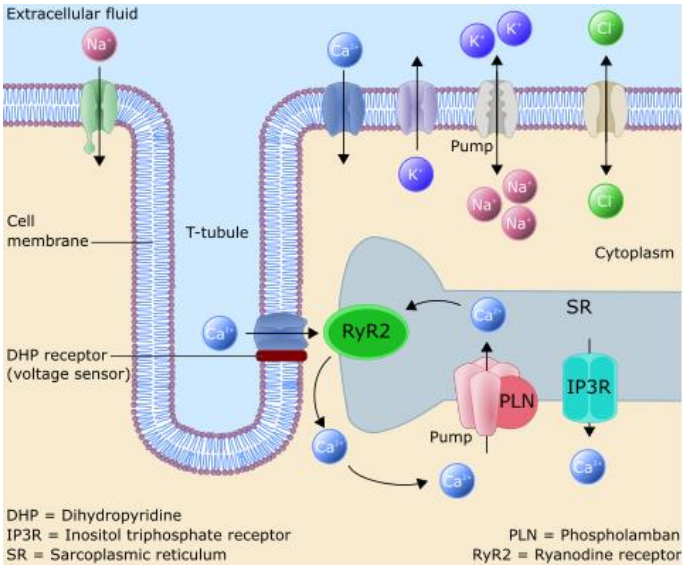


Sarcolemma

This is the cardiomyocyte membrane and is a bilipid membrane.

There are **T-tubules** that allow rapid depolarisation of the sarcolemma to rapidly penetrate the myocyte. T-tubules are short and broad, encircle the sarcomeres at the Z-lines and lie close to a single terminal SR cisterna to form a diad. DIFFERENT TO SKELETAL MUSCLE!

There is also the **sarcoplasmic reticulum (SR)** that sequesters, stores and releases the majority of the calcium ions that are required for muscle contraction. Important SR proteins include:

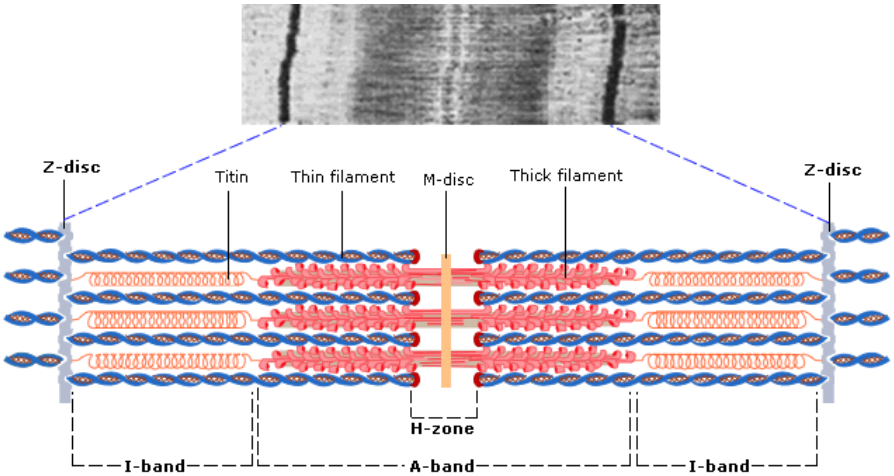


- **RyR2 (Ryanodine Receptor):** intracellular Ca^{2+} channel that is activated by Ca^{2+} and is inhibited by Dantrolene and Ryanodine. Calcium in the cytosol induces further release of calcium – known as **Ca^{2+}-induced Ca^{2+} release** (**CICR**)
- **Inositol trisphosphate receptor (IP3R):** glycoprotein complex that functions as a Ca^{2+} channel
- **Phospholamban (PLN):** pentamer that inhibits SR Ca^{2+} ATPase (SERCA). Once phosphorylated by protein kinase C, PLN effect is reversed.

There are a number of other membrane proteins that regulate the movement of ions across the sarcolemma and the sarcoplasmic reticulum through different mechanisms.

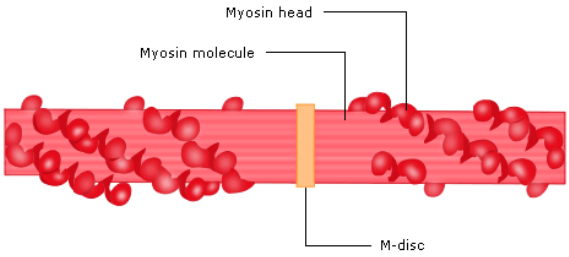
Sarcomere

This is the basic contractile unit of muscle:

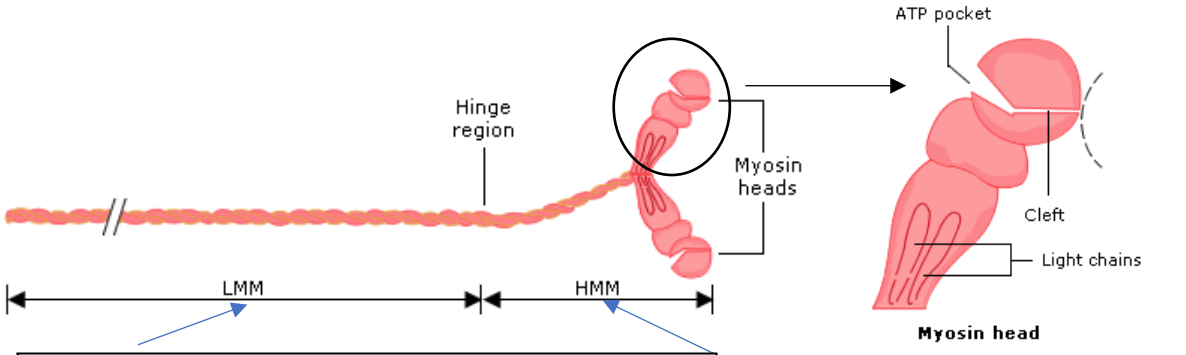


Thick Filaments

They are usually 300nm in length and 10nm in diameter. Composed of >200 molecules of the dimeric protein – **myosin**.



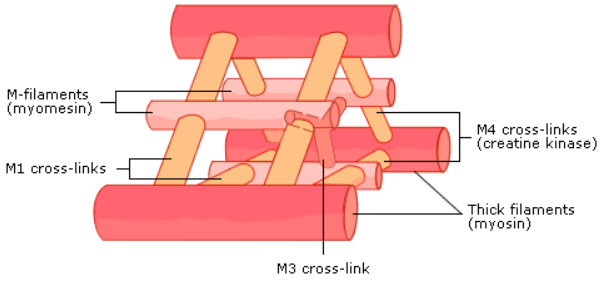
Myosin: Arranged in a helical fashion that the globular heads form 6 rows. There are 2 regions of myosin separated at a hinge:



LMM composed of light meromyosin (LMM) and heavy meromyosin (HMM), are arranged in an alpha helix with a functionally flexible hinge region

HMM comprises two globular heads, each composed of three segments (see above)

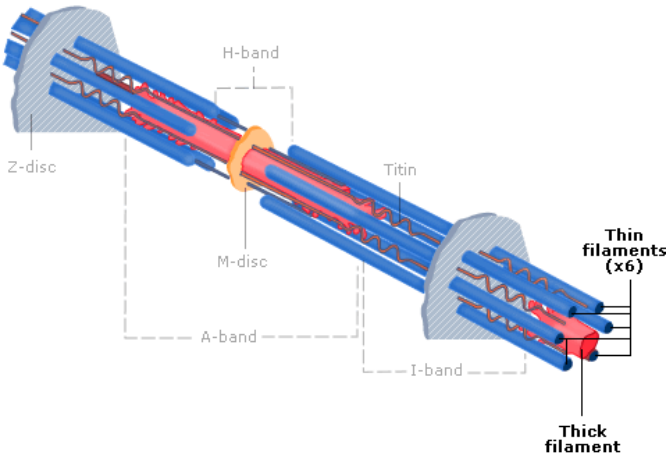
M-Disc: Adjacent thick filaments are joined at the M-disc by creatine kinase, *M-protein and myomesin to form a stable lattice*.



Titin: Is another protein associated with the thick filaments and is the largest known polypeptide (~3,800 kD). Bound to the M-lines and Z-lines, it spans half the length of the sarcomere (see sarcomere image). Titin possesses an elastic amine (N-terminal) region that allows **stabilisation of myosin** and **limitation of sarcomere stretching.**

Myosin binding proteins C and H: Hold the thick filaments together and to modulate actin-myosin interaction under the influence of cyclic AMP.

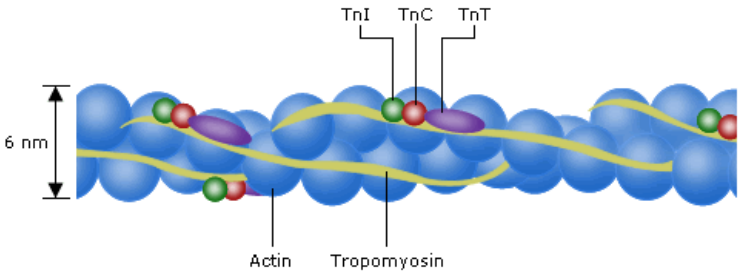
Thin Filaments



Composed of actin, tropomyosin and troponin. They are **anchored at the Z-disc** with a-actinin, nebulette, filamin and other proteins.

Actin: The most abundant cytoplasmic protein in mammalian cells. They exist as **G-actin monomer** (globular) and **combine to form the F-actin polymer** (Filamentous). 2x F-actins wind in a helix pattern. Actin monomers have a

binding site for myosin head interaction.



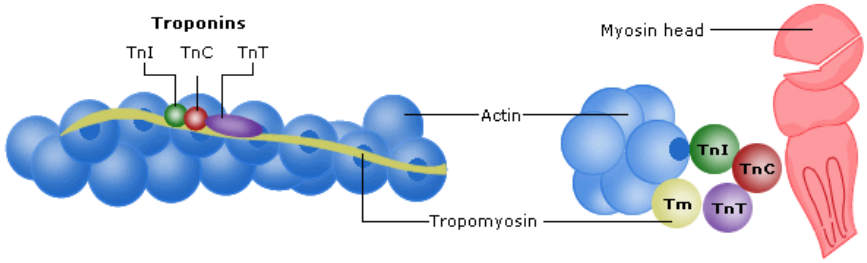
Tropomyosin: consists of 4 alpha helices and winds around the actin polyfilament to block the binding of myosin heads to actin.

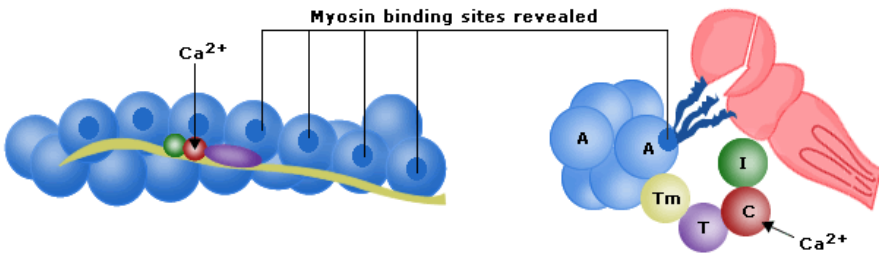
Troponins: All form clusters spaced out along the tropomyosin. Three types:

- **TnI** = inhibitory actin-binding
- **TnC** = calcium-binding
- **TnT** = tropomyosin-binding

Contraction

- ***In resting muscle***, tropomyosin (Tm) overlays the myosin binding sites on actin, with a single tropomyosin molecule that spans 7 actin subunits. This position is locked by TnI and TnT.
- ***Calcium release from SR*** binds to TnC – unbinding of Tropomyosin from actin.
- ***Myosin binding*** to 1 actin subunit causes full displacement or tropomyosin and allows additional heads to bind.
- ***Calcium is sequestered*** and tropomyosin rebinds to actin to prevent further cross linkage.



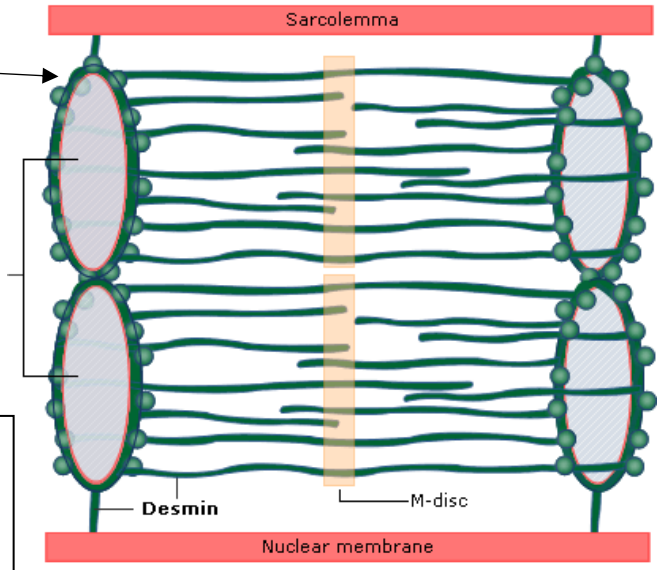


Z-Disc:

Desmin, a 52 kD intermediate filament protein that encircles the z-disc and connects to the z-disc of adjacent sarcomeres.

Z-disc composed of numerous structural and regulatory proteins, i.e. enzymes

Desmin also **attaches** to the **intracellular surface of the sarcolemma** and **links the sarcomere to** the nucleus, mitochondria and other **organelles**

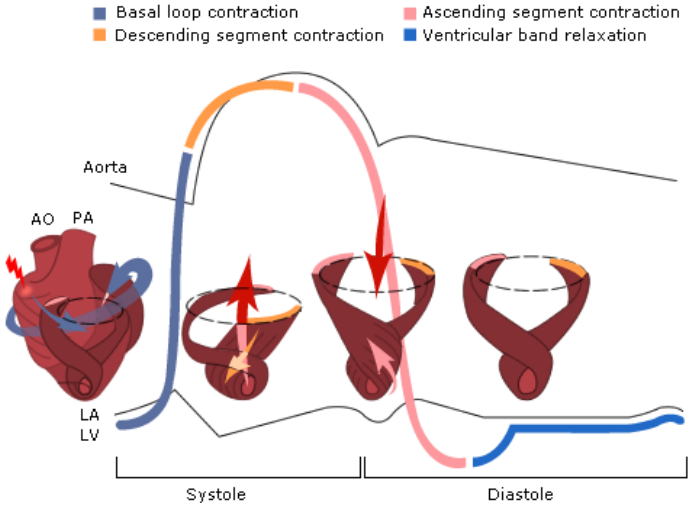


It is thought that Desmin is involved in force transmission and longitudinal load bearing and plays an important role in the maintenance of the structural and mechanical integrity of cardiac myocytes.

Functional Organisation

Connective tissue provides a spongiform scaffold for cardiomyocytes, blood vessels and nerves. **Capillary density** in cardiac **muscle is 3000/cm²**, compared to <1000/cm² in skeletal muscle. It also provides an **endothelial surface area for gas exchange 5x greater** than that found in skeletal muscle.

The muscle fibres (4-6 myocytes in thickness) are arranged in distinct tightly adhered laminae which are separated from adjacent laminae by an extracellular collagen matrix. These muscle fibre bands are arranged in multiple directions allowing ascending, descending and rotational forces to be generated during ventricular systole – ***helical ventricular band model***.



The Cardiac Cycle

(07b_02_04)

The cardiac cycle involves the dimensions of **time**, **pressure** (LA, LV and aorta) and ventricular blood **volume.**

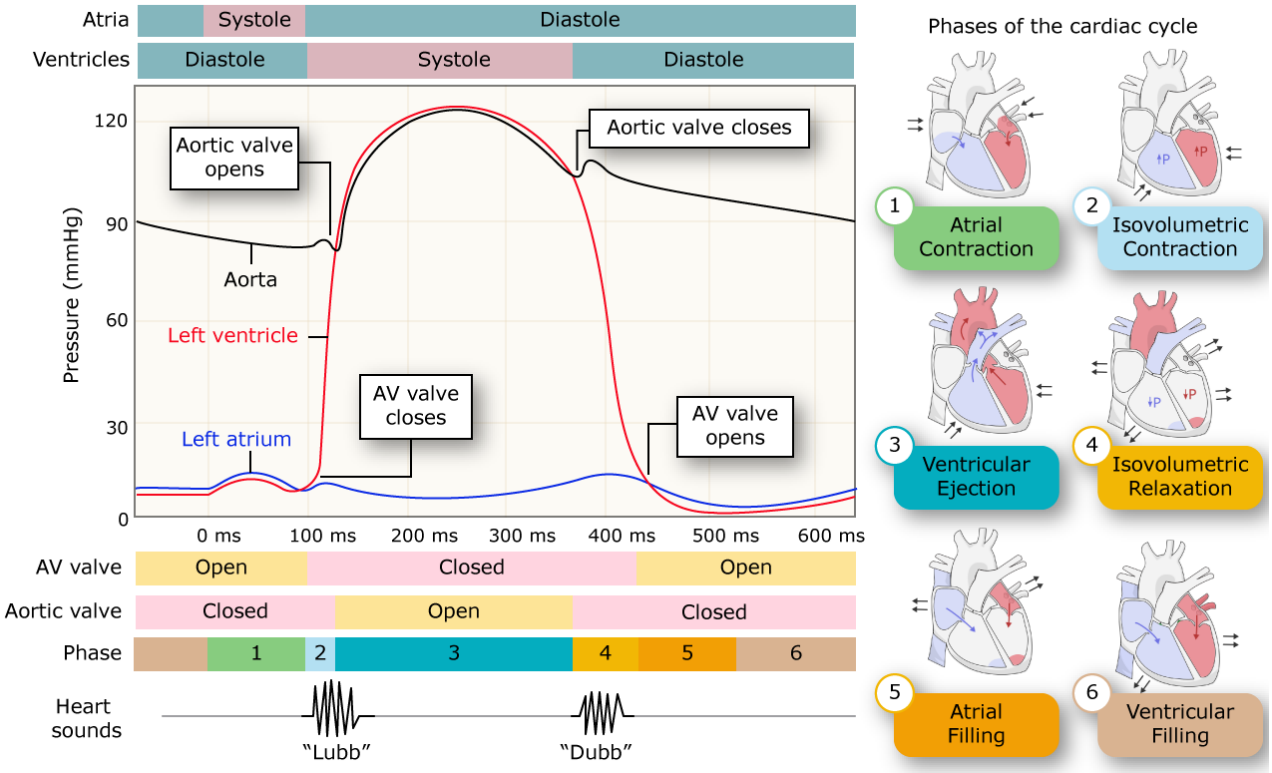
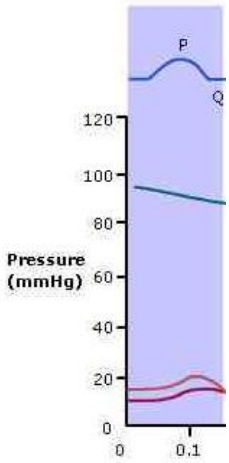


Diagram above is the complete cardiac cycle which can be split into 6 or 7 stages:

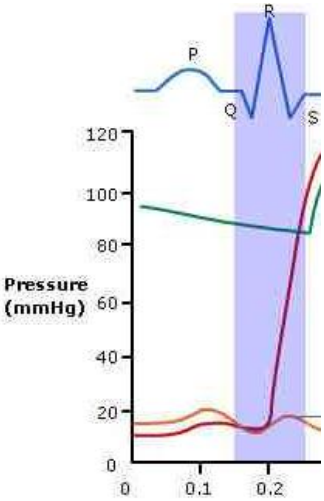


STAGE 1: ATRIAL SYSTOLE **Atrioventricular valves are open** **P-wave**

Pressure in the atria are greater than that of the ventricles – blood is forced through the AV valves.

Atrial contraction only accounts for 20% of ventricular filling at rest but can be as much as 40% if tachycardic due to less time at diastole.

The small increase in atrial pressure is represented as the **a-wave** of a jugular pulse and the **x descent** is present following the fall of atrial pressure on completion of contraction and the atrioventricular valves close.



STAGE 2: ISOVOLUMETRIC CONTRACTION

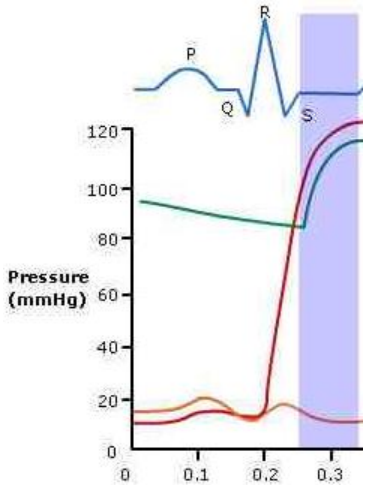
All valves closed

QRS complex

Ventricular depolarisation → ventricular contraction → rapid increase in ventricular pressure > atria but < outflow tract. AV valves close.

Known as isovolumetric as there is no change in the volume of the heart whilst all valves are closed

Atrial pressure increases as the AV valves bulge back seen as the **c-wave** in the jugular pulse.



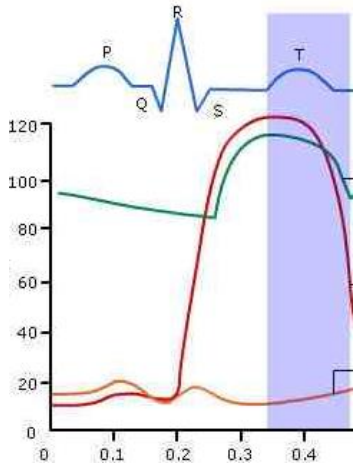
STAGE 3: RAPID (VENTRICULAR) EJECTION

Aortic & pulmonary valves open, AV valves remain closed

ST segment

Intraventricular pressure exceeds that in the outflow (aorta/pulmonary a.) so the valves open and ejection of blood resumes. This is the stage of **maximum velocity blood flow** and the ventricular volume subsequently decreases.

Atrial volume increases from passive filling but pressures initially decrease as the base of the atria is pulled downward and so the atrial size expands.



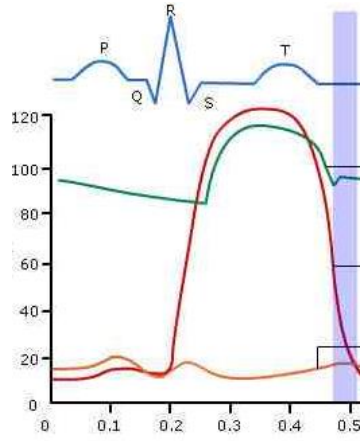
STAGE 4: REDUCED (VENTRICULAR) EJECTION

Valves as stage 3

T wave

Ventricular repolarisation initiates its relaxation and rate of emptying falls. Although ventricular pressure falls below the outflow tract, the **kinetic energy of propelled blood** allows continual ventricular emptying.

Atrial pressures rise as a result of continued filling.



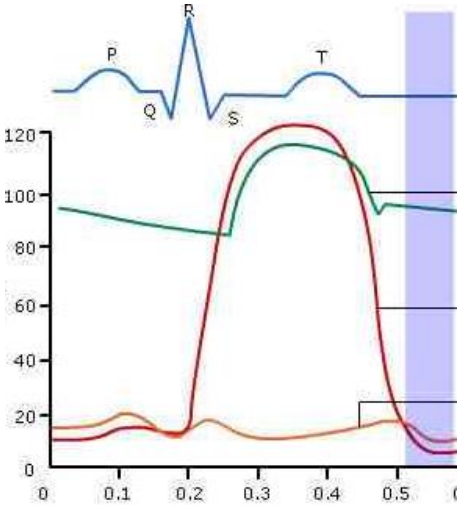
STAGE 5: ISOVOLUMETRIC RELAXATION

All valves closed

Neither the ventricular pressure nor the kinetic energy is enough to allow blood flow to the outflow tract so valves close. Valve closure is associated with a **characteristic notch** caused by a brief flow reversal → closure of the valves → **elastic recoil** of the vessel walls

Ventricular "*end systolic/residual*" volume remains constant throughout this phase at approximately 50ml

As the atria fill further, the pressure increases against the closed AV valve representing the **v-wave** of the jugular pulse.



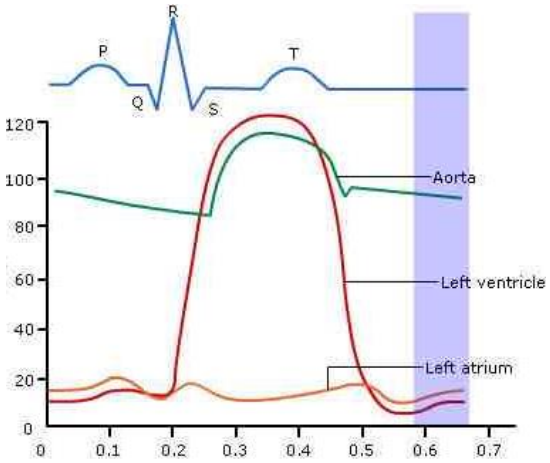
STAGE 6: RAPID (VENTRICULAR) FILLING

AV valves open

Ventricular pressures are lower than atria so the AV valves open. As atria are at maximal pressures, the **flow is rapid** especially with the help of diastolic suction of the ventricles as it actively relaxes.

The rapid fall in atrial volume and hence pressure is represented as the **y-decent** on the jugular pulse waveform.

A **3rd heart sound** may be heard due to tensing of the chordae tendineae and the atrioventricular ring which support the valve leaflets. This is pathological except in children



STAGE 7: REDUCED (VENTRICULAR) FILLING

Valves as with stage 6

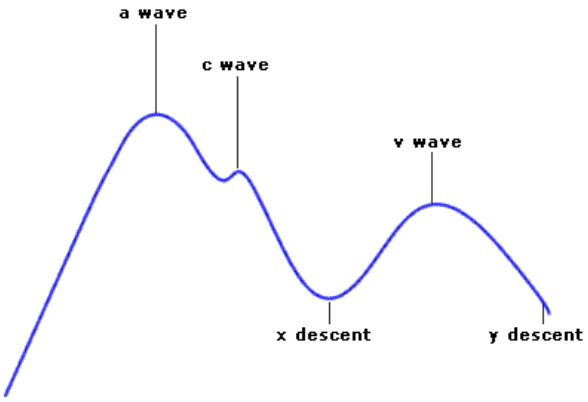
Pressure gradient between ventricles and atria decreases and therefore they fill more slowly. The compliance of the ventricles reduces as they fill with more blood.

Jugular Vein Waveform

The waveforms have been previously described in the cardiac cycle but in summary:

a wave: Atrial contraction causes a reflux of blood into the venae cavae increasing the pressure to 3-5 mmHg Not present in AF.

c wave: Formed when the cusps of the AV valves bulge back into the atria during isovolumetric contraction and from transmitted pulsation from the carotid arteries



x descent: Following the c wave, the atrial pressure drops rapidly as the atria relax and the atrioventricular ring and base of the ventricle are pulled down during the early rapid ejection phase

v wave: In ventricular systole, the pressure rises due to filling of the atria from the continued venous return of blood to the heart on closed AV valves

y descent: This pressure drop represents the AV valves opening and blood rapidly leaving the atria into the relaxed ventricle in diastole

Pressure (peak and trough) levels

	Pressure (mmHg)			Pressure differential (mmHg) between aorta and ...	
	Aorta	Left ventricle	Right ventricle	Left ventricle	Right ventricle
Systole	120	121	25	-1	95
Diastole	80	0	0	80	80

Heart Sounds

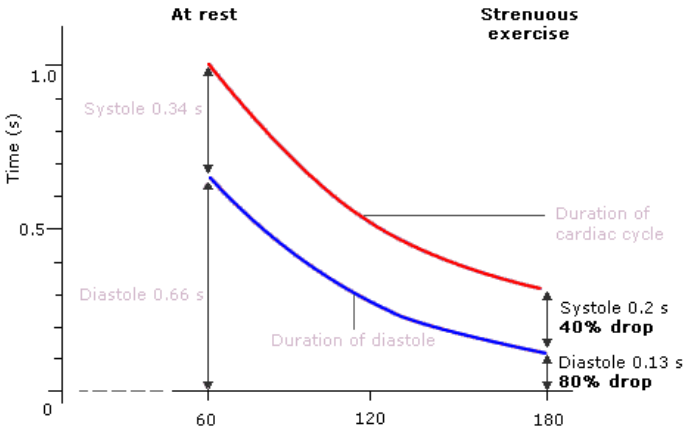
1st and 2nd heart sounds are both split as the L sided heart valves close just prior to the R sided heart valves.

3rd heart sound: may represent tensing of the connective tissues that support the valve cusps. It commonly occurs in conditions with **ventricular dilation**

4th heart sound: occurs during atrial systole. It represents vibration of the ventricular wall during atrial contraction and occurs in conditions associated with **'stiff' ventricles**

Effect of Heart Rate

As HR increases, cardiac cycle phases shorten disproportionately with diastole shortening more than systole. During **short diastole**, most **ventricular filling still occurs in the early rapid filling phase** and atrial systole contributes to more filling.



With **further increases in heart rate**, the **diastolic phase becomes too short** to ensure adequate filling of the ventricle and **cardiac output cannot be sustained**. Therefore, oxygen supply to the myocardium declines because most coronary blood flow occurs in diastole. Those with coronary artery disease experience this state at a HR <180.

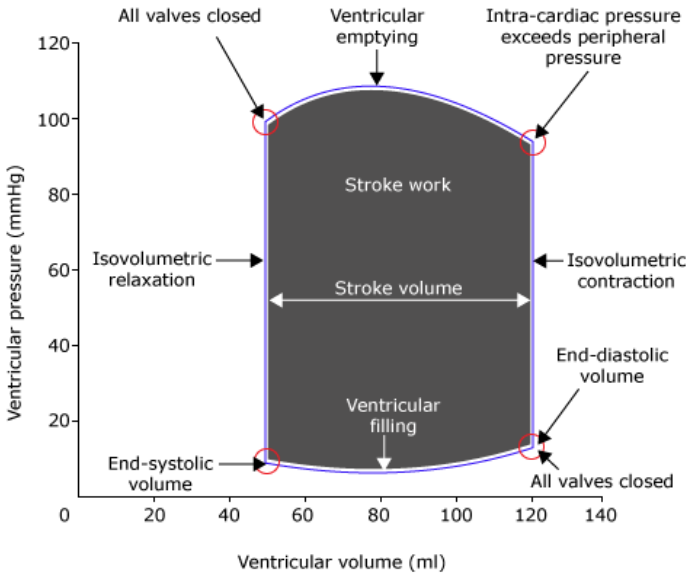
Pressure-Volume Loops

This is a way in which the cardiac cycle can be analysed and is generated by plotting **ventricular volume (x-axis)** against **ventricular pressure (y-axis)**

The cycle runs **anti-clockwise.**

Stroke volume is the EDV – ESV and therefore represented by the width of the curve.

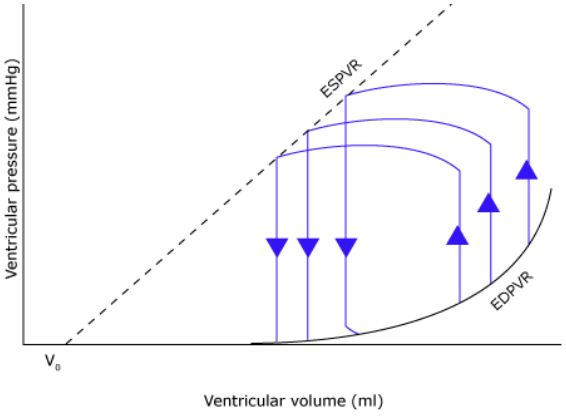
Stroke work is represented by the area in the loop (measured in J)



If multiple flow pressure loops are created for the **left ventricle**, a line can be drawn along the beginning of the end-diastolic volume and beginning of the end systolic volume. The slope of these curves is **reciprocal of the ventricular compliance**.

ESPVR = end-systolic pressure-volume relationship

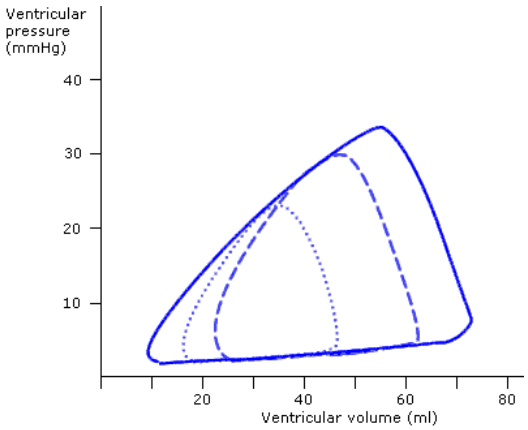
EDPVR = end-diastolic pressure-volume relationship



The right ventricular shape differs as ejection from the RV occurs earlier in systole before peak R ventricular pressure (poorly defined isovolumetric contraction phase).

The pulmonary circulation has high capacity and low pressure so ejection continues whilst the RV pressure falls (up to 60% volume ejected following peak systolic pressures).

As the RV pressures are lower, the stroke work is less than that of the LV.



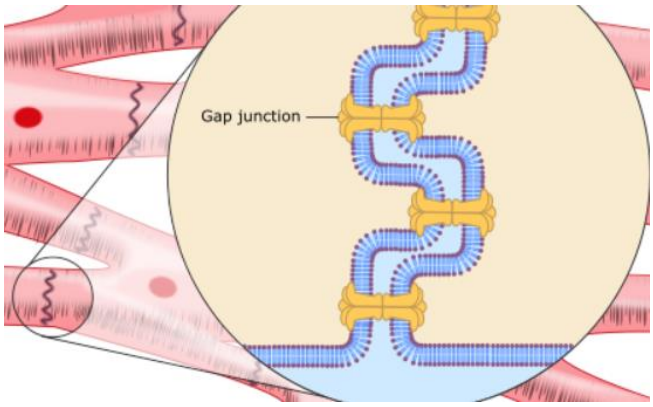
Both RV and LV pressure-volume loops change according to differing pathology.

Cardiac Electrophysiology

(07b_02_05)

The heart is made up of **2 tissues: contractile myocytes** and **specialist cells** responsible for action potential generation and conduction.

Myocytes are arranged end-to-end separated by **gap junctions** which allow passage of the action potential throughout the **syncytium**.



Cardiac Myocyte Action Potential

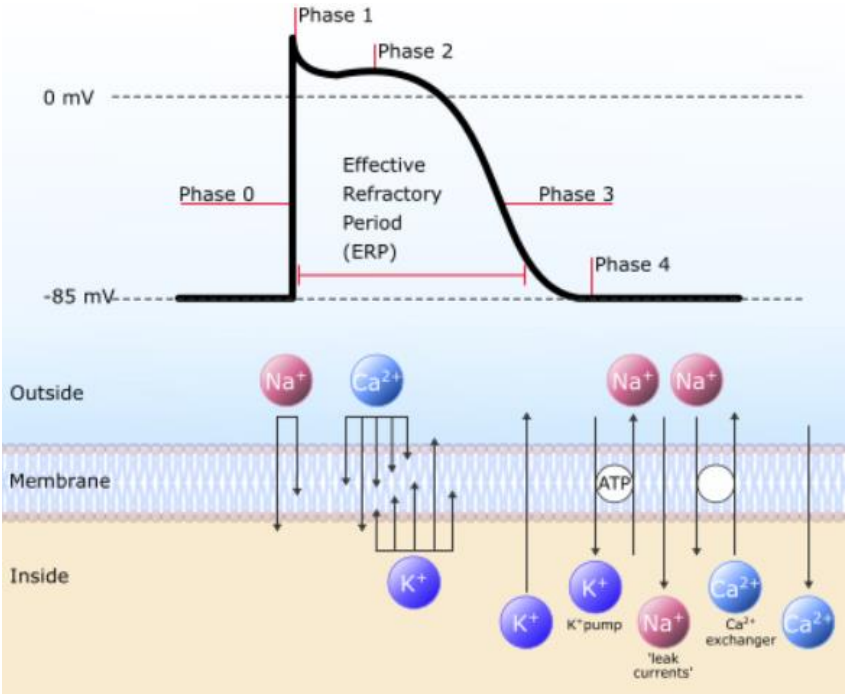
Phase 0: Depolarisation from **sodium ion influx** into the cell

Phase 1: Sudden **closure of sodium channels** and opening of **potassium channels for efflux.**

Phase 2: Plateau absolute refractory period where **Ca^{2+}** flows **into** the cell to help balance the potassium efflux. This prevents tetanic contraction.

Phase 3: Relative refractory repolarisation through **Ca^{2+} channel closure** and ongoing **K^+ efflux**

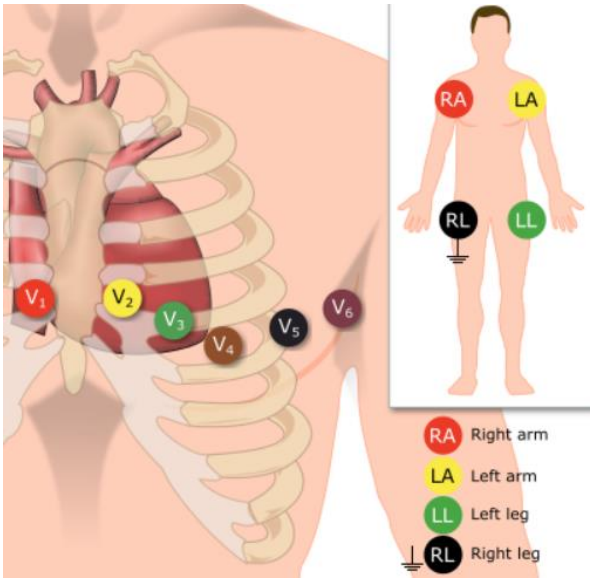
Phase 4: Resting membrane potential created through the action of Na^+/K^+ ATPases.



ECG

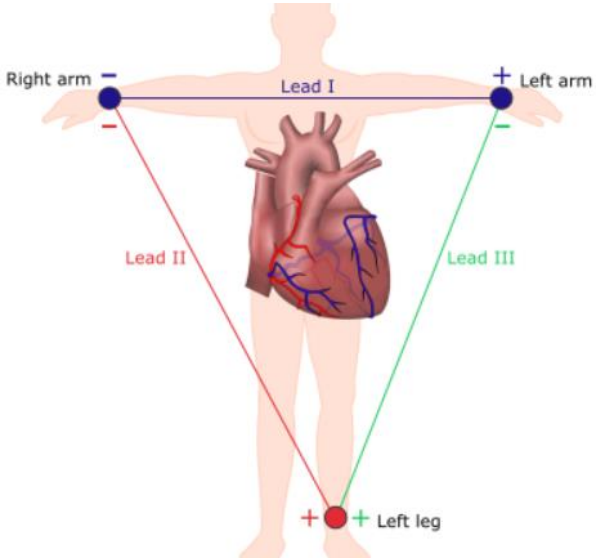
If you imagine the heart as an accumulation of myocardial cells, each cell forms an action potential which can be measured and has a **potential** and a **direction**. Through mathematical vector analysis, measurement in line with the fibre will show the classic shape as above but perpendicular will show a flat trace (isoelectric). All the action potentials will add up and can be measured and analysed.

Electrodes are placed so that the potential **between 2 electrodes** are able to be measured. The LV has the largest muscle mass so its action potential predominates.



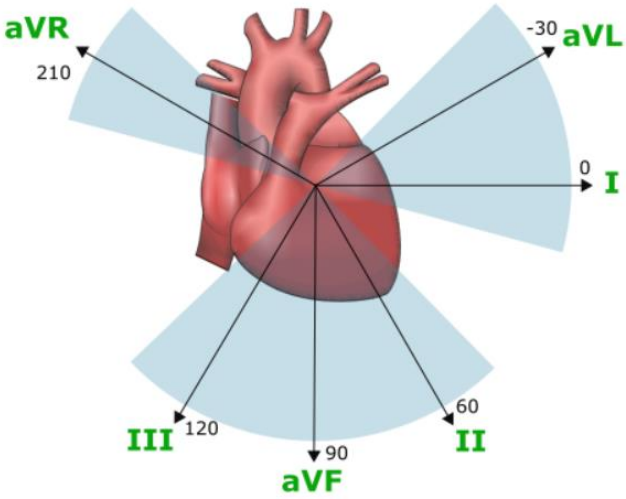
Einthoven's triangle is a representation of the potentials measured **between bipolar** (measures between 2 leads) **leads** giving 3 directions for vector analysis:

- Lead I: LA-RA
- Lead II: LL-RA
- Lead III: LL-LA



Unipolar leads compare the potential of one of the limb leads to a common neutral lead:

- aVF: LL-Common
- aVR: RA-Common
- aVL: LA-Common



From the understanding of the position of the leads and interpretation of the ECG regarding the QRS deflection, one can calculate the **cardiac axis**. This is represented as leads I and aVF in the module as these have a pure x and y-axis deflection respectively.

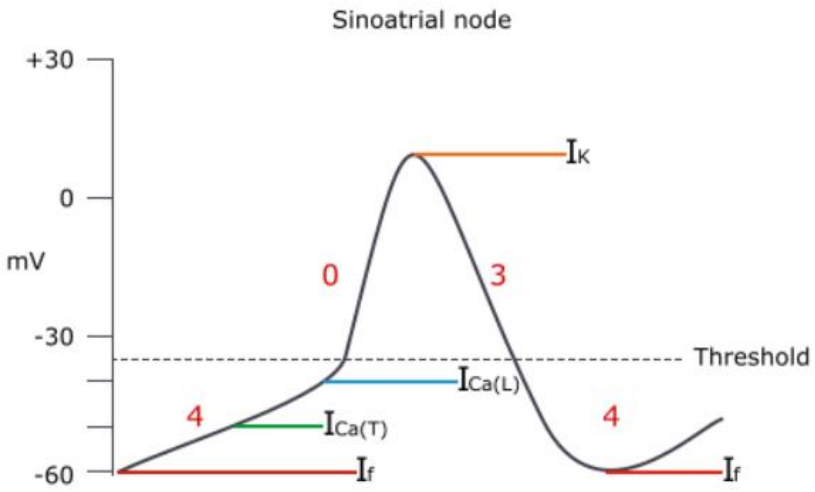
The **normal axis** is **between -30° and +90°.**

Pacemaker Action Potential

Phase 4: At -60mV **slow inward Na^+ 'funny' currents** for gradual depolarisation.

At -50mV, **T-type Ca^{2+} channels open** allowing Ca^{2+} influx for further depolarisation.

At -40mV, **L-type Ca^{2+} channels open** to allow further influx till the **threshold potential** of ~-35mV is reached.



Phase 0: L-type Ca^{2+} channels create a slow rise in action potential to depolarisation at +10mV.

Phase 3: Closure of the Ca^{2+} channels and opening of K^+ channels to allow potassium efflux. This returns the AP to the resting potential of -60mV – necessary for reactivation of phase 4 ion channels.

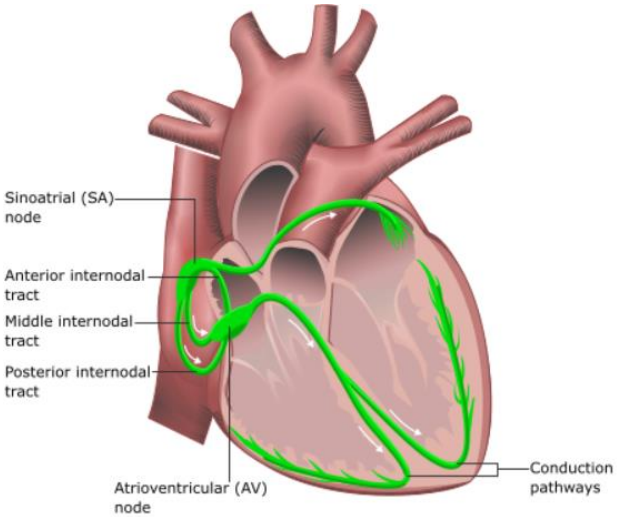
The SAN has the highest rate of spontaneous depolarisation than any other pacemaker cell. Therefore, it is the primary pacemaker. If the SAN fails, other pacemakers will trigger to prevent electrical standstill.

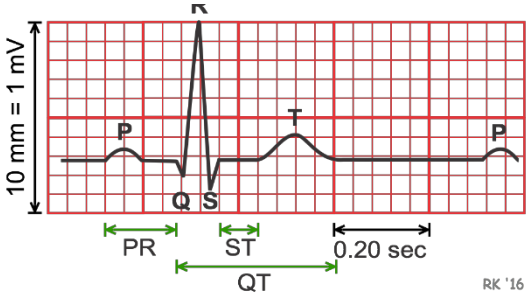
Cardiac Conduction System

The **SAN** lies in the **posterolateral wall** of the **R atrium**. 3 internodal tracts connect the SAN to the **AVN** which lies in the **R atrium behind the tricuspid valve**. (**P-wave**)

The atria and ventricles are otherwise electrically insulated from each other by **fibrous tissue**.

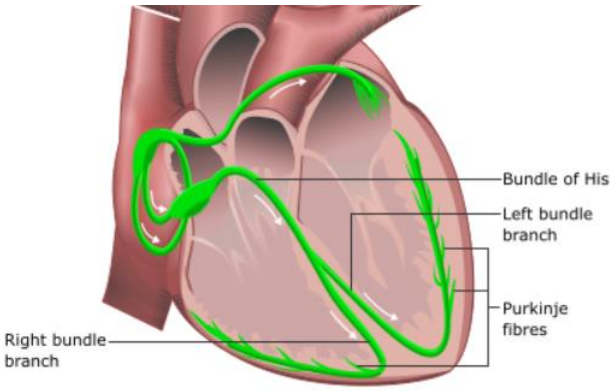
The **delay in contraction** between the atria and ventricles is essential to allow adequate time for ventricular filling from atrial contraction and is achieved by the AVN. This is achieved by a **reduction in the number of gap junctions** between the adjacent cells and transmission in a **unidirectional manner**. (**PR interval**)





The next tracts are **AVN → bundle of His → L and R bundle branches → Purkinje fibres**. The LBB divides into anterior and posterior fascicles.

The Purkinje fibres exist throughout the ventricular syncytium and are **large** allowing **very fast transmission** of APs. (**QRS complex**)



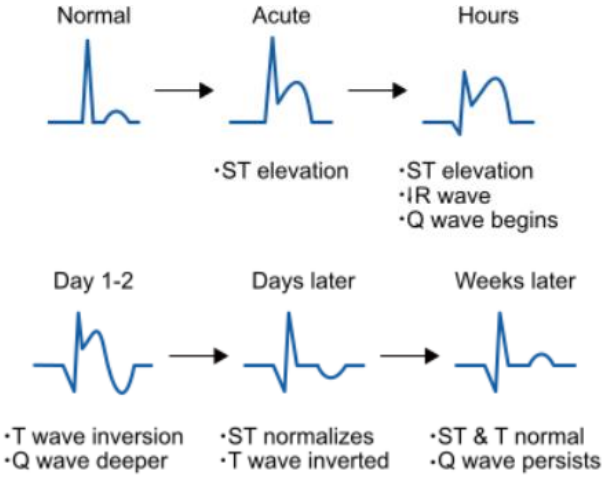
T-wave represents repolarization of the ventricles. This does not require involvement of the Purkinje system so is slower and less coordinated than the phase of depolarisation. This is why the T-wave has a lower peak and a longer duration.#

Coronary Artery Perfusion

Pathological ECG complex changes

The ECG may be used for investigating confirmed/possible ischaemic damage to the myocardium. A **sequential change** occurs in the ECG complex during a developing **myocardial infarction**:

The ST segment is affected due to a voltage gradient being produced between sections of damaged and non-damaged myocardium.

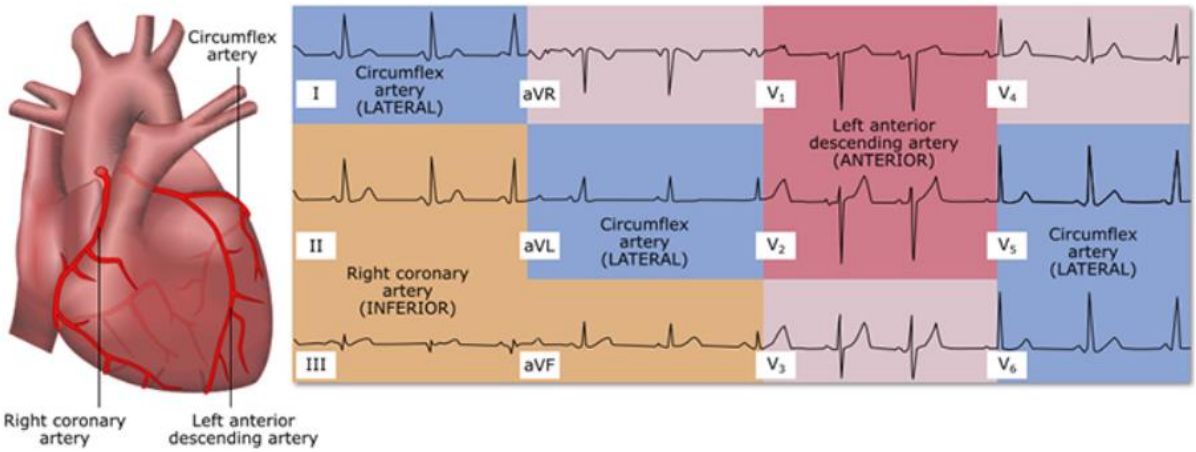


- **ST elevation:** Transmural infarction
- **ST depression:** Ischaemia

Myocardial Territories

Each lead on the ECG examines the flow of current in a specific vector. The posterior and far lateral portions of the myocardium are poorly represented in the normal '12-lead' ECG.

The **coronary artery blood flow** supplies particular parts of the myocardium and hence can be represented on the 12-lead ECG trace as shown. The ECG changes described above will be shown in the territories supplied by the coronary artery which is occluded.



- ***Right Coronary Artery*:** Supplies the **right atrium and ventricle** and **posterior septum**. This is the **inferior territory** represented by **leads II, III and aVF**

Left Coronary Artery splits into the:

- ***Left Anterior Descending Artery*:** supplies the **anterior and apex of the left ventricle** and **anterior portion of the interventricular septum**. This is the **septal and anterior territory** represented by **leads V1 and V2, V3 +/- V4**.

1.

- ***Circumflex Artery*:** Supplies the **left atrium** and **posterior portion of the left ventricle**. This is the **lateral territory** represented by **leads I, aVL, V5 and V6**.

Further information on the ECG and arrhythmia physiology can be found on the e-LA module. Click here for the link if using these notes electronically.

(07b_02_06)

Central and Autonomic Regulation of Cardiac Function

(07b_02_07)

The regulation of cardiac function is a dynamic process responding to constantly changing demands on the heart and occurs via multiple homeostatic mechanisms altering:

- Heart rate
- Stroke volume
- Vascular resistance
- Changes in circulatory volume (indirect)

Measurement of cardiac function is defined as either/or:

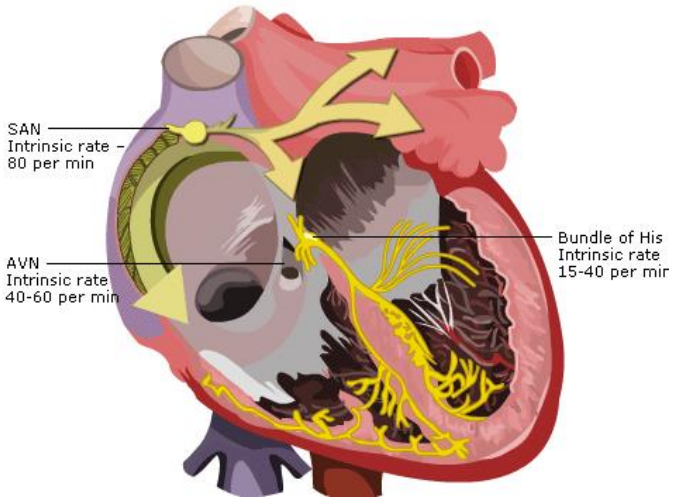
Cardiac output = Stroke Volume x Heart Rate

Cardiac Index = Cardiac Output (L/min) / Body Surface Area (m^2)

Cardiac Intrinsic Autorhythmicity

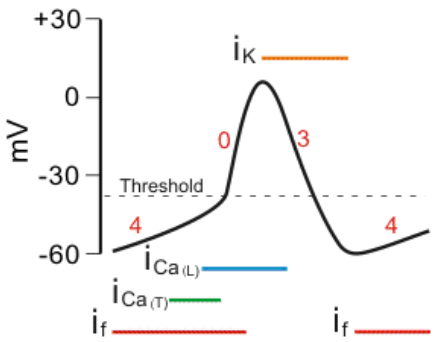
The **sino-atrial node** (**SAN**), **atrio-ventricular node** (**AVN**) and the **bundle of His** all have intrinsic pacemaker activity. The SAN has the greatest intrinsic rate of rhythmical discharge and is the normal pacemaker of the heart and is therefore said to have **dominance**.

This is controlled by central, autonomic and humoral mechanisms.



Autonomic Control of the Sinoatrial Node

The initial potential (not resting) is at -50 to -70mV and decays spontaneously over time. Once it reaches around -50mV, an action potential is initiated. The **rate of decay of phase 4 of the pacemaker potential determines the rate of action potential initiation.**

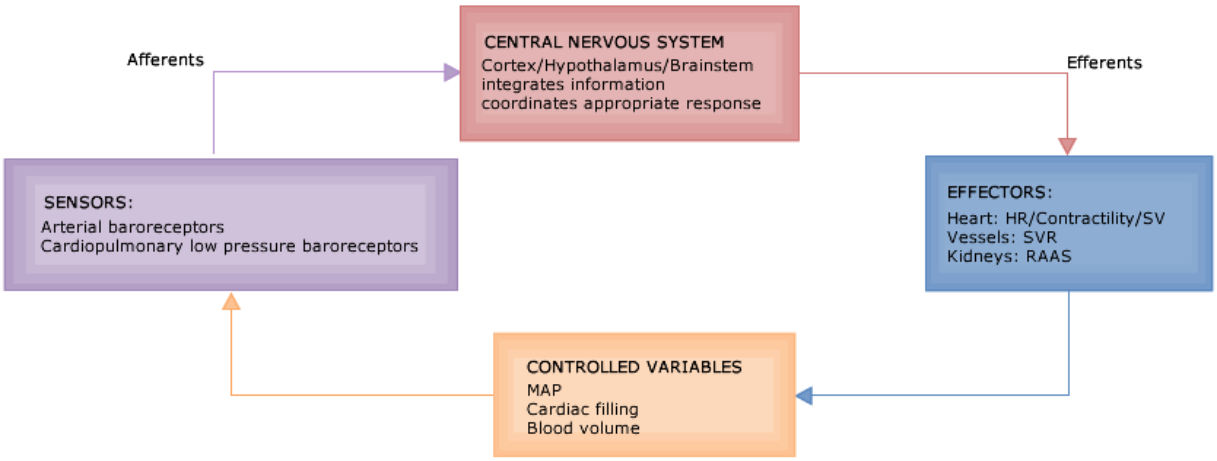


Sympathetic nervous system increases sodium and calcium permeability → ***more positive membrane potential*** *and* ***shortening of phase 4 of SAN and AVN length of decay to threshold*.**

Parasympathetic nervous system increases membrane permeability of potassium to the SAN, AV junctional fibres and the AVN → ***more negative membrane potential*** and ***lengthening of phase 4 AVN length of decay to threshold***

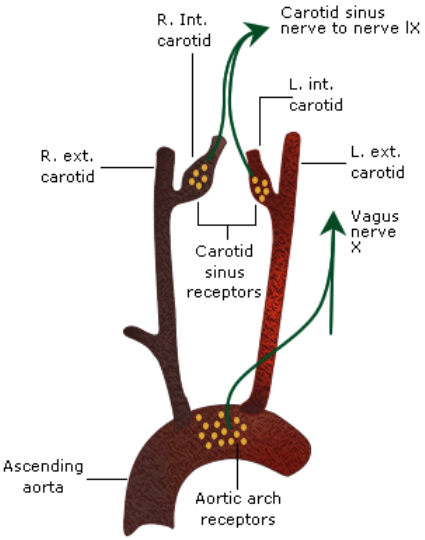
Nervous System Control of Cardiac Autorhythmicity

Nervous system regulation of cardiac autorhythmicity, HR and contractility have the following 4 principle elements to its control:



BARORECEPTORS

These mechanoreceptors measure the magnitude and the rate of rise of **stretch** and are the most important *short-term regulators of MAP* through reflex arcs.



High pressure baroreceptors: These are found in the **carotid sinus** (*adventitia of the right and left internal carotid arteries*) and **aortic arch** (*adventitia of the transverse arch of the aorta*)

- *Afferents*: Glossopharyngeal (IX) and Vagus n. (X) → Nucleus tractus solitarius (NTS) (in dorso-medial medulla).
- *Efferents*: Regulated by the SNS and PNS of Vagus n. via NTS

Increased MAP → baroreceptor activation → Inhibition of SNS and **Activation of PNS** → Decreasing HR, SV, SVR → decreased MAP.

Opposite occurs if decreased MAP.

Low pressure baroreceptors: Located predominantly in the atria, ventricles and coronary arteries

Myelinated veno-atrial baroreceptors: Located in the veno-atrial junctions, these mainly signal **central blood volume** and is triggered through **increased cardiac filling** via myelinated **vagal afferents** → paradoxical increased **SNS stimulation** → **reflex tachycardia** to shift blood from a potentially congested venous system to the arterial system → **BAINBRIDGE REFLEX**.

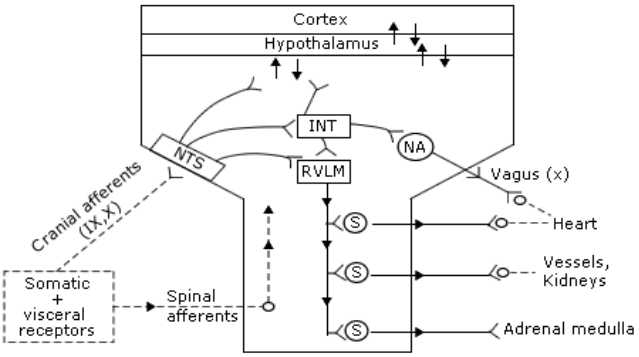
Non-myelinated baroreceptors: Located in the left ventricle, atria and pulmonary artery. Non-myelinated fibres responsive to stretch → Vagus and SNS nerves → Reflex bradycardia and vasodilation. *NB the activity of these receptors are weak*.

Coronary artery baroreceptors: Located in in the adventitia of coronary arteries → Vagus n.

CENTRAL CONTROL PATHWAYS

There are multiple areas of control but the **nucleus tractus solitarius** is the principle relay centre. The only part not labelled in the diagram on the right is the limbic system and periaqueductual grey that has some control over the cardiac function.

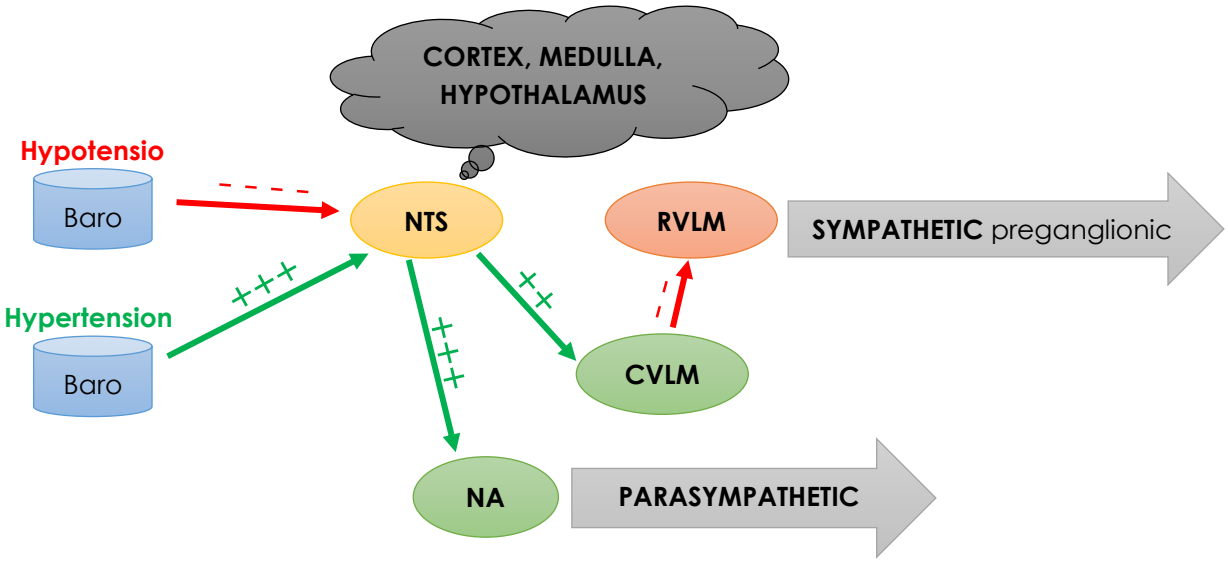
The autonomic nervous system and hormones provide the main efferent pathways from the CNS to the heart.



RVLM - Premotor sympathetic nerve cells (rostral ventrolateral medulla)
NA - Preganglionic parasympathetic nerve cells (nucleus ambiguus)
INT - Interneurons
NTS - Nucleus tractus solitarius
S - Preganglionic sympathetic nerve cells in the intermediolateral column of the spinal cord

Nucleus Tractus Solitarius (NTS)

Principle relay centre: Receives afferent impulses predominantly from baroreceptors and relays information to the spinal cord, medulla, hypothalamus and cerebral cortex for cardiac function.



- **NTS = Nucleus Tractus Solitarius**
- **NA = Nucleus Ambiguus**
- **CVLM = Caudal Ventrolateral Medulla**
- **RVLM = Rostral Ventrolateral Medulla**

The cortex, medulla and hypothalamus modulate the NTS response from the baroreceptors and is thought to be the mechanism that baroreceptors are reset in chronic hypertension.

Periaqueductal Grey (PAG)

located in the midbrain is important in the regulation of cardiac function, especially in the flight and fight response:

- **Lateral areas:** responsible for vasoconstriction and hypertension
- **Ventrolateral areas:** responsible for vasodilatation and hypotension

Hypothalamus, Limbic System and Cortex

Hypothalamus: STRESS → Stimulation of RVLM → Increased sympathetic outflow & inhibition of baroreceptor reflex at NTS

Limbic system: Emotion such as fear and rage → activation of hypothalamic stress response

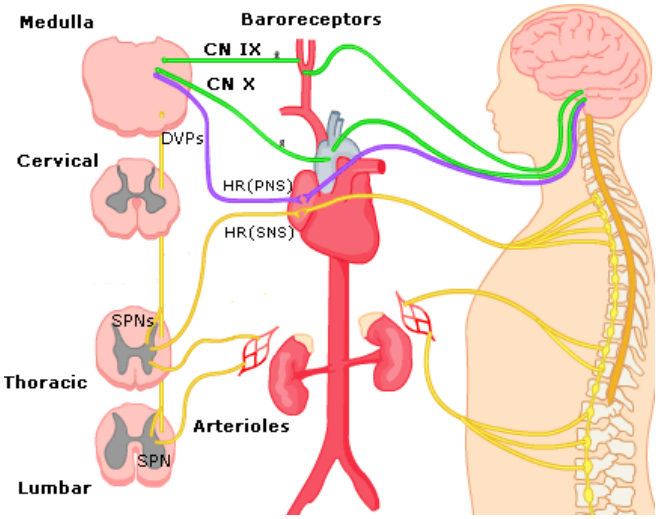
Cerebral cortex: Multiple connections to hypothalamus, limbic system and NTS.

AUTONOMIC REGULATION OF CARDIAC FUNCTION

At rest both the PNS and SNS are active continuously modifying cardiac function.

PNS inhibition of intrinsic **cardiac pacemaker** activity **predominates at rest** → 50-100bpm.

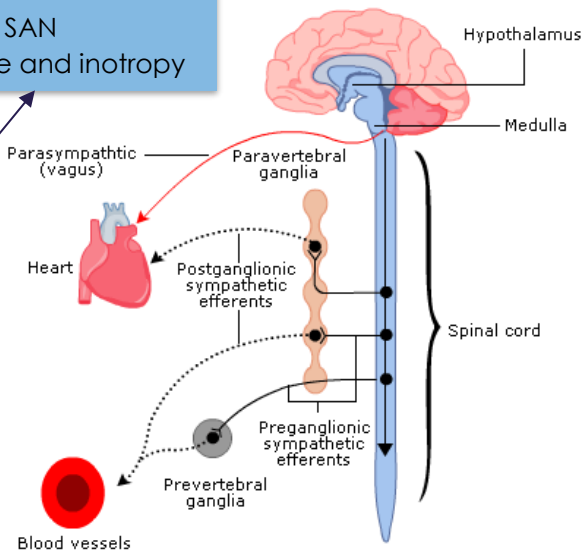
Changing PNS and SNS balance predominates the physiological changes in heart rate – less so the contractility. The **PNS is more rapidly changing** than the SNS and therefore responsible for more rapid control of heart rate.



Remember:
1. R ganglia is closer to SAN
2. L ganglia – L ventricle and inotropy

Sympathetic Nervous System

Originates as preganglionic fibres from the intermediolateral columns of the upper thoracic spinal cord, T1-T5. Right paravertebral ganglia predominantly influence chronotropy. Left paravertebral ganglia predominantly control inotropy. The long post-ganglionic fibres innervate all parts of the heart & vasculature.

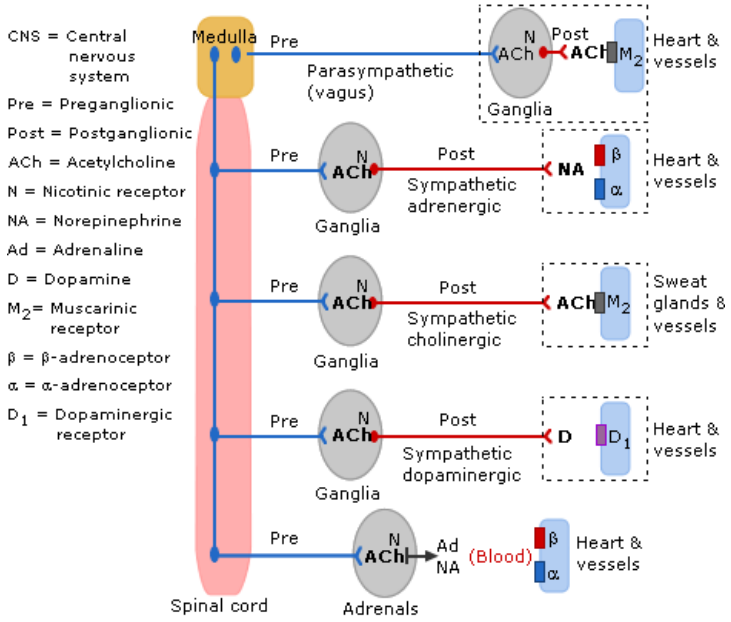


- **Chronotropic Effect:** increases membrane sodium and calcium permeability of the SAN and AVN → ***more positive membrane potential*** and ***shortening of phase 4 of SAN and AVN length of decay to threshold***. This may increase up to 200bpm in an adult.
- **Inotropic Effect:** increases membrane calcium permeability (especially plateau of phase 2) → Increases calcium store of sarcoplasmic reticulum → increased free systolic concentration of calcium → increased **actin-myosin crossbridges** → increased force of contraction.
- **Lusitropic Effect:** Shortened duration of contraction and increased rate of cardiac relaxation which serves to preserve the diastolic interval which is needed for ventricular filling

Parasympathetic Nervous System

Right vagus predominantly innervates the SAN and the left vagus the AVN. Unlike the SNS the actions of PNS are predominantly confined to the atria.

- **Chronotropic Effect:** increased membrane permeability of potassium to the SAN, AV junctional fibres and the AVN → ***more negative membrane potential*** *and* ***lengthening of phase 4 AVN length of decay to threshold.*** Strong vagal stimulation may lead to complete arrest of intrinsic SAN and AVN autorhythmicity and the ventricles may stop beating for 5-20 seconds until the intrinsic autorhythmicity of the bundle of His fibres develop a rhythm of their own and cause ventricular contraction at a rate of 15-40 beats per minute – known as **Ventricular escape**.
- **Inotropic Effect:** less marked than that of the SNS due to reduced paucity (quantity) of PNS fibres. Can reduce ventricular contraction strength by as much as 20-30%.



ANS Pathway	Preganglionic NT	Postganglionic NT	Chronotripic Effects	Inotropic Effects	Lusitriopic Effects
SNS	Acetylcholine	Heart and Vessels: - Noradrenaline (NA) - Adrenaline (AD from adrenals) Sweat gland: - Acetylcholine (Ach)	↑HR	↑Contraction	↑Relaxation
ANS	Acetylcholine	Acetylcholine	↓HR	↓Contraction	-

Hormonal and Metabolic Regulation of Cardiac Function

(07b_02_08)

The key metabolic factors regulating cardiac function are:

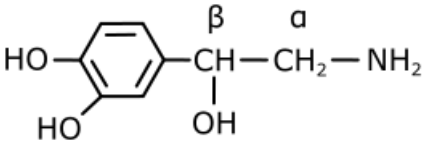
- Electrolytes
- Acidosis
- Hypoxia
- Temperature

ADRENAL MEDULLA HORMONES

Adrenaline and **Noradrenaline** are released from the adrenal medulla in the ratio of 4:1.

Activity at Receptors						
Agent	**α_1**	**α_2**	**β_1**	**β_2**	**Dopaminergic**	**SUMMARY**
Dopamine	+ +/+ + +	?	+ + + +	+ +	+ + + +	
Adrenaline	+ + + +	+ + + +	+ + + +	+ + +	0	**α = β**
Noradrenaline	+ + +	+ + +	+/+ +	+/+ +	0	**α > β**

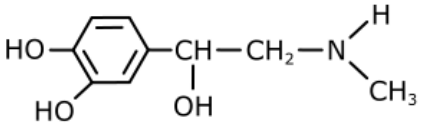
α_1 adrenoreceptors mediate **vasoconstriction** vie activity on increase in intracellular calcium via IP3 channels on vascular smooth muscle. **Noradrenaline** principally acts on these receptors and allows: vasoconstriction in the skin, splanchnic vascular bed as well as arteries and veins → increased preload and afterload → Increased MAP → baroreceptor mediated reflex bradycardia.



Noradrenaline

β_1 adrenoreceptors mediate an **increased heart rate and contractility** → increased cardiac output and myocardial oxygen demand.

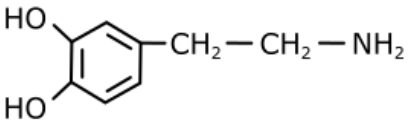
β_2 adrenoreceptors mediate vasodilatation through vascular smooth muscle relaxation via increase in cAMP. Predominantly seen in the skeletal muscle vascular bed. ***Adrenaline*** mainly acts on these receptors.



Adrenaline

HYPOTHALAMIC / POSTERIOR PITUITARY HORMONES

Dopamine



(See action on receptors above). It is a precursor of noradrenaline and functions as a neurotransmitter and neurohormone.

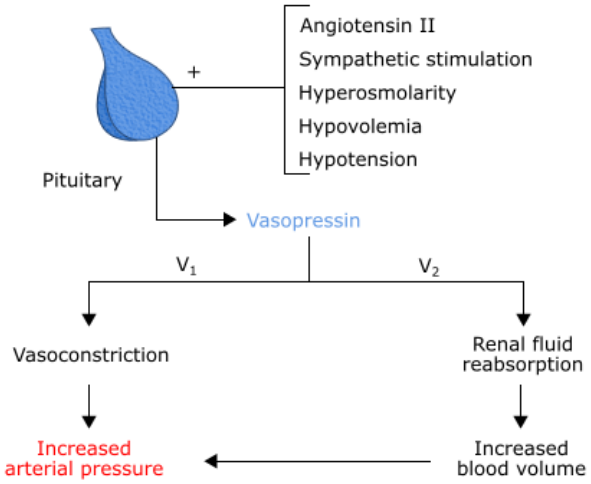
DA1 receptors mediate vasodilatation and are found in the renal, mesenteric and cerebral circulations. **+cAMP**

DA2 receptors inhibit noradrenaline release and are presynaptic, like a2-receptors. **-cAMP**

DA3-DA5 receptors exist in much lesser amounts but are associated with N&V.

Vasopressin aka **Anti-Diuretic Hormone (ADH)**

Released in response to the triggers seen on the diagram as well as pain, stress, emotion and exercise which induces SNS stimulation. It regulates cardiac function through 2 principle receptors:

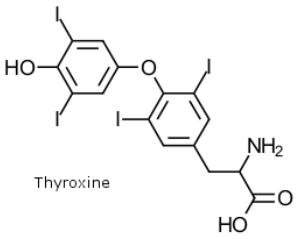


V2: Water retention and plasma volume expansion

Located in the **basolateral membrane of the cortical and medullary collecting ducts** of the kidneys. It allows increased water permeability of the apical membrane and hence increases preload. **+cAMP**

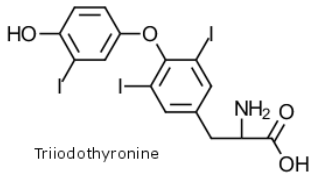
V1: Vasoconstriction and increased peripheral vascular resistance

Located in the ***vascular smooth muscle of arterioles*** and causes an increase in intracellular calcium → increased MAP and hence afterload. **IP_3 & DAG**



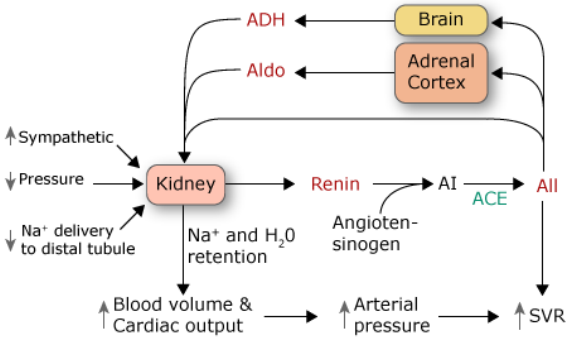
THYROID HORMONES

Thyroid hormones (***T4 thyroxine*** and ***T3 triiodothyronine***) cause an increase in metabolic rate as well as having direct chronotropic and inotropic effects on the myocardium, resulting in increased heart rate, stroke volume, and therefore a rise in cardiac output.



RENIN-ANGIOTENSIN-ALDOSTERONE PATHWAY

Renin release is stimulated by hypotension, SNS and reduced sodium delivery. Renin is a ***proteolytic enzyme*** that splits angiotensinogen → Angiotensin I. **ACE** found predominantly in the pulmonary capillaries converts it to ***Angiotensin II*** which has 3 effects:



1. **Aldosterone secretion** → increased sodium reabsorption
2. **Direct vasoconstriction**
3. **Increased thirst** and hence water intake by direct hypothalamic action
4. **Increased ADH secretion**
5. **Increased sympathetic drive**

***Chronic cardiac failure*:** compensatory changes in the endocrine systems regulating cardiac function occur, and renin, aldosterone and vasopressin secretion is reset. Higher plasma concentrations of these hormones persist after the cardiovascular parameters return to normal, which results in a **cycle of increasing sodium and water retention**, and worsening cardiac failure.

ATRIAL & BRAIN NATRIURETIC PEPTIDE

Involved in the regulation of preload, afterload and cardiac output. They can also be used as markers of heart failure. Its production is stimulated by excess wall stress and stretch of the atria and ventricles.

- ***ANP*:** secreted from cardiac atrial cells
- ***BNP*:** secreted from cardiac ventricle cells

Overall, they decrease blood volume and cardiac output through the following mechanisms:

1. Reduction in preload through water and sodium excretion

Dilation of the renal afferent arteriole and **constriction of the renal efferent** arteriole. This increases filtration pressure and GFR. They also **directly inhibit sodium absorption** in the renal collecting ducts. **ANP** also **inhibits renin secretion and aldosterone release** from the adrenal cortex.

2. Reduction in afterload through a reduction in systemic vascular resistance

Vasodilatation of blood vessels that leads to reduced SVR occurs in part due to the suppression of renin release.

Metabolic Regulation of Cardiac Function

ELECTROLYTES: Calcium and potassium are amongst the most important electrolytes which directly affect cardiac function

- **Hypocalcaemia & *Hypermagnesaemia***
  - Reduces cardiac contractility and causes peripheral vascular dilatation → decreased cardiac output and reduced MAP.
  - Prolongation of the QT interval → increased risk of ventricular dysrhythmias.
- **Hypercalcaemia**
  - Increased myocardial contractility and irritability → HTN may occur together with a reduced QT interval and bundle branch block
- **Hyperkalaemia**
  - Raises the cardiac resting membrane potential, e.g. from -90 mV to -80 mV and reduces the duration of the cardiac action potential → slowed conduction from the SAN to AVN, PR interval prolongation, bradycardia and even asystole.
  - Hyperkalaemia is associated with a dilated and flaccid heart

ACIDOSIS: Hydrogen ions compete with calcium ions → reduced contractility and cardiac output. By the same mechanism, there is vasodilatation and a reduction in MAP

TEMPERATURE: Heart rate increases by 10 beats per minute for every 1°C rise in temperature, with cooling producing an opposite effect. Transiently increased myocyte contractile strength occurs from moderately increased temperatures. However, if pyrexia is prolonged, this exhausts the metabolic reserves of the heart and is associated with a decline in contractility.

Control of Cardiac Output

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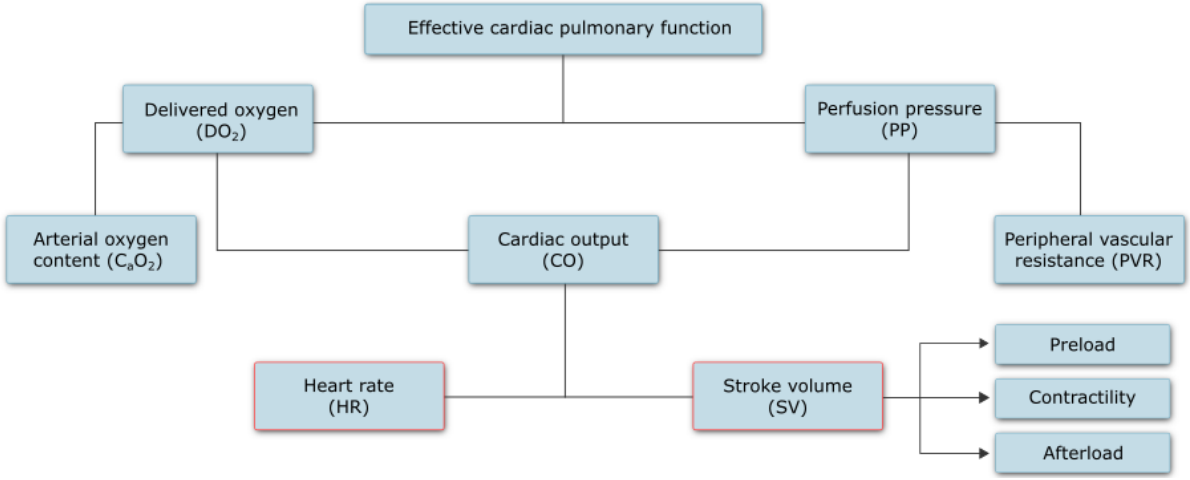
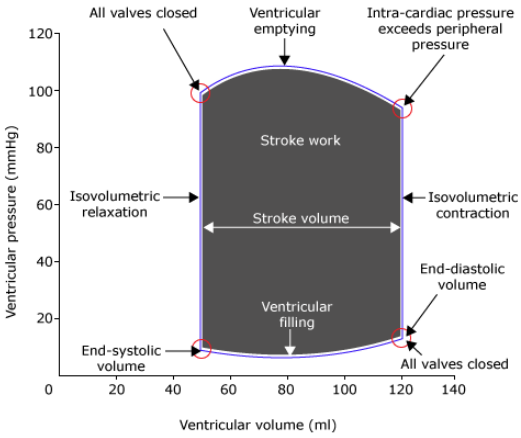
The primary aim of the cardiopulmonary system is to **deliver oxygen to the tissues** (**DO_2**) at an adequate **perfusion pressure** (**PP**).



The amount of oxygenated blood that leaves the heart depends on **arterial oxygen content** (**C_aO_2**) and **cardiac output** (**CO**). Transport of this blood to the tissues is dependent on **PP** which itself is determined by **CO** and **peripheral vascular resistance** (**PVR**).

Ventricular Pressure-volume Loop

As a recap see diagram right.



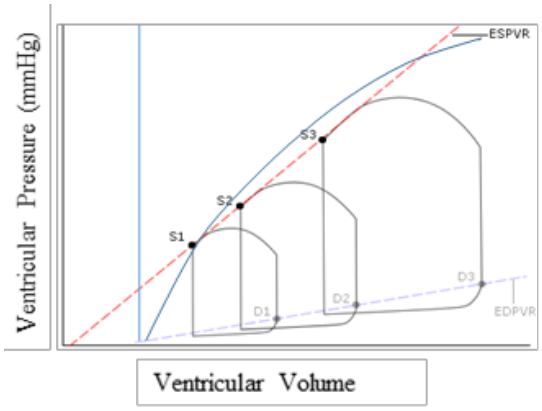
ESP = End-systolic point (correlates to the volume at end of systole)

EDP = End-diastolic point (correlates to the volume at end of diastole)

EDPVR = shifts up and to the left in ventricular diastolic dysfunction such as ischaemia and ventricular hypertrophy (less compliant)

ESPVR = Shifts up and to the left with increasing force of contraction with positive inotropic drugs and sympathetic stimulation

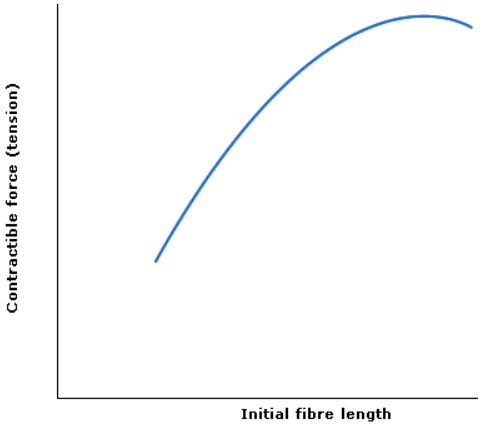
ESPVR = **Shifts down and right with negative inotropic drugs** i.e. b-blockers and inhalational anaesthetic agents.



Frank-Starling Curve

Describes the intrinsic ability to change its contractility according to continuously changing venous return.

In an isolated muscle fibre, as the length of fibre lengthens, the tension developed during contraction increases until it reaches a maximum – after which, there is no further tension produced with increasing fibre length. This relationship is more sensitive in cardiac muscle than with skeletal muscle. The figure on the right demonstrates this phenomenon:



The **Frank-Starling Law** therefore states "**the contractile force of a cardiac muscle fibre is proportional to the initial fibre length**"

NB the varied parameters that may appear on the x-axis:

- *Central venous pressure (CVP)*
- *Right ventricular end diastolic pressure (RVEDP)*
- *Left ventricular end diastolic pressure (LVEDP)*
- *Right ventricular end diastolic volume (RVEDV)*
- *Left ventricular end diastolic volume (LVEDV)*

…and on the y-axis:

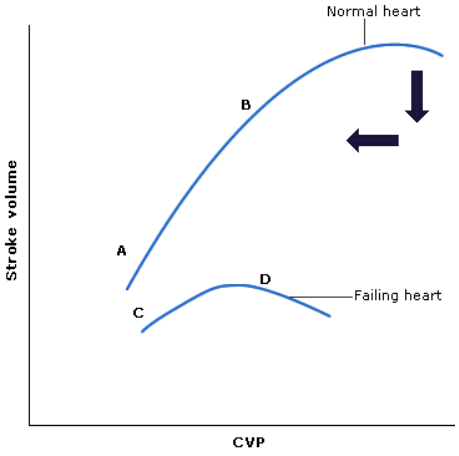
- *Stroke volume (SV)*
- *Stroke work (SW)*
- *Right ventricular end systolic pressure (RVESP)*
- *Left ventricular end systolic pressure (LVESP)*

Fluid Challenge

The Ventricle normally works on the steep part of the curve. A fluid challenge will increase venous return and therefore increase the fibre length. The increasing fibre length in turn increases the contraction force – and hence the stroke volume.

In **cardiac failure**, ventricular function is altered becoming flatter and lower and the ventricle works in the flatter section. The **fibres are overstretched** due to pathological dilation.

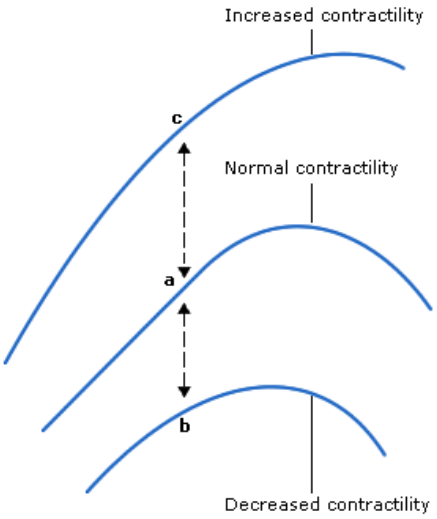
Fluid challenges represented from A→B and C→D.



Effect of Ventricular Contractility

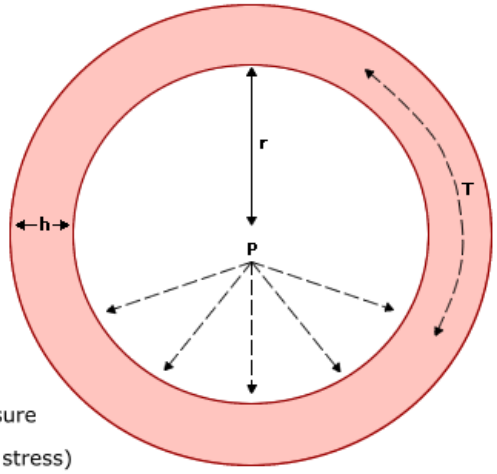
For a given CVP (initial fibre length) the **increase or decrease in contractility subsequently increases or decreases the stroke volume** (tension developed) provided afterload also remains unchanged.

Overall, the stroke volume is dependent on the interaction between preload, contractility and afterload.



Laplace's Law

In a living human it is not practically possible to measure the resting fibre length and tension developed in the ventricular wall CVP, EDV and EDP are all related to resting fibre length so may be used as an indirect index of resting fibre length on the x-axis



For the case of tension, **Laplace's law** had been developed as a way to obtain an **indirect index of tension only in elastic spheres**. This needs the presumption that the ventricle exists as a sphere. From the equation on the right, the following is assumed:

$P = \frac{2Th}{r}$

P = Internal pressure

T = Tension (wall stress)

h = Wall thickness

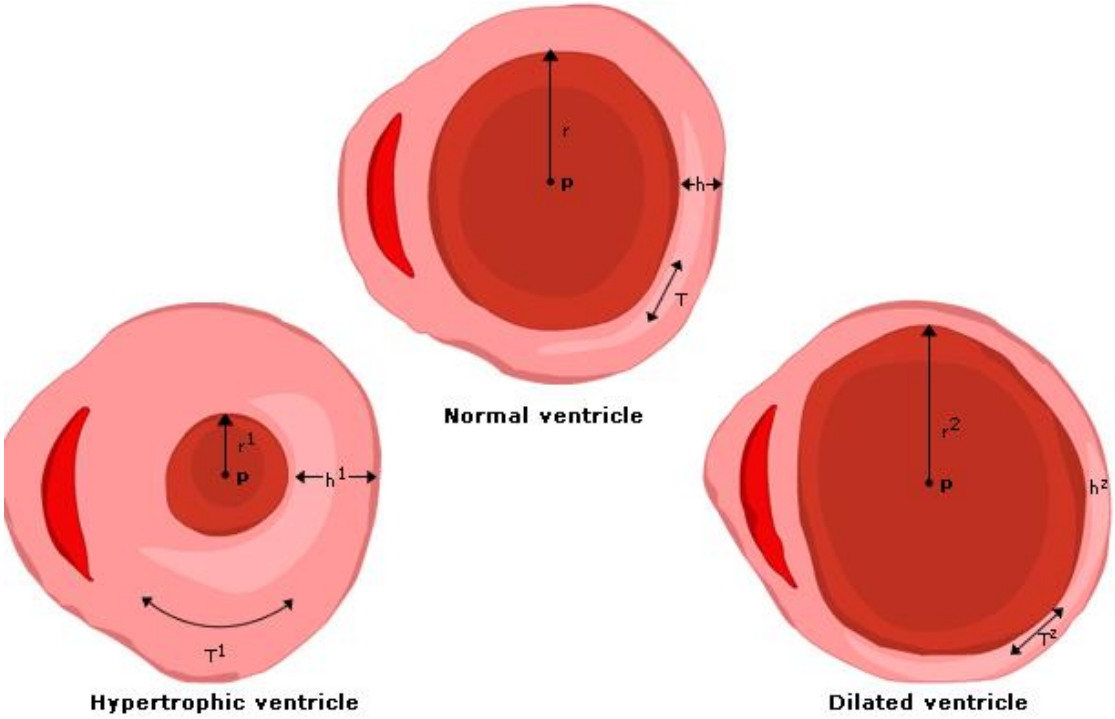
r = radius of sphere

Intraventricular pressure (P) ∝ tension (T)

Therefore, intraventricular pressure (RVESP, LVESP) can be used as an indirect index of tension on the y-axis of ventricular function curve.

Differing Ventricular States

If Pressure (P) is constant, then T is proportional to $\frac{r}{h}$. This means that the tension is dependent on the radius and wall thickness.



Therefore, the hypertrophic ventricle needs much less tension than the dilated ventricle to create the same pressure. Likewise, if pressure was constant in both, the ventricular wall tension will be much less in the hypertrophied ventricle. **Increased wall tension means higher oxygen consumption by the ventricles.**

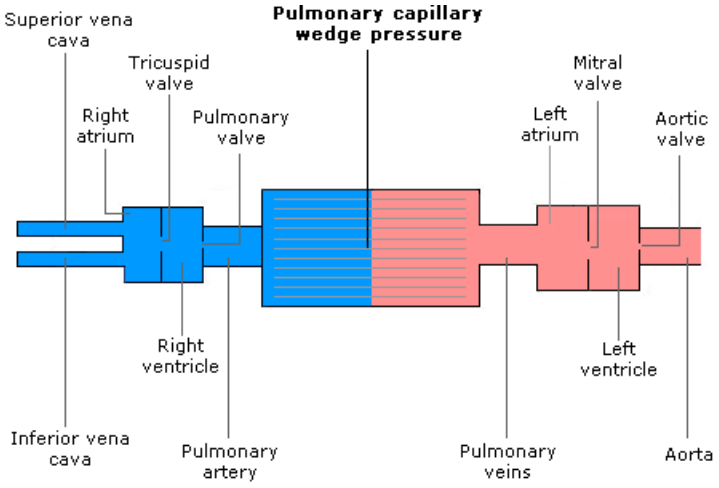
Control of Cardiac Output

This is controlled by multiple mechanisms that control **heart rate** and **stroke volume**.

1. STROKE VOLUME

Preload

End diastolic volume is an indirect measure of presystolic fibre length and is related to end diastolic pressure by the EDPVR (which is not entirely linear). In practice, this can be estimated by measuring the **pulmonary capillary wedge pressure** (**PCWP**) or **pulmonary artery diastolic pressure** (**PADP**). The right side of the heart is reflected by CVP.



The diagram on the right shows that there is a relationship between CVP, PCWP, EDP, EDV and preload. This however gets **distorted** with **abnormal ventricular compliance, valvular pathology, pulmonary HTN, positive intrathoracic pressure.**

CVP≈RAP ≈RVEDP ≈PADP ≈PCP ≈PVP ≈LAP ≈LVEDP

Afterload

A surrogate of muscle fibre tension during ventricular contraction and is therefore measured through **Laplace's law** aka ***End-systolic intraventricular pressure***. Indices such as systemic vascular resistance (**SVR**) and mean arterial pressure (**MAP**) relate well to intraventricular pressure in the normal heart. See the equation on the right. From this one can see that arterial pressure and ventricular pressure during systole follow each other and are indirect indices of ventricular wall tension. Normal ventricle contractility increases with afterload, through the **Anrep effect.**

$$P = \frac{2Th}{r}$$

$$SVR = \frac{(MAP\text{-}CVP)x80}{CO} \quad dyn \cdot s/cm^5$$

Contractility

Describes the power of the ventricle for a given preload and afterload. SV, SW, RVESP and LVESP are indices of contractility. It is increased by:

1. Sympathetic stimulation and parasympathetic blockade
2. Inotropes
3. Increased levels of Ca^{2+}

And decreased by the opposite.

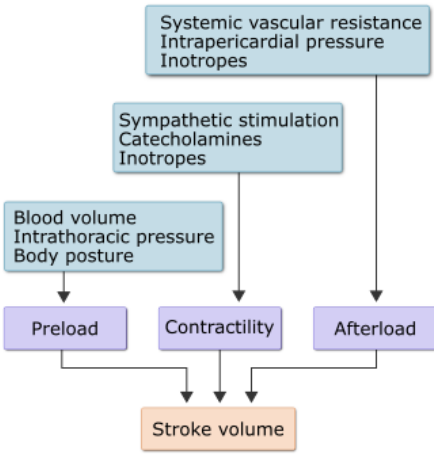
Anrep effect: increase in afterload should normally lead to a reduction in SV as the ventricle has to pump the blood against higher resistance but in reality, an increase in afterload usually causes a rise in SV initially due to increase in contractility.

2. HEART RATE

Normally the HR is controlled by spontaneous, i.e. autonomic control, depolarization of SA node cells in the right atrium, which itself is under control of medullary nuclei and higher centres in the hypothalamus and cortex.

See previous lectures on neurological control of cardiac output. A completely denervated heart beats at a rate of more than 100 bpm, if blocked with atropine, there will be unopposed sympathetic activity and therefore would be an increased heart rate.

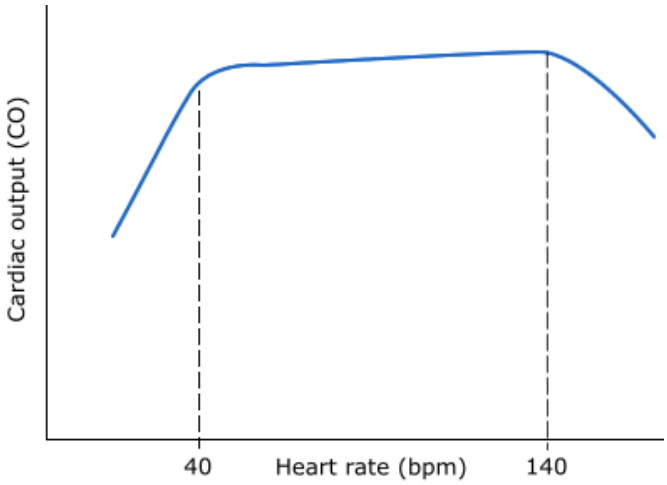
IN SUMMARY:



Cardiac output is affected by both heart rate and volume. However, they are dynamic in relationship i.e. at very high HR there is reduction in both systolic time and diastolic time. However, the latter is affected more and so limits the filling time and SV for ventricles.

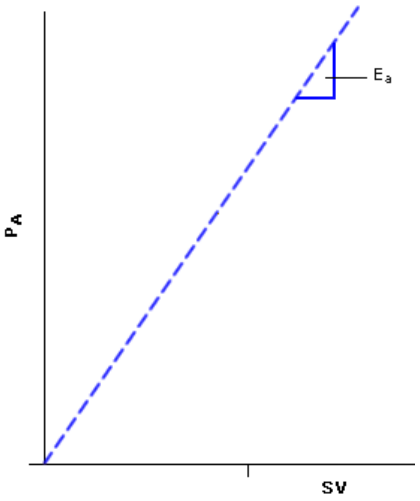
Treppe Effect aka (*Bowditch effect*)

Increase in inotropy, i.e. contractility, that occurs **in response to an increase in HR**. This usually occurs from an HR of 40-140 bpm. The reduced diastolic time in response to an increase in HR leads to more available intracellular calcium for excitation-contraction coupling.



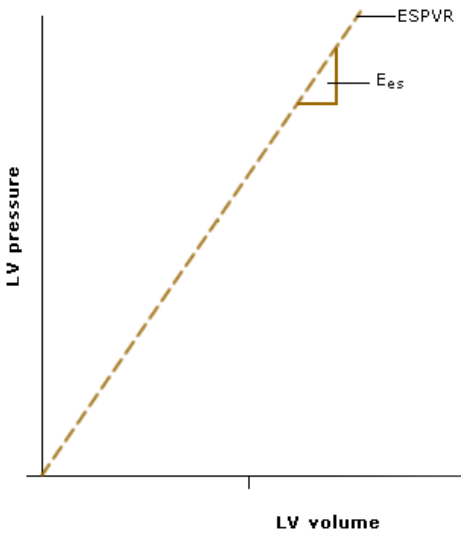
Cardiovascular Coupling

LV performance is affected by the opposing arterial load. The interaction between LV and arterial system is known as **ventriculo-arterial coupling** and is illustrated by plotting the ventricular elastance (E_{es}) and arterial elastance (E_a) on the same diagram.



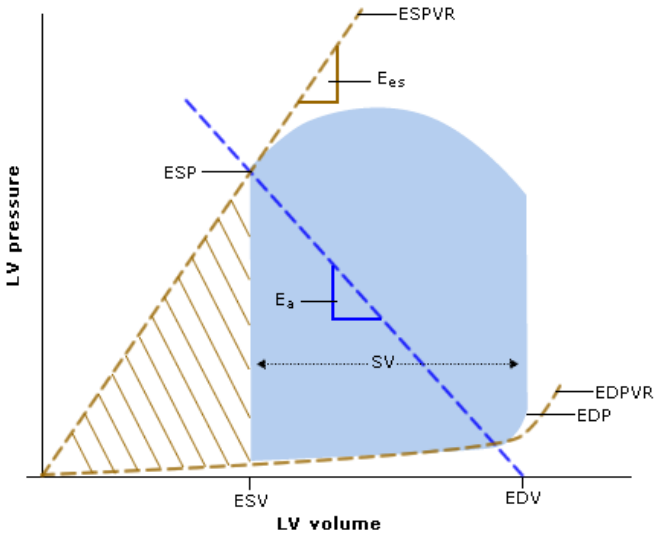
- E_{es} is determined by the slope of the ESPVR (see diagram below)

ELASTANCE = 1/Compliance



- Ea is measured from the arterial afterload that is imposed on the LV and is expressed by the arterial end-systolic pressure (Pa)
- stroke volume (SV) relationship (*displayed on the graph above*).

As the ventricular and arterial pressure oppose each other when they are displayed on the same diagram, equilibrium lies at ESP, at which point the ventricle pressure is equal to the arterial pressure. In this diagram E_a is the negative slope of the line joining the EDV and ESP points.



Changes in arterial pressure: results in a shift of the position of ESP. When preload (EDP) is kept constant, then displacement of ESP tells us the degree to which ventricular contractility or arterial elastance is responsible.

Maximum work occurs when $E_{es} = E_a$, though this is not an efficient state. **Maximum mechanical efficiency** or optimal ventriculoarterial coupling occurs when:

$$E_{es} = 2 \times E_a$$

Mismatch or uncoupling of these opposing forces may lead to ventricular dysfunction, as with, for example, vasodilatation due to sepsis.

Control of Systemic Blood Pressure

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Systemic blood pressure is a major determinant of blood flow distribution to all organs.

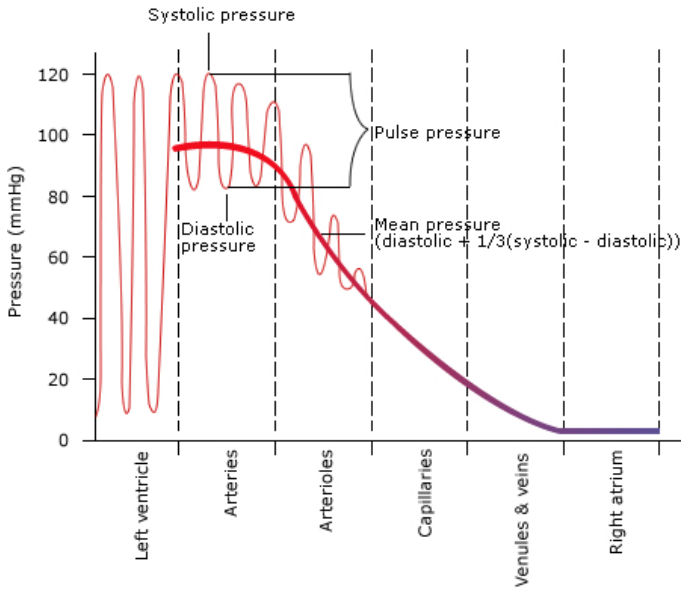
- Fluid always travels from areas of high pressure → low pressure unless flow kinetic momentum momentarily sustains flow i.e. in cardiac cycle.
- Blood flow is also limited by the resistance of the vascular system.

$$\text{Flow} = \frac{\text{Pressure}}{\text{Resistance}}$$

Physiological Determinants

BP = CO x SVR

Capillary fluid shifts also affect BP. The exchange of fluid across the capillary membrane between the blood and the interstitial fluid affects BP very quickly. There is a balance between hydrostatic and oncotic pressure. Low BP leads to reduced hydrostatic pressure resulting in fluid moving from the interstitial space into the circulation in an attempt to restore the circulating volume.



Autonomic Nervous System

Most rapid regulator of blood pressure. All **blood vessels receive sympathetic innervation** with **arterioles** being most densely innervated. They cause:

- Vasoconstriction at the end of vessels in all parts of the body (**tonic discharge**). With reduced rate of SNS discharge, there will be vasodilation.
- A chronotropic and an inotropic effect in the heart, which increases CO
- Suppression of vagal (parasympathetic) tone, so that when the vagus is cut the result is tachycardia

Some resistance vessels also receive vasodilatory cholinergic fibres with no tonic discharge.

Vasomotor Centre

Describes a group of **neurons in the medulla oblongata** controls the SNS discharge control of the cardiac function. The following are factors that affect the activity of the vasomotor centre:

DIRECT STIMULATION:	EXCITATORY INPUTS:	INHIBITORY INPUTS:
• CO_2 • Hypoxia	• The cortex via the hypothalamus • Pain pathways • Carotid and aortic chemoreceptors	• The cortex via the hypothalamus • The lungs • Carotid and aortic and cardiopulmonary baroreceptors

***Chemoreceptors*:** Located in the **carotid** and **aortic bodies**. These respond to **hypoxia** and **hypercarbia** by increasing the blood pressure.

***Somatosympathetic Reflex*:** The pressor response to stimulation of somatic afferents. Pain i.e. increases blood pressure.

***Baroreceptors*:** Mentioned in previous lectures. **Cardiopulmonary baroreceptors** are located in the **low-pressure part of the circulation** (right and left atrium, inferior vena cava, superior vena cava and pulmonary veins).

Humoral Mechanisms

Catecholamines: Adrenaline and noradrenaline are produced by and released from the adrenal medulla in response to sympathetic stimulation

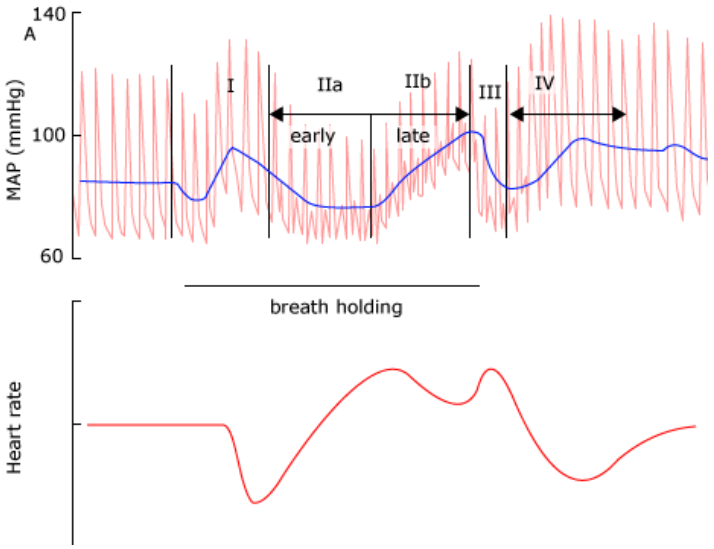
RAS: Renin production is fast whilst aldosterone production is slow

Endothelial locally produced vasoactive substances:

- **Thromboxane A_2** promotes platelet aggregation and vasoconstriction
- **Prostacyclin** inhibits platelet aggregation and produces vasodilatation
- **Nitric oxide** is synthesized by the endothelium from L-Arginine and is a potent vasodilator
- **Adenosine**, **bradykinin** and **histamine** are other vasodilating substances
- **Endothelins** are the most potent naturally occurring vasoconstrictors in the body

VALSALVA MANOEUVRE

This is a description of forced expiration & increased intrathoracic pressure against a closed end i.e. glottis/syringe and the response to blood pressure. This is split into 4 phases:



PHASE I: BP increases at onset of straining and increased intrathoracic pressure → increases thoracic aortic pressure

PHASE IIa: BP begins to drop due to compression of cardiac chambers & pulmonary veins → preload and reduction in CO

PHASE IIb: Baroreceptor reflex kicks in and causes SNS output to increase BP via tachycardia and SVR

PHASE III: Valsalva released, glottis opens and intrathoracic pressure returns to normal. **Thoracic aorta is decompressed** and **pooling of pulmonary blood** causes an immediate, brief fall in MAP.

PHASE IV: Preload is restored against a toned vasculature from the baroreceptors reflex initiated in phase II of the Valsalva. This causes an overshoot of BP and bradycardia with eventual return of BP to normal.

Baroreceptors incompetence/autonomic neuropathy: Would result in a

1. **Continuing BP drop in phase IIb**
2. **No overshoot of BP in phase IV.**
3. **Phase IV has no reflex bradycardia.**

Max HR in phase IIb/Min HR in phase IV

If >1.5, shows good autonomic function

Orthostasis and Haemorrhage

On standing, the BP drops and there is neuroendocrine reflex with an initial baroreceptor reflex which causes:

- Tachycardia by reducing the vagal tone
- Stroke volume remains depressed due to volume redistribution; CO falls
- Peripheral resistance increases by 30-40% due to sympathetically mediated vasoconstriction
- Mean arterial pressure is not only restored by vasoconstriction but actually raised by 10-14%

During the next half-hour the **orthostasis-induced rise in capillary filtration pressure** causes 12-13% **fall in plasma volume**. This **reduces systolic BP** and **elicits a further increase in HR**. To compensate for the fall in plasma volume, the **excretion of salt and water is reduced** through the **RAA system** and **reflex vasopressin secretion**

During haemorrhage, the reduced circulating volume influences the cardiopulmonary stretch reflex, arterial baroreceptor reflex and increase in arterial chemoreceptor activity due to acidosis and poor receptor perfusion.

Peripheral Circulation

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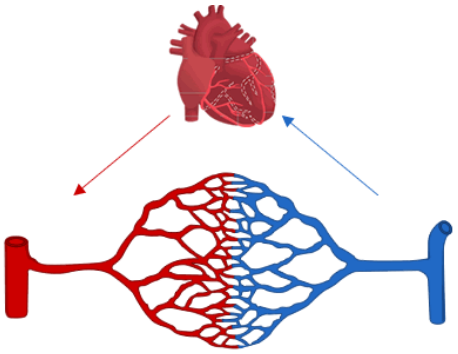
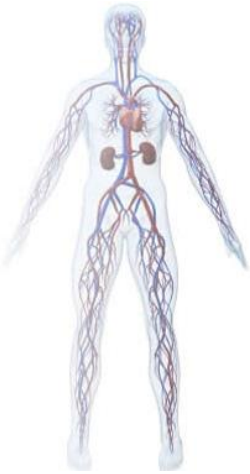




Gross and Functional Anatomy

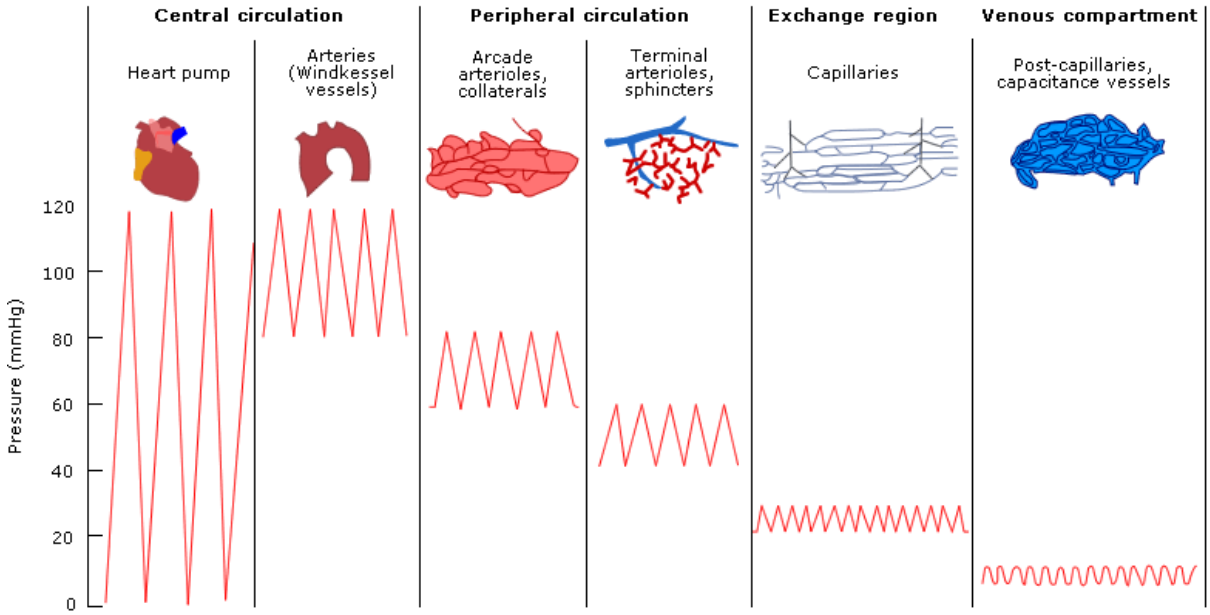
Arteries - transport blood away from the heart and are muscular and elastic to allow conversion of the intermittent pulsatile ejection of blood from the heart into a steady flow with a constant pressure: the **Windkessel effect**

Arterioles – main resistance vessels due to small diameter and ultimate control of MAP. Terminal arterioles provide the largest mean pressure drop (75 mmHg to 38 mmHg). Subject to **tonic discharge of SNS** and has the greatest control of flow.

Capillaries – provide the selectively permeable site of exchange of fluids, gases, electrolytes and macro molecules. So numerous and provide such a large cross-sectional area they present almost no resistance to flow. Flow to capillary beds is controlled by the proximal arterioles.

Venules – resistance vessels that regulate the capillary hydrostatic pressure and is also controlled by the same local and systemic factors that control arterioles.

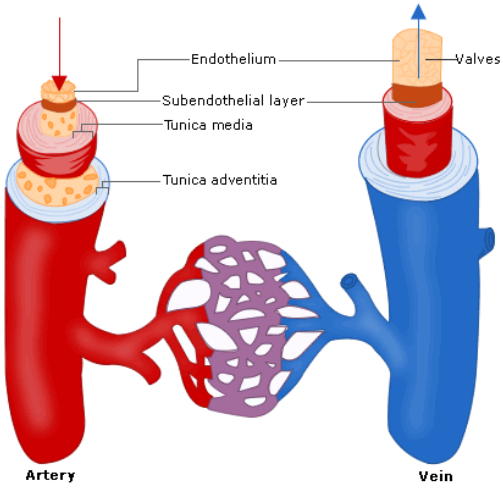
Veins – transport blood toward the heart, have valves and act as capacitance vessels i.e. they contain the majority of the blood volume at rest.



Structure

Both arteries and veins have the same 3-layer structure, starting from the innermost:

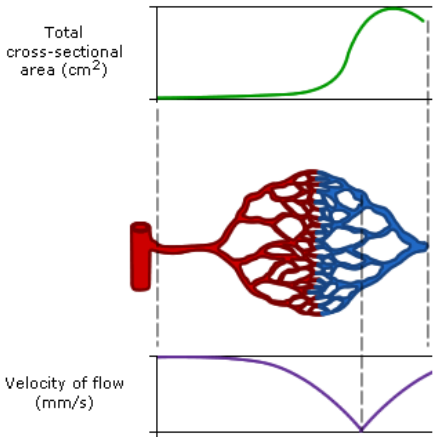
1. **Tunica Intima** – Single layer squamous endothelial cells surrounded by connective tissue.
2. **Tunica Media** – circularly arranged elastic fibres and connective tissue and the vascular smooth muscle which controls the size of the lumen
3. **Tunica Adventitia –** made entirely of connective tissue and contains the nerves that supply the vessel and, in larger vessels, the vasa vasorum



Function

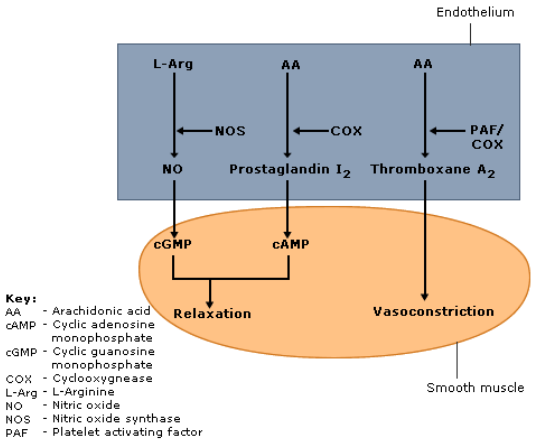
Arterial system – converts **high-velocity pulsatile flow**, at the level of the ascending aorta (1m/s), to the optimal **low-velocity steady flow** in the capillary bed (0.01cm/s). This is done through:

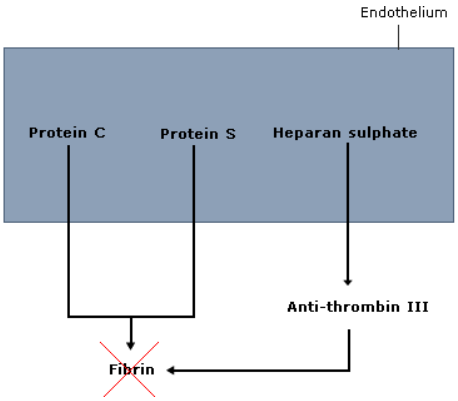
- **Regulation of diameter** through autonomic, hormonal or metabolic responses.
- Aorta has **low resistance distensible walls** so the phasic pressure from the L ventricle is reduced. Along with the **elastic recoil in diastole**, the pulsatile nature is smoothened (the **Winderkessel** effect)
- Branching of arteries **increases cross sectional area** resulting in a **fall in MAP and mean velocity**.

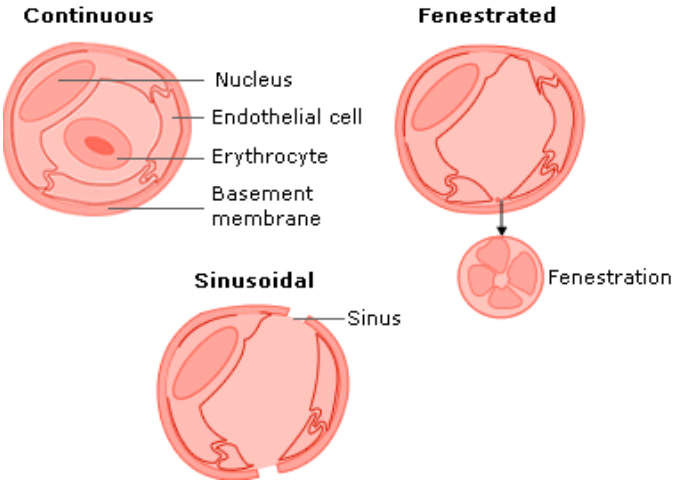


Vascular Endothelium – Has a number of functions:

1. Regulates **basal vasomotor tone** through controlled release of:
    a. **Vasodilators** i.e. nitric oxide (NO) and prostaglandin I_2 (PGI_2 aka prostacyclin)
    b. **Vasoconstrictors** i.e. endothelins and platelet activating factor (PAF)
2. Presents a **smooth surface** to encourage laminar flow
3. **Regulates growth of surrounding connective tissue**
4. Acts as a **non-thrombogenic surface**, secondary to **expressing heparan sulphate, protein C and protein S**







Capillary Endothelium – Unlike arteries and veins, they consist of a **single layer of endothelium** (thinner than those of the arteries and veins) and some **connective tissue** and form a **selectively permeable membrane** (greatest at the venous end of the capillary) to allow exchange of molecules in the extravascular space. 3 types:

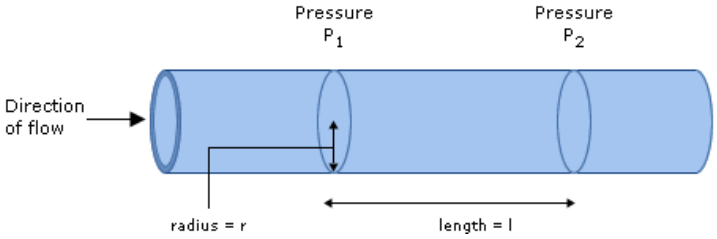
1. **CONTINUOUS:** Uninterrupted lining so only water gases and ions can diffuse through i.e. brain
2. **FENESTRATED:** Pores allow small molecules to diffuse through
3. **SINUSOIDAL:** Larger proteins, white and red cells are able to pass through located in the bone marrow and liver

During diseased states i.e. sepsis, vascular permeability increases and is known as **third spacing** and is mediated by histamine, prostaglandins and interleukins. **Oedema results** when this increased fluid and protein loss overwhelms the absorptive capacity of the lymphatic system.

PHYSICS/PHYSICAL FACTORS

Poiseuille's Equation

Relates the flow (Q) of a liquid in a fixed length (*l*) and radius (*r*) vessel according to pressure change (*P2-P1*) and liquid viscosity (η):

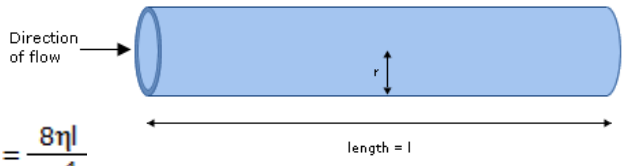


$$\text{Flow (Q)} = \frac{\pi(P_2 - P_1)r^4}{8\eta l}$$

NB, flow must be laminar for the correct application of Poiseuille's equation

Resistance

Describes the impediment to flow offered by a vessel. Hydraulic resistance to flow is determined by the following equation:



$$\text{Resistance (R)} = \frac{8\eta l}{\pi r^4}$$

From this, you can see the vessel diameter is the single most important factor in controlling MAP. The type of flow (laminar/turbulent) also determines the resistance to flow.

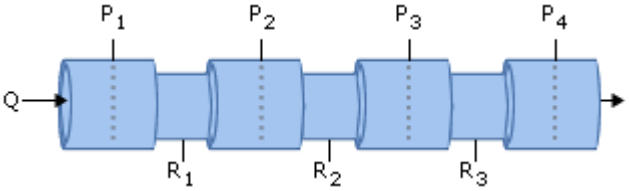
From this the overall equation is:

Flow = Change in Pressure/Resistance

Series vs. Parallel

SERIES:

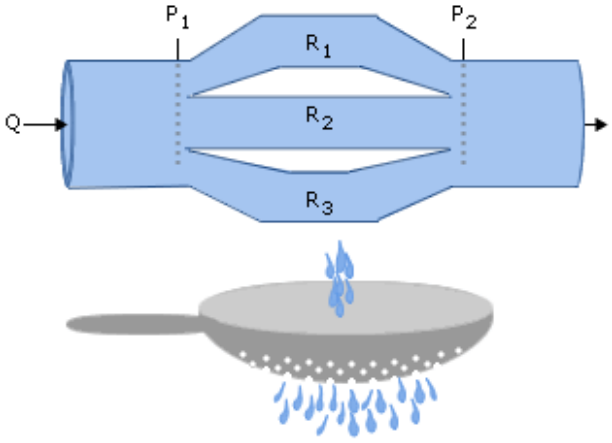
If the driving (change in) pressure across each resistance is 3 mmHg and the flow is 1ml/min, from the equation above, the total resistance would be 3/1 + 3/1 + 3/1 = 9mmHg.min.ml^{-1}



PARALLEL:

Total resistance is calculated by $1/R_p$. For example if Q = 1ml/min and ΔP = 3mmHg and resistance is equal across all parts of resistance, then total resistance is 3 by the equation divided by 3 (3 parallel circuits) or 1 mmHg/ml/min:

1/3 + 1/3 + 1/3. PRACTICE!



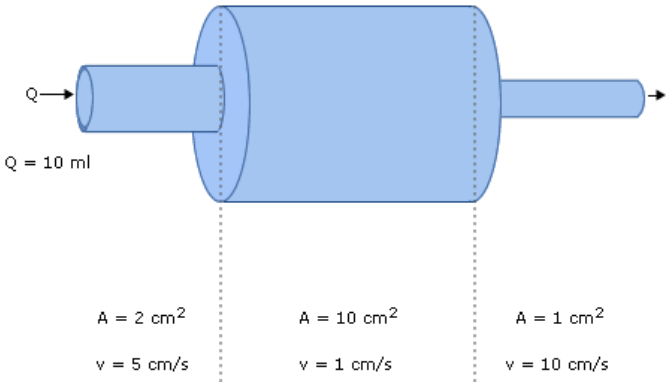
Resistances in parallel

Velocity and Flow

Velocity (v) = distance/time
Flow (Q) = volume/time

They are related together by the cross-sectional area (A) of the vessel by the equation:

$$Q = vA$$

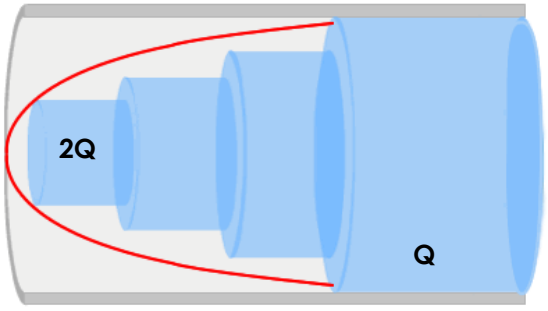


The smallest cross-sectional area to receive the entire stroke volume is the aorta in which the velocity is the highest at 0.93m/s

In the capillaries, where the total cross-sectional area is 1000 times that of the aorta, velocity is 0.05 cm/s. Gross reduction in blood velocity enables efficient exchange of gases and metabolites across the short length of capillaries.

Laminar and Turbulent Flow

Laminar Flow: all the elements of the fluid move in streamlined layers parallel to the vessel wall. The central core of fluid has the highest velocity and each cylinder of fluid around this central column is progressively slower until the outermost layer, in contact with the vessel wall is stationary. This profile of flow velocity is known as a **Parabola.**



NB high viscosity fluid reduces the ability to create streamlined flow

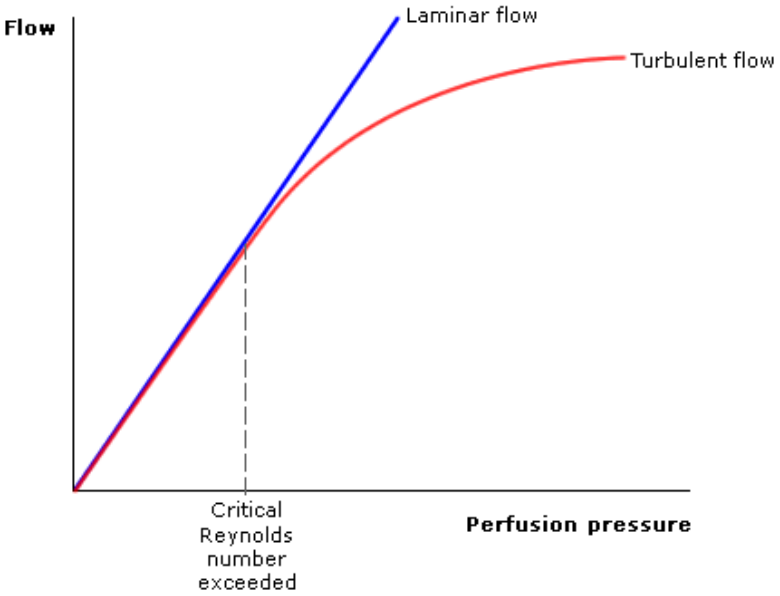
Turbulent Flow: Describes a state when blood velocity is high and a point is reached when flow is no longer directly proportional to pressure as fluid moves discordantly and the frictional resistance to flow increases. Flow will only increase in proportion to the square root of pressure

$$Q \propto \sqrt{\Delta P}$$

REYNOLDS number: used to determine whether flow is likely to be laminar or turbulent by identifying the point when flow is no longer proportional to the driving pressure according to driving pressure.

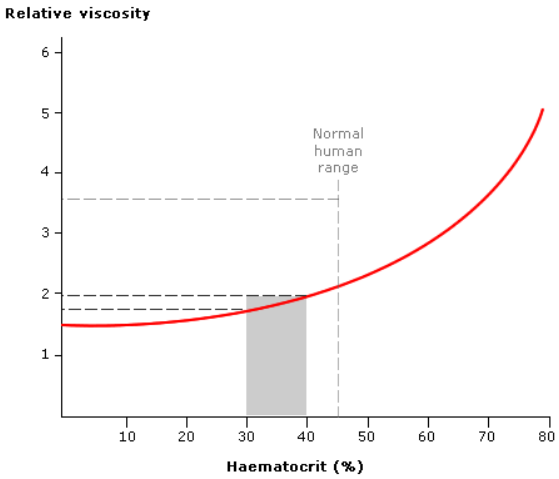
At **high Reynolds numbers**, the above equation apples when **turbulent flow dominates**. **Below the Critical Reynolds number** (2000), **Poiseuille's law is accurate**.

Reynolds number increases in low viscous states.



Viscosity

Relates to the internal friction of adjacent fluid layers during laminar flow. In the blood, this is affected by:



- **Haematocrit:** viscosity of blood relative to water is 3.6 and increases in a non-linear fashion with increasing haematocrit.
- **Temperature:** 2% more viscous for every degree centigrade decrease in temperature
- **Flow rate:** The lower the flow rate, the increased interactions between RBC (e.g. rouleaux formation) and between RBC and plasma proteins.
- **Vessel Diameter:** In arterioles and capillaries, the **Fahroeus-Lindqvist effect** leads to a reduction in the haematocrit relative to larger vessels as cells tend to occupy the central axial stream, therefore, there is an offset of the reduction of velocity in capillaries through effective reduction of haematocrit. In capillaries, blood viscosity is no greater than that of plasma. *See the grey box on the above graph.*

Mean Arterial Pressure

MAP = (CO x SVR)

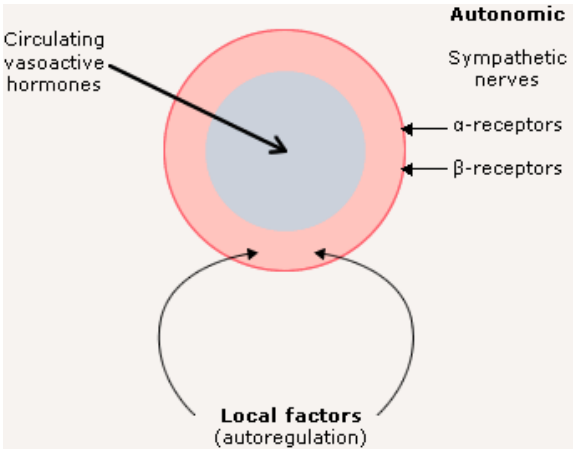
This is usually measured directly or indirectly. A geometrical mean may be calculated from the direct intra-arterial trace or indirectly with NIBP using the formula on the right.

$$MAP = P_d + \frac{P_s - P_d}{3}$$

Control of BP is covered in the previous lecture notes.

Control of Smooth Muscle Tone

Local control of blood flow by the tissues (autoregulation) through vasodilation is by:



Metabolic control:

- Falling levels of oxygen → vasodilation
- Rising levels of hydrogen and potassium ions
- Rising lactic acid concentrations
- Adenosine production
- Prostaglandin and histamine

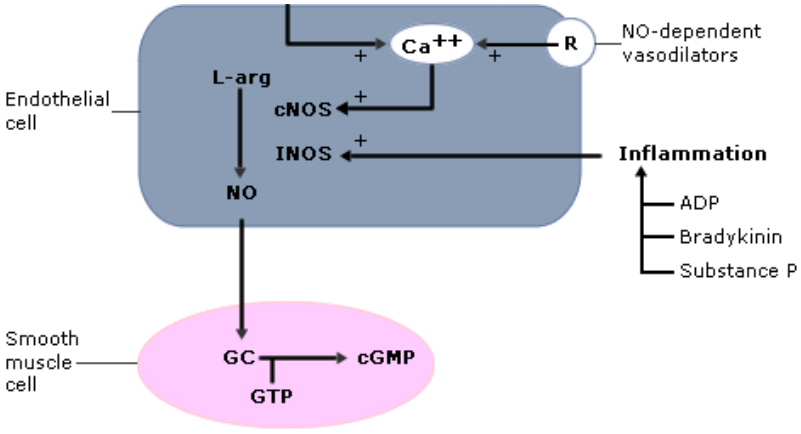
Myogenic control: where passive stretching of the smooth muscle wall by increased pressure or volume can result in either reflex vasoconstriction (negative feedback) or further vasodilation (positive feedback)

Vasoactive Chemicals

NO and endothelins are the main regulators of basal vascular tone. It is only when vascular function is disturbed that PGI_2 and PAF come in to play.

Endothelial NO: NO **binds to guanylyl cyclase** to cause **smooth muscle relaxation.** It is constitutively active but can be further induced by agonists like:

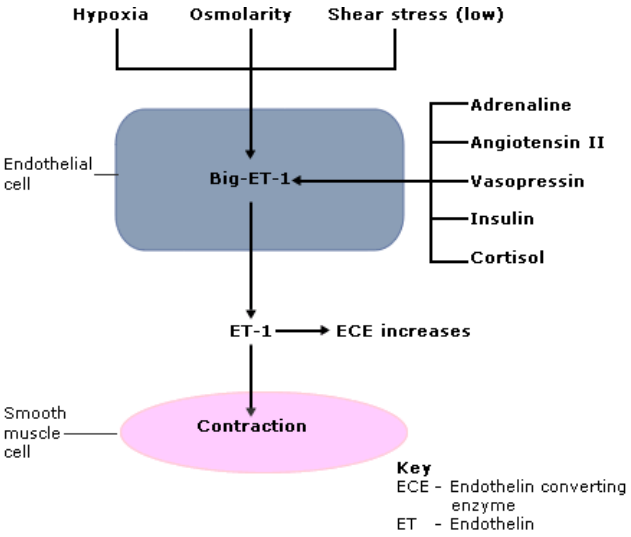
- thrombin, ADP, bradykinin and substance P.
- Increased shear stress also induces NO production.



Key

Ca	Calcium
cNOS	Constitutive NO synthase
cGMP	Cyclic guanosine monophosphate
GC	Guanylyl cyclase
GTP	Guanosine triphosphate
L-arg	L-Arginine
NO	Nitric oxide
R	Receptor

Endothelin 1 (ET-1): Hypoxia, ischaemia and shear stress induce production of endothelin 1 (ET-1) by endothelial cells. Catecholamines and noradrenaline potentiate its effect.



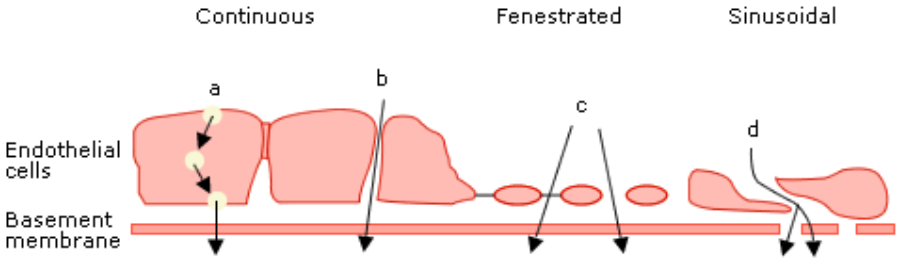
Systemic factors in control of smooth muscle tone has been covered in previous lectures.

Autoregulation has the following few features:

- Low neurogenic control of the supplying arteriole's smooth muscle
- Nearly constant blood flow over a wide range of mean blood pressure
- Most evident in skeletal, cardiac and cerebral circulations
- High degree of local control of vascular resistance i.e. tissue metabolic requirements

Capillary Exchange

There are several different mechanisms of exchange through a capillary membrane including.



1. Diffusion

Fick's Law of diffusion says that the movement of a molecule across a membrane is directly related to the diffusion constant across a barrier, the surface area available for diffusion and the concentration gradient. Diffusion is the major route of exchange for gases (O_2 and CO_2) and lipid-soluble substances (e.g. anaesthetic agents). Some exchange of fluid and electrolytes also occurs by diffusion

2. Convection (bulk flow) *together with diffusion are the most common methods of transport*

Occurs via pores or clefts in the endothelial cells (b, c & d). Most evident in the **renal glomerular capillaries**. This method of exchange follows **Starling's law**. Altered by changes in the pressure driving forces (whether hydrostatic or osmotic) and in the size of pores or intercellular clefts (e.g. as occurs in sepsis)

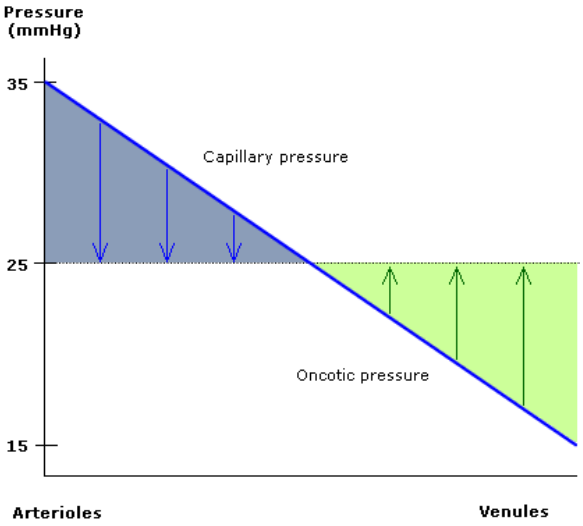
3. Vesicular Transport: **Translocation of macromolecules across the capillary endothelium (a)**

4. Active Transport: **Only into cells i.e. vascular endothelial cells and not into interstitial fluid.**

Starling's Forces

Describes the forces governing transport via filtration: **Hydrostatic pressure** and **Oncotic pressure**.

In the normal state, there is a balance of forces so there is no net gain or loss of fluid in the interstitial fluid (ISF). If excess fluid remains in the ISF, it is drained back to the circulation via the lymphatic system.



Oncotic Pressure

Exerted by osmotic pressure of plasma proteins. They also have a negative charge and therefore hold cations in the lumen (**Gibbs Donnan effect**). This value remains constant along most of the capillary beds.

As not all protein is effective in retaining water, the effective capillary oncotic pressure is lower than the measured oncotic pressure. The **reflection coefficient** is a correction method of measurement between 0-1 where 1 is no difference between the measured and effective (more likely in capillaries with tight endothelial junctions). Closer to 0 in hepatic capillary beds.

Oedema

Produced by expansion of interstitial fluid (ISF) and occurs secondary to one of the following:

- Disruption of capillary haemodynamics, e.g. a rise in capillary hydrostatic pressure, a fall in capillary oncotic pressure or increase in capillary permeability
- Obstruction to lymphatic flow
- Retention of IV or dietary sodium and water by the kidneys

Lymphatics

Multiple roles including: Immune system transport of **antigen presenting cells** and **lymphocytes**, absorbs and transports fatty acids and fats (as chyle) from the digestive tract to the circulatory tree and removes any excess fluid and proteins that have crossed from the capillaries into the interstitial fluid (10% vs the 90% recovered by Starling's forces). Hydrostatic pressure of fluid in the interstitium opens spaces between the cells of the lymph vessel walls to allow lymph to enter. Then, as the interstitial pressure drops, these spaces close. Lymph nodes act to filter lymph prior to its return to the systemic circulation by way of the thoracic duct and the superior vena cava.

Circulation to the Skin

Unlike the brain, heart and skeletal muscle, which exhibit autoregulation where local metabolic influences tend to override neural ones, the circulation of the skin is under powerful sympathetic control. Contains arteriovenous (AV) anastomoses through which blood may be shunted to the **venous plexuses**, entirely **bypassing the capillaries**. These AV anastomoses are controlled by reflex influences from temperature receptors and centres in the anterior hypothalamus.

Differential Blood Flow to Organs

Region	Blood flow (ml/min)	Arteriovenous oxygen difference (ml/L)	Oxygen consumption (ml/min)
Brain	750	62	46
Liver	1500	34	51
Kidneys	1260	14	18
Skin	462	25	12
Skeletal muscle	840	60	50
Whole body	5400	46	250

Coronary Circulation

(07b_02_12)

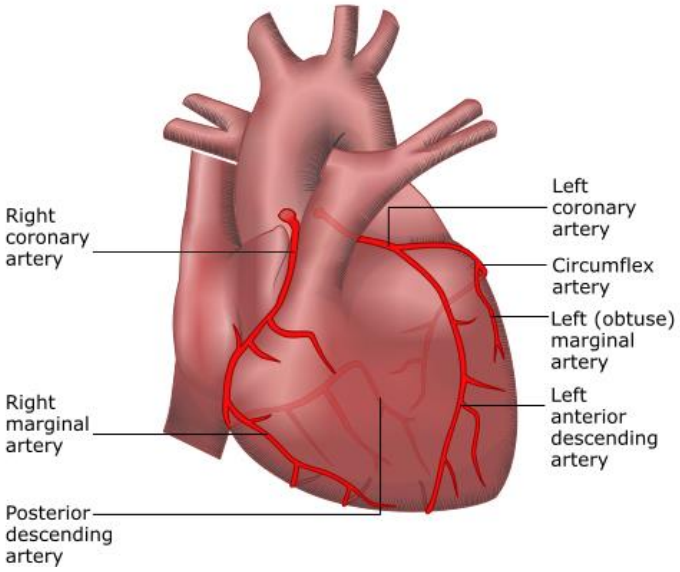
Anatomy

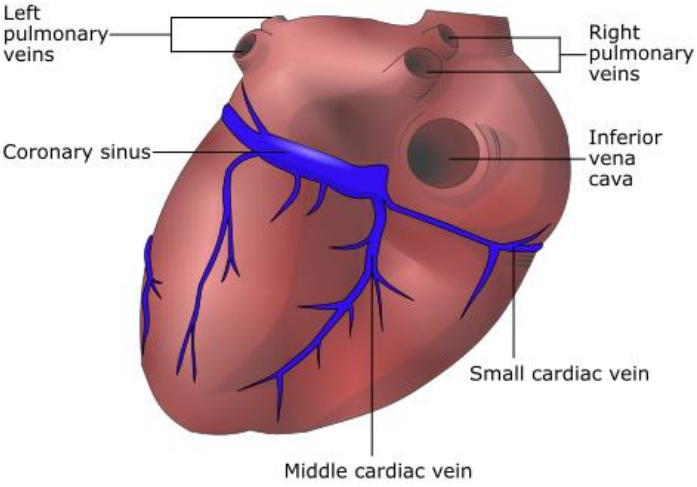
Coronary Arteries

Coronary arterial dominance is defined by the vessel which gives rise to the posterior descending artery (PDA), which supplies the myocardium of the inferior 1/3rd of the interventricular septum. 30% of cases, dominancy arises from both L and R coronaries.

The **R coronary artery** generally supplies blood to the right atrium and ventricle. Dominance in 50%.

The **L coronary artery** generally supplies blood to the left atrium and ventricle. It divides into the LAD and circumflex a. Dominance in 20%.





Coronary Veins

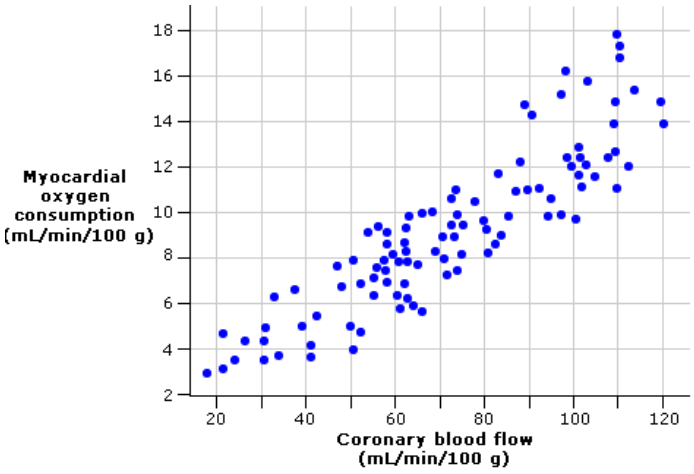
Venous blood **returns predominantly through the coronary sinus.** Small amounts drain directly into the heart chambers via **Thebesian** veins

If drained into the L ventricle, it will result in a physiological shunt.

Coronary blood flow and Myocardial Metabolism

Under resting conditions, the oxygen consumption of the myocardium is around 8-10 ml/100 g and PO_2 of the coronary sinus is relatively constant meaning oxygen extraction is about 60% at rest so does not increase much. Therefore, **increased oxygen demand is dependent on coronary blood flow** as seen in the graph.

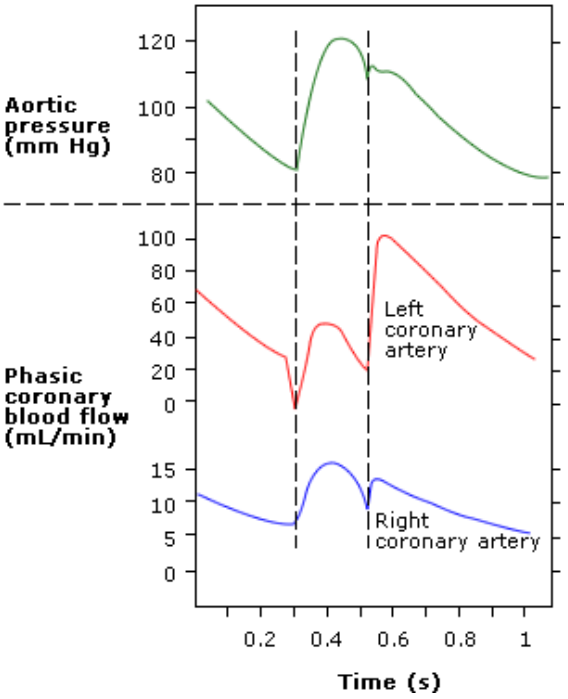
However, during increased pressure states in the L ventricle, oxygen demand is much higher so cardiac efficiency decreases – i.e. in patients with HTN.



Phasic Flow

Blood flow in coronary arteries depends on the **pressure difference between aortic pressure and extravascular myocardial pressure**. Therefore, flow to the L coronary artery is hugely affected during ventricular systole whereas the R coronary a. is not as markedly reduced. **L coronary a. blood is mainly supplied during diastole**.

With **increased HR, diastole time is reduced** and is therefore **counteracted by vasodilation** in response to higher metabolic activity.



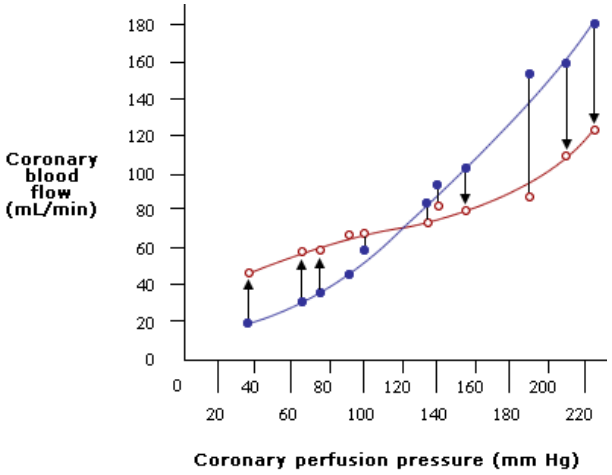
Regulation of Blood Flow

As stated before, increasing coronary a blood flow is the main method of increasing oxygen supply to the myocardium and is mainly regulated by **changing vascular tone:**

- **Increased metabolic activity → Reduced coronary vascular resistance**
- **Increased aortic pressure → Increased coronary vascular resistance**
  - Autoregulation occurs between aortic pressures of 40-160 mmHg

The graph shows an abrupt change in perfusion pressure through catheterisation independent of cardiac work resembled by the closed blue circles and the subsequent return towards baseline blood flow indicated by open circles.

The mechanism is unclear but may include neural/humeral factors, local metabolism and vascular endothelium involvement.

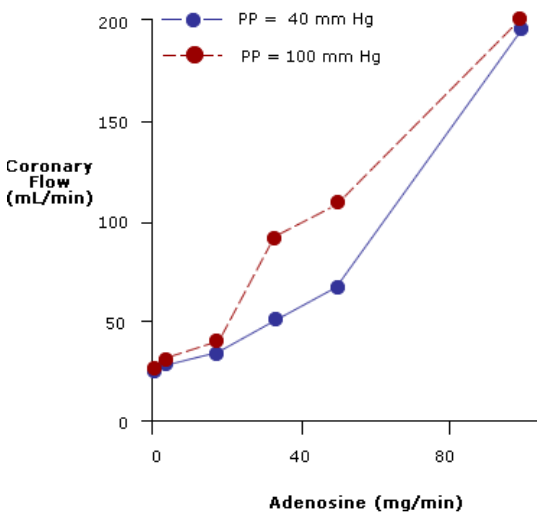


Local Metabolism

One mediator for increased coronary blood flow due to metabolic changes is **adenosine**. When **oxygen concentration decreases** with **higher metabolic activity**, the regeneration of **ATP is reduced**, and **adenosine accumulates** and causes vasodilatation.

Opening of **ATP sensitive potassium channels** is triggered directly by adenosine and through reduced ATP. This is a 2nd key factor for vasodilation.

NB this effect is seen in the denervated heart

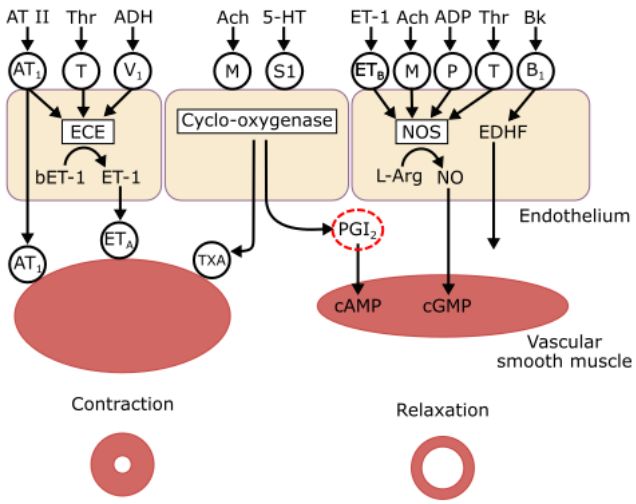


Vascular Endothelium

Vascular smooth muscle relaxation (vasodilatation) is mediated by **nitric oxide** (**NO**) stimulated by endothelin, ACh, adenosine, bradykinin, shear stress and hypoxia (far right of image). NA and Dopamine inhibit NO.

Prostacyclin is also vasodilatory stimulated by pulsatile flow and shear force, hypoxia, adenosine diphosphate (ADP) and adenosine triphosphate (ATP), serotonin and thrombin.

Endothelin is the main vasoconstrictor acts via the endothelin receptor type A stimulated by thrombin, noradrenaline, adrenaline, vasopressin, hypoxia and shear stress.Neural and



Endothelium-derived vasoactive substances

Ach	=	acetylcholine
ADH	=	anti-diuretic hormone (vasopressin)
ADP	=	adenosine diphosphate
AT II	=	angiotensin II
bET-1	=	big endothelin-1
Bk	=	bradykinin
cAMP	=	cyclic adenosine monophosphate
cGMP	=	cyclic guanosine monophosphate
ECE	=	endothelin converting enzyme
EDHF	=	endothelium-derived hyperpolarizing factor
ET-1	=	endothelin-1
5 HT	=	serotonin
L-Arg	=	L-arginine
NO	=	nitric oxide
NOS	=	nitric oxide synthase
PGI_2	=	prostacyclin
Thr	=	thrombin
TXA	=	thromboxane A2

Receptors

AT_1	=	angiotensinergic
B_1	=	bradykinergic
$ET_{A/B}$	=	endothelin A/B receptor
M	=	muscarinic
P	=	purinergic
S1	=	serotonergic
T	=	thrombin receptor
V_1	=	vasopressin receptor 1A

Neurohumeral Factors

α-receptors – induces coronary a. vasoconstriction

β-receptors – induces coronary a. vasodilatation

However, direct neural effects are not as strong on coronary resistance as metabolic control of blood flow which is why blood flow is increased with increased cardiac work secondary to SNS stimulation of the heart.

The net effect of the neural influence seems to be constriction rather than dilatation as denervation of the heart is initially followed by a reduction in coronary vascular resistance.

Pulmonary Circulation

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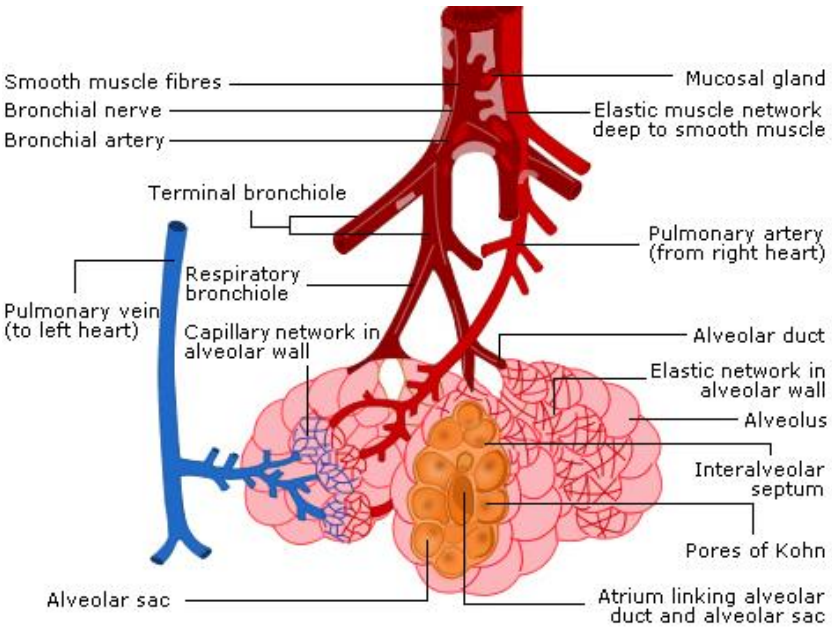
Anatomy – Recap

Diagram indicating the relationship between the pulmonary circulation and the respiratory system.



Pulmonary arteries

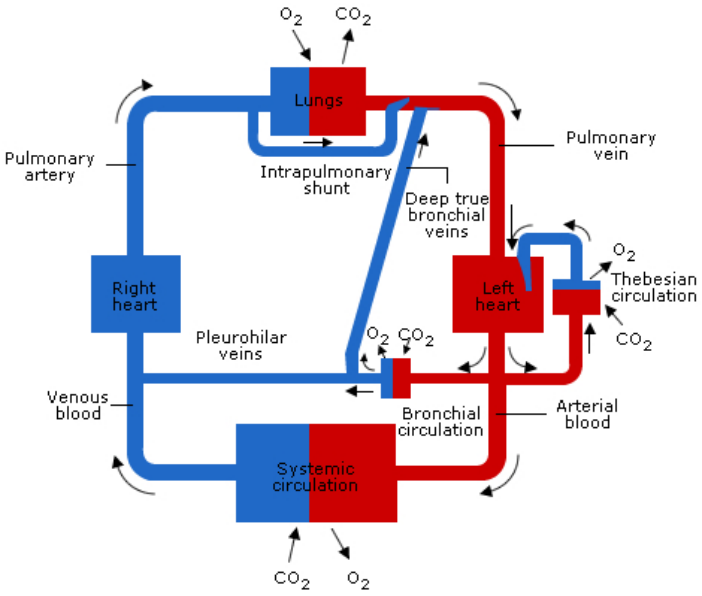
Are thinner than systemic and have less smooth muscle – therefore there is a reduced capacity for vasoconstriction and they are **highly distensible**. This way, the pulmonary circulation can accommodate great variations in cardiac output with little pressure change and R ventricular strain.

The pulmonary capillaries provide a large surface area (50-70m^2) for gas exchange when aligned with the alveoli.

Bronchial Arteries

Originate from the thoracic aorta and supplies blood to up to the terminal bronchioles and have the same characteristics as systemic arteries. Bronchial veins return blood into:

- The R atrium via azygous veins
- The L atrium via pulmonary/bronchial veins
  - Causes mixing of venous blood and physiological shunt
  - Also accounts for the larger cardiac output on the L side vs. R side of the heart.
  - In pulmonary HTN → increased bronchial a. flow → arterial desaturation

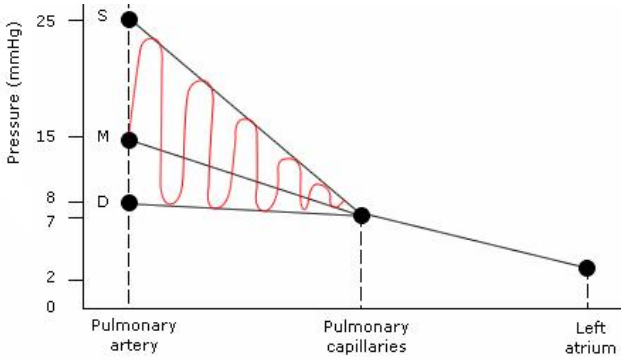


Pulmonary Vascular Resistance (**PVR**)

Pulmonary artery pressure – L atrial pressure

PVR = Driving pressure / Cardiac output

Considering the pressure drop between the pulmonary a and the L atrium is 10mmHg and the cardiac output is 5L/min, the PVR is 2mmHg.min/L or 160 dyn.sec.cm^{-5}. Pressure variations occur in the pulmonary arteries. PVR decreases with an increase in pulmonary blood flow (cardiac output) in order to maintain good pressure and is vital to protect the R ventricle and to prevent pulmonary oedema. This is done by the following 2 mechanisms:



1. **Recruitment –** Previously unperfused pulmonary capillaries are opened, mainly in the upper part of the lung (low capillary pressure)
2. **Distension** – of the thin walled pulmonary vessels → widening.

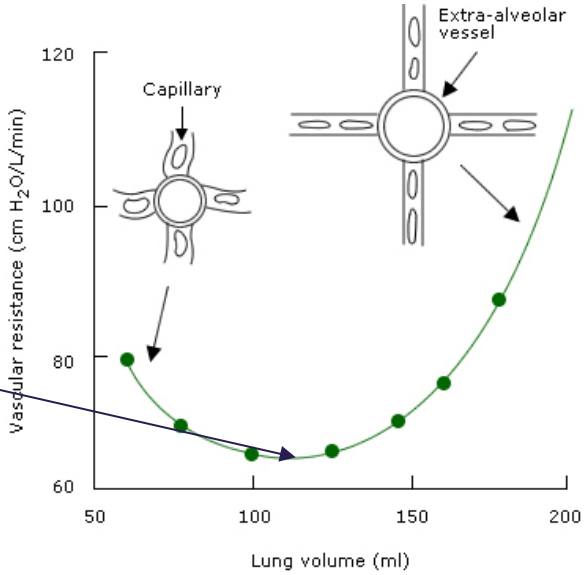
Effect with Lung Volume

Not fully understood. However, it is known that PVR may increase with either:

- **High lung volumes** as septal capillaries stretch with the thinning alveolar wall
- **Low lung volumes** due to extra-alveolar vessel narrowing.

Pulmonary vascular resistance is lowest at FRC.

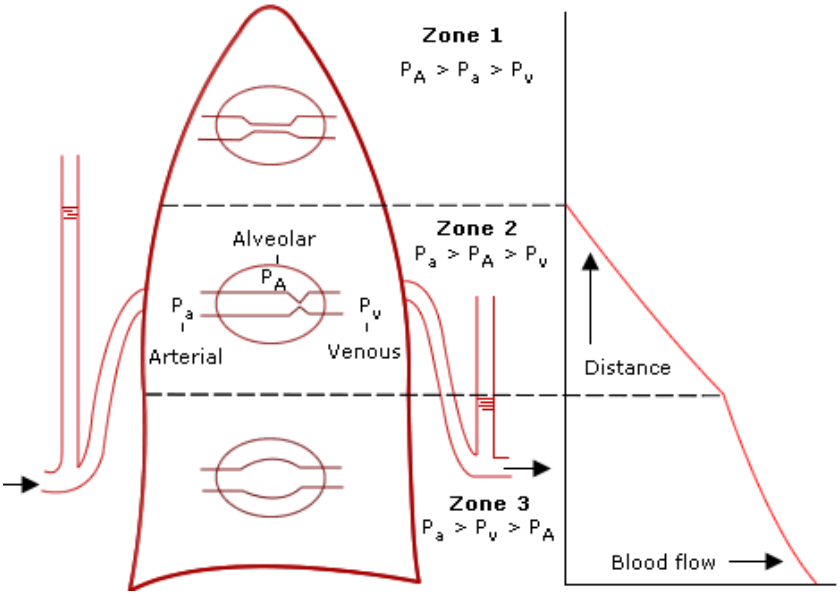
Therefore, FRC is the lung volume at which the net effect of lung tissue on septal capillaries and extra-alveolar vessels is such that there is the least PVR.



Normal Pulmonary a. pressures = 8-20mmHg @ rest. >25mmHg indicates pulmonary HTN.

Blood Flow Distribution

Gravity specific because of the **low pressure** and the **distensibility of the pulmonary vasculature**. The below diagram describes the influence of gravity on the upright lung.



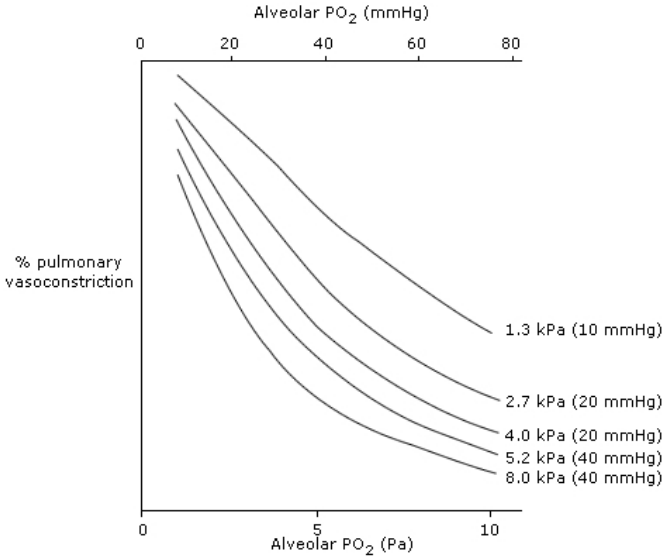
ZONE 1: Intravascular pressures are reduced compared to the main pulmonary artery according to the hydrostatic level. Alveolar pressure exceeds venous and arterial pressure and results in **no blood flow**: **$P_A > P_a > P_v$**

ZONE 2: Same hydrostatic level as the main pulmonary artery, the blood pressure might fall below alveolar pressure only during diastole and result in **intermittent blood flow** so that **$P_a > P_A > P_v$**

ZONE 3: In the lower part of the lung, arterial pressure is augmented by the hydrostatic pressure and exceeds alveolar pressure during the entire cardiac cycle, which produces **continuous blood flow**, so **$P_a > P_v > P_A$**

Hypoxic Pulmonary Vasoconstriction

With falling oxygen-concentration in the alveoli, vasoconstriction of the adjacent blood vessels occurs and blood flow is **diverted to better aerated areas** resulting in a better **matched ventilation-perfusion ratio**. This mechanism is not fully understood but thought to be from intermediary increases in NO and direct hypoxic effects on vascular smooth muscle.



In chronic hypoxia, there is proliferation of vascular smooth muscle cells which may lead to pulmonary HTN.

Maximal hypoxic vasoconstriction occurs during the foetal period to limit pulmonal blood flow to less than 15% of cardiac output

Physiology of Exercise and CPEX Testing

(07b_02_14)

Initiation of Exercise

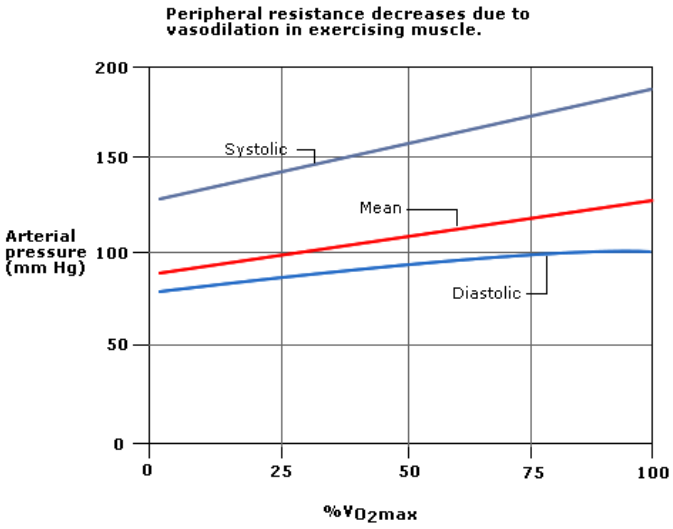
Anticipation of exercise produces increased O_2 demand by the resp and CV systems. Therefore, there is increased CO and vasodilation for O_2 delivery through:

- **Vasodilatory metabolites** H^+, K^+, PO_4^{3-}, AMP and adenosine override NA.
- **Improved oxygen dissociation** through increased temperature and reduced pH.
- **Increased muscle blood flow** from 4ml/100g to 100ml/100g muscle/min.

Protective Reflexes

Limiting damage to body systems during exercise i.e. limiting stretch, temperature increases and BP.

- As the $\%VO2_{max}$ increases, MAP also increases linearly
- Rise in temperature opposed through sweating and increased peripheral blood flow



CV changes

In elite athletes, CO can increase to 40L/min from baseline 5L/min. The blood pressure paradoxically rises despite the increased blood flow to muscles through a rise in **cardiac output**: In mild jogging and **aerobic exercise** – the increased CO is derived mainly from an **increased stroke volume** whereas in running and **resistance training** – the increased CO derives also from an **increase in HR**.

Stroke Volume: Increases to a maximum of 110-115ml/beat:

1. **Increased ventricular filling** from increased **venous return** increasing the preload.
2. **Secretion of catecholamines** increases inotropy and increases **ejection fraction**

Heart Rate: Increases as a reflex of the **SNS stimulation** and **vagal inhibition** and maintained via the ANS and CO_2 action on the medulla. Linear increase with $\%VO2_{MAX}$.

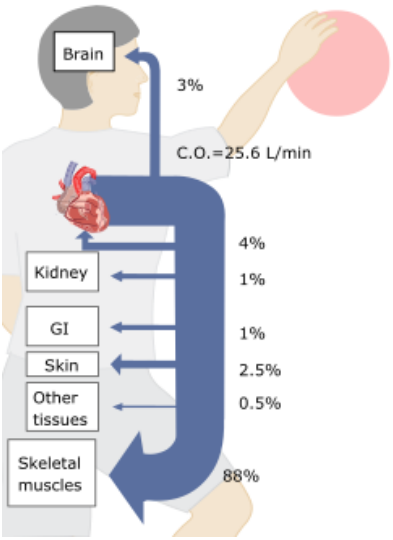
Maximum HR = (220 – age) +/- 11

In trained athletes, the CVS function is the limiting factor for O_2 delivery to the tissues and in strenuous exercise, HR and SV increase to 90% of their maximum. **Endurance training** improves the maximum CO through myocardial hypertrophy whilst resistance training has little effect on max CO.

Redistribution of Blood Flow

During exercise **blood** is **diverted to skeletal muscle** and **active tissues** and **away from inactive tissues** such as the digestive system. Skin flow changes according to heat dissipation.

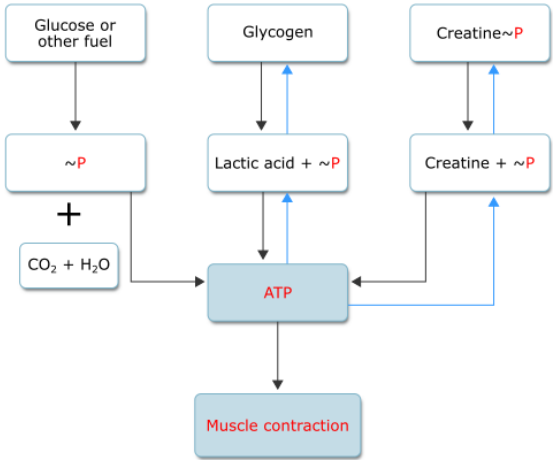
This occurs through **local mediators** and **directly through the SNS**.



Energy Sources

Immediate: Each mole of ATP releases 7.3 kcal. Only small amounts of ATP need to be stored to allow 1-2s of immediate exercise. ATP needs to therefore be **synthesised rapidly** and sourced from the **phosphocreatine system** and minimally from the **adenylate kinase reaction** (ADP + ADP → ATP + AMP).

At rest, this energy is produced from oxidative metabolism of glucose and fatty acids. Glucose metabolism has been covered earlier.

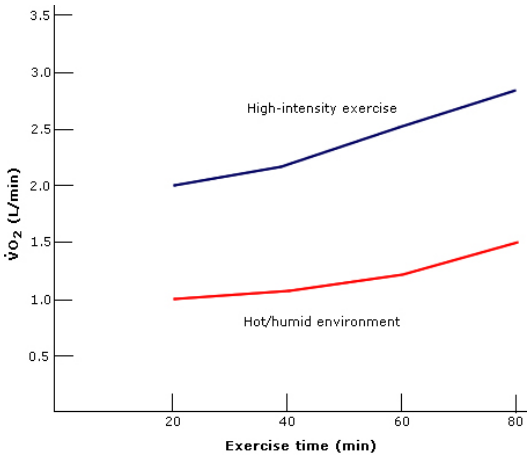


Fatty Acid metabolism: Fats are denser than glycogen – if all energy was stored as glycogen, body weight would increase by 50kg. **Fatty acids** undergo **β oxidation** and then enter into the Krebs cycle. Synthesis of ATP from fats are **slow** and is therefore the dominating substrate for ATP production at rest.

CHO stores are used for the beginning of exercise but with **prolonged exercise, fat metabolism** becomes important (see below). *Protein* stores are only used in endurance sports or during starvation. In summary, **exercise lasting:**

- **High intensity exercise 2-20s:** ATP from ATP-phosphocreatine system
- **Intense exercise >20s:** ATP from glycolysis
- **Intense exercise >45s:** ATP from PC, glycolysis and aerobic systems.
- **Exercise >10mins:** ATP from aerobic metabolism in a steady state.

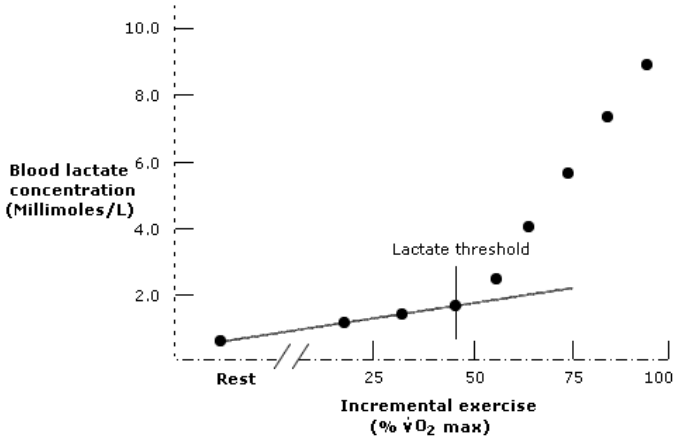
Hot/humid environments/ high intensity exercise: VO_2 steady state is not achieved and O_2 uptake drifts over time (see figure)



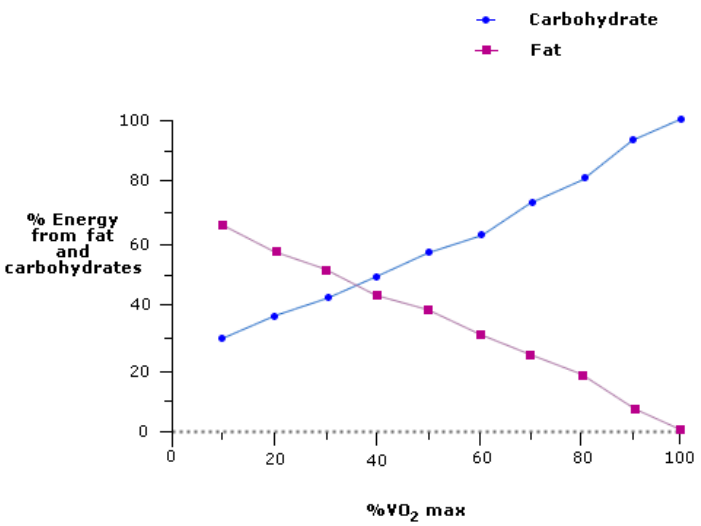
Lactate Threshold

This is the **anaerobic threshold** and is where **blood lactate suddenly rises**. It is used as a marker of exercise intensity. It may occur through:

- Low muscle oxygen
- Accelerated glycolysis with failure of mitochondrial hydrogen shuttle to keep pace of glycolysis resulting in excess NADH in sarcoplasmic reticulum favouring conversion of pyruvate to lactic acid.
- Recruitment of fast-twitch fibres
- Reduced rate of lactate removal



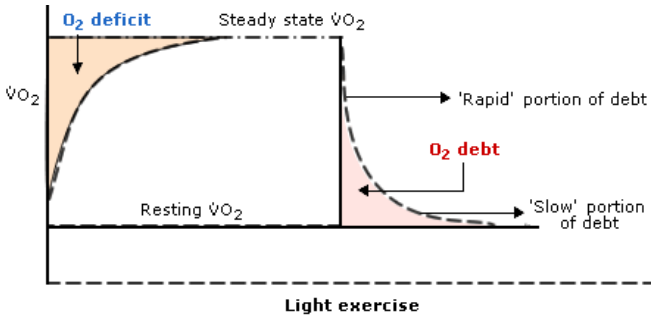
CROSSOVER CONCEPT: Describes the **shift from fat to CHO** substrate as exercise intensity increases due to the **recruitment of slow-twitch fibres** and **increasing levels of adrenaline**. The **shift reverses** in **prolonged exercise.**



Respiratory System

Oxygen Consumption

From resting state, oxygen uptake must increase rapidly and reaches steady state in 5mins. **Increased O_2 uptake lags behind O_2 consumption** resulting in **O_2 deficit** at the beginning of exercise suggesting anaerobic metabolism contribution to total ATP production. **Training increases VO2** at the **beginning of exercise** enabling earlier transition to steady state.



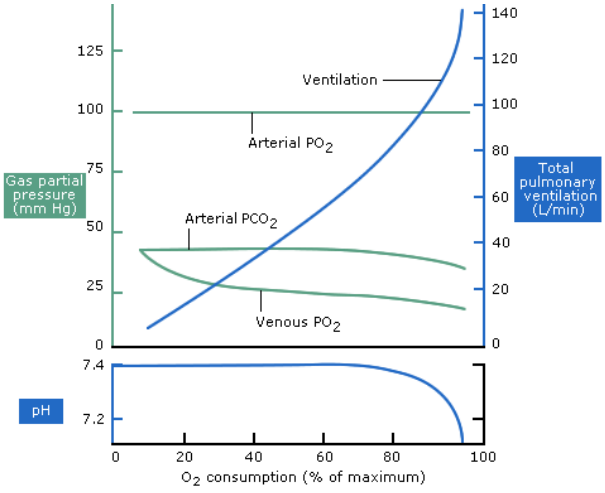
Fast portion of O_2 debt is the re-synthesis of stored phosphocreatine and the replacement of muscle and blood stores of O_2. The **slow portion of O_2 debt** is the removal of lactic acid.

Ventilation

Ventilation can increase from 5 L/min to >100 L/min. It **increases linearly with increasing exercise** until maximal exercise intensity is reached but **ventilation is not the limiting factor**. Hb is fully saturated in a healthy individual and therefore, ventilation does not limit oxygen delivery. Resting O_2 consumption in an average man is 250 ml/min and can increase to 5 000 ml/min in an endurance athlete.

PO_2 and PCO_2 change very minimally with the latter only slightly decreasing when approaching $VO2_{MAX}$. This is likely to be compensatory for a **drop in pH at maximal exercise intensity**.

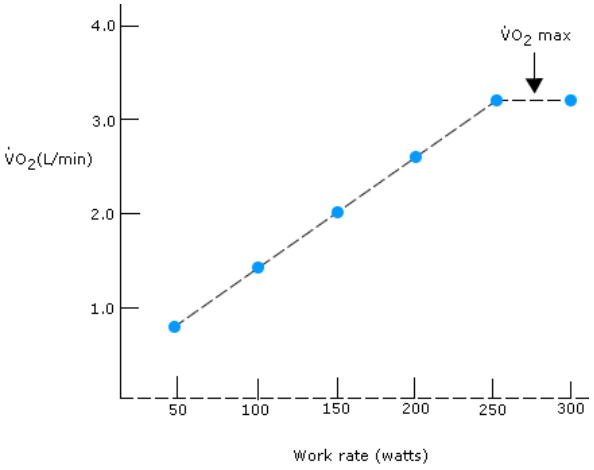
Therefore, it is likely that a number of regulatory systems tighten the control of these parameters through feedback to the CNS. At the **onset of exercise**, the **oxygen deficit accumulates**, leading to an accumulation of pyruvate which cannot enter the Krebs cycle (no oxygen). The **lactic acid produced increases $PaCO_2$**, this accumulation **quickly stimulates ventilation** and reduces $PaCO_2$.



Incremental Exercise

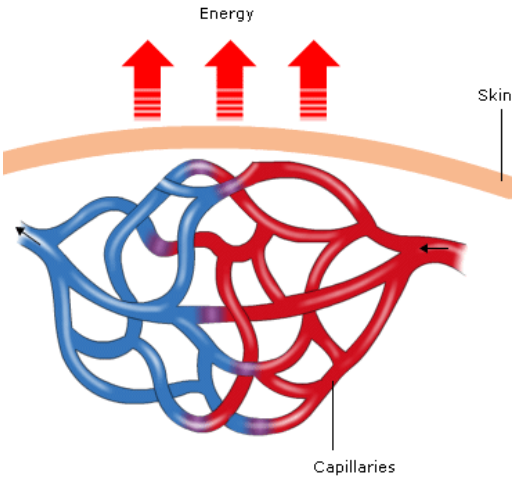
O_2 uptake increases linearly until $VO2_{MAX}$ is reached defined as the **plateau of oxygen uptake** at a **certain work rate.** At sea level, $VO2_{MAX}$ is influenced by:

1. **CVS ability** to deliver O_2 to tissues
2. Ability for muscles to use O_2 for ATP production aerobically. This is not due to mitochondrial numbers but **capillary density.**



Temperature Regulation

Max efficiency of conversion of energy nutrients to muscular work is 20-25% and the rest is released as heat. The **hypothalamus** is responsible for its regulation and heat is lost via **loss of vasoconstrictor tone** increasing convection of heat out via skin. Also, through **sweating and evaporation** and **expiration of hot air**. These mechanisms become more ineffective in hot and humid climates → **heat exhaustion**: weakness, exhaustion, headache, dizziness and eventually collapse, and coma.



RESPIRATORY PHYSIOLOGY

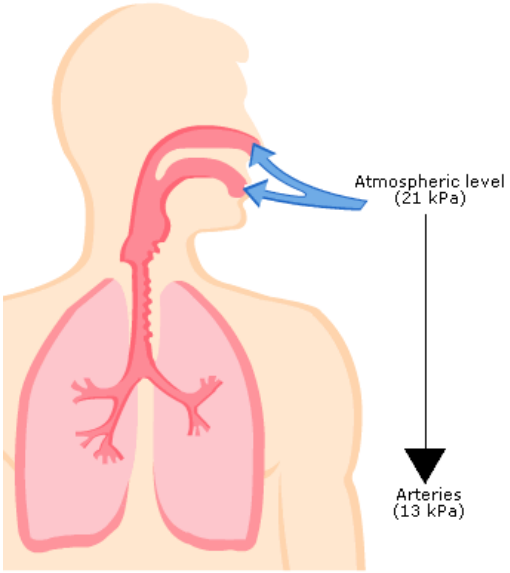
The following link is a YouTube resource that has West respiratory physiology lectures

West Physiology Lecture Videos

Gaseous Exchange: Oxygen

(07b_03_01)

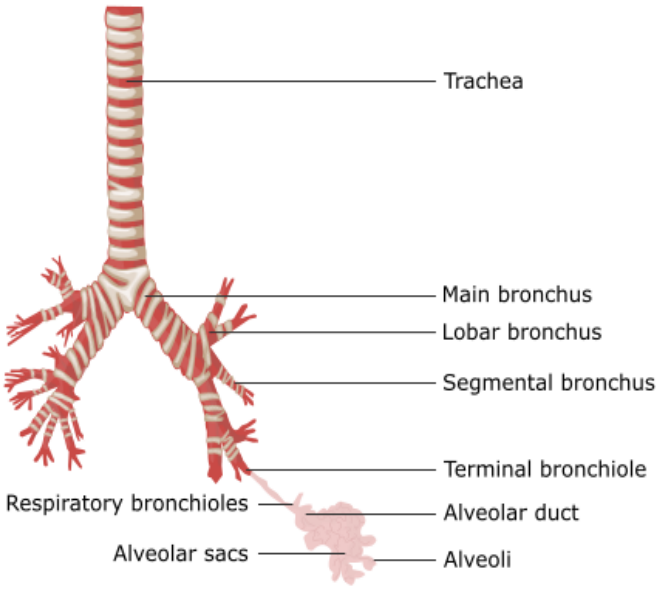
Oxygen Cascade describes the transfer of oxygen from the atmosphere to the arterial blood and occurs along a concentration gradient. Normal partial pressures are:



Dry atmospheric air	P_iO_2 = 21.2 kPa (159.2 mmHg)
Alveolar gas	P_AO_2 = 14 kPa (10.5 mmHg)
Arterial blood	P_aO_2 = 13.3 kPa (100 mmHg)
Mixed venous blood	P_vO_2 = 5.3 kPa (40 mmHg)

Airway Anatomy

This is divided into 2 zones according to the capabilities of gas exchange:



1. CONDUCTING ZONE

From generations 1-16 of **trachea to terminal bronchioles**. Does not include the alveoli and can hold 150ml of gas. It functions to allow bulk flow during inspiration and expiration and also humidification of the inspired air

2. RESPIRATORY ZONE

From generations 17-23 of **respiratory bronchioles to alveolar sacs** allowing 3000ml of gas. There is no bulk flow so gas can only move down a concentration gradient i.e. by diffusion

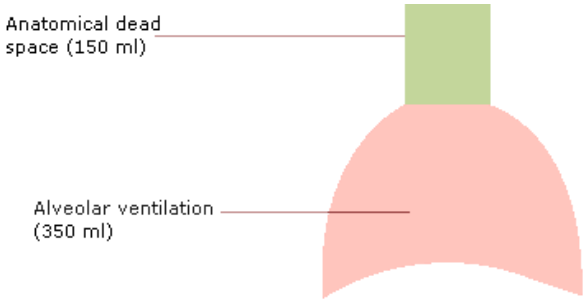
Gas exchange

The ideal alveolus is perfused and ventilated normally. Factors that affect gas exchange are:

- **Dead space:** The proportion of tidal volume not involved in gas exchange
- **Diffusing capacity:** The volume of gas that can transfer across a membrane per unit time
- **Shunt:** The proportion of the blood entering the left side of the heart that has bypassed the oxygenation process of the lungs.

1. DEAD SPACE V/Q ~ ∞

There are 2 types: anatomical dead space and alveolar dead space.



Anatomical Dead Space

Consists of the **conducting zone** and the **upper airways** – nose and pharynx. Equates to around 150ml per breath. Therefore, alveolar minute ventilation (AMV) can be calculated from:

AMV = (TV-DSV) x RR

Alveolar Dead Space

The **proportion of AMV that does not take part in gas exchange** due to entering either **unperfused** alveoli (where no gas exchange occurs) or **under-perfused alveoli** (where gas exchange is incomplete).

	Anatomical dead space	Alveolar ventilation
Timing in exhalation	Early expiration	End of expiration
Respiratory exchange?	No	Yes
% Oxygen	21	15
% Carbon dioxide	0	5

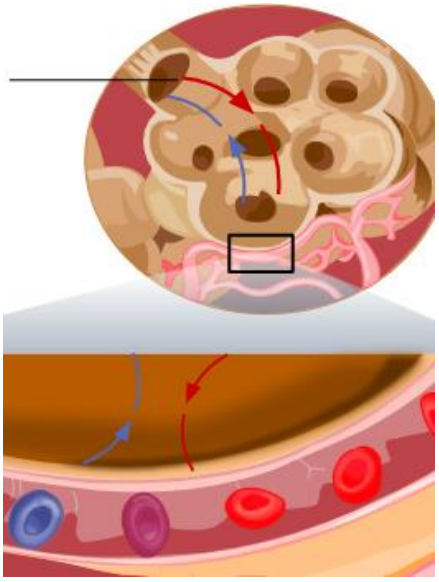
Physiological dead space (DS) = anatomical DS + alveolar DS

2. DIFFUSION CAPACITY

The variables involved in gas exchange between the alveolar membrane and erythrocytes within the pulmonary capillaries include and calculated using **Fick's law of diffusion**:

$$\text{Flow of gas} \propto \frac{A}{T} .D (P_1\text{-}P_2)$$

1. Surface area of the lungs (A)
2. Diffusion constant for oxygen (D)
3. Thickness of the capillary and alveolar membrane (T)
4. Partial pressure gradient between alveoli and blood. (P1 and P2 respectively)



Oxygen diffusion is around 250ml/min and equilibration across the membrane is rapid at around 0.25s (250ms). Considering it takes 750ms for RBC to transit during gas exchange, it allows enough time for full oxygenation – even when transit time is shortened following exercise or periods of increased oxygen requirements. It is therefore known as a **diffusion limited** process.

3. SHUNT V/Q ~ 0

This is divided into 4 different types of shunt:

- **Normal extrapulmonary shunt**
  - Part of the bronchial circulation
  - Thebesian drainage, from heart muscle directly into the left ventricle
- **Normal pulmonary shunt**
  - Areas of lung with V/Q >0 and <1
- **Pathological extrapulmonary shunt** i.e. heart disease (mainly congenital)
- **Pathological pulmonary shunt**
  - Pneumonia
  - Atelectasis

The term **venous admixture** describes the calculated amount of mixed venous blood required to be mixed with pulmonary end capillary blood to produce the observed difference between arterial and alveolar PO_2.

Ventilation/Perfusion (V/Q) Ratio

The V/Q ratio gives a global figure for the entire lung and should ideally be 1.

- **Alveoli ventilated but not perfused**: ratio of ∝
- **Alveoli perfused but not ventilated** (*Increased shunt fraction*)**:** ratio of 0
- Normal AMV = 4000 ml; Normal CO = 5000 ml; Therefore, Normal V/Q = 0.8

Even though the V/Q ratio differs in individual alveoli, multiple need to have a ratio of 0 to have an overall venous admixture.

Alveolar Air Equation

As the alveolar air cannot be sampled, it may be calculated with the following equation which takes into account that it is affected by amount that is delivered and rate of removal by circulation. It considers that:

1. **$P_aCO_2 = P_ACO_2$**
2. **Alveolar and arterial CO_2 are less affected by changes in the V/Q ratio**
3. **Water vapour effect on gas exchange**
   a. Inspired $PO_2 = F_iO_2$ x (Barometric pressure – Saturated vapour pressure of water @ 37 degrees Celsius)
   b. Inspired $PO_2 = F_iO_2$ x ($P_B - P_{H2O}$)

$$\text{Alveolar } P0_2 = \text{Inspired } P0_2 - P_aC0_2 \left(\frac{P_i0_2 - P_E0_2}{P_EC0_2} \right)$$

Alveolar-Arterial (A-a) Gradient

In healthy lungs this is **<2kPa**. With more venous admixture, the A-a gradient will increase.

Total Oxygen Content

98% of oxygen is carried on Hb and does not contribute to the PO_2 unlike the dissolved O_2 content. At normal P_aO_2 of 100 mmHg (13.3 kPa) = 0.3 mlO_2/100 ml

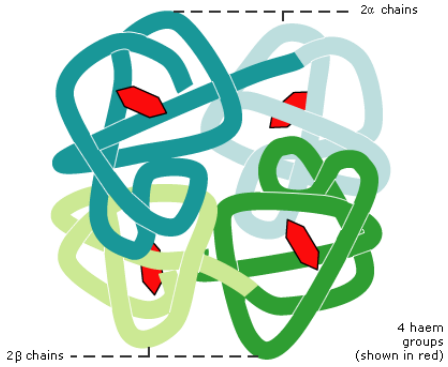
Total O_2 carrying capacity = (Hb X 1.34 x S_aO_2) + dissolved O_2

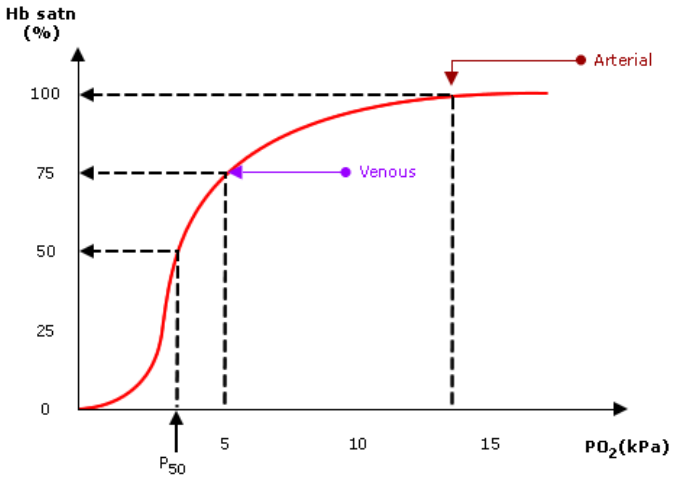
Traditionally calculated as **mlO_2 per 100ml blood** (Dissolved O_2 = k * pO_2 Where k= 0.023 if units are O_2 /100ml or 0.023 if units O_2 /litre). Approximately 20ml O_2/100ml.

Haemoglobin

Its production is regulated by **erythropoietin** regulated by the kidneys. Consists of 4 subunits with a central **haem group**. Oxygen binds to the Fe^{2+} ion and changes conformation of the protein.

As each oxygen molecule binds, the shape changes and therefore increases the ease of oxygen binding by the 2nd and 3rd molecules. However the 4th molecule is difficult to bind. This can correspond to the **haemoglobin-oxygen dissociation/saturation** curve:





Normal PaO_2: 13kPa

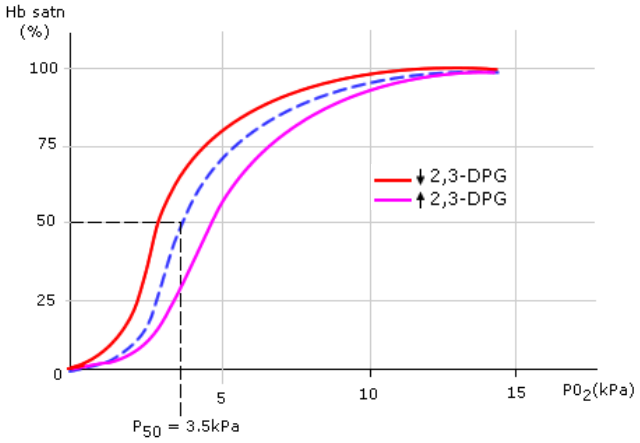
Normal PvO_2: 5.3kPa (P_{75})

P_{50} is the point at which the Hb saturation is 50% or 50% of binding sites are occupied and usually is around 3.5 kPa. This is a reference point to describe the position of the curve under different conditions.

BOHR EFFECT: Describes the effect of **pH on the oxygen dissociation curve.** The shifting of the curve to the right causes a reduction in the affinity of haemoglobin to oxygen helping oxygen delivery to tissues.

- **Acidosis/Increased PCO_2:** Induced by carbon dioxide production in metabolically active tissues – shifts the curve to the right so that for a given kPa, more oxygen is dissociated and visa versa in the lungs. **Lung CO_2:** removal of carbon dioxide shifts the curve to the left in order to increase affinity of Hb to oxygen to aid loading
- **Temperature Rise:** Curve shift to the right in response to heat produced by muscles.

- **2,3-diphosphoglycerate:** Produced as a side product of glycolysis and increases in anaemic states and at low oxygen tension and present in erythrocytes. It **binds to β chains of Hb** to reduce oxygen affinity.



Oxygen Delivery

Tissue Oxygen Delivery (DO_2) = blood O_2 content x cardiac output

Normally is 200ml/L x 5L/min = **1000ml/min**

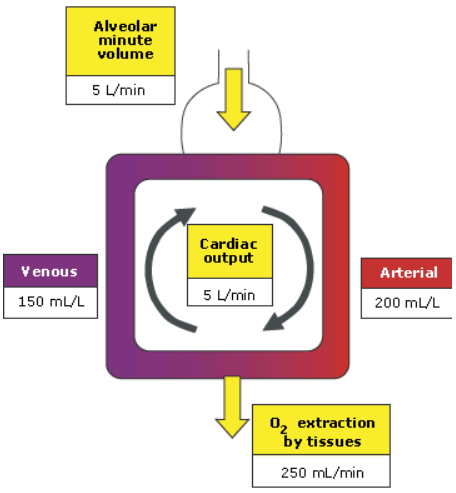
Oxygen Consumption (VO_2)

Oxygen consumption (VO_2) refers to the amount of oxygen utilized by the body for producing energy and is approximately = 250 ml/min at rest. It is calculated from the difference between arterial and venous oxygen content and the cardiac output:

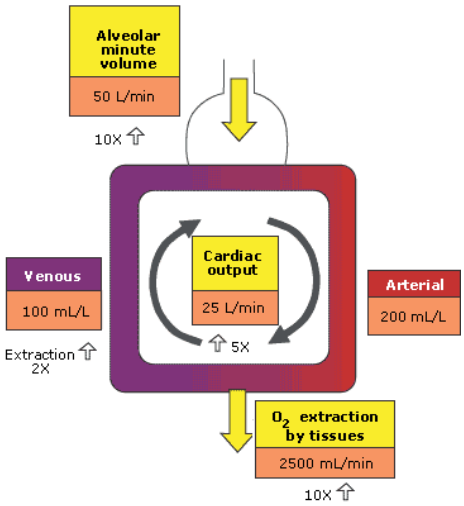
VO_2 = cardiac output x (CaO_2-CvO_2)

Oxygen content in arterial blood (C_aO_2) is most accurately estimated using the haemoglobin bound oxygen and dissolved oxygen. 1g of Hb can hold 1.34ml O_2 and the solubility of O_2 in the plasma (0.23ml/L/kPa) i.e.

O_2 Content= (Hb x 1.34 x SaO_2) + (0.23 x pO_2) = (150x0.98x1.34) + (0.23x13) = **200mL/L**



Content is multiplied by the cardiac output to give a delivery rate and the **rate of consumption (VO_2)** at rest will be 25% of this → corresponding to venous saturations of 75% (LEFT). This can subsequently increase to 10x this value during exercise helped by increased cardiac output, oxygen extraction by tissues and alveolar minute volume (RIGHT).



Gaseous Exchange: Hypoxia & Hypo-/Hypercapnia

(07b_03_02)

Hypoxaemia: Defined as an arterial PO_2 below normal levels

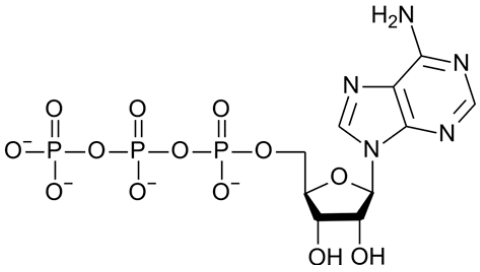
Tissue Hypoxia

Describes when the PO_2 within the cells is insufficient to allow normal aerobic metabolism to provide energy for cellular functions. This might occur with a normal arterial PO_2. Classified into 4 groups:

1. **Hypoxic Hypoxia:** Any cause of reduced oxygen availability to haemoglobin:
    a. High altitude – low oxygen tension
    b. Hypoventilation, V/Q mismatch or reduced O_2 diffusion in lung i.e. emphysema/CCF
2. **Anaemic Hypoxia:** any cause of reduced oxygen carrying capacity in the blood
    a. Reduced erythrocyte count – blood loss or marrow suppression
    b. Reduced haemoglobin concentration – Iron deficiency
    c. Abnormal haemoglobin – Sickle cell/thalassaemia
    d. Reduced oxyhaemoglobin binding – carbon monoxide binding
3. **Ischaemic/Stagnant Hypoxia:** Hb and PO_2 levels are normal but DO_2 is reduced so more O_2 is extracted leading to an increase in arterio-venous O_2 difference:
    a. Reduced cardiac output – hypovolaemic or primary cardiac failure
    b. Vascular abnormalities i.e. thromboembolic or AV shunting
4. **Histotoxic Hypoxia:** Oxygen is delivered but cells unable to utilise it i.e. cyanide poisoning

Cellular Metabolism

ATP is the high energy compound most used for cellular processes and is produced from catabolism of carbs, fat and protein. 3 phases in aerobic metabolism:



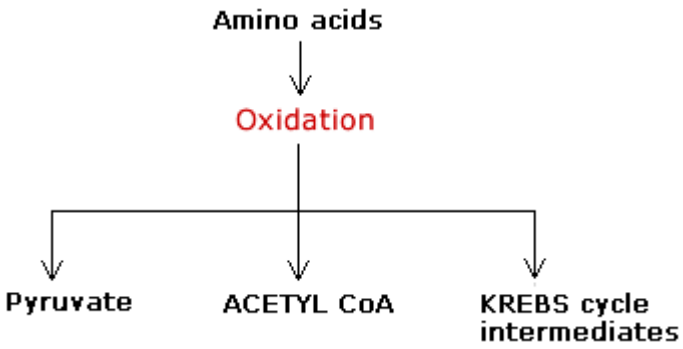
- **Phase 1:** production of two carbon compounds
- **Phase 2:** Citric Acid Cycle
- **Phase 3:** Electron Transport chain

PHASE 1

Small components of metabolic fuels are initially processed to produce 2 carbon compounds for phase 2 reactions:

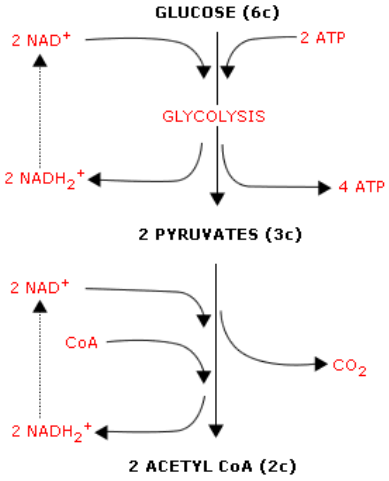
FREE FATTY ACIDS: Undergo **β-oxidation** to produce **Acetyl CoA**

AMINO ACIDS:



GLUCOSE:

Glycolysis occurs in the **cytoplasm. Oxidative decarboxylation of pyruvate to acetyl CoA** occurs in the **mitochondria**



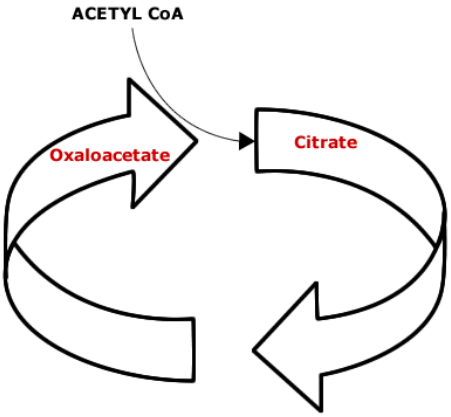
PHASE 2 – Citric Acid Cycle

Acetyl CoA enters the **Citric Acid Cycle** aka the Krebs Cycle.

It combines with **oxaloacetate** to produce **citrate** following which, there are a series of intermediary compounds to produce high energy compounds and carbon dioxide. The last compound produced is oxaloacetate – hence a re-cycle. Compounds created:

- 2 ATP
- 6 $NADH_2^+$
- 2 $FADH_2$
- 4 CO_2

Product of 1 glucose molecule or x2 cycles of the Citric Acid Cycle (x2 Acetyl CoA per glucose molecule)



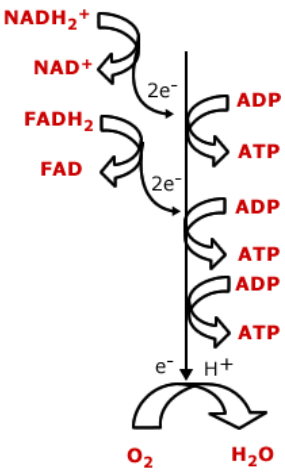
$NADH_2^+$ and **$FADH_2$** are reduced molecules containing high energy electrons.

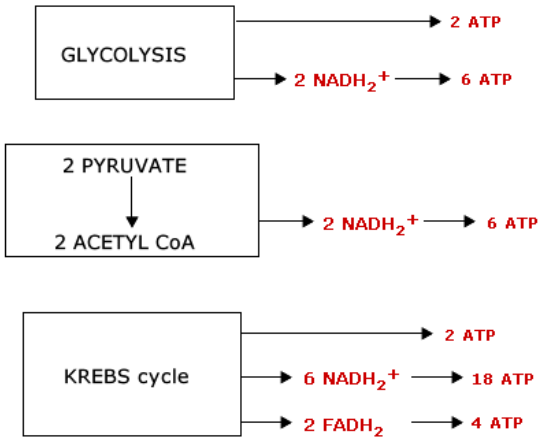
PHASE 3 – Electron Transport Chain

Oxidisation of the reduced molecules which releases ***electrons*** and ***energy***. The energy is utilised for oxidative phosphorylation of ADP → ATP.

- ***$NADH_2^+$*** enters at the beginning of the chain → converts 3 molecules of ADP
- ***$FADH_2$*** enters further down the chain → converts 2 molecules of ADP

Oxygen is the final electron acceptor in the chain and combines with hydrogen ions to produce water. Without the presence of oxygen, phase 3 is unable to commence.





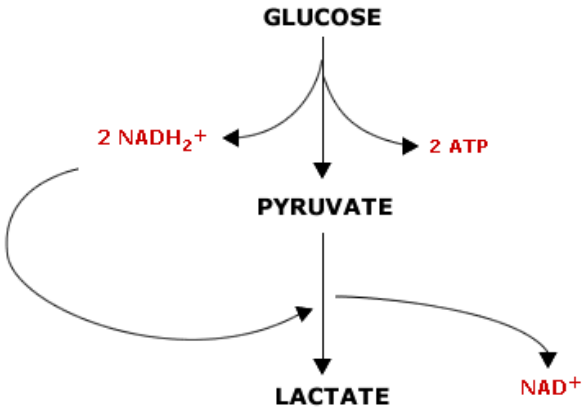
Summary

All three phases of metabolism is performed in aerobic respiration. A total of **38 ATP** is produced per molecule of glucose.

Anaerobic metabolism is the absence of oxygen and therefore:

1. Electron transfer chain stops
2. $NADH^+$ and FAD is not re-formed so the Citric Acid Cycle ceases

Only glycolysis is available and the $NADH_2^+$ is used to convert pyruvate to lactate. Therefore, **2 ATP** is produced per molecule of glucose.



HYPOXIA

There is only 90s of ATP supply within the body and mitochondrial PO_2 will be used for the 3 phases till it reaches a level of 0.4kPa. Once oxygen stops, the electron transfer chain ceases and inefficient metabolism of glucose commences.

Cellular Hypoxia therefore causes a switch to anaerobic metabolism resulting in:

1. **Fall in available ATP** → insufficient energy for cell functions i.e. Transport, muscle contraction and enzyme production.
2. **Fall in intracellular pH** → further inhibition of chemical reactions requiring a narrow pH band

➔ OVERALL LOSS OF CELL FUNCTION

Tissue Specific consequences of loss of cellular function

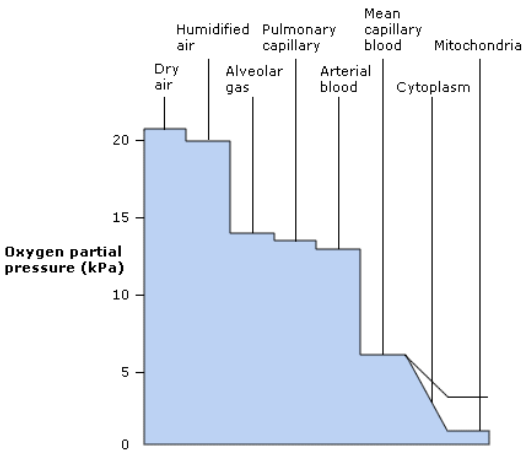
Muscle: Failure of production of high energy compounds results in failure of muscle fibre contraction.

Neurones: Ions are unable to move against an electrochemical gradient and therefore, the electrical potential gradient is not maintained and signal propagation ceases

Brain: Cells are most sensitive to hypoxic damage as rely entirely on oxidative phosphorylation of glucose for energy → 2 mins = irreversible cell damage → 4 mins = cell death.

Critical PO_2

Mitochondrial PO_2 needs to be >0.4kPa for aerobic respiration to commence – **Pasteur's point** Maintenance is dependent on oxygen delivery, tissue perfusion and consumption. Further to the oxygen cascade on the previous lecture, the following diagram describes PO_2 from capillary to mitochondria. Intracellular PO_2 may reduce to 0.5–2 kPa following barriers to diffusion. Therefore, a short period of poor oxygen delivery will result in mitochondria approaching critical PO_2.



Compensatory Mechanisms for Hypoxia

Divided into Early and Late.

EARLY Compensation:

1. Changes in Hb/O_2 affinity i.e. Bohr Effect

Anaerobic metabolism **reduces the pH of tissue** and therefore the oxygen dissociation curve shifts to the right allowing easier dissociation of oxygen to tissues. Within hours 2,3-DPG is produced which has the same effect.

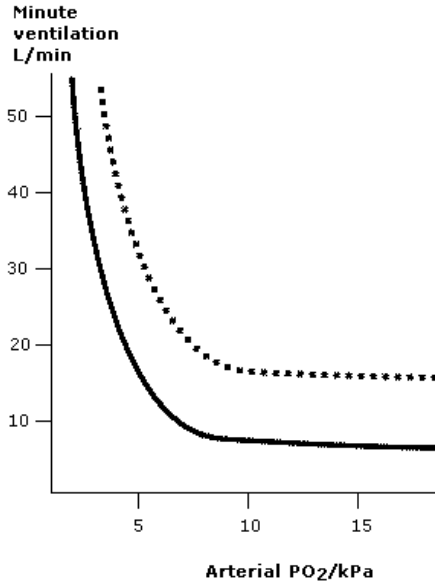
2. Local arteriolar vasodilation **which allows better perfusion and delivery. Stimulated by:**

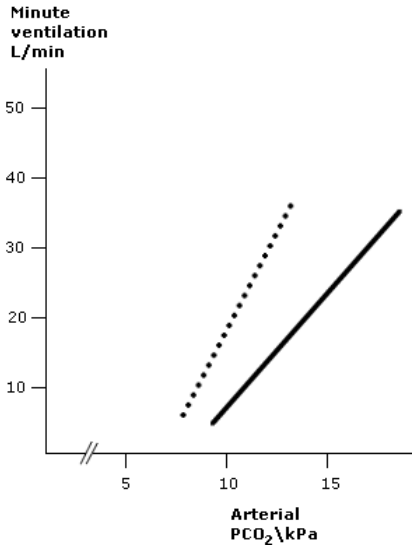
↓PO_2 ↓pH ↑PCO_2 ↑ local metabolites e.g.adenosine, K^+

3. Ventilatory Compensation

Mediated by ***peripheral chemoreceptors*** in the Type I cells of the carotid bodies responding to a lower oxygen tension. It responds to both hypoxia and hypercarbia:

HYPOXIA: Has no effect until **PaO_2 < 7kPa.** If hypercarbic also (dotted line), the ventilatory response across all ranges of PaO_2 increases:





HYPERCARBIA: Linear increase in minute ventilation with increasing $PaCO_2$ with enhancement with hypoxia (dotted line). With normal PO_2, minute ventilation rises by 15-20 L/min per kPa rise in $PaCO_2$.

4. Cardiovascular Compensation

Mediated by the ***peripheral chemoreceptors*** also from low oxygen tension. Hypotension may also cause this due to **stagnant hypoxia** and leads to:

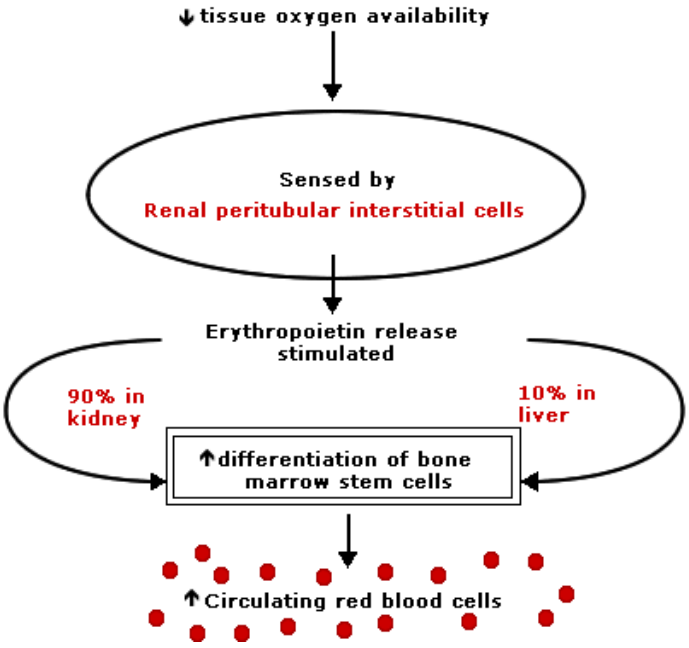
- **Vasoconstriction**
- **Tachycardia**

→ Improved tissue perfusion through **increased CO** and **BP**

LATE Compensation:

Occurs with prolonged periods of hypoxia i.e. high altitude, chronic lung disease and anaemia.

This causes an increase **erythropoietin production** which occurs within hours. However the resulting **increase in erythrocytes** take 3-5 days and allows an increased oxygen carrying capacity of the blood and hence increased DO_2.



Tissue Specific Responses to Hypoxia

BRAIN: utilises 20% of total body O_2 consumption. Cerebral blood flow (CBF) is maintained constant over a mean arterial pressure range of 50–150 mmHg. A fall in PO_2 below 6.7 kPa leads to exponential increases in CBF from local vasodilation and lactic acidosis production.

CORONARY: Covered in previous lecture but in summary, as oxygen extraction is very high, increases in coronary blood flow is required and achieved through arteriolar dilation from local metabolites, low oxygen tension and myogenic control of arteriolar tone.

PULMONARY: Hypoxic pulmonary vasoconstriction to balance V/Q ratio. Locally mediated through inhibition of NO production, vasoconstrictor production and direct effect of hypoxia on vascular smooth muscle.

CARBON DIOXIDE HOMEOSTASIS

As CO_2 is very soluble, it equilibrates across the alveolar wall rapidly. End tidal CO_2 may be lower that $PACO_2$ due to mixing with other expired gases. Factors that affect $PaCO_2$ include:

1. **Alveolar Minute Ventilation** (removal)
2. **Tissue Metabolism** (production)

NB this excludes V/Q mismatching due to its fast membrane exchange rate

Alveolar CO_2

Proportion of alveolar CO_2 can be calculated from the following equation:

$$\%\ \text{alveolar}\ PACO_2 = \frac{CO_2\ \text{output} \times 100}{\dot{V}A}$$

In an adult this would normally be 200/4000 = 5%. Therefore, doubling the minute ventilation would halve the $PACO_2$

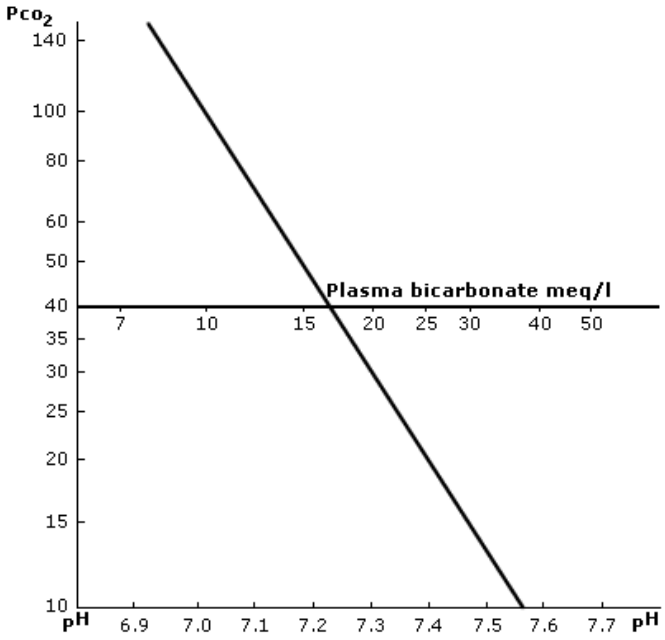
$$CO_2 + H_2O \leftrightarrow H_2CO_3 \leftrightarrow H^+ + HCO_3^-$$

pH can be described as the **inverse log[H⁺]** or by the

Henderson-Hasselbach Equation Which relates the concentration of CO_2 to that of it's base (bicarbonate) in solution:

$$pH = pKa + Log \frac{[HCO_3^-]}{[CO_2]}$$

Therefore, the pH can be maintained by CO_2 elimination or by HCO_3^- / H^+ balance in the kidney. This is illustrated in the **modified Siggaard-Andersen nomogram:**



Hypocapnia

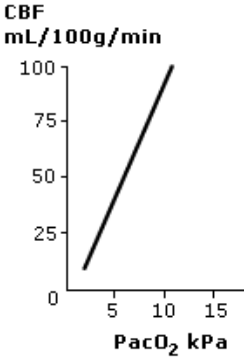
May be *primary respiratory* caused by **hypoxia**, **anxiety** or **pain** or it may be *compensatory* in response to a **metabolic acidosis**.

Hypercapnia

There are 4 main causes:

1. **Increased inspired CO_2** from re-breathing or additional exogenous CO_2.
2. **Primary respiratory** from hypoventilation or increased dead space
3. **Increased CO_2 production** i.e. in anaesthesia without compensatory rise in mechanical ventilation and in sepsis and malignant hyperthermia
4. **Compensatory** to a metabolic alkalosis i.e. hypokalaemia, vomiting, additional bicarbonate. This is however limited by the hypoxic effect if PaO_2 < 7kPa.

Effects of Hypercapnia



1. NEUROLOGICAL

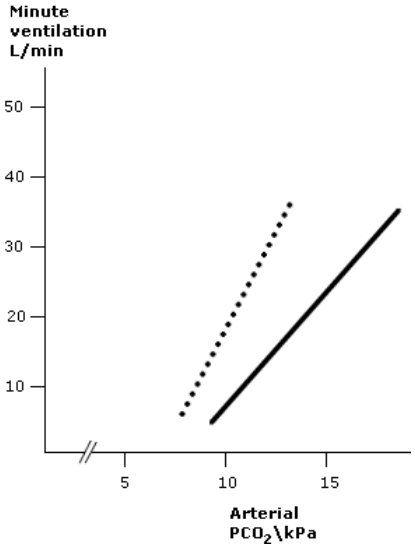
Increased cerebral blood flow (CBF) secondary to **vasodilatation and increased mean arterial pressure**. CBF increases by approximately 7-15 ml/100g/min for each kPa increase in $PaCO_2$. It is most sensitive to blood pH as CBF normalises following prolonged hypercapnia when pH normalises.

The increased vasodilation also causes **increased intracranial pressure**.

Narcosis occurs – reduced consciousness – usually at levels of **$PaCO_2$> 12 kPa** and mediated by pH.

Autonomic effects from increasing catecholamines.

2. RESPIRATORY



Increased minute ventilation is mediated through **central chemoreceptors** located in the medulla close to the respiratory centre. The BBB is more permeable to CO_2 and dissolves in the CSF to dissociate into H^+ ions which is much slower to move back across the BBB. Therefore, due to the **differing permeability of CO_2 and H^+** it is much **more sensitive to a respiratory than metabolic acidosis.**

Pulmonary Vasoconstriction occurs with an alveolar PCO_2 > 7 kPa and may subsequently **alter the V/Q ratio**.

Raised alveolar PCO_2 may also cause a **dilutional alveolar hypoxia** which may be compounded by a **shift of the Hb/oxygen dissociation curve to the right**.

3. CARDIOVASCULAR

Direct Effects

- **Myocardial contractility is impaired** but is usually overwhelmed by increased circulating catecholamines (see below). In severe acidotic states, the direct effects prevail
- **Arterial vasodilation** caused directly from acidosis → flushed skin and bounding pulses
- **Arrhythmias** may occur due to altering potassium levels in the myocardial conducting tissue.

Catecholamine Effects – induced by acidosis and release from the adrenal medulla

- **Myocardial contractility increases** from catecholamines.
- **Arterial vasoconstriction** in milder acidotic states
- **Tachyarrhythmias**

4. BIOCHEMICAL

Acidosis from hypercapnia induces **potassium leak from cells** and an **elevated serum potassium**. It also causes: **ionised → unionised calcium**.

Gaseous Exchange: Hyper-/Hypobaric Pressures

(07b_03_03)

Hypobaric pressures = Pressures below normal atmospheric pressure. Increasing altitude causes hypobaric conditions but with normal FiO_2 and vapour pressure of water in the alveoli resulting in **progressive alveolar hypoxia** & **hyperventilation**. Barometric pressure @ 5000m approx 50kPa.

Hyperbaric pressures = Pressures above normal atmospheric pressure. Respiratory gases become dense and nitrogen develops narcotic anaesthetic properties at 4atm. On decompression, gases in closed spaces expand and bubbles may form in blood or tissues causing **decompression sickness**, 'the bends'.

Alveolar Air Equation

This is mentioned previously. It is quantified by the difference between ***oxygen supplied (PiO_2)*** and the ***oxygen used metabolically in the body (arterial PCO_2/RQ)*** where RQ is the respiratory quotient (ratio of CO_2 produced to O_2 consumed).

$$P_AO_2 = inspiredPO_2 - \frac{arterialPCO_2}{RQ}$$

RQ is assumed to be 0.8.

Calculating PiO_2

Inspired PO_2 = F_iO_2 x (Barometric pressure – Saturated vapour pressure of water @ 37 degrees Celsius)

Sea level: barometric pressure is 101kPa and the SVP of water is 6.3 kPa at 37 degrees. Therefore, PiO_2 = 19.89 kPa.

Location	Altitude(ft)	Altitude(m)	Barometric Pressure (kPa)	Barometric Pressure (mm Hg)	Inspired PO_2 (kPa)	Inspired PO_2 (mm Hg)
Sea level	0	0	101	760	19.9	150
Ben Nevis	4 000	1 220	88	659	17.0	127
Aviation (cabin Pressure)	6 000	1 830	81	609	15.7	118
	8 000	2 440	75	564	14.4	108
	10 000	3 050	70	523	13.0	100
	20 000	6 100	46	349	8.4	63
Everest	30 000	9 150	30	226	5.0	37
Military Aircraft	50 000	15 300	11	87	1.1	8

At 63,000ft, inspired PO_2 = 0 and body water would boil. Military aircraft may fly at up to 70 000 ft with a cockpit pressure of 30 000 ft. Pilots therefore need oxygen

Although aircrafts fly up to 35,000ft, the cabin is pressurised to about 5,000-6,000 ft and may cause problems in susceptible people. Patients with an O_2 saturation of less than 92 % on air can be given a pre-flight 15 % hypoxic challenge test, to simulate a cabin pressure of 8 000 ft.

Acclimatisation and Adaption to Altitude

Acclimatisation

Rapid ascent to a summit will result in an **initial increased hypoxic drive** to respiration but this is short lived and returns to normal within hours.

Long term restoration of P_AO_2 is through hyperventilation and hypocapnia (see alveolar gas equation) → **respiratory alkalosis** → **renal bicarbonate excretion** and a **metabolic acidosis** which increases the respiratory drive. Therefore, the peripheral and central chemoreceptors are important in the process.

The rate of acclimatisation varies with species and affected by the rate of ascent – it may take days to weeks for humans.

Haemoglobin

Increased concentration to preserve oxygen content and carrying capabilities - (operation Everest II found Hb increased from 13 to 17 g/dl). Also, an **increased production of 2,3 DPG** but the shift is **counteracted by respiratory alkalosis**

Adaptation

Andean llama have haemoglobin similar to human foetal haemoglobin, which favours loading and unloading of oxygen at low ambient pressures.

Humans residing and born in high altitudes have **polycythaemia** (up to a Hb of 22) and a **reduced ventilatory response to hypoxia.** The highest workers in the world work in the Andes, at 19 000 ft. They prefer to live at lower altitude and climb every day.

Mountain Sickness

Acute Mountain Sickness (AMS) usually occurs above 6000ft. When mild, may cause dyspnoea, headache, nausea, fatigue and sleep disturbance associated with Cheyne-Stokes respiration.

The severity of AMS is **related to the speed of ascent** as 25 % of tourists arriving in the Andes by air suffer mountain sickness cf less in climbers who have a slower rate of ascent.

Severe AMS

High altitude pulmonary oedema (**HAPO**) associated with exercise and caused by **excessive pulmonary vasoconstriction**. 1% of climbers suffer and there is a high mortality if untreated. Symptoms include a *persistent cough productive of a white, watery or frothy fluid*.

High Altitude Cerebral Oedema (**HACO**) where the individual may suffer a coma or hallucinations.

TREATMENT: Immediate descent and **nifedipine** or **acetazolamide**.

Chronic Mountain Sickness is found in populations living at altitude. Symptoms/Signs include:

- Poor hypoxic response to ventilation
- CO_2 retention
- Polycythaemia
- Cyanosis
- Clubbing

HYPERBARIC PRESSURE

Pressure in water increases by one atmosphere per 10 m of descent below the surface.

Submariners are trained to escape from 100 ft. They must keep their glottis open and exhale as the gas in their lungs expands.

Depth (m)	Pressure (atm)	Pressure (kPa)
10	2	200
20	3	300
50	6	600
55 (limit for breathing air)	6.5	650
200	21	2 100
330 (limit for SCUBA diving)	34	3 400
3 820 (Titanic)	383	38 300

Symptoms of Rapid Decompression

- Barotrauma in any air-filled space, such as the lungs or ear
- Arterial air embolus
- Potentially permanent neurological damage
- Bubbles forming in vessel poor tissues (cartilage) with avascular necrosis

Hyperbaric Oxygen Treatment

Describes the administration of 100 % oxygen at a pressure of 2-3 atm given for 1-2h daily through a tight-fitting mask for up to 30 days. Why?

- **Arterial oxygen** only **increases marginally** (19 to 25 ml/dl)
- **Venous oxygen increases dramatically** at 3atm vs 1atm. As tissue oxygen approximates with venous oxygen, it aids with **tissue hypoxia**.

Indications: anaerobic infections and CO poisoning.

Interesting points at high pressure:

1. Density of gases increase. Air at 4 atm is four times as dense as at atmospheric pressure. This leads to increased ventilatory resistance and increased work of breathing
2. Nitrogen narcosis starts to occur at about 4 atm (30 m). Divers breathe a mixture of helium and oxygen because of its reduced density and to avoid nitrogen narcosis
3. Lower concentrations of oxygen are needed, as PiO_2 is maintained by high pressure. A concentration 2.5 % oxygen is required at 20 atm pressure to maintain a PiO_2 of 50 kPa
4. Breathing 100 % oxygen will produce oxygen toxicity, convulsions, chest pain and irreversible acute lung injury

Management of Decompression Sickness

Slow decompression through slow ascent helps prevent decompression sickness. Commercial divers may take several days to decompress.

Decompression sickness is treated by **emergency recompression in a hyperbaric chamber** and the administration of oxygen

Anaesthetic Implications

Hypobaric Pressures:

- **Pressurization and depressurization in aeroplanes occurs suddenly** during take-off and landing. This may cause sudden **expansion of air filled spaces** within a patient, such as bullae and pneumothorax.
- Valves in breathing systems may stick
- Inappropriate light anaesthesia because of inadequate vaporization of volatiles due to low temperature, which accompanies the altitude
- Theoretically, boiling point of volatiles will fall but they have still been used safely in altitude.

***Hyperbaric Pressures*:** To be aware of the complications of nitrogen narcosis and sudden decompression and to treat appropriately.

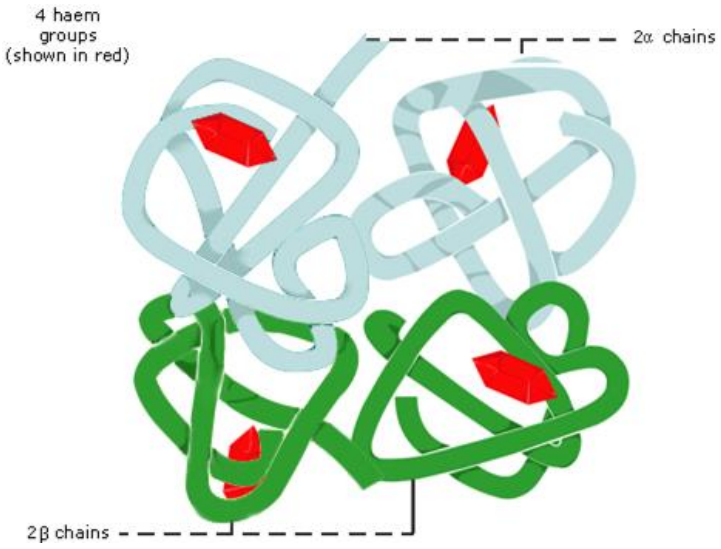
Haemoglobin and Oxygen Carriage

(07b_03_04)

Haemoglobin is the most well understood protein in our body but despite this, attempts to create artificial proteins similar has been unsuccessful.

Structure

Composed of **four polypeptide globin chains**, each containing a haem molecule made up of **protoporphyrin** ring with a central iron atom in the ferrous state (Fe^{2+}). Has a **quaternary structure** of 65,000 Da.



Definition of Quaternary protein:

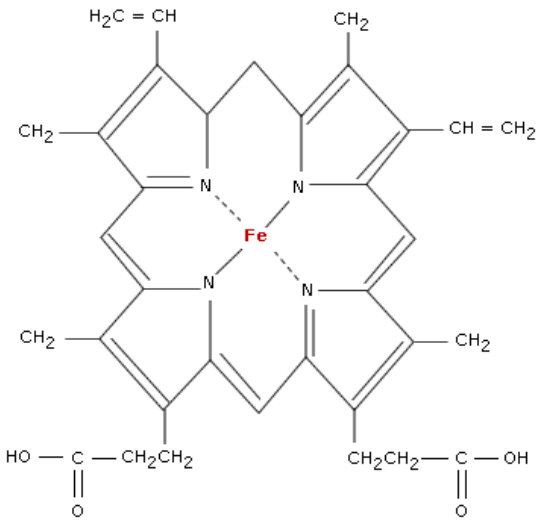
1. ***Primary*** *– sequence of aa's*
2. ***Secondary*** *– angles created in aa chain to form differing angles*
3. ***Tertiary*** *– Changes in the full shape of the protein i.e. crevasse for haem binding region created*
4. ***Quaternary*** *– interaction between* the protein chains (i.e. 4 globin chains)

Alpha Globin Chains: *Consists of 141 amino acids. Genes for which are on* ***chromosome 16***

Beta Globin Chains: *Consists of 146 amino acids. Genes for which are on* ***chromosome 11****.*

In depth…

Organic part is **protoporphyrin** which is made of 4 pyrrole rings which give the **haem** its red colour. This is illustrated in the diagram with iron centrally.



Iron in its ferrous state(Fe^{2+}) **forms 6 bonds**:

- **4 with nitrogen atoms**
- **1** to a **histidine "proximal" residue** located on the globin chain to form 1 subunit of haemoglobin
- 1 with an oxygen molecule (O_2)

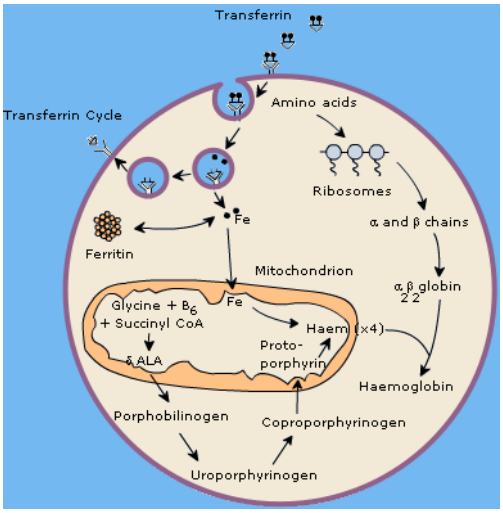
Near to the oxygen binding site, there is another **distal histidine** which acts to perform 2 important functions:

1. Prevents oxidation of Fe^{2+} to Fe^{3+} by other haem groups on Hb molecules
2. Prevents irreversible binding of CO to ferrous iron

Forms of Haemoglobin

- **HbA** – composed of **2 alpha (α) and 2 beta (β) chains** → 95% of adult Hb
- **HbA2** – composed of **2 alpha (α) and 2 delta (δ) chains** → 2.2-3.5% of adult Hb
- Little efficiency in oxygen carriage
- **HbF** – composed **of 2 alpha (α) and 2 gamma (γ) chains** → <1% of adult Hb
    - 50-95% of baby haemoglobin and has a very high affinity to allow greater oxygen transfer in a more general hypoxic environment. Levels decline 6 months post-natal

Haemoglobin Turnover



***SYNTHESIS*: Occurs in **mitochondria** of RBC where:**

1. **Protoporphyrin** is synthesized from the condensation of glycine and succinyl coenzyme A.
2. Protoporphyrin **combines with iron** in the ferrous state (Fe^{2+}) **to form haem**.
3. **Globin chains are formed** in the ribosomes

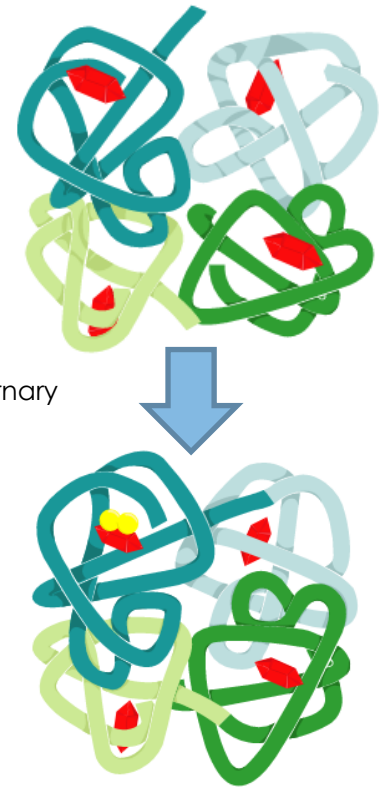
4 of the above will produce 1 haemoglobin molecule

***REMOVAL*: removed by the **reticulo-endothelial system**:**

1. **Globin chains** are **broken down** to amino acids and re-enter the amino acid pool
2. **Iron is reused by bone marrow** to synthesise haemoglobin
3. **Protoporphyrin ring** is opened to form biliverdin which is then metabolised to **bilirubin**.
    a. **Binds to albumin** and carried to the liver to be **conjugated** with glucuronic acid and is **excreted in the bile** and then into the small bowel.
    b. GI tract: Bilirubin converts to either **stercobilin** – some of which may be reabsorbed and excreted in the urine as **urobilinogen**.

Oxygen Binding

Allosteric: Binding of O_2 to one haemoglobin subunit increases the affinity of other subunits for O_2.



As one molecule of oxygen binds to haem, it **pulls the ferrous group into the plane of the porphyrin ring** to flatten it. This causes **change in ionic interactions** holding the 4 subunits together (quaternary structure) and they reform in a different position **altering the quaternary structure** to help facilitation of oxygen binding.

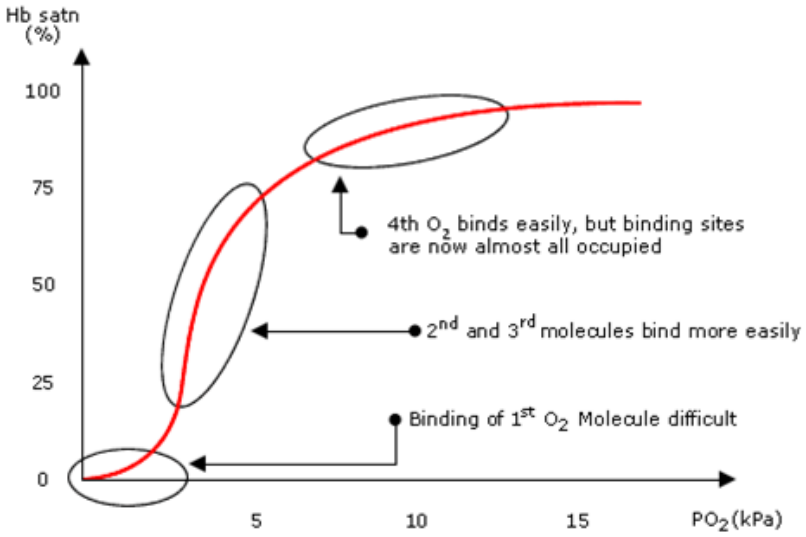
The diagram on the right shows the process of change from **Deoxygenated (tense) → Oxygenated (relaxed)**

Hüfner Constant: This is the amount of oxygen in ml, carried by each gram of haemoglobin. Based on Hb's molecular weight, this value should be theoretically 1.39 ml/g but the in-vivo experiments have revealed a value of **1.34 ml/g** and is due to other forms of haemoglobin which have a reduced affinity for O_2.

Oxy-haemoglobin Dissociation Curve

The affinity of haemoglobin for oxygen is lowest (as explained above) for the 1st oxygen molecule to bind.

Venous oxygen saturation is usually 75% which means that only the final molecule typically binds and unbinds from haemoglobin making it very efficient.



P_{50} is the point at which the Hb saturation is 50% or 50% of binding sites are occupied and is a reference point to describe the position of the curve under different conditions:

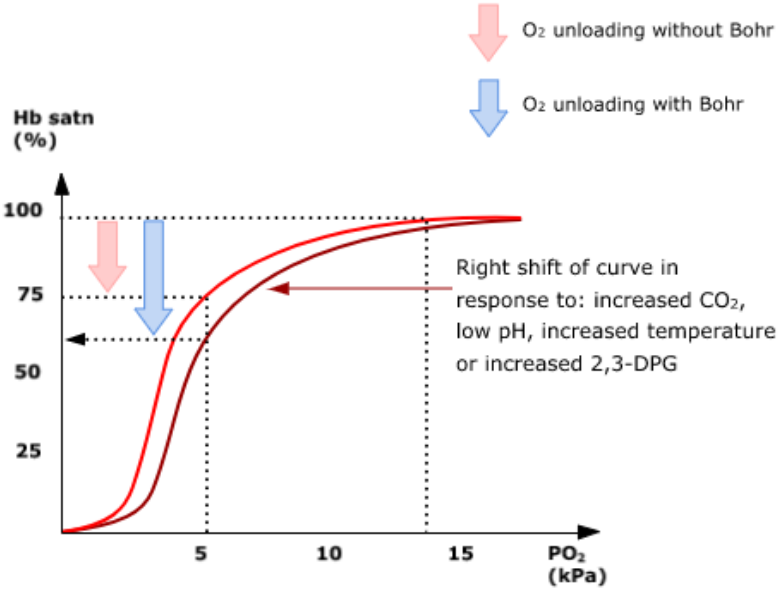
- HbA is 3.5 kPa
- HbF is 2.5kPa

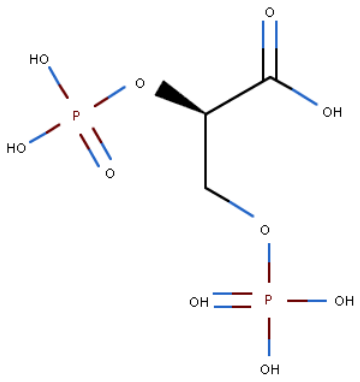
Factors Affecting Hb-O_2 Binding

Bohr Effect describes the ***reduced affinity*** of haemoglobin to oxygen in the presence of a low pH.

'Double Bohr Effect' helps increase foetal oxygenation. Uptake of CO_2 by mother from foetal blood shifts:

- Maternal Hb-O_2 curve to right
- Foetal Hb-O_2 curve to left





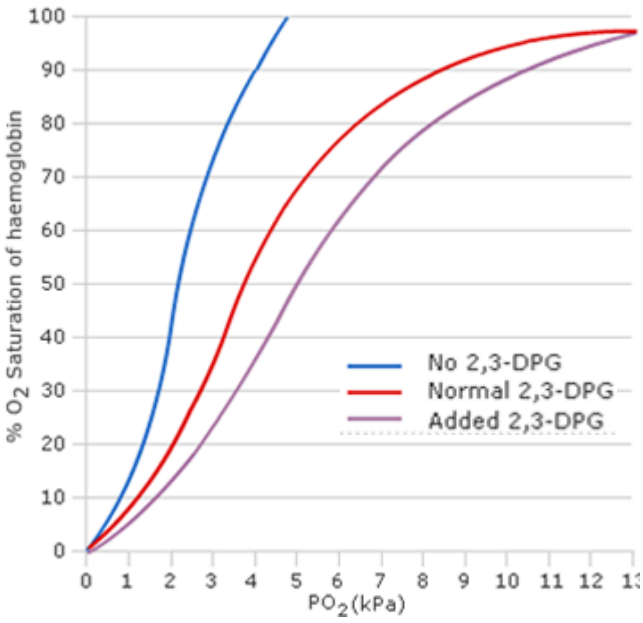
2,3-Diphosphoglycerate

Highly anionic organic phosphate which promotes the allosteric deoxyhaemoglobin state. It is **produced by a side-shunt reaction in glycolysis and is present in large quantities in the erythrocyte**. It **binds to beta globin chains** of deoxyhaemoglobin altering its protein conformation.

Its production is promoted in anaemic states.

2,3-DPG in Altitude

In hypobaric environments, there is a lower inspired PO_2 and there is an increase in 2,3-DPG concentration causing right shift to the curve. This is counteracted by the respiratory alkalosis induced by hyperventilation.



2,3-DPG in Blood Transfusion

Stored blood quickly loses 2,3-DPG and its ability to deliver oxygen. In the storage solution SAG-M (saline, adenine, glucose and mannitol) 2,3-DPG levels are very low after 14 days. O_2 is not delivered to the tissues efficiently as the oxy-haemoglobin dissociation curve of stored blood is shifted to the left.

Abnormal Haemoglobins

This can result from:

- The absence or abnormality of a globin chain
- Abnormal ligand bound to the haem group
- Oxidised Fe^{2+} molecule

1. GLOBIN CHAINS

Genetic defects in haemoglobin are the most common of all genetic disorders.

Impaired Production of Globin Chains:

THALASSAEMIAS: Abnormal ratios of α and β chains are produced (rather than equal quantities). With excess of globin chains comes precipitation resulting in **haemolysis** and **anaemia.**

- **α-thalassaemia:** Usually as a result of DELETIONS of 1 or all 4 of the alpha genes on chromosome 16. Varies in clinical severity according to number of deletions – 4 = death
- **β-thalassaemia:** gene mutation and results in reduced production of β chains and becomes apparent between 3-6 months of age when the switch to HbA occurs. The excess α chains combine with whatever β, δ or γ chains are present, forming **excess HbA2** and **HbF**.

Abnormal Structure of the Globin Chain: (*haemoglobinopathies*)

SICKLE CELL DISEASE: Caused by a **single base mutation** in the β globin gene where **valine is substituted for glutamic acid** at position 6 of the β globin chains.

- **Homozygote disease HbSS:** both β globin genes are abnormal which results in **sickle cell anaemia** The P_{50} is lower than normal and the oxy-haemoglobin dissociation curve is shifted to the left.
- **Heterozygote (trait) HbAS:** clinically much less severe

NB, many other haemoglobinopathies exist including HbC which are not considered further here.

2. ABNORMAL LIGANDS and Fe2+ OXIDATION

Other ligands may combine with iron – most commonly **carbon monoxide** which has an affinity to haemoglobin 300x greater than oxygen. It shifts the oxy-haemoglobin dissociation curve to the left and also reduces the availability of binding sites for O_2.

Iron can be **oxidised from the ferrous (Fe^{2+}) to the ferric (Fe^{3+})** form which is unable to bind with oxygen. This may occur in the following scenarios:

1. Haemoglobin naturally **scavenges nitric oxide,** therefore If a patient is treated with nitric oxide
2. If a patient is **treated with prilocaine** or **nitrates**

CO2 Carriage in Blood and Acid-Base Equilibrium

(07b_03_05)

Transportation of carbon dioxide from its site of production in the tissues to the lung is vital to maintain pH for optimal cellular function as most are entirely reliant on the actions of the thousands of protein molecules found within the cell membrane sensitive to the pH.

Buffer systems: minimise changes in the free H^+ concentration and are usually comprised of a **weak acid** and **weak base in equilibrium**. Strong acids are not able to hold an equilibrium. There are 3 main systems existing in the blood. Phosphate contributes a little in the extracellular fluid.

Buffer system	Capacity mmol H^+ per L
Bicarbonate	18
Haemoglobin	8
Plasma proteins	1.7
Phosphate	0.3
Total	**28**

Carriage of Carbon Dioxide

***In blood*:** CO_2 is produced from the Citric Acid Cycle and diffuses down a gradient into the blood to get to the alveoli. Total 510ml CO_2/L of blood. 3 systems in which it is carried in the blood:

1. PHYSICAL SOLUTION

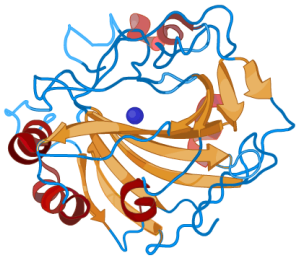
The contribution of dissolved CO_2 to the total carriage of it in the blood is very small where there is no carbonic anhydrase (see below). CO_2 is moderately soluble in water and the equation is defined by **Henry's law of solubility** where the solubility coefficient value is dependent on temperature**:**

$$PCO_2 \times \text{solubility coefficient} = CO_2 \text{ concentration in solution}$$

2. BICARBONATE IONS

This is a 2-stage reaction (there may be a final dissociation at pH>9 but biologically irrelevant):

- **Stage 1:** dissolved carbon dioxide hydrates to form carbonic acid (**$CO_2 + H_2O \leftrightarrow H_2CO_3$**)
- **Stage 2:** carbonic acid dissociates into bicarbonate (**$H_2CO_3 \leftrightarrow H^+ + HCO_3^-$**)



Carbonic Anhydrase (CA): Stage 1 requires catalysis using **carbonic anhydrase** as CO_2 reaction with water without it will take several minutes. There are multiple isoenzymes of carbonic anhydrase located in other tissues & organs.

It is a **zinc containing** low molecular weight enzyme. The zinc is the basis of the catalysis:

1. Zinc +H_2O → Zn-OH^- species
2. Histidine molecule removes H^+ from the Zn and transfers it to the buffer molecules
3. Zn-OH^- + CO_2 → ZnHCO_3^- → dissociation from zinc.

Very fast and limited only by the presence of surrounding buffers to provide/remove H^+ ions to/from the enzyme.

Acetazolamide inhibits carbonic anhydrase non-specifically and has been used to show that >98% CA activity must be blocked before there is any discernible change in carbon dioxide transport. It is used in acute mountain sickness.

Quantification of Bicarbonate production

Through **mass effect,** the equation of stage 2 will be as follows where **k=equilibrium constant**:

$$\frac{[H^+] \times [HCO_3^-]}{[H_2CO_3]} = K \Rightarrow [H^+] = K\frac{[H_2CO_3]}{[HCO_3^-]} \Rightarrow [H^+] = K\frac{\alpha PCO_2}{[HCO_3^-]}$$

H_2CO_3 cannot be measured so the term is replaced by "*proportional to* PCO_2"so all can be measured.

$$pH = pK + \log\frac{[HCO_3^-]}{\alpha PCO_2}$$

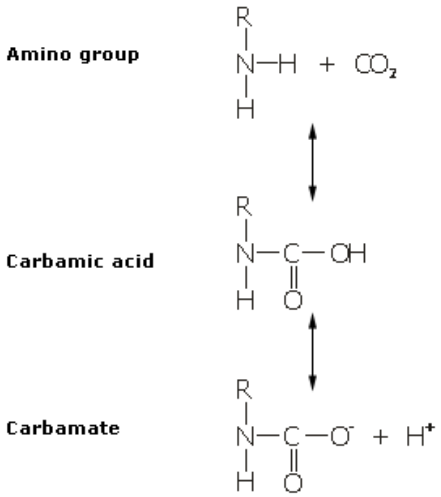
Henderson-Hasselbalch equation where logarithms of each reciprocal is taken. pK is 6.1 but varies with temperature and pH.

3. CARBAMINO CARRIAGE

Formation of Carbamic Acid from combination with uncharged **amino groups**. This is also a buffer as may dissociate into **carbamate.**

Without an amino group, this reaction cannot occur so is limited to the ends of protein chains and the side chain amino groups found in **lysine** and **arginine**.

CO_2 competes with H^+ to complete this reaction so is **pH dependent**.



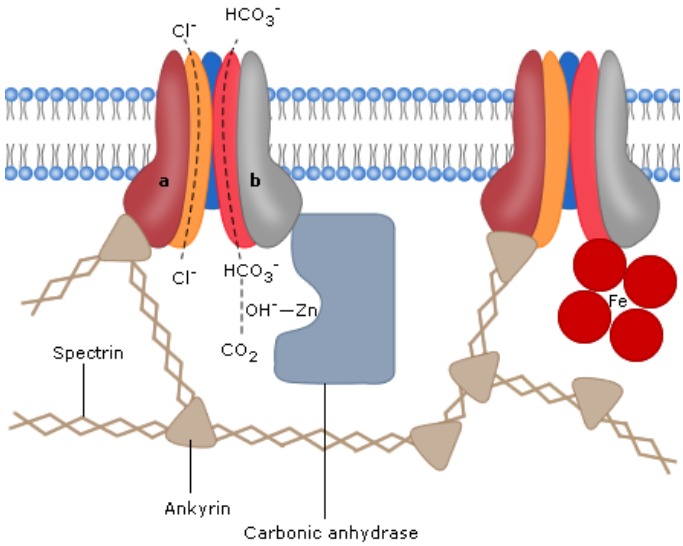
Almost all blood carbamino carriage is in combination with haemoglobin. Deoxyhaemoglobin is about 3.5 times as effective as oxyhaemoglobin, this being the major component of the Haldane effect:

Haldane Effect: An Important principle. The **proportion of carbamino carriage of CO_2 in venous blood is higher than arterial blood**. This is because the haemoglobin once deoxygenated is a better buffer and can bind carbon dioxide. The **affinity for haemoglobin to CO_2 progressively improves along the systemic capillary**. Conversely, in the pulmonary capillaries, oxygenated blood dissociates more CO_2.

Hamburger Shift

Within the red blood cell, as well as the haemoglobin buffering described above, there is another method of buffering known as the:

Hamburger Shift: Excess HCO_3^- ions produced in the RBC are actively exported in exchange for Cl^- ions **facilitated by the 'Band 3' membrane protein**. This is a ping pong effect rather than simultaneous exchange.



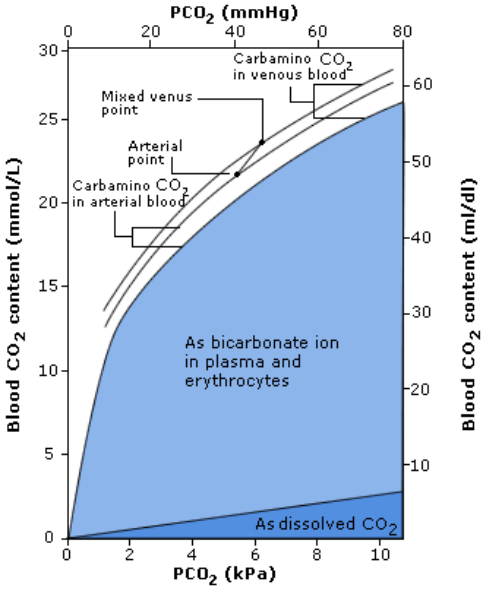
Band 3 is **attached to the RBC cytoskeleton** and therefore, an inherited defect results in **hereditary spherocytosis** making the RBC spheroidal in shape and fragile.

Band 3 is **loosely bound to carbonic anhydrase** facilitating transport of HCO_3^- out the cell through direct transfer. This allows a greater amount of HCO_3^- to be produced from CO_2.

Dissociation Curve of CO_2

As there are 3 methods of transport of CO_2 the dissociation curve is derived from blood CO_2 content relative to PCO_2.

From this graph you can quantify the proportions of each method of CO_2 transport and it is also noticeable the larger amount of carbamino carriage in venous blood although being very small. Therefore, it is an important contributor in vivo.



Effect of Temperature on CO_2 transport

***Hypothermia*:** As with all gases, **CO_2 becomes more soluble in water** such that maintenance of the same PCO_2 in blood when hypothermic will require a greater total CO_2 content. It also **reduces the ionisation of water into H^+ and OH^- ions** encouraging alkalinisation of blood.

Animals respond in 2 ways:

1. ***pH stat hypothesis*** – maintenance of pH in hypothermia is achieved by hypoventilation
2. ***Alpha-stat hypothesis*** – allowing the pH to rise but proteins continue to function. The buffering state of histidine is important as ionisation stays normal throughout (although should change with temperature) – The pK of histidine changes with temperature. Demonstrated most in poikilothermic animals

There is controversy of either mechanism predominating in humans.

Pulmonary Ventilation: Volumes, Flows, Dead Space and Preoxygenation

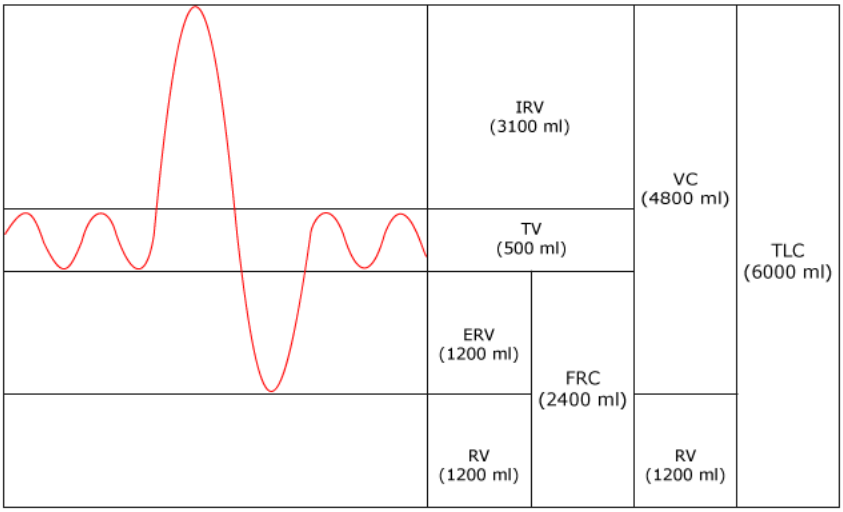
(07b_03_06)

Lung Volumes

Capacity is the sum of 2 or more volumes:

The FRC, RV and TLC are unable to be measured using a standard spirometer and can only be measured using a **helium dilution** or a **body plethysmograph**.

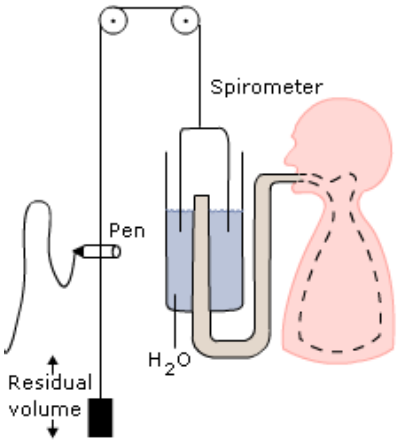
This spirometer graphic shows average values for an 70kg healthy male.



Measurement of Lung Volumes

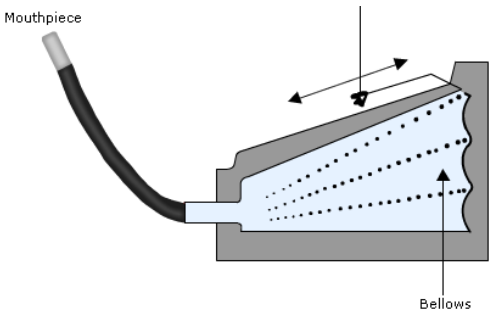
1. WATER SEALED SPIROMETER

This is a very historical apparatus which has been superseded by modern versions. The patient blows into a closed chamber in contact with water, as he inhales/exhales, the water displaces and allows movement of a pencil on moving paper:



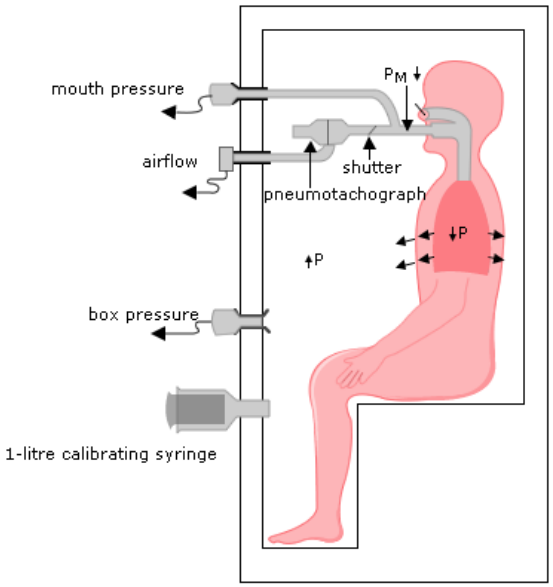
2. DRY SPIROMETER

The simplest example is that of one with bellows attached to a pen and as the bellows expand/collapse, it moves the pen:



Boyle's Law: *at a constant temperature, the pressure and volume of a gas are inversely proportional to one another*

3. BODY PLETHYSMOGRAPHY



The patient is placed in an **airtight box** and the initial pressure and volume of the box is measured (P1 and V1). The patient breathes through a mouthpiece going outside of the box to a specific lung volume (usually FRC). At the point of FRC, a **shutter in the mouth piece closes** so the patient is unable to inhale causing the **chest volume to increase with respiratory effort.** This volume will indicate a reciprocal increase in pressure change (P2) of the box according to **Boyle's law** (**k=PV)**

Therefore, though this volume is unquantified, it can be measured the following equations:

P1 x V1 = P2 x (V1 - change in lung volume)

Initial airway P x initial lung V (FRC) = inspiratory airway P x inspiratory V of chest

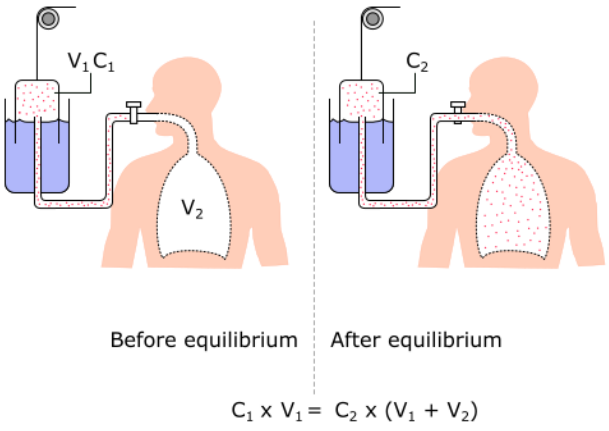
Inspiratory volume of the chest = FRC + change in lung volume. Once the change in lung volume and the inspiratory volume of the chest is calculated, the FRC can be calculated also.

4. HELIUM DILUTION

The patient **breathes air with a known concentration of helium from FRC** in a closed system containing a spirometer.
The CO_2 produced during the test is absorbed by soda lime and replaced with oxygen. The helium is then distributed through the lungs (not obstructed units making its measurement less valuable to the body plethysmography).

Concentration x Volume = amount

Similar to the equation above, the change is calculated and from this, the FRC can be measured.



Helium is used due to its very low solubility and little lost through absorption to blood.

5. NITROGEN WASHOUT

The subject breathes 100% oxygen from FRC (end expiration) in a closed breathing circuit connected to a spirometer. After several minutes, the measurement of nitrogen is taken at FRC and this will be the same amount present at the start of the test (**FRC x atmospheric nitrogen concentration (79%)**)

FRC x 0.79 = 40,000ml with **5% Nitrogen**

FRC x 0.79 = 40,000 x 0.05

FRC = 2000/0.79 = 2531ml

Again, like helium dilution, obstructed units are not calculated.

Functional Residual Capacity

The lung volume at barometric pressure where the national tendency of the rib cage to spring out is balanced by the tendency of the lung to collapse. Typically, it is around 2500ml. This is an important measurement for anaesthetists as it has 4 key functions:

1. **Oxygen Reservoir**
2. **Prevention of Airways Collapse**
3. **Optimal Compliance**
4. **Optimal pulmonary vascular resistance**

Oxygen Reservoir

Oxygen consumption is around 250ml/min. Alveolar concentration of oxygen at sea level is 15%. Therefore, available oxygen is 0.15 x 2500ml = 375ml. Therefore, there is normally **90secs** of **oxygen reserve**.

Pre-oxygenation with 100% oxygen: The alveolar concentration will be increased to 90%. Therefore, oxygen reserve will be 2500 x 0.9 = 2250. 2250/250 = **9 minutes of oxygen reserve**.

The lower the FRC, the lower the oxygen reserve available

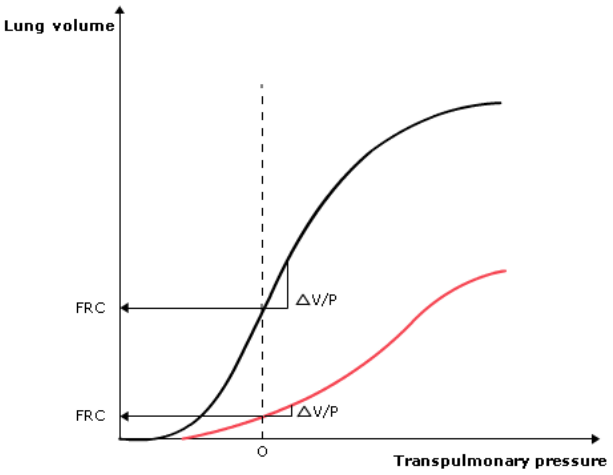
Prevention of Airways Collapse

Closing Capacity (CC): volume in the lungs at which its smallest airways, the bronchioles, collapse. In fit patients, this is always less than the FRC. If the FRC is reduced or the CC is increased, there may be airway collapse close to breathing at tidal volume. CC may increase in **smoking, asthma** and **age**. This is the rational for using **PEEP.**

Optimal Compliance

FRC is at a point where the **balance between** the **tendency of lungs to collapse and the rib cage to spring out** and in a healthy individual, is at the steepest part of a lung volume vs transpulmonary pressure curve where compliance is greatest.

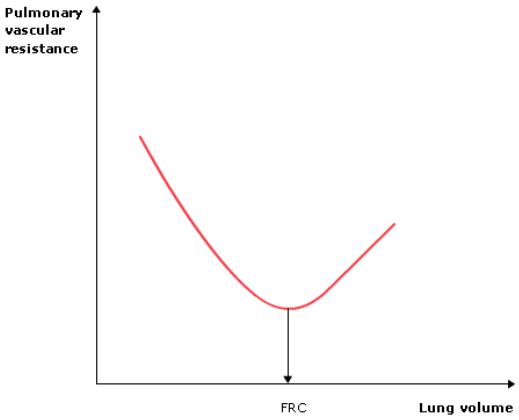
The **red** line represents a patient with **reduced FRC** with added **restrictive lung disease** so to overcome inspiration, requires a greater increase in work of breathing due to fall in compliance.



Pulmonary Vascular Resistance

This is lowest at FRC allowing optimal pulmonary blood flow:

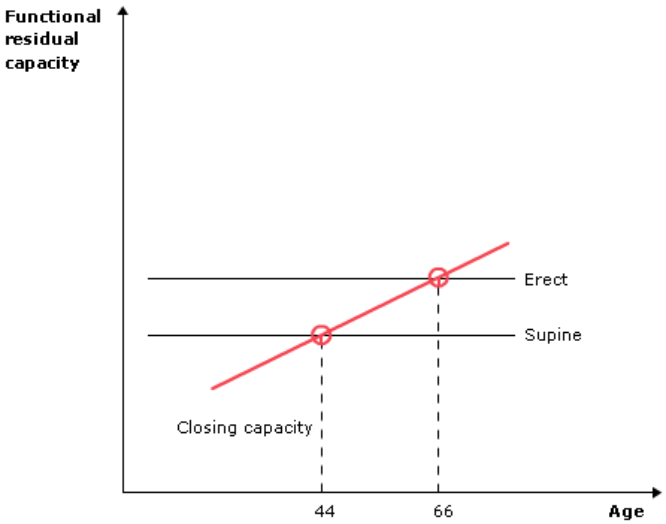
Click here for the pulmonary circulation module which contains further information on this principle.



Factors Affecting FRC

Increase	Decrease	No effect
Height Male gender Asthma Emphysema Intermittent Positive Pressure Ventilation (IPPV)	Obesity Anaesthesia Supine positioning Kyphoscoliosis Lung fibrosis Pregnancy	Age – although closing capacity is increased (see figure right)

As mentioned above, there is no associated change in FRC with age but there is an increase in closing capacity. This will affect ventilation positionally after a certain age.



Dead Space

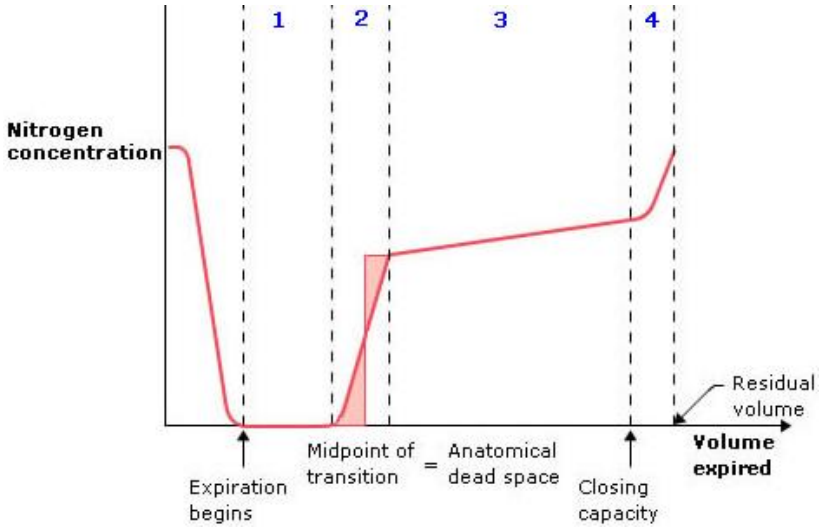
This is the volume of inspired air **not taking place in gas exchange** and typically is **30% of tidal volume**. As mentioned previously: **AV = TV-DSV**

Physiological dead space = Anatomical Dead Space + Alveolar Dead Space

In the anaesthetised patient, apparatus dead space must be taken into account including the volume of any external equipment, such as HMEFs, mainstream capnometry, face masks etc.

Fowler's Method

This is a method of **measuring anatomical dead space**. An inspiratory breath to vital capacity of 100% oxygen is taken and measurement of **Nitrogen from beginning expiration (1)** occurs.



1. No nitrogen is seen as it contains only oxygen from the vital capacity inspiration (**dead space gas**)
2. This is a **mix of alveolar gas and dead space**. The horizontal distance from beginning of expiration to the midpoint of transition is the **Anatomical dead space**.
3. Nitrogen from **pure alveolar gas** that was present prior to inspiration of oxygen. Its gradient depends on partial emptying of separate alveoli (**alveolar time constants**) – increased in obstructive disease.
4. Represents **closing capacity.** As lower alveoli are best ventilated, the oxygen breath would have filled in the base alveoli initially which would also have emptied first (2 & 3). At closing capacity, the lower airways collapse and the exhaled gas comes from the upper alveoli instead (which had contained the N_2 not mixed with VC O_2). Thus, the N_2concentration rises.

Bohr Equation

This is a method of **measuring physiological dead space**. All expired CO_2 is assumed to come from alveolar gas. Therefore:

Alveolar CO_2 Concentration x Alveolar Ventilation = Mixed expired CO_2 Concentration x Tidal Volume

$$F_ACO_2 \times \dot{V}_A = F_ECO_2 \times \dot{V}_T$$

$$\text{As } \dot{V}_A = \dot{V}_T - \dot{V}_D$$

$$F_ACO_2 \times (\dot{V}_T - \dot{V}_D) = F_ECO_2 \times \dot{V}_T$$

Where VT = Tidal volume, VA = Alveolar ventilation and VD = Dead space volume.

$$\dot{V}_D/\dot{V}_T = (F_ACO_2 - F_ECO_2)/F_ACO_2 \quad \textbf{OR} \quad \dot{V}_D/\dot{V}_T = (P_aCO_2 - P_ECO_2)/P_aCO_2$$

As concentration is proportional to the alveolar partial pressure which is proportional to arterial CO_2.

The VD/VT ratio is approximately 0.2-0.3 in most mammals

Flow Volume Loops

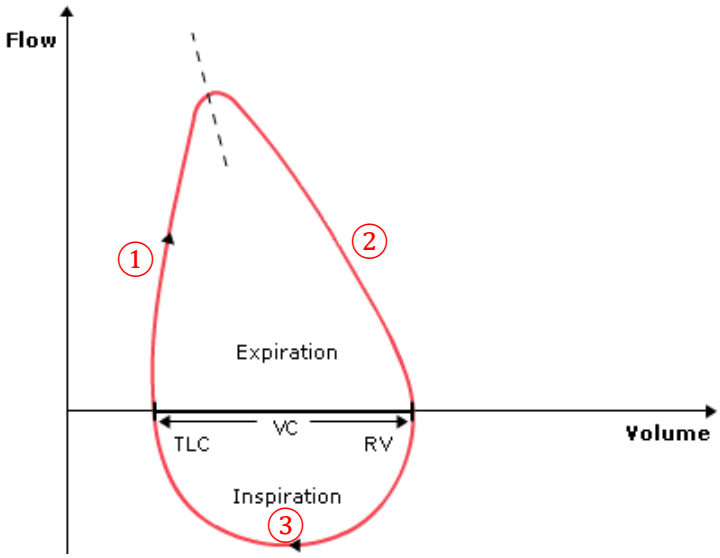
This is a **graphical illustration** of a patient's **spirometric efforts**.

Volume and flow is measured from within the machine so that inspiration is measured as reducing volume and negative flow.

Point 1: From TLC at max inspiration to **initial expiration** with max flow from elastic recoil - explosive. The dotted line resembles the peak expiratory flow.

Point 2: **Continuing expiration** to Residual volume with reducing flow rates.

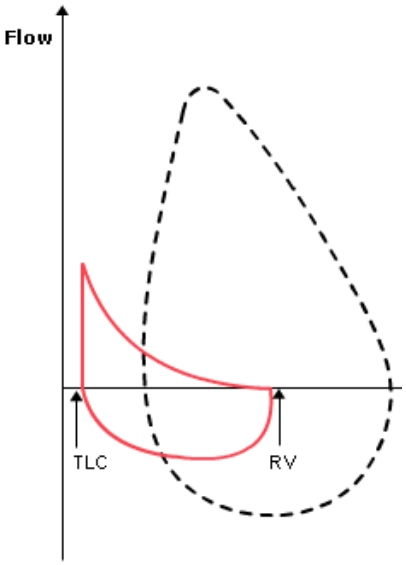
Point 3: **Beginning – end inspiration.** It does not reach instant maximal flow as the inspiratory respiratory muscle contraction is slower than the pure elastic recoil of expiration but occurs at midpoint of VC.

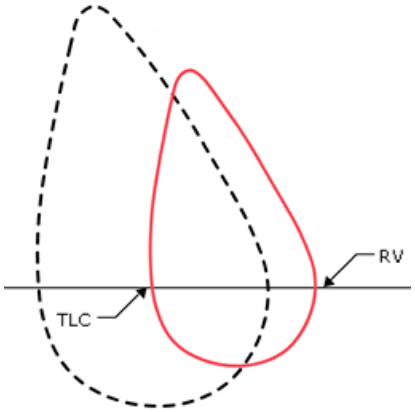


Obstructive Disease

Changes in the flow-volume loop:

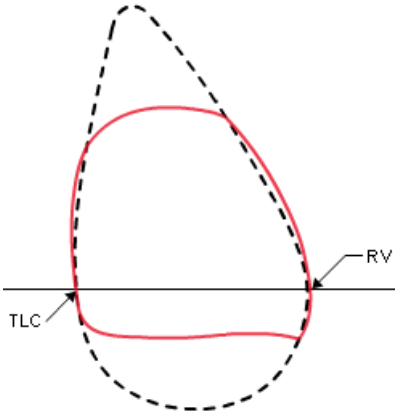
1. **TLC is raised** due to lung hyper-expansion
2. **RV is increased** due to gas trapping
3. **Flow at expiration** is reduced due to loss of elastic lung tissue
4. Point 2 develops a scalloped-out appearance as **small airways collapse rapidly** creating an early decline in flow
5. **VC** is reduced





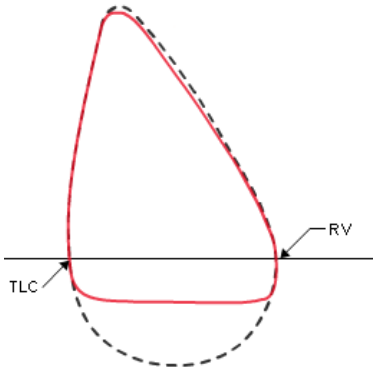
Restrictive Disease

1. **Both TLC** and **RV are reduced**
2. Flow rates on expiration is comparably increased due to increased elastic recoil holding the airways open



Fixed Upper Airway Obstruction

Lung volumes are unchanged but **flow rates are decreased on the inspiratory and expiratory loops**

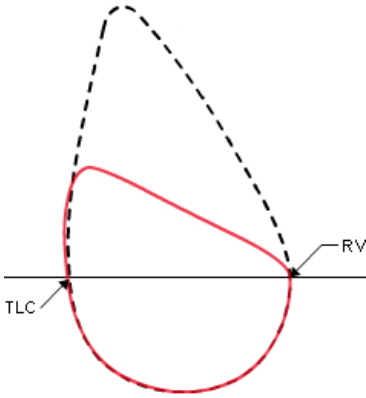


Variable Extrathoracic Airway Obstruction

i.e. unilateral vocal cord paralysis. **During inspiration**, the paralysed vocal cord is drawn inwards resulting in a **reduced flow** but the paralysed cord can be blown aside during expiration:

Variable Intrathoracic Airway Obstruction

i.e. tracheomalacia. During inspiration, the trachea is held open due to negative intrapleural pressure resulting in a near normal inspiratory loop but the there is a lack of support to the trachea in expiration so the trachea collapses resulting in **reduced expiratory flow**.



Ventilation / Perfusion Abnormalities

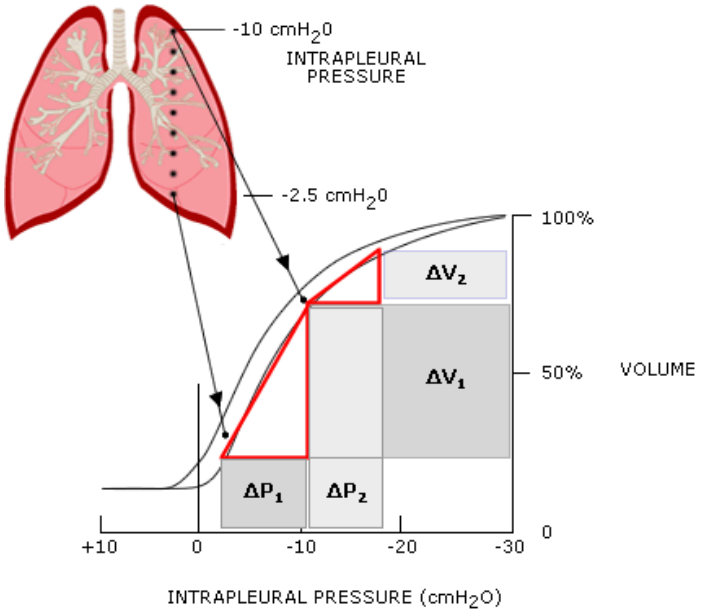
(07b_03_07)

Ventilation

There is better ventilation of the lower dependent zones. To balance the increased weight of lungs at the base, there is a higher (less negative) intrapleural pressure.

The curve gradient reflects the compliance. At the **bases**, the alveoli are relatively compressed (low resting volume) and require less pressure change to inflate open – high compliance (unlike the apices which are more inflated).

Regional ventilation = change in volume per unit resting volume. Inflation pressure is lower at the bases as is resting volume, hence a greater change is seen when compared with the apices.



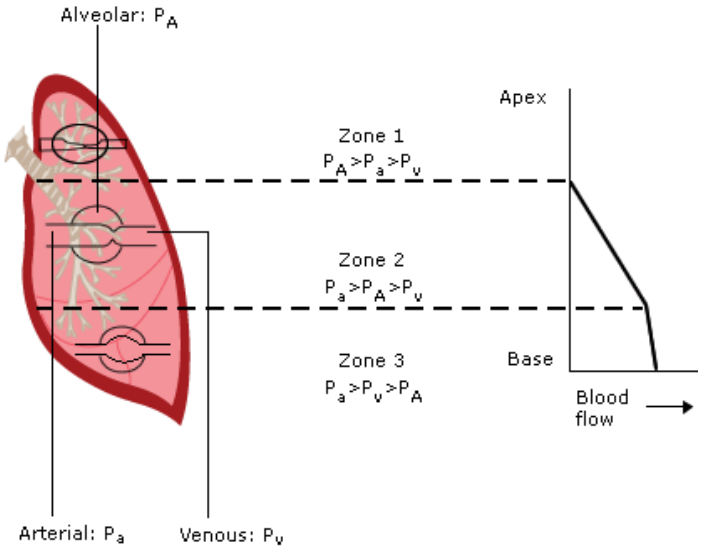
Perfusion

Blood flow is better in the bases than the apices due to gravity assisted hydrostatic pressure.

Zone 1 describes capillaries compressed in the apices providing no flow. This is not normally present but will occur with IPPV or reduced arterial pressure. This area is **alveolar dead space**

Zone 3 venous pressure exceeds alveolar and **perfusion is determined by the arteriovenous gradient**.

Low lung volumes – compression of extra-alveolar vessels → reduction of blood flow. Resistance becomes important for blood flow. This region is sometimes known as **zone 4**.



Impairment of Gas Exchange

Alveolar PO_2

This is determined by 2 factors:

1. **The rate of oxygen removal from the blood** (metabolic rate)
2. **The rate of addition of oxygen to the blood** (alveolar ventilation)

If **alveolar hypoventilation** occurs, PaO_2 decreases and $PaCO_2$ increases. REMEMBER THE ALVEOLAR GAS EQUATION.

Shunt

Refers to blood passing from arterial circulation to venous with passing through ventilated lung. Normally there is a small amount of shunt from:

- Bronchial arteries draining directly into pulmonary veins
- Coronary venous blood draining into the left ventricle via Thebesian veins

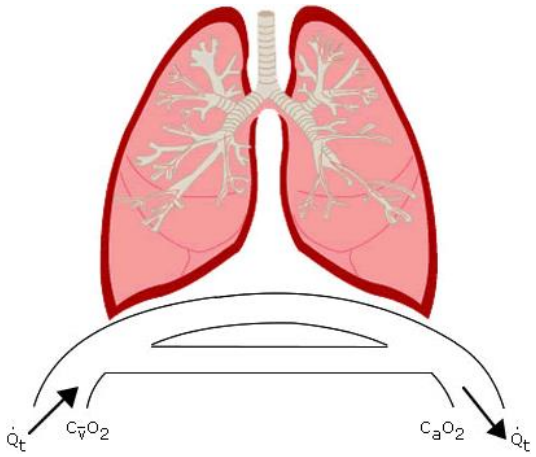
Shunt Equation:

Theoretically, **with no shunt**:

Qt = blood flow which will be the same in the afferent as the efferent.

C_vO_2 – Mixed venous oxygen content. Total amount of oxygen entering the system = Qt x C_vO_2

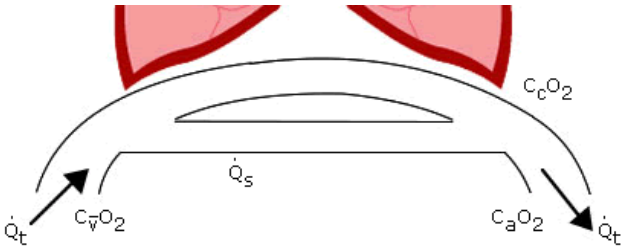
C_aO_2 – Arterial blood oxygen content. Total amount of oxygen leaving the system = Qt x C_aO_2



With a shunt:

Qs = blood flow through the shunt with the same oxygen content as C_vO_2.

C_cO_2 is the end capillary blood oxygen content and is calculated from P_AO_2 and the O_2 dissociation curve



C_aO_2 then becomes the sum of the oxygen content in the C_vO_2 and the C_cO_2.

Therefore: $(\dot{Q}_t \times C_aO_2) = (\dot{Q}_s \times C_{\bar{v}}O_2) + ((\dot{Q}_t - \dot{Q}_s) \times C_{c'}O_2)$

Rearranged to form: $$\frac{\dot{Q}_s}{\dot{Q}_t} = \frac{(C_{c'}O_2 - C_aO_2)}{(C_{c'}O_2 - C\bar{v}O_2)}$$

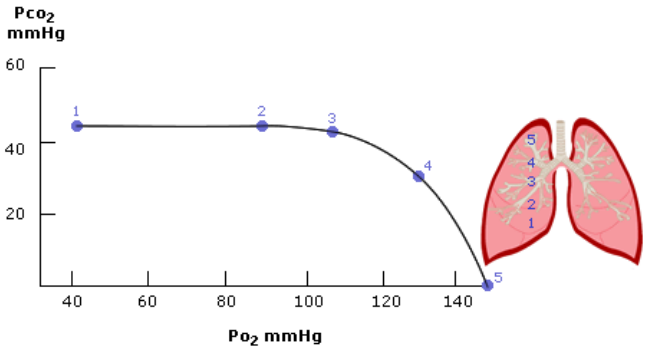
Unlike oxygen, there is **no change in $PaCO_2$ with shunt** due to the reflex **increase in ventilation**.

Ventilation/Perfusion Ratio

At point 1 (lung base) the V/Q ratio < 1 due to perfusion > ventilation → **shunt**

At point 5 (lung apex) the V/Q ratio >1 due to ventilation > perfusion → **alveolar dead space**

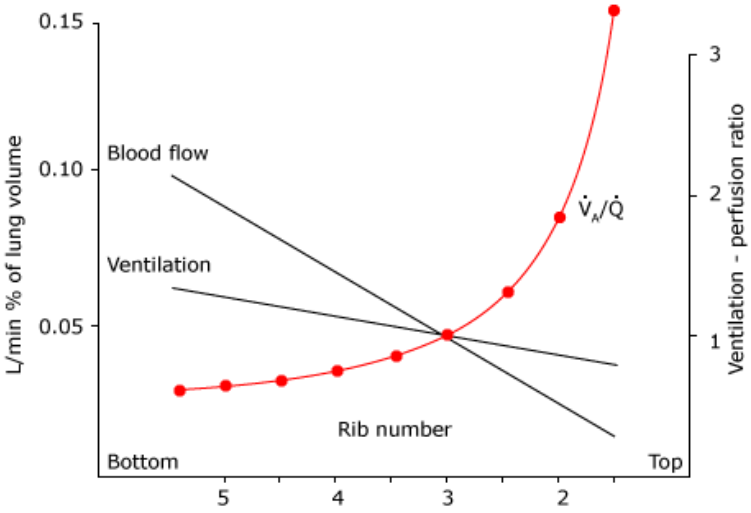
This graph shows the how the alveolar concentration of gases varies in different regions of the lung.



Alveolar-Arterial Oxygen Gradient

In comparison, the **perfusion changes from the base to apex of the lungs to a greater extent than the ventilation**. The PO_2 of oxygen is higher at the apex than the base as the increased perfusion (reduced V/Q ratio) at the base means more O_2 is exchanged. Therefore:

Alveolar-arterial oxygen difference: is about 4mmHg as the above physiology tends to lower the PO_2 and raise the PCO_2 in arterial blood when compared to the composition of alveolar gas. In lung disease, this gradient is much increased.



Measurement: It can be calculated from the following equation:

$$P_AO_2 - P_aO_2 = {}_{A\text{-}a}\text{ difference}$$

This is measured from blood gas analysis and the P_AO_2 is calculated with the alveolar gas equation.

Causes of Increased A-a Gradient

LOW V/Q RATIO

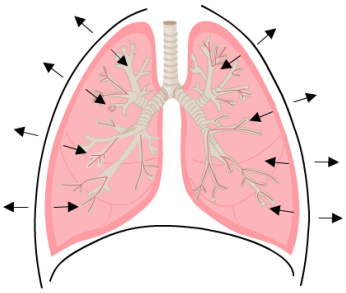
The biggest effect is seen in conditions which reduce the V/Q ratio i.e. in shunt or reduced ventilation. If there is some ventilation, the problem can be overcome by increasing FiO_2. If there is complete obstruction of ventilation to part of the lung – increasing FiO_2 will be less effective.

HIGH V/Q RATIO

Also increases the A-a gradient to a lesser extent and may be caused by i.e. PE or hypotension. Causes **Alveolar dead space** and increases P_AO_2 relative to PaO_2.

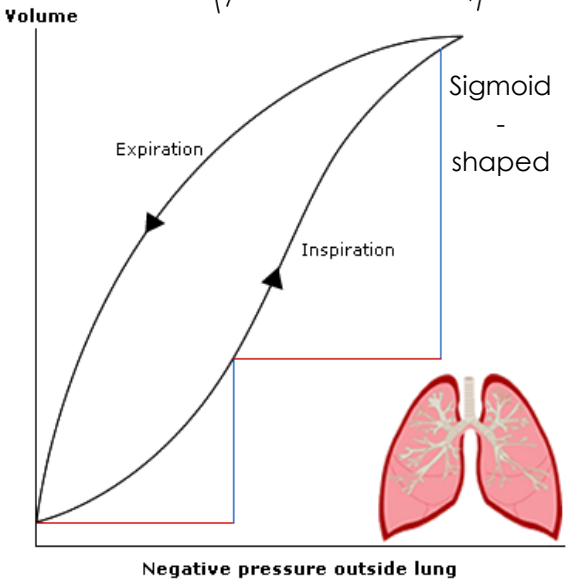
Mechanics of Ventilation and the Effect of IPPV on the Lungs

(07b_03_08)



Work of Breathing: energy spent on overcoming airway resistance and the elasticity of the chest wall and lungs. These forces are balanced at rest (FRC). This only occurs during inspiration as expiration is passive through elastic recoil.

Pressure-Volume Curve: Plotted by measuring lung volume at different pressures. The area inside the inspiratory and expiratory traces resembles the work of breathing. Both limbs do not follow the same trace and is therefore known as **hysteresis** (due to elastic properties of the lungs).



Compliance: This is the relationship between the volume displaced and the pressure change and can be measured by the gradient of the slope of a pressure volume line. The same in inspiration as expiration. In the lungs, it tells us how easy it is to inflate the lungs. Nor**mal value is 200 ml/cm H_2O**. May be **decreased in**:

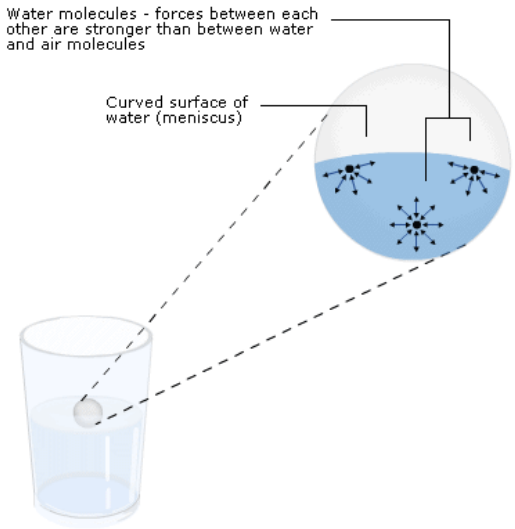
- **Raised pulmonary venous pressure**
- **Alveolar oedema**
- **Fibrotic Lung Disease**

It is **increased by age** and **emphysema**.

Specific Compliance: Lungs of different sizes will have different compliance (elephant > mouse). Therefore, specific compliance is per unit volume and shows the property of lung tissue independent of body size (elephant ~ mouse).

Surface Tension

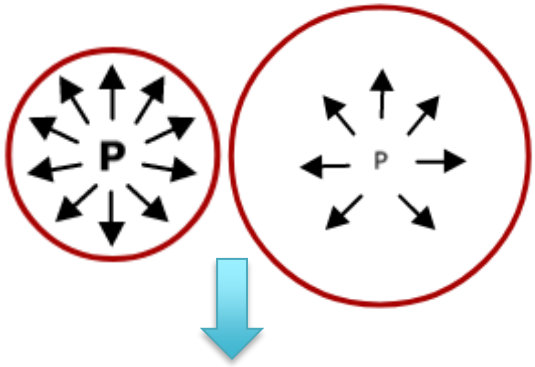
Arises as forces of adjacent water molecules are stronger than water and gas. This is responsible for the meniscus visible at the surface of a liquid.



Laplace's Law

The **surface tension** is defined as the **force acting across an imaginary line 1 cm long on the surface of a liquid**. This generates a pressure that can be calculated from Laplace's law:

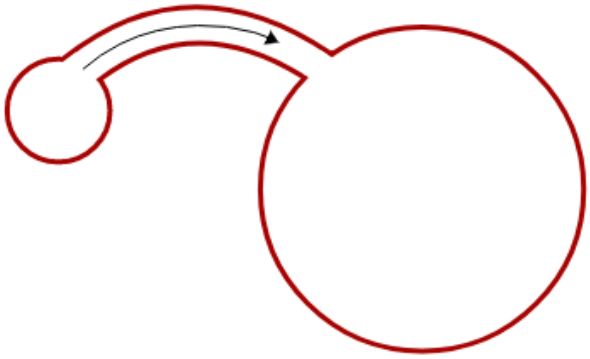
$$P = (4 \times T) / r \text{ (radius of the sphere)}$$



The numerator changes to 2 if only one surface is involved unlike the bubble imaged. From this equation, you can predict higher pressures to be generated from smaller spheres:

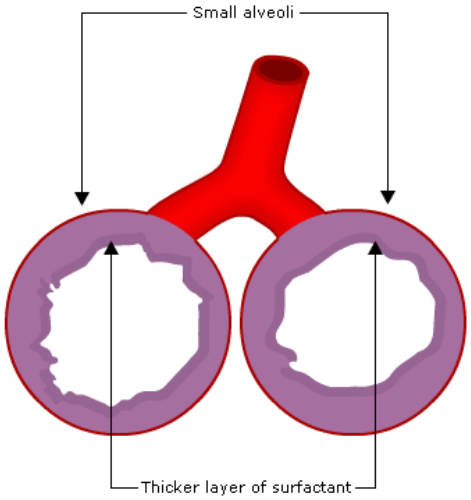
Surfactant

In the case of alveoli, there are a wide range of sizes and therefore, the volume should transfer from the smaller to the larger to equilibrate pressure:



This **does not happen due to surfactant** which is produced from **Type II alveolar epithelial cells.** Surfactant **reduces this surface tension** by **opposing the normal attraction forces of the surface molecules** making ***Laplace's law obsolete***.

In the **smaller alveoli** with smaller volumes, the **surfactant molecules are pushed closer together** making the **repellent forces greater** to give **better stability** and **preventing them from emptying**. It also **reduces their compliance**.



The presence of surfactant within the alveoli **prevents them from collapsing at end expiration**. Connective tissue lines the outside of alveoli to prevent over-distension and allows greater support.

Surfactant is a **phospholipid** containing **dipalmitoyl phosphatidyl choline**

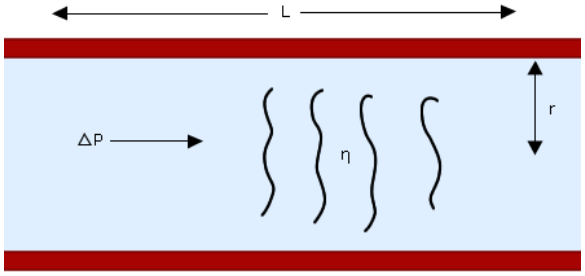
Gas Flow

Similar to liquid, flow depends on the pressure differences at the beginning and end of a tube. As the **flow rate increases** there will be a **change from laminar to turbulent gas flow**.

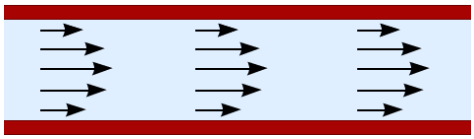
Hagen-Poiseuille's Law

Another equation…describing the **effect of variables on airway resistance** and hence **gas flow** where η = viscosity

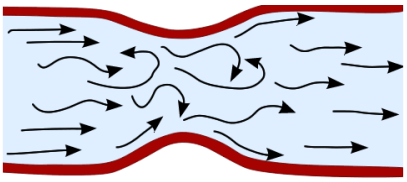
$$R = \frac{8\eta l}{\pi r^4}$$



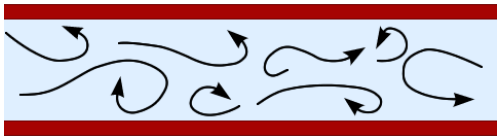
Laminar Flow: No disruption between layers of flow. Occurs at low velocity smooth walled airways. The driving pressure (ΔP) is the alveolar – barometric pressure. Resistance is determined by **viscosity** of the gas rather than its density.



Transitional Flow: Some characteristics of both laminar and turbulent flow. Occurs at bifurcation of airways and in airway narrowing (reduction in radius)



Turbulent Flow: Chaotic movement of the gas and occurs at **high flow rates** typically in the larger airways. In this situation the **density** of the gas (d) is more important than the viscosity (η) in resistance. The physiological advantage is that turbulent flow will cause particles in the inspired gas to collide with & get trapped in the mucus on the walls of larger airways and not get to the alveoli.



Reynolds Number is a way to predict when turbulent flow is likely to occur and calculated from:

$$Re = (2 \times velocity \times density \times radius) / viscosity$$

When it is 2000, turbulent flow begins which is why it is more common in larger airways with high velocity flow. In lower density gases i.e. helium, laminar flow is more likely.

Respiratory Function Tests

Spirometry has been described previously, A flow volume curve shows initial high flow in expiration which then declines independent of effort due to compression of the airways by intrathoracic pressure.

This table shows typical values for a white 45-year-old female who is of height 165cm.

Measurement	**Normal (predicted)**	**Obstructive disease**	**Restrictive disease**
$FEV_{1.0}$	2.60 L	1.43 L	1.24 L
FVC	3.03 L	2.5 L	1.35 L
$FEV_{1.0}$ / FVC	86%	57%	92%
50% - 80% predicted = mild disease 30% - 50% = moderate disease Below 30% = severe disease			

Changes according to **ethnicity, gender, height** and **age.** Not weight!

Control of Breathing

(07b_03_09)

Aims of the regulatory system of breathing:

1. Achieve uninterrupted involuntary rhythmical breathing
2. Regulate $PaCO_2$ and PaO_2 within tight limits
3. Ensure minimal effort to obtain (close to) full saturation of haemoglobin
4. Allow involuntary/voluntary overriding in different situations

Basic elements of the breathing system is split into the following:

SENSORS → MEDULLA → EFFECTORS

Respiratory Centre

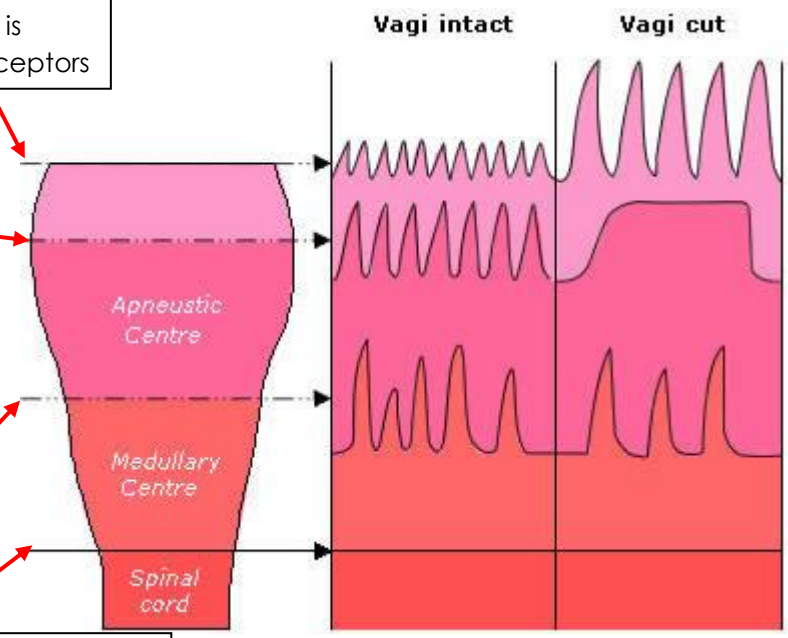
The method of identifying location had been from animal transection studies (now superseded by MRI and PET scans). The centre is split into **dorsal and ventral respiratory groups** known as the **Central Pattern Generator (CPG)**

Transection above pons – breathing is essentially normal. With vagotomy, there is removal of afferent input from stretch receptors

Transection mid pons – Increased depth of breathing (loss of inhibitory signals from upper pons). With vagotomy, **apneusis** occurs (prolonged periods of inspiration interrupted by occasional expiration)

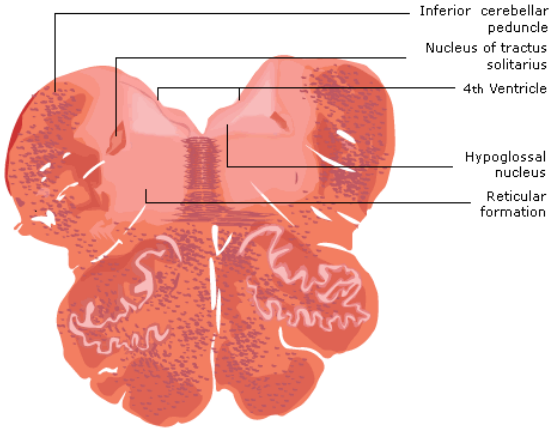
Transection above medulla – Maintained breathing but irregular. Vagotomy has little effect – the **basic rhythm generator** is at the level of the medulla or below.

Transection below medulla – Cessation of breathing. **Basic rhythm generator** is in the medulla

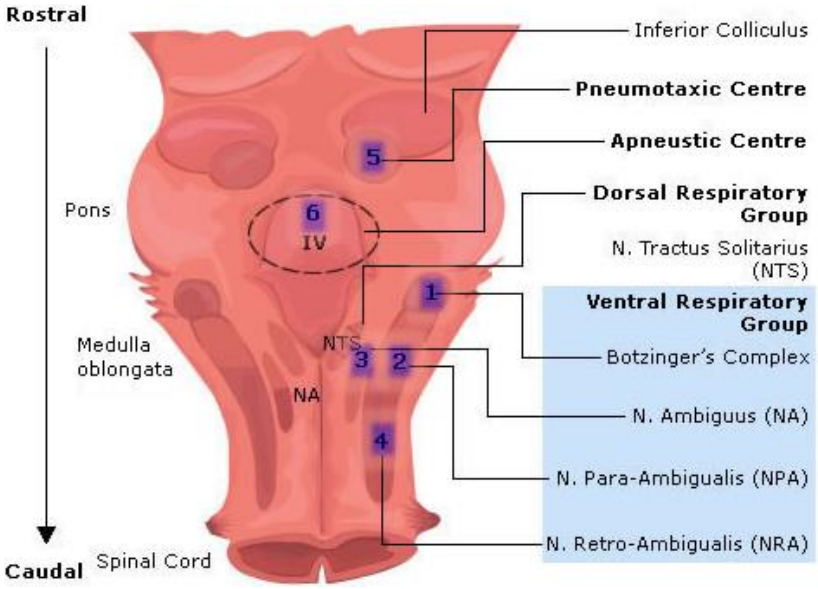


Dorsal Respiratory Group (DRG)

Is located on the **floor of the 4th ventricle** near the **tractus solitarius**. Predominantly consists of **inspiratory neurones** (phrenic and intercostal muscle neurones). It sends UMNs to the anterior horn cells on the contralateral side of the spinal cord and is primarily concerned with the **timing of the respiratory cycle**.



Ventral Respiratory Group (VRG)
Consists of 4 nuclei that are involved in controlling the muscles of respiration. As shown in this diagram:

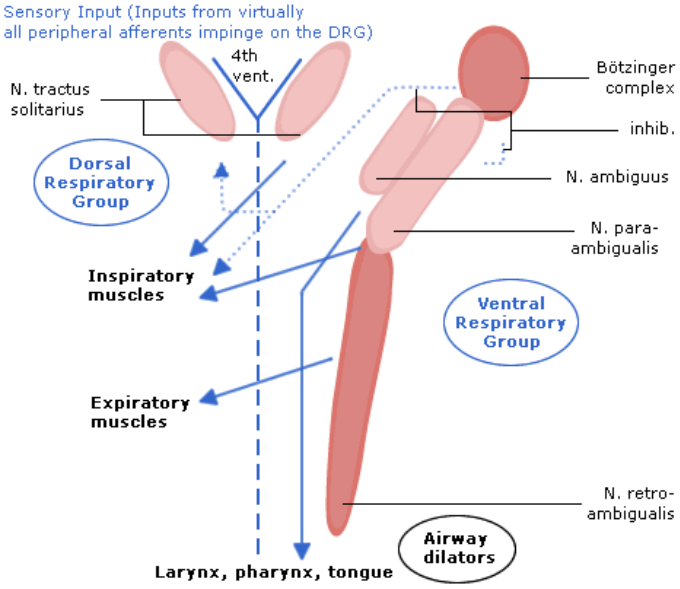


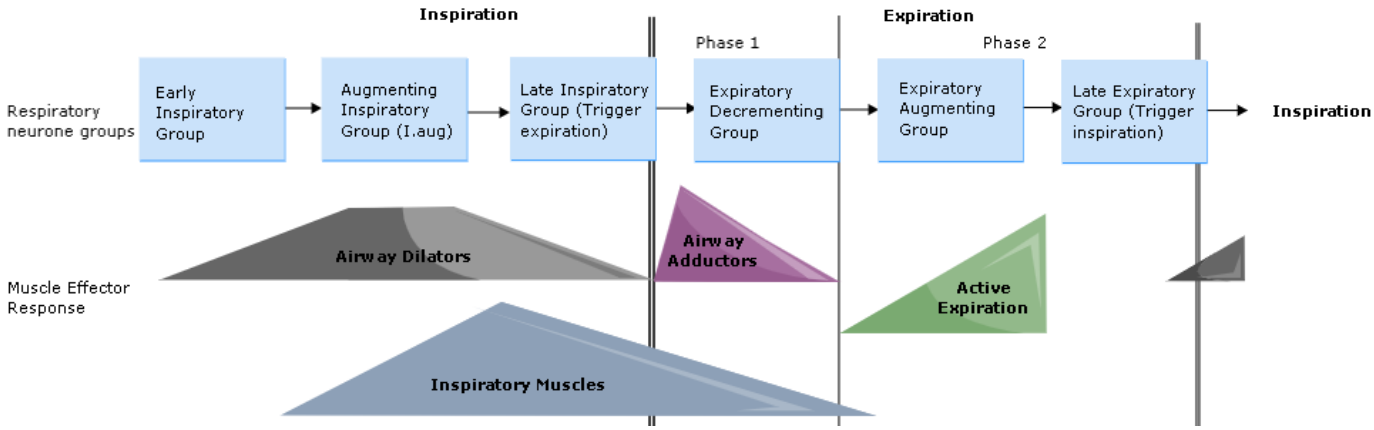
Pontine Respiratory Group (PRG)
Contributes to the fine control of respiratory rhythm through interaction with the medullary respiratory neurones in a multi-synaptic pathway.

Putting it all together…
From this diagram:

- Almost all sensory inputs go to the DRG.
- **Darker shaded** parts are **expiratory**
- **Light shaded** parts are **inspiratory**
- Dotted lines are inhibitory
- Note the fibres running contralateral and cross the midline

The current theory (cf the traditional) is that there is a complex interaction between all neurones to create groups of function and the oscillation is driven by lung afferents and chemoreceptors that are integrated within the medulla:





Expiration is split into 2 phases:

Phase 1 – the inspiratory muscles have reducing tone and there is passive exhalation. The larynx initially brakes the expiratory gas flow.

Phase 2 – Inspiratory muscles are silent and if required, expiratory muscles will be recruited.

Biochemistry of Signal Generation

***Central Pattern Generation*:** With the **I. augmentation neurone**, there is a **slow membrane depolarisation** that allows a **spontaneous discharge** by a similar mechanism to pacemaker cells using a combination of potassium and calcium. Switched off using the activation of calcium-dependent potassium channels.

***Inhibitory Neurones*:** Work through hyperpolarisation of the target cells making them much harder to depolarise.

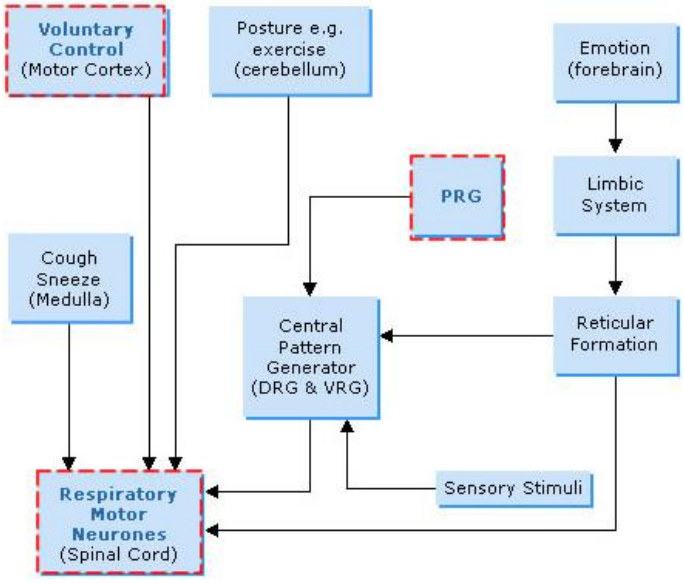
Other Connections to the CNS

Voluntary Control: From the cortex can bypass the CPG altogether but not indefinitely as involuntary systems eventually force their way back in i.e. in **breath holding** a combination of hypoxia, hypercarbia and impulses from the diaphragm muscle override.

Respiratory Motor Neurones: 3 groups of neurones converge on the anterior horn cells:

1. Comes from the DRG and VRG (**CPG**) – concerned with the inhibitory and excitatory output
2. **Voluntary control** of breathing
3. **Involuntary non-rhythmic respiratory control** (e. g. coughing, swallowing, hiccups)

Additional input to the respiratory motor neurones come from the **cerebellum** and from the **reticular activating system**, 'informing' the respiratory motor neurones of the body's posture, and relaying information about level of arousal.



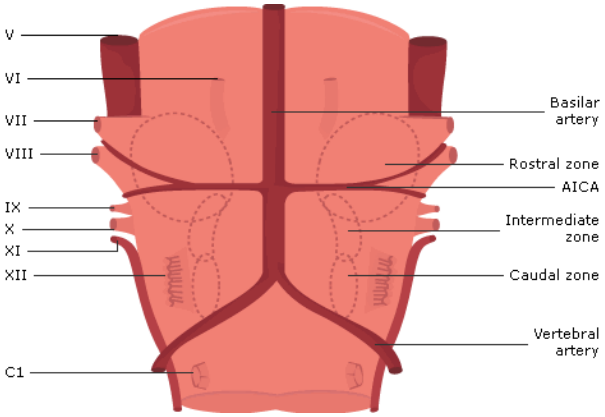
Chemoreceptors

Central chemoreceptors: Are sensitive to **$PaCO_2$** through **hydrogen ion concentration in CSF**. This is the most important single driver of ventilation

Peripheral Chemoreceptors: Sensitive to **PaO_2, $PaCO_2$** and **H^+ ions** in the **periphery**.

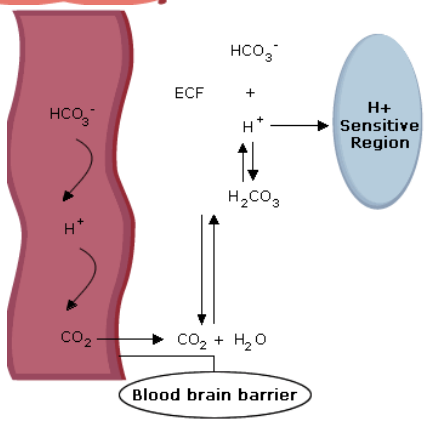
Central Chemoreceptors

***LOCATION*:** They are present 0.2mm below the **antero-lateral (ventral) surface of the medulla** (distinct from the DRG) located close to the origin of cranial nerves IX and X. Crossed by the ant. Inf. Cerebellar a. (AICA). 2 areas: one rostral and one caudal with an intermediate zone to connect the 2.



Mechanism of Action

1. CO_2 diffuses through the BBB
2. Dissociation to H^+ ions occur (impermeable through the BBB)
3. Sensed by the chemoreceptors



80% of response to CO_2 occurs in central chemoreceptors and the response occur relatively slowly over 1-3mins from the time of change of $PaCO_2$. *NB there may be an additional effect from the indirect increase in cerebral blood flow in response to a fall in pH*.

Normal pH of CSF is 7.32 as it has lower protein content and **less buffering capability**. Therefore in:

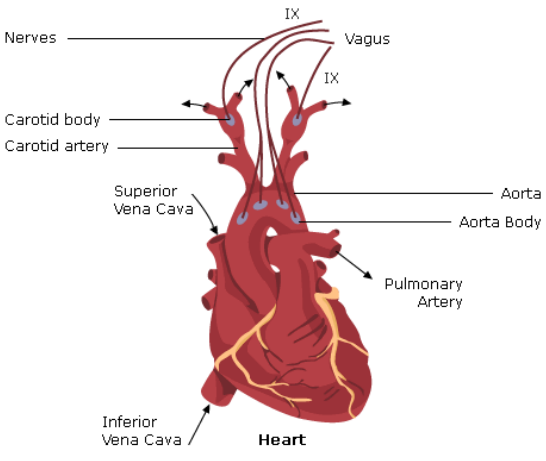
Chronic Compensation: With chronic raises in CO_2 and hence CSF $[H^+]$ concentration, **HCO_3^- is transported across the BBB**. This may take hours – days to take place and eventually blunts the resultant increase in minute ventilation. Eventually individuals **lose their response to hypercapnia** and **rely on the response to hypoxia.**

Other factors which reduce central chemoreceptor response to CO_2: Sleep, increased age, genetic and racial factors, training (athletes and divers have a low CO_2 sensitivity), drugs (opiates, barbiturates).

Peripheral Chemoreceptors

- **Carotid Bodies** responsive to **PaO_2, $PaCO_2$** and pH.
- **Aortic Bodies** sensitive to **PaO_2, $PaCO_2$** but not pH. (Less important than carotid bodies)

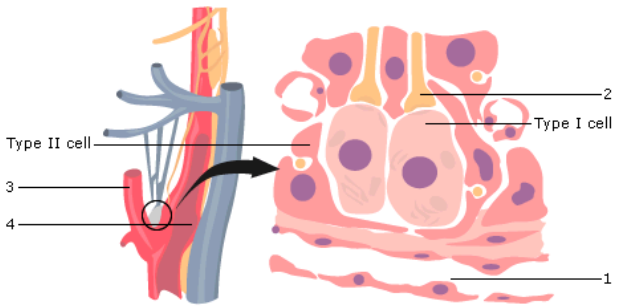
Peripheral chemoreceptors are the **main receptors responsible for the increase in ventilation in response to hypoxaemia**.



Carotid Body Structure

Located at the **bifurcation of both carotid arteries** as a small cluster of chemoreceptor cells. Receives 40x more blood flow than cerebral blood flow. It is made up of 2 types of **glomus cells:**

- **Type I (chief) – Chemoreceptive cells** in synaptic contact with afferent nerve endings of the sinus nerve (branch of CN IX)
- **Type II (sheath) –** Resemble glial cells that act as **supporting cells**

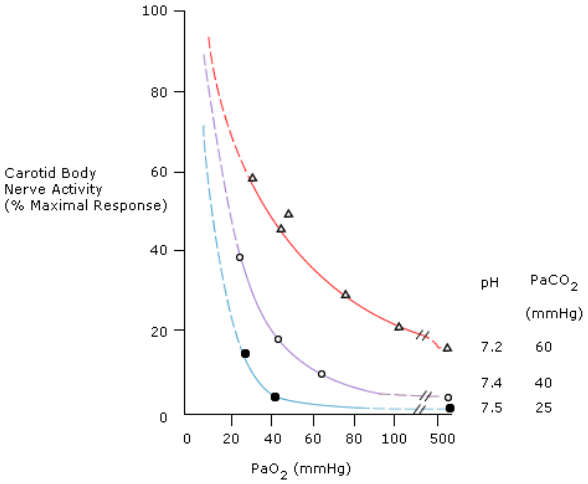


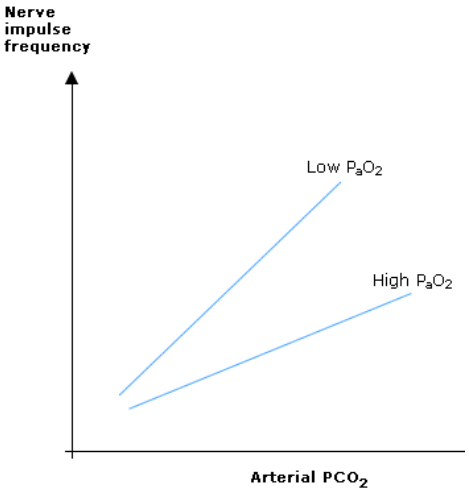
Key:

1. Fenestrated capillary sinusoid
2. Nerve afferent - connects Type 1 cells with DRG via C. IX
3. External carotid artery

Response to PO_2

Due to large blood flow (2L/100g/min) across the carotid body, there is a negligible arterial–venous O_2 difference in spite of a high metabolic rate (8mlO_2/min/100g) and is therefore ideally situated to sample changes in PaO_2. Type I cells **detect graded changes to ATP concentration** from minor changes of PaO_2 and responds **within a few seconds** with a fall in PaO_2 < 7.89 kPa augmented by acidosis and raised $PaCO_2$.





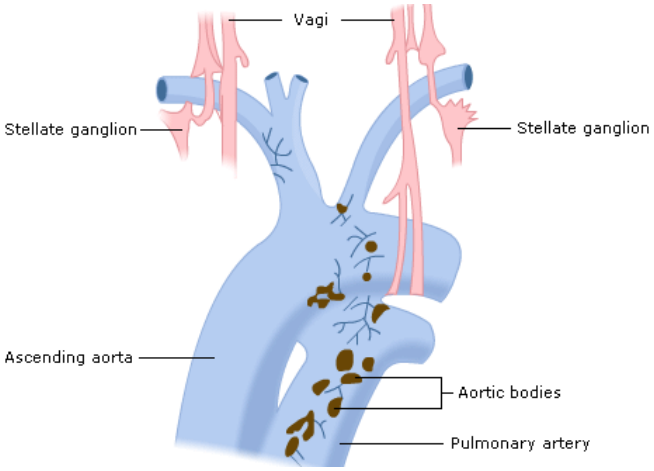
Response to PCO_2 and Hypotension

Response to $PaCO_2$ is **dependent upon carbonic anhydrase** which suggests it **responds to [H^+]**. It accounts for less than 20% of responses to $PaCO_2$ but is **very rapid** and is the **only response to rapid alterations in $PaCO_2$.**

Hypotension increases ventilation through **stagnant hypoxia**.

Aortic Body

Located above and below the aortic arch and responds more weakly than the carotid bodies. Blood supply is not as high as carotid bodies so they have a greater arterial-venous oxygen difference and will respond to changes in oxygen content rather than changes in arterial oxygen tension. Aortic bodies are therefore more **attuned to actual oxygen delivery i**ncluding a reduced haemoglobin and hypoxaemia



Pulmonary Stretch Receptors

An example of **slowly adapting stretch receptors (SARs)** – responds slowly to stimulation (**distension of the lungs**) and continues firing as long as the stimulus continues. Located in the **smooth muscle cells** of the **large airways** and transmit impulses **via Vagus (X) nerve** to the respiratory centres which is sustained with **lung inflation**. Functions to:

- Terminate inspiration via the Hering-Breuer reflex
- Strong stimulation → Expiratory neurones

It is more important in infants as in normal tidal volumes in adults, SARs are not activated but only in exercise where the tidal volume is increased. Little effect on breathing following lung transplantation

Irritant Receptors

Rapidly adapting receptors which fire rapidly to stimulus but effects are diminished if the stimulus is constant. They respond to mechanical and chemical irritation such as noxious gases/cigarette smoke/dust/cold air. Located in **epithelial cells of large airways** – especially at the level of the **carina** through the **Vagus (X) nerve.** Also located in the **nose and upper airways** and the cause for **laryngospasm**. They cause coughing, sneezing, and bronchoconstriction

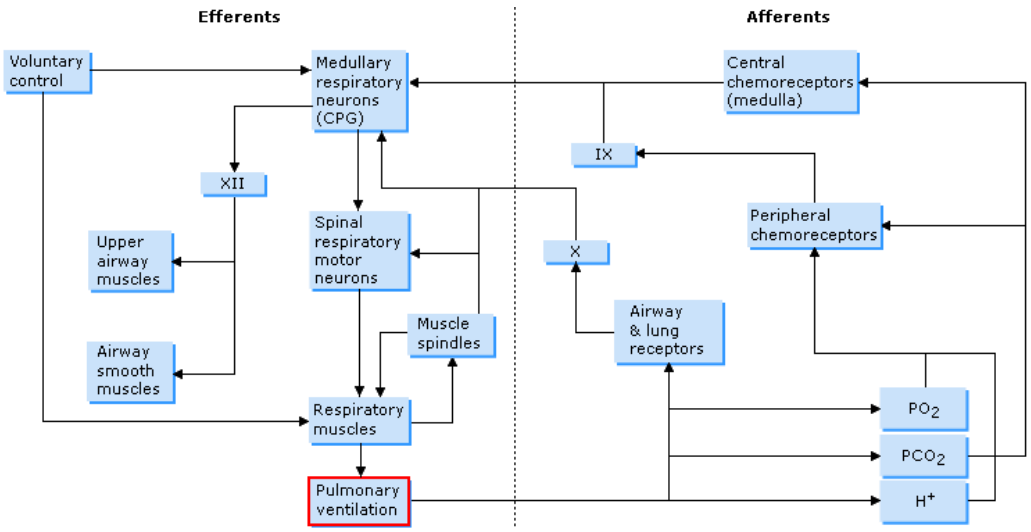
J-receptors (Juxta-capillary)

Located in **alveolar cell walls close to the capillaries**. They respond quickly to **chemicals in the pulmonary circulation** and is also **stimulated by oedema in the interstitium** (may play a part in pulmonary oedema) through the **Vagus (X) nerve**. They cause rapid, shallow breathing.

Other Receptors

1. **Joint and muscle proprioceptors** – thought to play a role during exercise where stimulation causes increased inspiration
2. **Muscle spindles** – responsible for the stretch reflex in muscle contraction. May influence the CPG in the medulla
3. **Arterial Baroreceptors** – Decrease in BP may result in hyperventilation
4. **Pain and temperature** – when stimulated → increased ventilation

Overall Integration



Ventilatory Failure and Oxygen Therapy

(07b_03_10)

Respiratory Failure (RF) is the inability to maintain normal arterial gas tensions. Defined through blood results. This can be split into type 1 and type 2 respiratory failure. Hypoxia secondary to cardiac failure is excluded from this definition

Ventilatory Failure (VF): Defined in terms of the normal clinical scenario of a patient with a pathological reduction in alveolar ventilation below the level required for the maintenance of normal alveolar gas tensions:

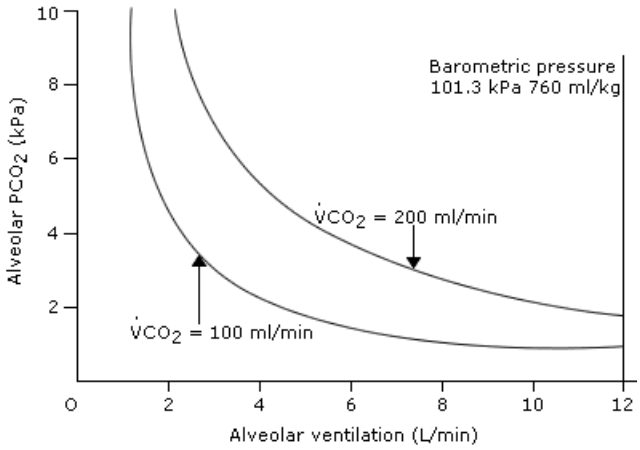
- **Type 1 –** secondary to V/Q mismatching with intrapulmonary shunting. Causes include pneumonia, asthma, oedema, PE, ARDS etc.
- **Type 2 –** commonly due to hypoventilation. Causes include COPD with VF and opioids.

In practice, the terms RF and VF are used interchangeably.

$PaCO_2$ and Alveolar Ventilation

It has an **inversely proportional relationship** if the volume of CO_2 produced (VCO_2) remains constant. 200ml CO_2/min is generated at rest by the average sized person

During exercise, although VCO_2 rises, the $PaCO_2$ actually decreases explained by the increase in alveolar ventilation to not only make up for the VCO_2 but also for the lactic acid increase (respiratory compensation for a metabolic acidosis).

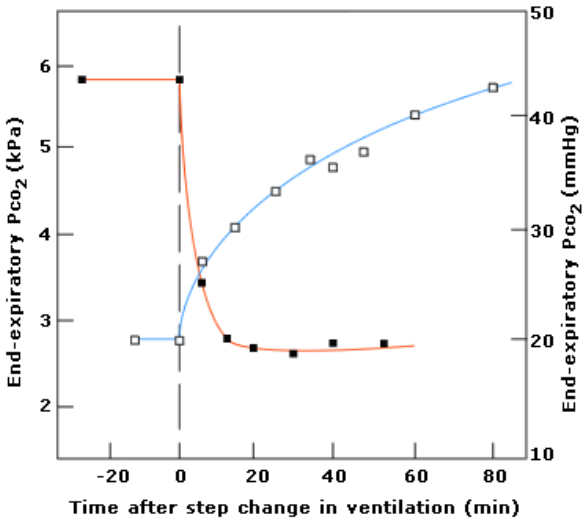


In chronic COPD, alveolar ventilation may not compensate for a rise in $PaCO_2$. This may be because of a greater proportion of dead space to alveolar ventilation. May also occur in acute situations i.e. pneumothorax.

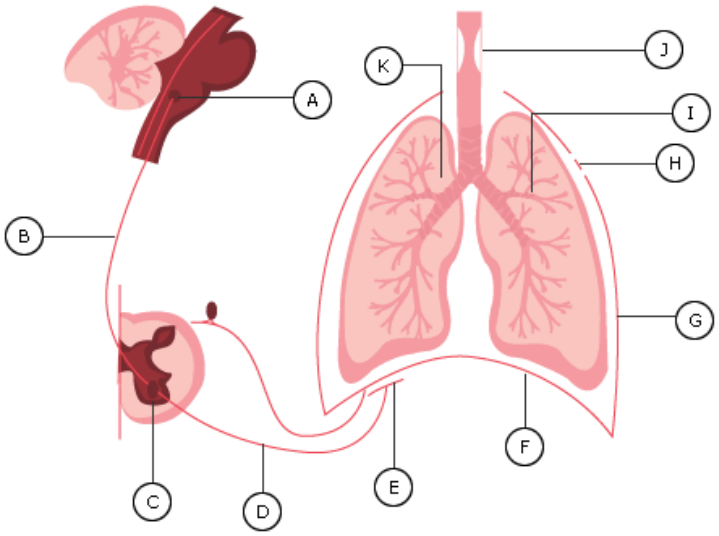
Stored CO_2

There is a **large body reservoir of CO_2 – approximately 120L** (100x that of oxygen). Therefore, when ventilation is out of sync with metabolic activity (VCO_2), these stores buffer any rapid changes in CO_2 levels unlike oxygen.

This buffer system is effective in hyperventilation states. However, with hypoventilation the increase in $PaCO_2$ can only be affected by the metabolic rate of production (assuming minute ventilation remains constant). This is why the rate of rise and fall of PCO_2 with ventilation is not mirrored:



Causes of Ventilatory Failure



A = Respiratory Centre may be depressed causing inadequate ventilatory drive. May be caused by hypercarbia, severe hypoxia, drugs, brain lesions

B = Upper motor neurones may be damaged through trauma or pathological tumours, syringomyelia, demyelination, brainstem strokes and AVMs. Phrenic nerve needs to remain intact C3,4,5.

C = Anterior Horn cells damaged in poliomyelitis.

D = Lower motor neurones affected in phrenic nerve injury, motor neurone disease, Guillain-Barre

E = Neuromuscular junction may be affected with **toxins/poisons** i.e. botulinum, ACh inhibition with organophosphorus compounds. Also affected in **autoimmune disorders** such as myasthenia gravis and Eton Lambert syndrome.

F = Respiratory muscles affected with mechanical splinting of the diaphragm, disuse atrophy and other muscular diseases

G & H = Lungs/chest wall with anything that affects the elasticity or structural integrity.

I = Increased small airway resistance i.e. asthma/COPD

J = Upper airway obstruction

K = Increased dead space

Hypoventilation

Although $PaCO_2$ is a more accurate indicator of ventilator sufficiency, the rate of rise in apnoeic patients will be the metabolic production – 0.4-0.8 kPa/min. In hypoventilation, this rate of increase will be less. As oxygen will also fall in a shorter time frame, $PaCO_2$ will not be noted to raise much and therefore measuring PaO_2 would be of more use in the acute setting.

$PaCO_2$ is therefore a better indicator of long term inadequacy of ventilation.

Intermittent Positive Pressure Ventilation (IPPV)

This is the application of positive pressure to facilitate ventilation. Ventilation of lung units depends on the compliance and resistance of the lung tissue. In:

- **Fast inspiration:** preferential gas flow to lung areas with short time constants
- **Slow inspiration:** Gas flow according to compliance
- **Expiration:** Passive where airway pressure falls back to ambient pressure + PEEP

Time Constant: the speed with which a particular system can respond to a specific rate of change. This is an exponential process.

Physiological Effects of IPPV

RESPIRATORY: Whilst it may include the following, PEEP can reduce these effects:

- **FRC and lung compliance falls;**
- **Intrapleural pressure changes** much more than with spontaneous ventilation (from approximately -5 cm H_2O in expiration up to +5 cm H_2O in inspiration) – higher risk of PTX.
- **Atelectasis** may occur in dependent lung tissue → increased dead space and A-a gradient.

CARDIOVASCULAR: All effects arise from an **increased intrathoracic pressure** which may result in reduced BP:

- **Increased pulmonary vascular resistance**
- **Reduced preload** due to reduced **venous return** and subsequent drop in cardiac output
- **Reduced compliance of L ventricle** → further reduction in R ventricular filling and preload
- **Raised CVP → reduced drainage of head and neck → Raised ICP** (bad for raised ICP!)

RENAL: These may include:

- Reduction in renal arterial BP and increased venous pressure → **reduced renal perfusion**
- **Reduced GFR** → stimulation of **RAS**
- **Vasopressin secretion**
- Reduced ANP secretion.

Urine output can drop up to 40% with sodium retention

OTHER:

- Ileus is common with prolonged IPPV
- Barotrauma risk
- Risks associated with the procedure of I&V

Oxygen Toxicity

Exposure to oxygen at a partial pressure > 2atm may result in convulsions and may be seen in hyperbaric oxygen therapy. Divers must be aware of this. Correlates with the **decreased concentration of GABA levels** state induced by oxygen in the brain.

Increased free radicals (atoms or molecules with unpaired electrons) which overwhelm natural defences may be caused by the reduction of molecular O_2 to oxide ions.

Pulmonary Toxicity

As pulmonary tissue sees the highest PO_2, they are at greatest risk of toxicity. The symptoms relate to the length of time exposed and the partial pressure – NOT concentration – of oxygen.

- **12-24 hours of 100% oxygen**
  - Substantial discomfort and tracheobronchial irritation
- **24-36h hours**: (*think lung trying to get rid of oxygen*)
  - Reduced compliance, vital capacity and diffusing capacity
  - Increased dead space and arteriovenous shunt
- **Changes or damage** after 24-36 hours:
  - Reduced mucous clearance
  - Endothelial damage with infiltration of macrophages and neutrophils
  - Potential decrease in surfactant and increase in capillary permeability

FiO_2 of 50% is safe over any period of time and to reduce risk of oxygen toxicity, the lowest FiO_2 should be used.

Other issues with oxygen therapy

Hypoxic Drive: Some patients depend on a hypoxic drive (very rare) and therefore oxygen therapy may cause reduced minute ventilation resulting in respiratory failure. Requires controlled O_2 therapy.

Absorption Collapse: Due to the high inspired concentration of oxygen, the nitrogen in the alveoli will be displaced and oxygen will diffuse out of the alveoli leaving it with no gas to keep it splinted open → collapse.

Non-respiratory functions of the Lungs.

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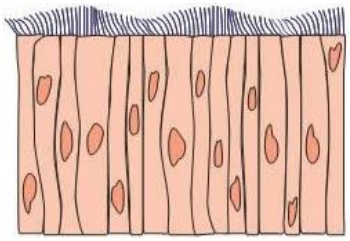
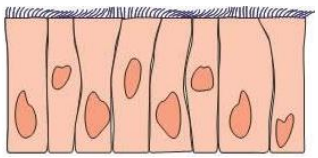
Warming and Humidification

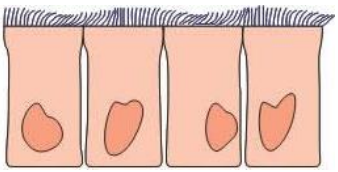
To avoid damage to the delicate alveolar epithelium, the respiratory epithelium must **warm and humidify air** as it passes along the airway



Pseudostratified ciliated columnar epithelium: Found in the nose, pharynx and trachea. Due to its spread of nuclei, it looks as if multiple layers of cells are present:



Ciliated columnar epithelium in a bronchus: Located in the bronchi:



Cuboidal epithelial cells: Located in the bronchioles. As the merge with the alveoli, they begin to flatten.

***Heat and Moisture Exchange*:** Provides the basis of which a HME filter is used:

During inspiration, cold air passes through the warmer airway lined fluid. Energy from the latent heat of vaporisation (from the mucous layer) cools the airway lined fluid.

During expiration, warm air condenses on the airways allowing re-warming.

***Efficiency of humidification*:** With nasal breathing at rest, there is a longer distance at which gas has to travel and therefore is more efficient and warming occurs at the level of the trachea. During exercise, warming occurs at the bronchioles. With turbulent flow at the corners of the mouth and at the nasal turbinates, air is closer to the airway lined fluid and humidification is more effective.

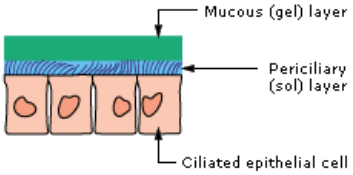
Production and control of airway lined fluid

Produced by epithelial and goblet cells. There are 2 distinct layers of airway lined fluid:

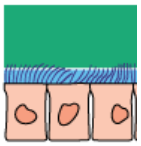
1. **Mucous 'gel' layer:** Secreted by **goblet cells** in response to irritation or infection. It contains several **very large, viscoelastic proteins called mucins**, which are sticky that forms a flexible gel which can also retain large amounts of water.
    a. Only occurs in large airways and exists as 'islands' in distal airways
2. **Periciliary 'sol' layer:** Produced by **ciliated epithelial cells** through active secretion of sodium and chloride and passive osmosis of water (origin of mutation for cystic fibrosis)

The periciliary layer must remain at the correct depth to allow cilia to beat efficiently and is controlled by the **active transport of ions by epithelial cells** and the **passive movement of water** between the mucous and periciliary layers and evaporation.

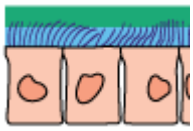
***Normal conditions*:** Tips of the cilia are brushing the mucous layer:



***Build up of mucous layer*:** Occurs with convergence of multiple distal airways to the larger airway and in smoking or infection. Excess water is all held in the mucous layer



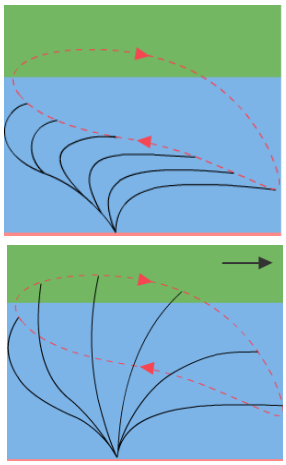
***Water Depletion*:** Systemic dehydration or from warm atmosphere – relatively increased rate of evaporation. Water is donated to the periciliary layer by the mucous layer through osmosis until the depth of the mucous layer is reduced by up to 70%



NB the periciliary layer always remains at the correct depth for cilia to beat.

Removal of inhaled particles by the lung

Mucociliary Escalator



Considering we breathe 10,000L air/day, it is inevitable that we will also inhale microscopic and macroscopic particles. **Trapping and inactivating** these particles is the next important function of epithelium. The **mucous layer is propelled cephalad** by beating cilia at 12-14x/s. 2 phases exist:

1. **Recovery Stroke** which occupies 75% of the cycle time. Slow movement away from the resting position by sideways action of cilium
2. **Effective Stroke:** Cilia grip the underside of the mucous layer and propel it the appropriate direction and then releases to prepare for the recovery stroke. This is coordinated with the cilia adjacent to it and so forth for more effective movement.

The mucous velocity averages at 4 mm/min at a steady rate. Correct depth of the periciliary layer is vital to get this system to work effectively so extreme environments may impede this process. The activity of cilia is easily affected by inhaled pollutants, tobacco smoke, anaesthetic agents and infection.

Effect of Particle Size

Inertial impaction when the momentum of the particle causes it to hit the wall of the airway and is affected by the velocity of the particle and by the turbulent flow in larger airways. These particles (PM_{10}) occur more in air pollution and are a major cause of airway disease in the urban population:

- **Large particles** (>10 µm) are deposited in the nose and pharynx and rarely pass beyond this
- **Smaller particles** (3-10 µm) penetrate further into the respiratory tract

Sedimentation occurs with slow gas velocity and deposition on the airway lined fluid. This occurs in the **small airways or alveoli** and the particles are **1-3µm in size.** Breath-holding following inspiration encourages this process. Fine particulate air pollution ($PM_{2.5}$) causes inflammation of the lung and elsewhere in the body.

Diffusion occurs with particles <1µm in size and have minimal contact with the airway or alveolar walls. I.e. in smoking where most is exhaled.

With inhalers, the particles range in size from 1-35µm and therefore, deposition occurs on the pharynx and systemic absorption from the alveoli occurs. MOA should be at bronchioles. With **microgravity** some of the large particles have less mass and may travel all the way into the alveoli. Particles in the alveoli can be cleared by **alveolar macrophages** which is activated to different extents according to the particle type – may cause cytokine release → inflammation → deposition of collagen → lung fibrosis.

Defence Against Inhaled Pathogens

This section describes the pathogens that bypass the mucociliary escalator. **Chemical inactivation** is a key defence system caused by:

- **Lysozymes:** destroys gram-positive bacterial cell walls excreted by neutrophils
- **Defensins:** Produced by epithelial cells and defend by **direct bacterial cell wall damage** or through attraction of other immune cells through acting as a **chemokine**.
- **Surfactant Protein A:** Acts as a chemokine through bacterial recognition in the alveoli.

Protease/Antiprotease System

Protease enzymes i.e. **elastase** or **metalloproteinases** are released in the lung in response to pathogens or tobacco smoke. Whilst they are powerful antimicrobials, they may also damage lung tissue but 2 mechanisms help prevent this:

1. **Confined to mucous layer** which avoids close contact with the epithelial cells
2. **Anti-protease enzymes** exist to inactivate it through conjugation e.g. a-1-antitrypsin (imaged)

Humoral Immunity

Large amounts of immunoglobulin is present in the airway lining fluid. The most abundant of which is **immunoglobulin A** and acts by preventing bacterial binding to the mucosa. **IgG predominates deeper** in the respiratory tract.

Cellular Immunity

Epithelial cells are immunologically active supported by macrophages. Neutrophils multiply in COPD and eosinophils and mast cells in asthma. Once the **epithelial cells are activated** they use a range of molecules to induce an inflammatory response and tissue healing:

- **Adhesion molecules** i.e. ICAM-1 in pulmonary capillaries for inflammatory cells
- **Chemokines** i.e. IL-8 recruit inflammatory cells to lung tissue
- **Cytokines** i.e. IL-1,6 & TNF amplifies the inflammatory response
- **Growth factors** i.e. TGF-β, EGF to stimulate cells responsible for repair i.e. fibroblasts
- **ECM proteins** i.e. collagen, hyalouronan to begin tissue repair.

The last 2 mechanisms may become overactive in prolonged/severe inflammation leading to **remodelling** of pulmonary blood vessels. This can cause **pulmonary HTN** or **excessive deposition of collagen** → irreversible **pulmonary fibrosis.**

Pulmonary Metabolism

The pulmonary circulation has other physiological functions apart from gas exchange:

- **Filter** for **particulate matter/gas** in the systemic circulation – prevents embolic disease
- **Metabolically active endothelium** for the processing of many compounds in the blood.

Endothelial Cells

70 m^2 of endothelial surface present in the blood vessels of the lung to act as an interface with the blood. Contains multiple **invaginations** and **projections** and contain numerous vesicles that abut against the cell surface (**caveolae**) which increases the surface area further to 126 m^2.

	Effect of passing through pulmonary circulation		
Group	**Activated**	**No change**	**Inactivated**
Amines		Dopamine Adrenaline Histamine	5-hydroxytryptamine Noradrenaline
Peptides	Angiotensin I	Angiotensin II Oxytocin Vasopressin Atrial natriuretic peptide	Bradykinin Endothelins
Arachidonic acid derivatives	Arachidonic acid	PGI2 (prostacyclin) PGA2	PGD_2 PGE_2 $PGF_{2\alpha}$ Leukotrienes
Purine derivatives			Adenosine ATP, ADP, AMP
Steroids	Cortisone		Progesterone Beclamethasone
Basic drugs		Morphine Isoprenaline	Fentanyl Lidocaine Propranolol

Tight junctions are found between the endothelial cells, forming part of the physical barrier between blood and the alveoli. Although there is a sparsity of organelles involved with metabolic activity i.e. mitochondria and smooth endoplasmic reticulum, they still have a high metabolic activity

***Enzymes*:** There are 2 groups of enzymes existing on pulmonary endothelial cells:

1. **Membrane bound** – responsible for Angiotensin I → Angiotensin II. Also for the inactivation of bradykinin and adenosine
2. **Cytoplasmic** – involved in metabolism of arachidonic acid products, degradation of prostaglandins and breakdown of 5-HT and other amines (see below).

Specific mechanisms of pulmonary metabolism

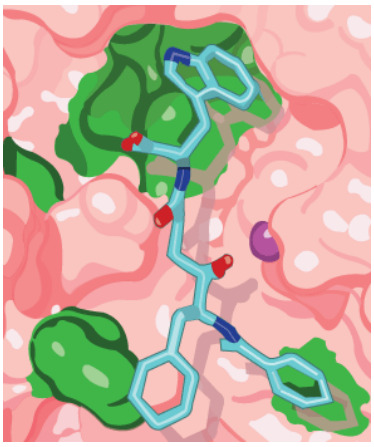
CATECHOLAMINES: Abundance of ***Monoamine oxidase*** and ***catechol-O-methyl transferase*** (non-specific) in the cytoplasm:

- 1/3rd Norad, 98% 5-HT is removed
- Adrenaline and dopamine are unaffected

The reason is from uptake of the cell membrane (selective metabolism) as it only takes up noradrenaline and 5-HT. This uptake also effects metabolism of other drugs:

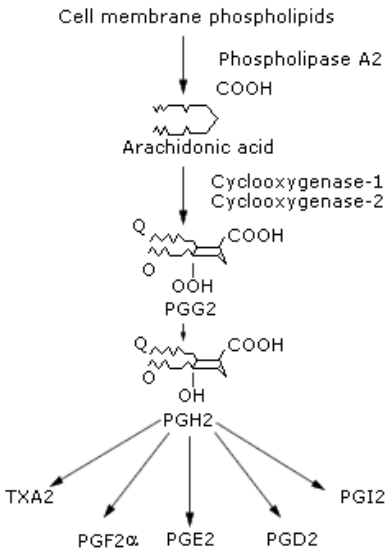
1. Basic drugs (pKa > 8) and lipophilic drugs tend to be bound by the pulmonary circulation while acidic drugs preferentially bind to plasma proteins
2. Drug binding in the pulmonary circulation may act as a first-pass filter for any drug administered intravenously
3. Fast or slow release of the bound drug is controlled by the pulmonary circulation. Rapid increase in levels may occur when the binding sites either become saturated, or when one drug is displaced by a different drug with greater affinity for the binding site

PEPTIDES: ACE is located on the surface of endothelial cells lining the inside of the caveola extending to the luminal projections. This allows 80% of Angiotensin I to be converted in one pass through the pulmonary circulation. ACE also effectively **removes bradykinin**. ACE is a:



- Zinc containing carboxypeptidase with two active sites located in deep grooves
- Once substrate is bound, zinc moiety then cleaves either a:
  - Phenylalanine-histidine bond (angiotensin I)
  - Phenylalanine-arginine bond (bradykinin)

ACE inhibitors bury themselves deep in the groove covering the active site



EICOSANOIDS: They are not stored pre-formed but are synthesised PRN by many cell types including the endothelium.

- Pathway starts through **activation of phospholipase** as a result of multiple different stimuli
- COX exists in isoform 1 and 2
  - COX-1: constitutive enzyme present at low concentrations
  - COX-2: Induced by inflammatory cytokines

NB COX 1 inhibition by aspirin may induce bronchospasm in asthmatics

The effects of eicosanoids are as follows:

Effect	Eicosanoid
Bronchoconstrictors	$PGF_{2\alpha}$,PGD_2, PGG_2, PGH_2 and thromboxane (all are more potent in asthmatic subjects)
Bronchodilators	PGE_1 , PGE_2 and PGI_2 (prostacyclin)
Pulmonary vasodilators	PGI_2 and PGE_1
Pulmonary vasoconstrictor	PGH_2 and $PGF_{2\alpha}$

The eicosanoids PGA_2 and PGI_2 may pass through the lung unchanged similar to the mechanism of catecholamines due to cell membrane uptake.

METABOLISM OF INHALED DRUGS: This is located in the **airway** of the lung which contains mixed function **oxidases** and **cytP450** systems – similar to hepatocytes. Steroids are broken down by this method

Endocrine Function

This is defined as a physiological secretion of a substance for action elsewhere in the body. This is minimal in the respiratory system and mainly occurs pathologically. However **nitric oxide** may be released for action in systemic capillaries in response to pulmonary endothelium association with haemoglobin.

NERVOUS SYSTEM PHYSIOLOGY

The Neuromuscular Junction (NMJ)

(07b_04_01)

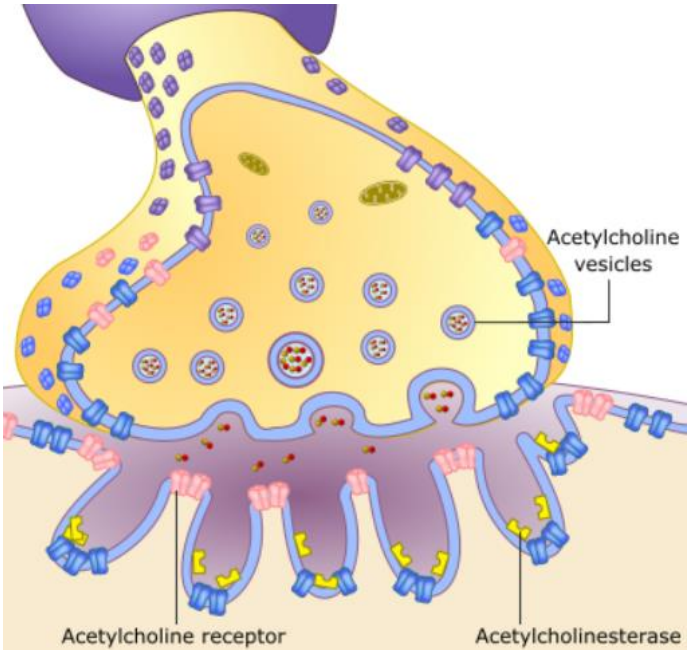
The Neuromuscular Junction

Skeletal muscles are innervated by large diameter Aα motor neurones which are **fast conducting**.

The **motor unit** is the ***number of muscle fibres controlled by one motor neurone***. It may contain 10-150 fibres. Smaller motor units exist in areas requiring fine control i.e. facial, extraocular and intrinsic laryngeal muscles.

The axon terminal adjoins to the **synaptic cleft** of width 20-30nm.

The **post-synaptic** membrane is located on the muscle and is folded to allow a large surface area. The **shoulders** contain **nACh receptors** and to bottom contains high concentrations of **acetylcholinesterase**.



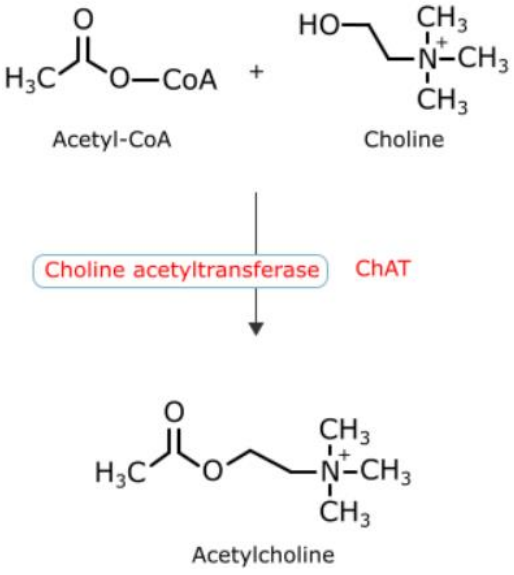
The described junction is known as the **motor end plate**.

Acetylcholine

This is the main neurotransmitter in the motor end plate. Below will describe the steps that allow muscle contraction.

Production

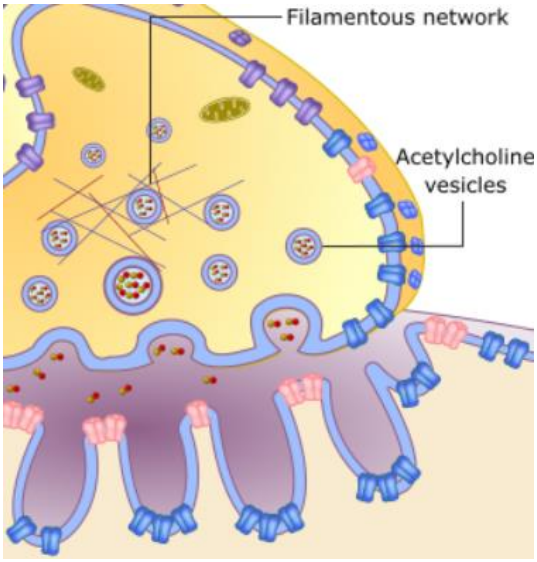
1. **Acetyl-CoA** is produced from ATP which itself is formed in the Krebs cycle
2. **Choline** comes mainly from recycled breakdown products of Ach and also dietary sources. Small quantities are synthesised in the liver.
    a. Choline is actively transported via the neurolemma
3. **Choline Acetyltransferase** increases the speed at which they combine to form ACh.



Storage

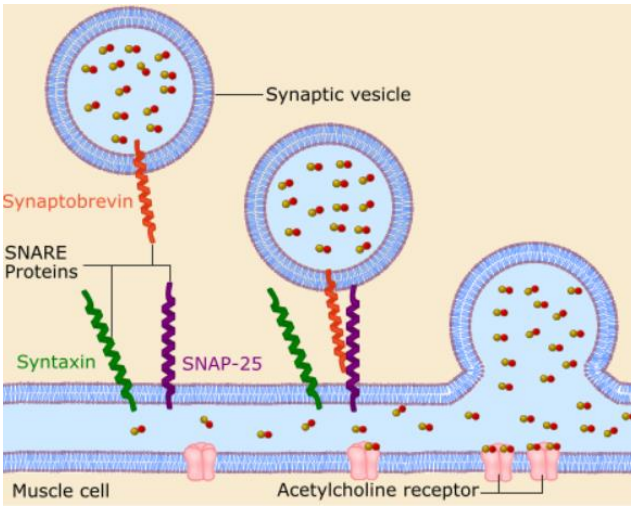
Absorbed into **storage vesicles** in the axon terminal. Each vesicle contains 10-12,000 molecules of ACh and the vesicles are arranged in **separate pools**:

- **Stationary Store:** 19% in pre-terminal part and are not immediately available
- **Reserve Pool aka VP1:** 80% tethered by a filamentous network of actin, synapsin and synaptotagmin ready for use in the case of repetitive nerve stimulation.
- **Immediate Pool aka VP2:** 1% adjacent to the release sites on the presynaptic membrane.

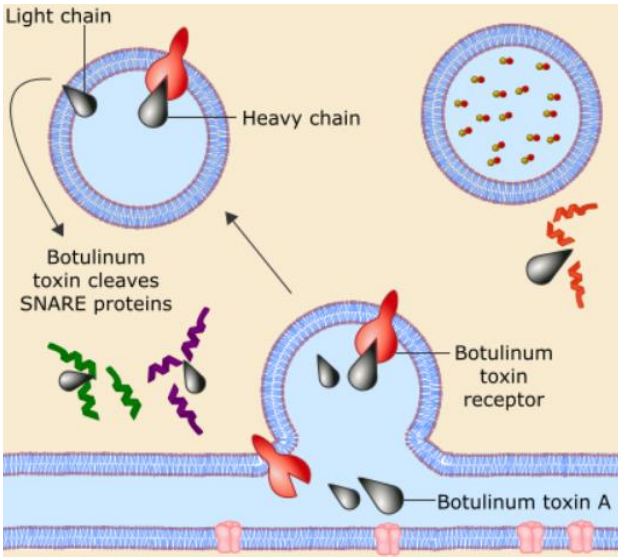


Triggered release

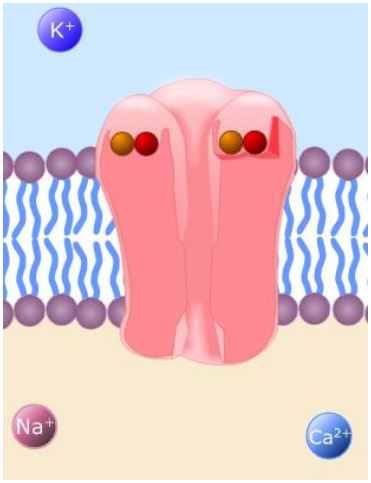
1. **Action potential** propagation occurs from the motor neurone axon to the **voltage-gated calcium channels** causing them to open. This allows an **influx of calcium ions:**
2. Calcium then **activates proteins** on the vesicular and presynaptic membranes collectively known as the **SNARE proteins** (individual proteins on image)
3. Once activated, the ACh vesicles dock to the presynaptic membrane and allow exocytosis of ACh into the cleft. There is also release of vesicles from the reserve pool.



***NOTE*: Botulinum toxin targets SNARE proteins** preventing ACh release into the cleft and hence causes a **flaccid paralysis.**



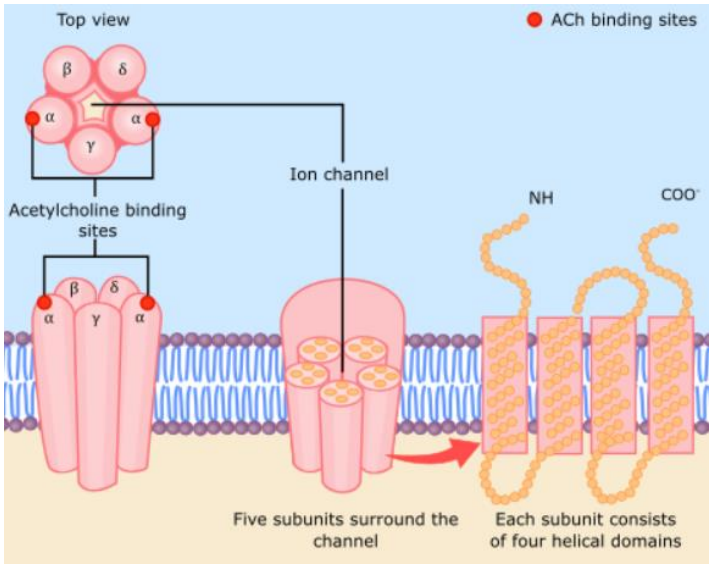
Postsynaptic end-plate potential



ACh diffuses across the synaptic cleft to **bind with nicotinic ACh receptors** allowing mainly sodium influx and depolarisation of the end-plate.

100-300 vesicles are required to **depolarise** the post-synaptic membrane. However, single vesicles are often released with insufficient numbers to cause an action potential – these are known as **miniature end-plate potentials**.

Nicotinic Acetylcholine Receptor



This is a **ligand-gated ion channel** comprised of **5 subunits** with a total molecular weight of 250kDa. Each subunit contains 4 α-helices that span the membrane.

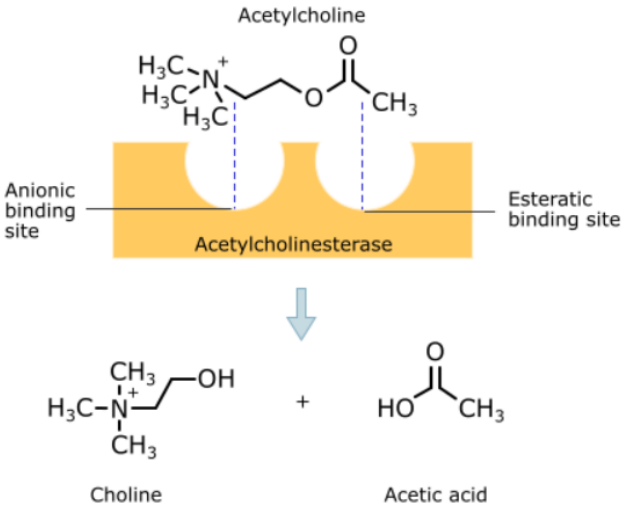
Adults: 2α; β; δ; ε subunits

Foetus: Pictured (same as adult but has a γ instead of an ε subunit). This is also the structure of **extra-junctional receptors**

For it to function, **2 ACh molecules** must bind to the **2 α-subunits** allowing a lumen to open 0.65nm wide. Allows Na^+, Ca^{2+} and K^+ movement.

ACh is **bound for 1-2ms** before being released back to the synaptic cleft.

Deactivation of ACh



Acetylcholinesterase (AChE) rapidly **hydrolyses** ACh once released from the receptor to prevent subsequent interaction with another receptor.

AChE is bound to collagen-Q on the bottom of the post-synaptic membrane and has itself, **2 binding sites:**

- **Anionic site:** negatively charged glutamate group forms a reversible bond with the quaternary amide group on ACh
- **Esteratic Site:** Contains serine amino acids which hydrolyses ACh into **choline** and **acetic acid**.

Choline is reabsorbed via presynaptic transporters where it is recycled.

Muscle contraction is covered on separate notes.

Smooth Muscle

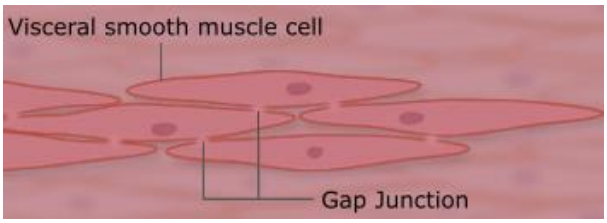
(07b_04_02)

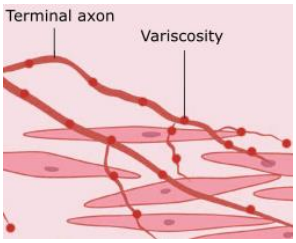
There are 2 types of smooth muscle:

1. **Visceral** i.e. single-unit found in large sheets in blood vessels and lining hollow viscera i.e. uterus, bladder, ureters, GIT.
2. **Multiunit** important for fine control i.e. iris. Also found in large arteries, bronchi and erector pili muscles.

Syncytial Function

Is found **only in visceral smooth muscle** cells and refers to the ability for an **action potential** to **propagate** to the next muscle cells. This occurs due to gap junctions.



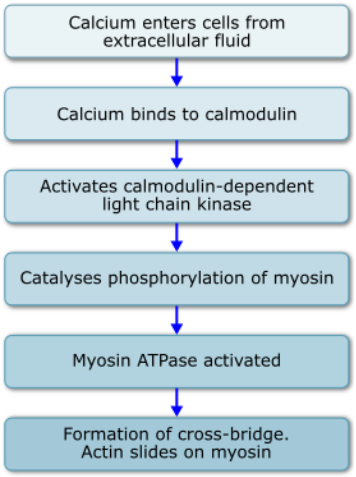


In contrast, **multiunit smooth muscle** do not have connecting gap junctions and each cell or fibre has its own nerve ending (think fine control)

Mechanism of Contraction

Initiation

Smooth muscle control is completely involuntary and **energy is derived predominantly from glycolysis**. Visceral smooth muscle is controlled spontaneously or by control from the ANS. It is initiated through a **rise in intracellular calcium** coming from the **extracellular fluid** rather than the SR through Voltage Gated and Ligand Gated calcium channels.

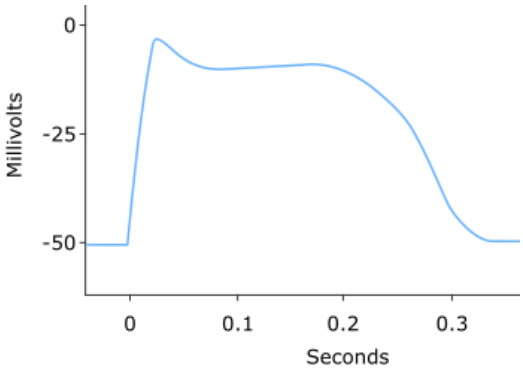


Cross-Bridge formation

Calcium binds to **calmodulin** to create a complex which activates **calmodulin dependent light chain kinase** which catalyses **myosin head phosphorylation** allowing **ATPase activation**. ATP is hydrolysed to provide energy for actin and myosin filaments to slide over each other.

Speed of Contraction

Takes longer to develop and lasts longer as it lacks T-tubules and sarcoplasmic reticulum – greater diffusion distance to fibres and reduced surface area. Mechanism is also slower in smooth muscle.



Mechanism of Relaxation

Requires the **dissociation of the calcium-calmodulin complexes** through active pumping out of the cytoplasm. Myosin is then dephosphorylated by **myosin light chain phosphatase**.

The actin-myosin cross-bridges remain intact at this point and is known as the **latch-bridge** mechanism to produce **sustained contraction with low energy expenditure**.

Plasticity

Refers to when the **initial increase in tension with stretching then decreases at the same length** and may even fall below the initial level of tension. Therefore, one cannot correlate stretch with tension in smooth muscle.

The bladder is a good example where as it stretches, compliance remains neutral from the plasticity effect. However, compliance does eventually reduce as it becomes more stretched over a certain point and the intravesicular pressure will increase

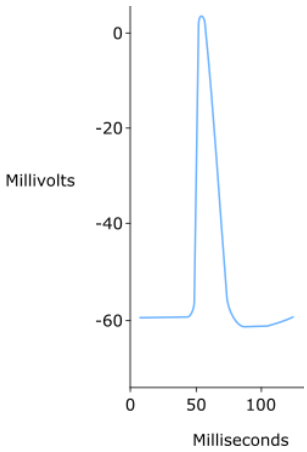
Visceral Smooth Muscle Function

It has an **unstable membrane potential** and therefore does not really have a resting potential. It is averaged at around -50mV which gets **lower** as the tissue is **active** and **higher** with **inactivity**.

Spontaneous Activity

Occurs from **oscillations in membrane potential** with inward currents of calcium independent of nerve supply. As mentioned above, when the threshold potential is achieved, the action potential propagates through syncytial function.

There are also cells that exhibit pacemaker potentials but its location is not fixed and occur in multiple foci which shift from place to place.



Nervous and Humoral Control

- **Parasympathetic Tone:** Ach released from PNS binds to **M3 mAChR** which are G-protein coupled and **activates phospholipase C** and **IP3** → increase **calcium influx.** Therefore, the PNS allows increased motility, secretion and relaxation of sphincters.
- **Sympathetic Tone:** Opposite effect. Through ***alpha receptors*:** IP3 and diacylglycerol → activated phospholipase C. ***Beta receptors*:** G-proteins → cAMP. For *some reason* this results in a decrease in free intracellular calcium.
- **Humoral factors:** Epinephrine and mechanical stress e.g.

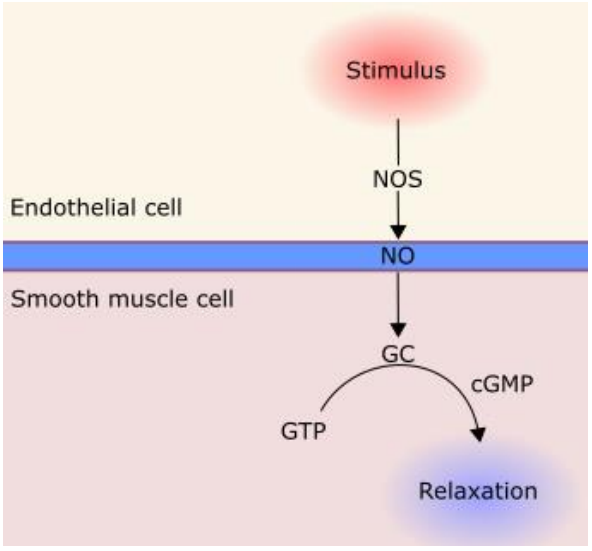
Multiunit Smooth Muscle Function

Non-syncytial. The action potentials ***don't tend to occur spontaneously*** and therefore, **control is achieved** via the **PNS** and **SNS** and humoral factors i.e. epinephrine and histamine. Each muscle cell has its own nerve endings to allow fine control due to well localised contractions.

Nitric Oxide Function

Acts to **relax vascular smooth muscle**.

1. **Stimuli** such as mechanical stretch and ACh cause an increase in **intracellular calcium** in the endothelial cell
2. **Nitric oxide synthase** (NOS) is subsequently activated to produce **nitric oxide.**
3. NO diffuses into smooth muscle to activate **guanylyl cyclase (GC)** → formation of **cyclic guanosine monophosphate (cGMP)** from **GTP**
4. Activation of **protein kinases** leading to a **fall in intracellular calcium** in the vascular smooth muscle cell and **muscle relaxation**



Summary of different muscle types

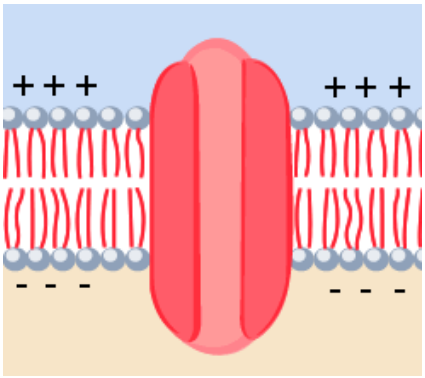
	SKELETAL & CARDIAC MUSCLE	SMOOTH MUSCLE
Source of calcium	Sarcoplasmic reticulum, i.e. intracellular	From the **extracellular fluid**. Sarcoplasmic reticulum is poorly developed
Calcium binds to	Troponin C	Calmodulin
Function of calcium-binding	**Removes inhibition of troponin I** and results in exposure of myosin-binding sites	**Ca^{2+}-calmodulin complex** activates myosin light chain kinase
Activation of myosin ATPase	**Does not require phosphorylation of myosin**	Requires **phosphorylation of myosin** which is catalysed by activated myosin light chain kinase
Contraction	Typically, brief and under voluntary control in skeletal muscle. Involuntary in cardiac muscle	Must last for **long periods**. Exhibits **plasticity** and latch-bridge mechanism
Stimulation of contraction	Nervous stimulation necessary to initiate contraction in skeletal muscle. Initiated by pacemaker cells or spontaneous in cardiac muscle	Spontaneous (visceral smooth muscle) or in response to nervous stimulation and humoral factors (visceral and multiunit smooth muscle)
Control	Voluntary in skeletal, involuntary in cardiac muscle	**Involuntary**
Resting membrane potential	Stable in skeletal muscle, unstable in cardiac pacemaker cell. May be unstable in cardiac myocytes	**No resting membrane potential** as potential tends to wander

Function of Nerve Cells

(07b_04_04 AN 07b_04_05)

The human body contains approximately 1 trillion neurones.

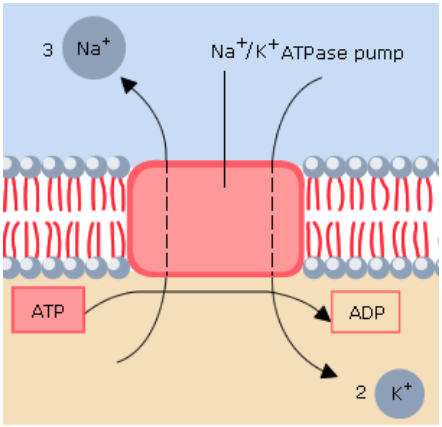
Resting Membrane Potential



The membrane is selectively permeable to certain ions. As a result, the **inside of the cell is negative** compared to the outside. Some ion channels are constantly open and some are gated. There are 2 types of gated channels:

- **Ligand-gated ion channel:** Ligand binds to the channel and it opens. This may be extracellular or intracellular ligands
- **Voltage-gated ion channel:** The alteration of resting membrane potential in the adjacent membrane opens the channel.

Maintenance of Potential



There are constantly open Na^+ and K^+ channels. There is also **Na^+/K^+ ATPases** which extrudes **3 Na^+** out of and uptakes **2 K^+** into the cell. Overall, there is a **net movement of positive ions out of the cell**. This takes up 70% of energy in the neurones and can be coupled to the movement of other molecules.

Gibbs and Donnan

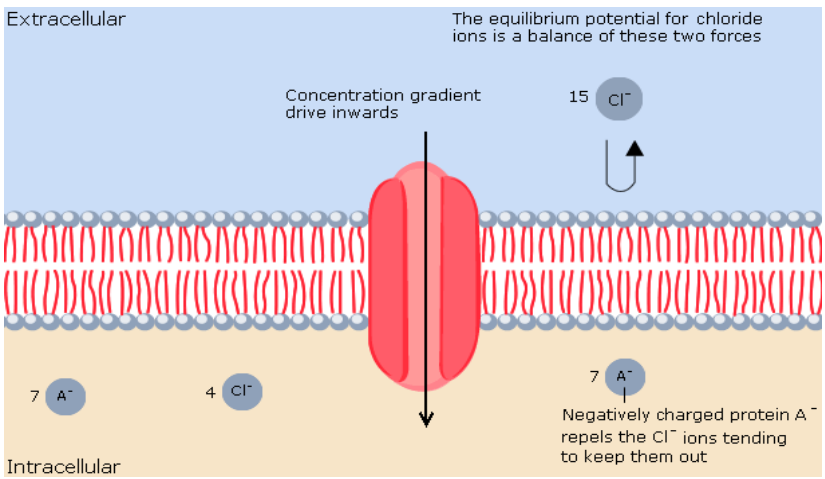
Showed that having an ion inside the cell that cannot cross the membrane will affect the distribution of ions. At equilibrium:

$$[K^+_{inside}] + [Cl^-_{inside}] + [A^-] > [K^+_{outside}] + [Cl^-_{outside}]$$

Because of the protein, there are more osmotically active particles in the cell than outside. Therefore, cells need the Na^+/K^+ ATPase pump to prevent cells to rupture.

Size of Membrane Potential

In neurones this is -70mV. This potential varies according to different excitable tissue as each ion has an equilibrium potential where the urge to move down a **chemical gradient** is **opposed** exactly by an **electrical gradient**. Chloride is shown Right.



Excitation and Nernst Equation

The **equilibrium potential** (electrochemical gradient) of the cell state is **calculated using the Nernst equation**. For example, there are more chloride ions outside of the cell but as the inside of the cell is negatively charged, the force is exactly opposed and no net movement occurs as mentioned above.

Nernst Equation: Example given to calculate the equilibrium potential of chloride. The **sum of all equilibrium potentials** of all the ions will be the **membrane resting potential** and determined by the **Goldmann equation**.

$$E_{Cl^-} = \frac{RT}{FZ_{Cl}} \ln \frac{[Cl^-_O]}{[Cl^-_I]}$$

R = Gas constant
T = Absolute temperature
F = Faraday constant
Cl^-_O = Cl^- concentration outside
Cl^-I = Cl^- concentration inside
Z_{Cl^-} = Valency of chloride ion

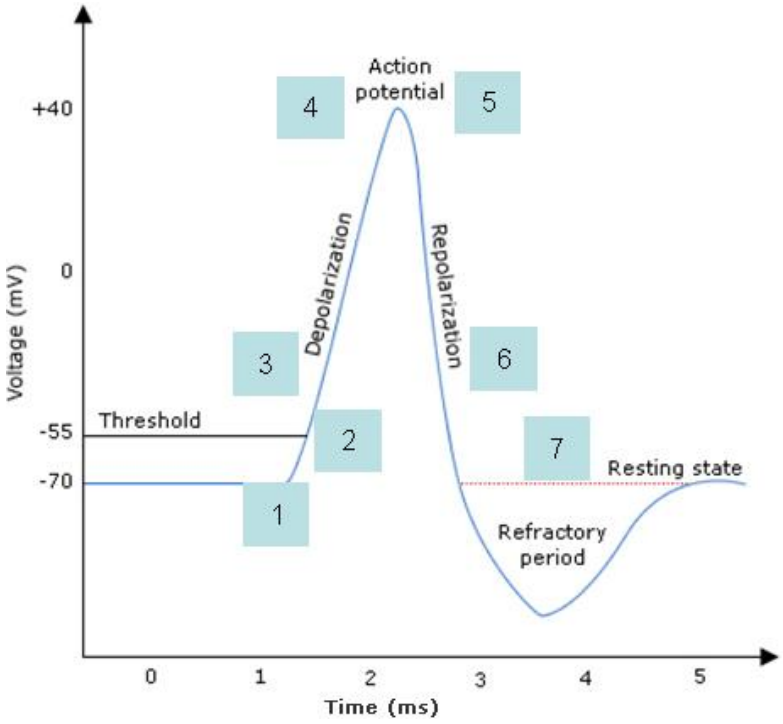
Action Potential

They pass from the **synaptic junction** to the **terminal end of the axon** and travel in one direction. This is known as **orthodromic conduction**. If starts in the centre of the axon, then the signal may propagate in both directions: **antidromic conduction** – however, it still travels in one direction as an action potential travelling back at the cell body will not trigger neurotransmitter release.

It has a **low threshold** and conduction is **self-propagating** (like a trail of gunpowder).

Steps

1. (+2) An above threshold **stimulus** occurs and
2. **Opens voltage-gated sodium channels** to move sodium inside the cell.
3. **Threshold** of -55mV is reached and the depolarisation is unstoppable. Each action potential alters the membrane potential ahead of it to bring it to threshold potential for **propagation.**
4. **Spike potential** with the junction between rapid depolarization to +35 mV and…
5. Rapid repolarisation
6. Rate of repolarisation slows after 70% of repolarisation is complete
7. Slight overshoot causes **hyperpolarisation** and a **refractory period.**



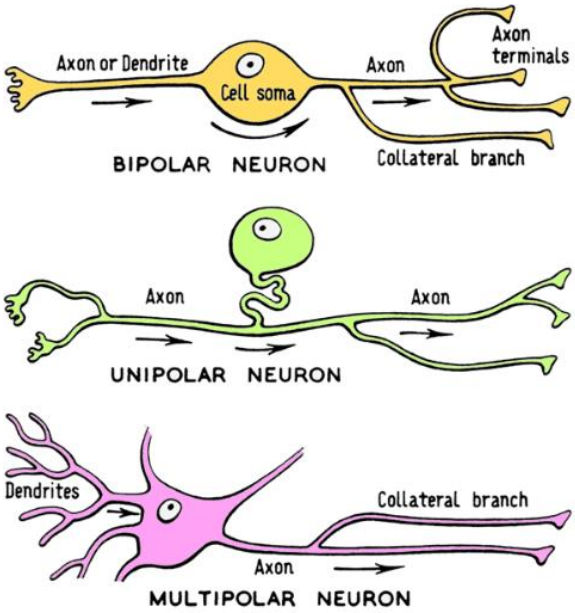
Neurones

Neurones conduct at different speeds with faster necessary for rapid responses and slower conduction as part of the large sensory world we live in.

Classification of Neurones

1. **Monopolar Neurone**: This has only 1 axon. Found in the ANS. The may be known as pseudo-unipolar if they originated bipolar embryologically.
2. **Bipolar Neurones** have 2 axons and associated with special sense organs.
3. **Multipolar Neurones** have 3 dendrites or more than 1 axon. These form the majority of the CNS.

They have an **axon hillock** which is the junction between the cell body and the axon.



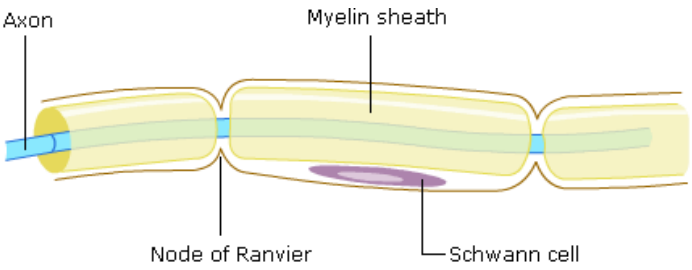
Synthesis in Neurones

Neurotransmitters are the means by which neurones communicate with each other and the effector organ. They are **synthesised** in the **endoplasmic reticulum** of the neurone cell body and travel through **axoplasmic flow** (down the axon to the synapses). This may be:

- **Antegrade flow:** Rapid flow from the cell body to the synaptic ends via **microtubules**
- **Retrograde flow: T**ransport of used vesicles from the synapse to the cell body and deposition in lysosomes

Anatomy of Axons

Whilst cell bodies are located in the CNS or ganglia, nerves are made up by axons which **vary in diameter** and speed is increased with myelination.



Myelin acts as an electrical insulator and is the **cell membrane of Schwann Cells**. The gaps between the Schwann cells are known as **Nodes of Ranvier**.

This is advantageous to perform **saltatory conduction** where action potential leaps from node to node and makes conduction faster than if unmyelinated.

Axon Classification

According to **diameter** (Type A, B and C) with further divisions and **speed:**

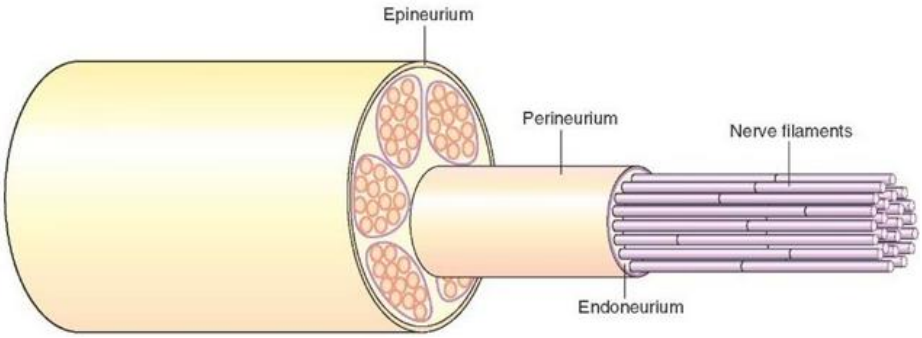
Speed increases with **size** and **myelination** so is in the same order as classification of size.

Fibre type	Function	Diameter (μm)	Conduction Velocity (m/s)
Aα	Proprioception **Motor** neurones	12-20 μm	70-120 m/s
Aβ	Touch, pressure (**sensory**)	5-12 μm	30-70 m/s
Aγ	**Motor** to muscle spindles	3-6 μm	15-30 m/s
Aδ	Pain, cold, touch (**sensory**)	2-5 μm	12-30 m/s
B	Preganglionic autonomic (**motor**)	<3	3-15 m/s
C	**Sensory**: Pain, temperature and postganglionic autonomic	0.3-1.3 μm	0.5-2.3 m/s

C-fibres are unmyelinated

Mixed Nerves

Nerves are surrounded by a **fibrous epineurium** consisting of **multiple neurones.** They may contain **axons of different sizes**. Therefore, detection through electrical recording will include (a sum of) all action potentials: there may be **multiple peaks** as individual axons conduct at **different velocities**.

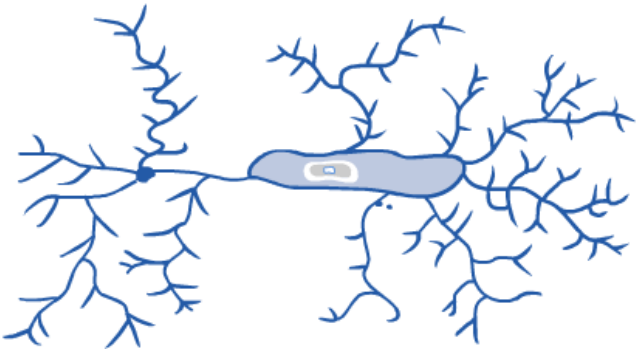


The Glia

There are 10-50x more glial cells than neurones and are important in **support** and **nurturing** of neurones (Schwann cells as an example) Recently has been found to be **signalling** through transmitter release. Persistent pain states may be associated with glial activity. They are classified into microglia, astrocytes and oligodendrocytes.

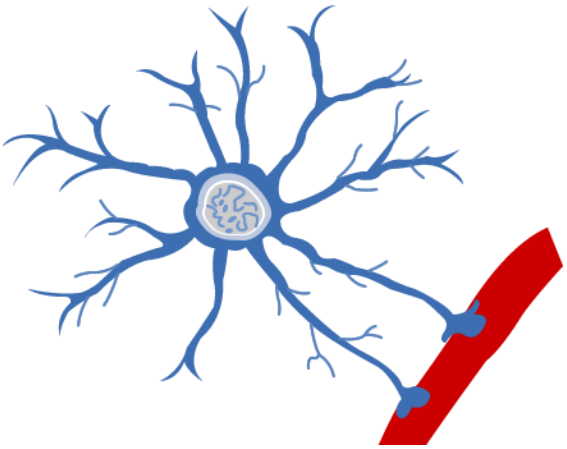
Microglia

These are the **scavenger cells** of the CNS and are derived from the immune system. These may be resident or from **monocytes**. They are activated and become **phagocytic** in response to **infection or inflammation**.



Astrocytes

There are 2 types:



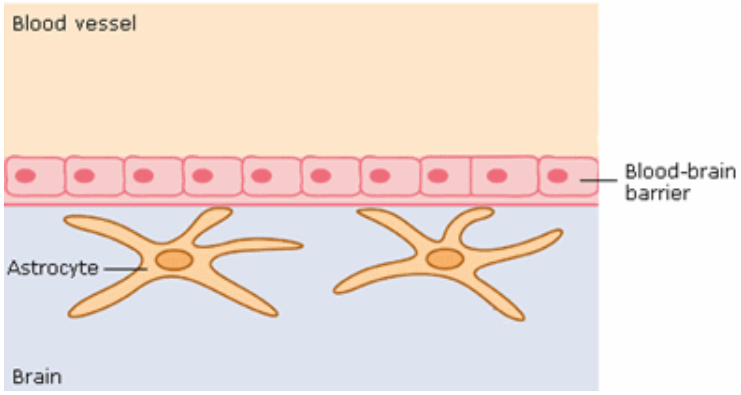
Fibrous Astrocytes which form the **white matter** of the brain and have multiple functions: to **support neurones**, **isolate synapses** and **absorption of neurotransmitters** that are released from synapses.

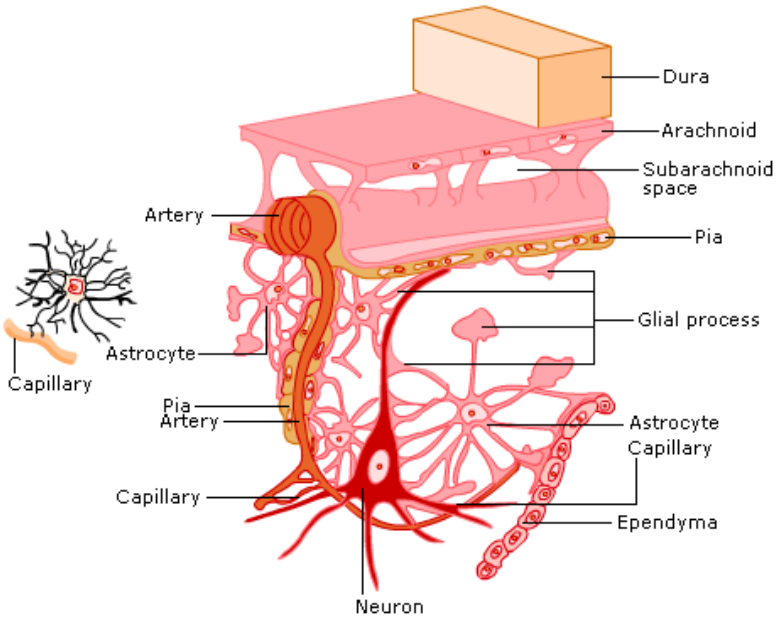
Protoplasmic Astrocytes maintain **electrolyte balance** and in particular K^+ levels. They have a membrane potential but do not generate action potentials (see image):

Astrocytes also produce **nerve growth factor**. They are integral to the formation of the BBB:

Blood Brain Barrier

This is created by **both astrocytes** and send processes around capillaries to prevent leakage of electrolytes and fluid into the interstitium. This is **not present** at birth but **forms in early life** as astrocytes grow and interact with capillaries. This is why **neonates are at risk of brain damage** from **neonatal jaundice**.





The Brain

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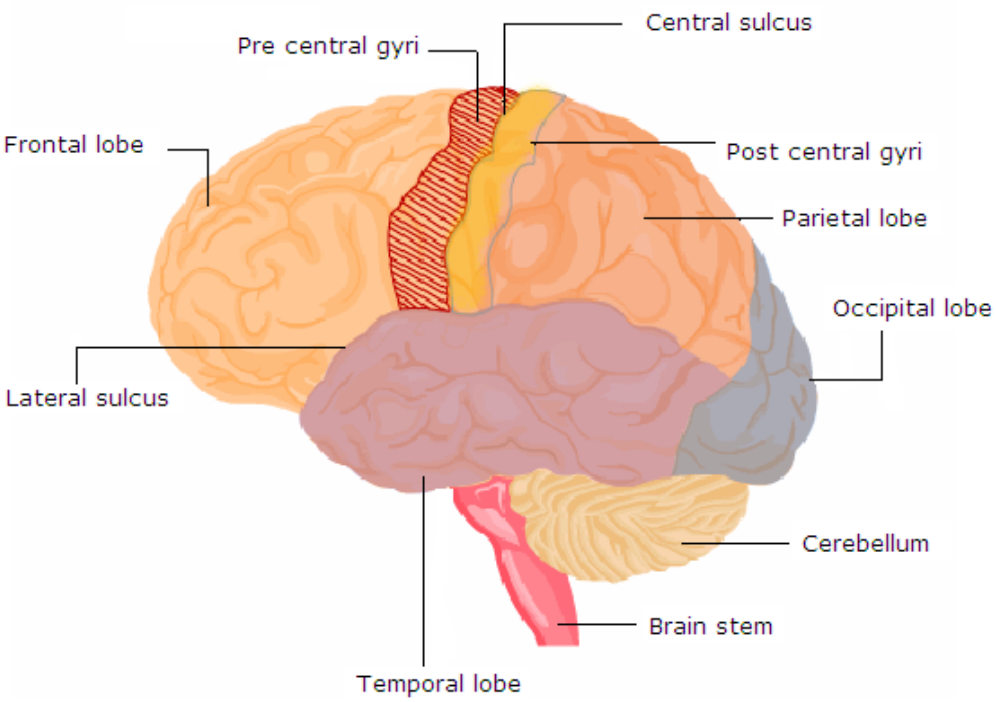
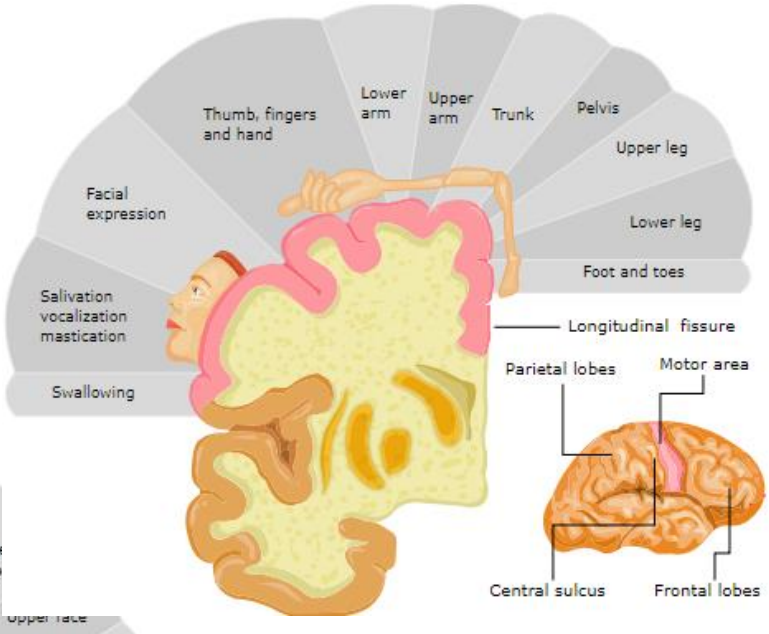
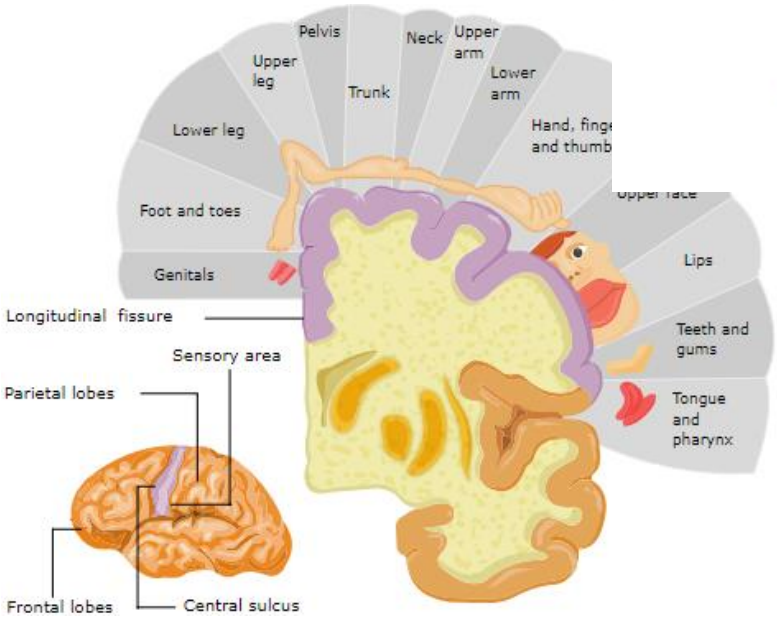


Frontal Lobe

Contains the **pre-central gyri** containing upper motor neurones involved in motor control. This is represented by the **motor homunculus**.





Parietal Lobe

Located posterior to the central sulcus and contains the **post-central gyri** which is the main sensory area. This is also represented by the **sensory homunculus**.

Temporal Lobe

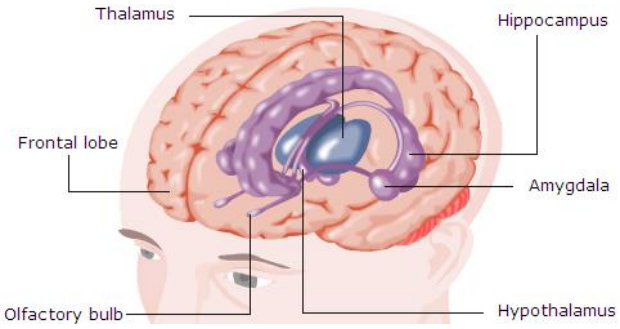
This is located anterior to the occipital lobe and inferior to the parietal lobe. It is involved with the **interpretation** and **association of auditory** and **visual information**. It plays a key role in the **organisation** and **comprehension of language** and in **memory recall and formation**.

Cerebellum

Located just above the brainstem and beneath the occipital lobes sitting at the base of the skull. It is responsible for **coordinating movements, controlling balance** and **posture** and receives afferents from joints, muscles, eyes, skin and the vestibular apparatus.

Limbic System

This is not an anatomically defined region – rather a **functional system** and receives interconnected fibres from the **temporal, frontal lobes**, **hypothalamus** and **thalamus**. It is involved with learning, emotions, behaviour and a variety of endocrine responses via the hypothalamus.



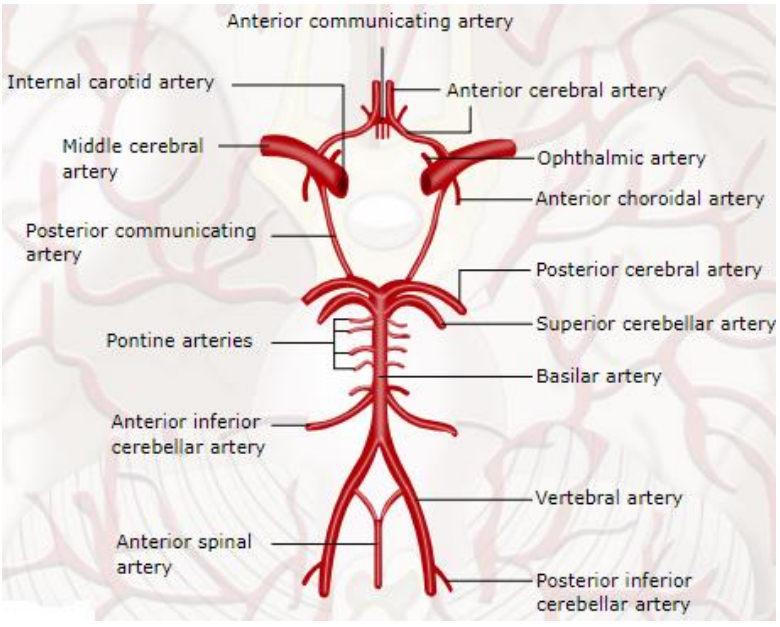
The **hypothalamus** has neural centres for thirst, hunger, body temperature and also helps to regulate fear, sleep, pain, wakefulness, pleasure, sexual arousal, anger and emotions.

Cerebral Blood Flow

Anatomy

Receives blood from **2 internal carotids** and from the **basilar formed from 2 vertebral arteries**.

There is little anastomotic flow between the two sides of the brain. The brain has a blood flow of 750 ml/min and a relatively high oxygen consumption rate when compared to the other organs.



The brain requires **glucose** as the main energy substrate (90%). In starvation, the brain utilises **ketones** as well. The brain has minimal reserves of energy substrates and oxygen so any occlusion of its blood supply can produce unconsciousness in 10 seconds.

Flow throughout the cerebrum is proportional to the area with highest metabolic requirements. Neuronal cell bodies are most metabolically active (grey matter):

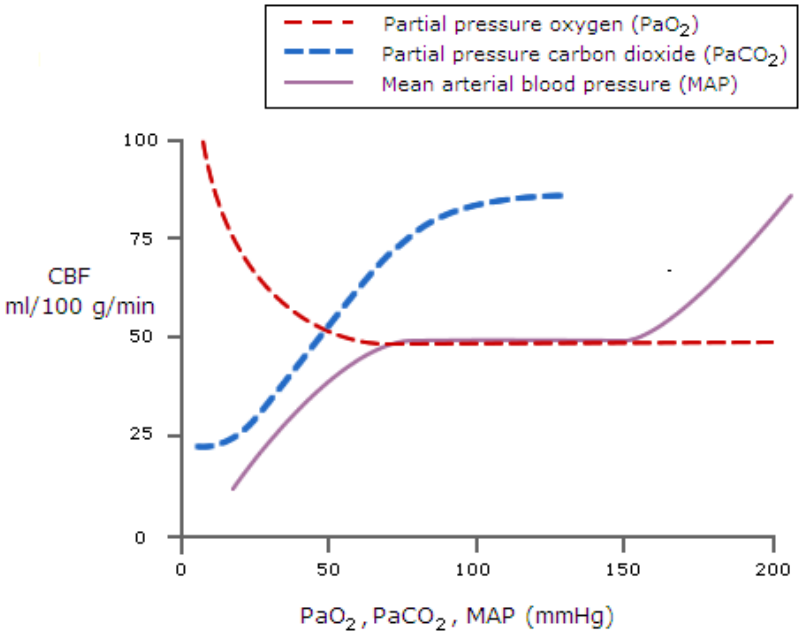
Cerebral blood flow	
Global	45-55 ml/100 g/min
Cortical (mostly grey matter)	75-80 ml/100 g/min
Subcortical (mostly white matter)	20 ml/100 g/min
Cerebral venous oxygen saturation	55-70%
Intercranial pressure (supine)	8-12 mmHg

<u>Autoregulation</u>

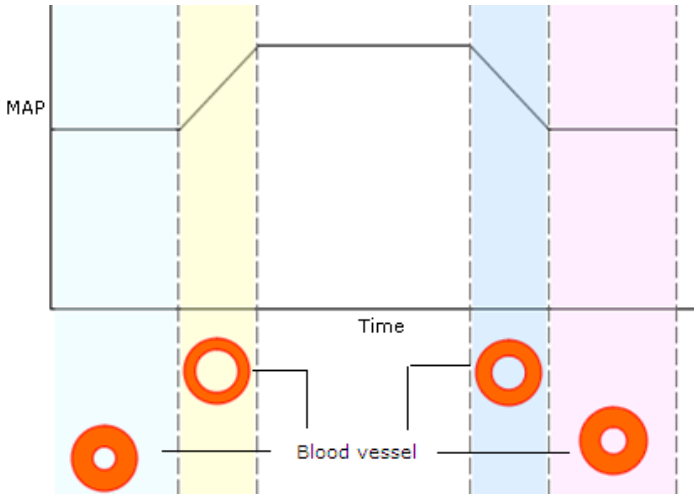
Maintenance of cerebral blood flow across ranging pressures. This occurs through 3 mechanisms:

1. **Mean Arterial Pressure** between 60-150mmHg
2. **$PaCO_2$** the graph is linear over the ranges used in clinical anaesthesia
3. **PaO_2** response is at high levels of O_2 at around 7-8 kPa.

There are 2 predominant theories as to why autoregulation occurs:

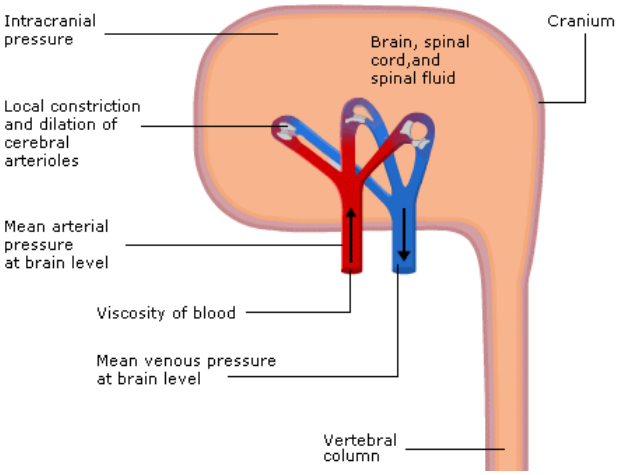


MYOGENIC THEORY: Occurs in response to the **stretching of blood vessels** as the endothelium detects the shearing force → contraction of smooth muscle. This continues until endothelial stretch is reduced back to normal. There is an overall **reduction in cross-sectional area** to return flow back to normal.



METABOLIC THEORY: Blood flow is **maintained** by the extravascular concentration of **metabolic vasoactive substances**. As metabolism of an organ increases, the production of vasodilatory molecules are increased. The subsequent **increased flow** washes out the **vasoactive substances** allowing vessel diameter to return to normal.

Intracranial Pressure



Monro-Kellie Doctrine

The brain and cerebral vessels are **enclosed within a rigid enclosure** and contains:

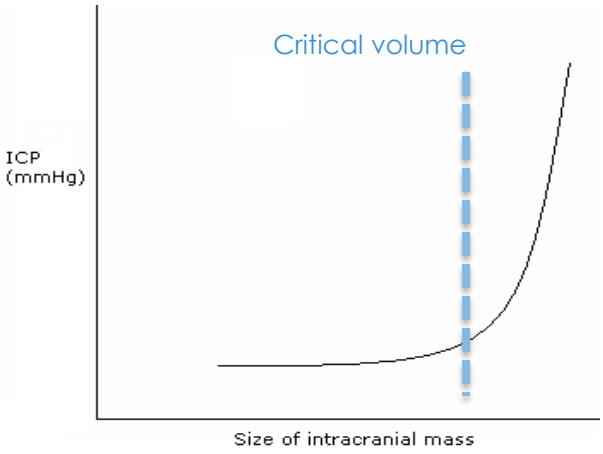
- 75ml of blood
- 75ml of CSF
- 1400g of brain

As these are **incompressible**, the **total volume** of all 3 remains **constant. If one component increases there must be a reduction in another.**

The cerebral perfusion pressure can be calculated from the following equation:

$$CPP = MAP - CVP - ICP$$

With a **space occupying lesion** (**SOL**) the increase in volume is **accommodated** through **fluid shifts out of the cranium**. However, the buffer mechanism change is limited as the **size increases** and **ICP rises** sharply after a point (critical volume). This results in a **reduction of cerebral perfusion pressure**.

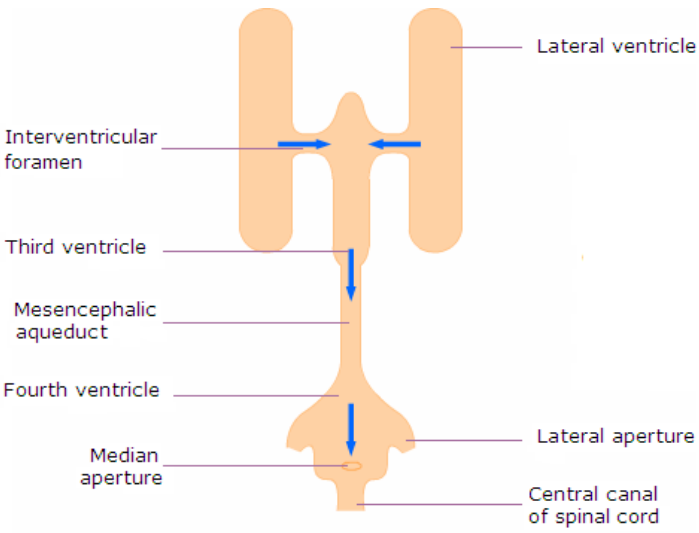


Cerebrospinal Fluid

Produced by the **choroid plexuses** of the lateral, 3rd and 4th ventricles. The rest is produced around blood vessels and ventricular walls. **550ml CSF produced/day**. **Total volume = 150ml.**

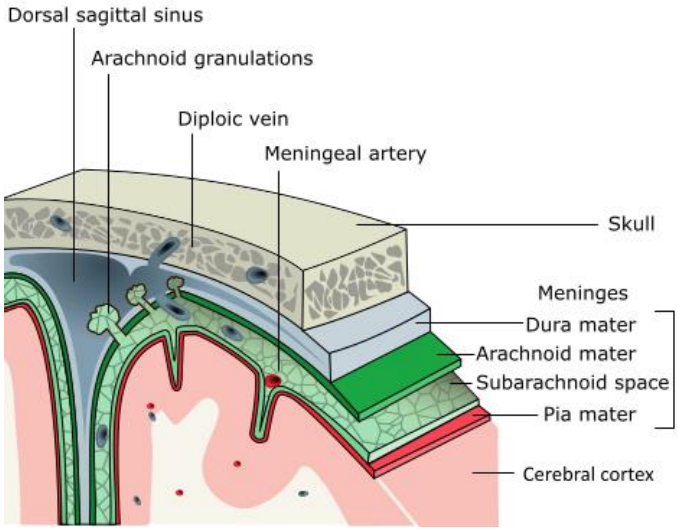
Flow

This occurs in the **subarachnoid space** which surrounds and supports the cranial structures. The CSF composition is identical to brain ECF but differs from plasma slightly with a pH of 7.3.



Substance	Units	CSF	Plasma
pH		7.3	7.4
Na^+	mmol/L	150	145
K^+	mmol/L	2.9	4.8
Ca^{++}	mmol/L	2.3	5.2
Mg^{++}	mmol/L	2.3	1.7
Cl^-	mmol/L	130	110
HCO_3^-	mmol/L	21	26
Phosphate	mmol/L	0.5	1.8
Protein	g/L	<0.4	70
Glucose	mmol/L	3.5	5
Osmolarity	mosmol/L	285	285

The CSF is then **absorbed** through the **arachnoid villi** into the **cerebral venous sinuses** of the brain. The arachnoid villi therefore act as one way valves for CSF drainage into the venous system.

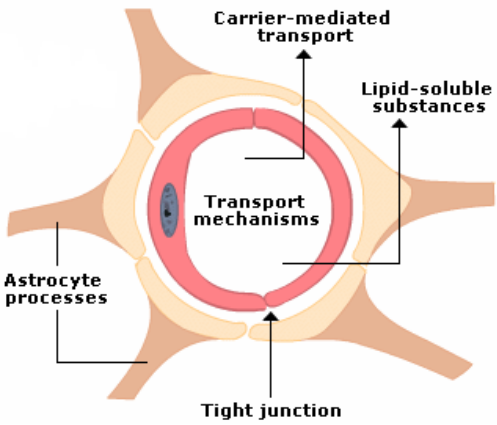


Functions of CSF include:

1. **Protection** along with the meninges. The CSF increases the net weight of the brain from 50g to 1400g.
2. **Chemical Buffer:** Buffers any changes in blood and plasma to protect sensitive neuronal tissue.
3. **Transport medium**: for nutrients, metabolites and neurotransmitters.

Blood Brain Barrier

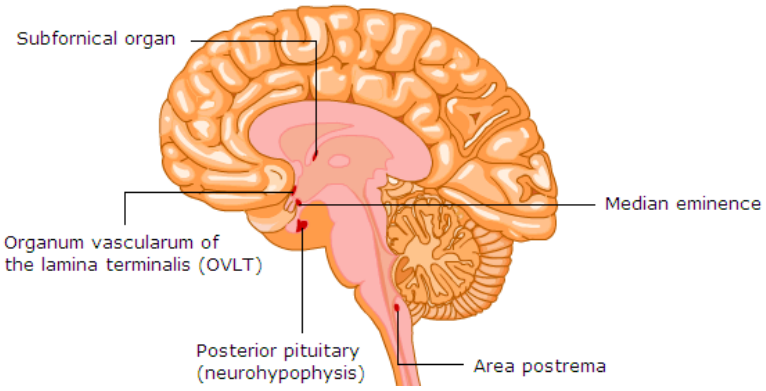
Formed from **specialised continuous capillaries** with extensive tight junctions with 2nm wide gaps between cells. There are **few cytoplasmic vesicles**. Therefore, little vesicular transport occurs but multiple transport mechanisms are present such as GLUT1 to carry hydrophilic solutes across the BBB. As mentioned above, the **foot processes of astrocytes** are also essential for its maintenance and regulation of ionic environment of the CSF.



Outside the Barrier

These areas are collectively known as the **circumventricular organs** and consists of:

1. **Posterior pituitary** and **medium eminence of hypothalamus**
2. **Area postrema**
3. **Organum vasculosum of the lamina terminalis (OVLT)**
4. **Subfornical** organ



These are important sites for communication with the CSF and between brain and peripheral organs through hormones.

Autonomic Nervous System

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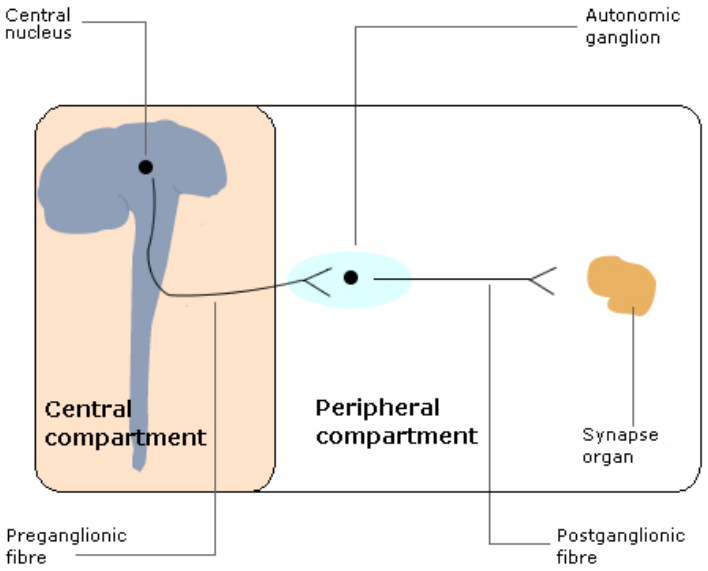
Structure of the ANS

Organised as **reflex arcs** with a sensory limb, integrating centre and a motor limb. The inflow and outflow **travel along the same nerves**.

Both the Sympathetic and Parasympathetic systems share similar organisation but with different locations.

The ANS provides us with **involuntary control** of body function. The SNS and PNS are antagonistic to each other yet complementary.

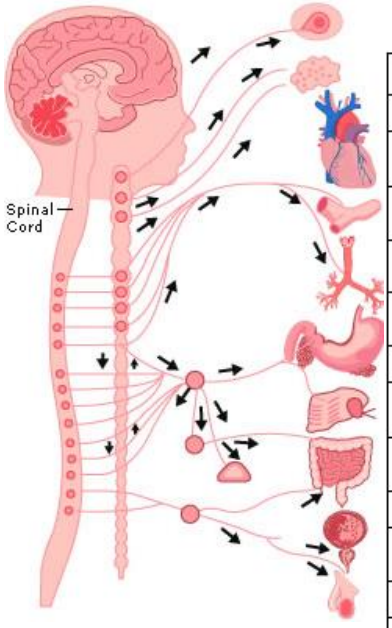


Sympathetic Nervous System

Involved in the **fight or flight** response.

The **preganglionic fibres** originate from the **thoracolumbar segments** of the cord, travel in the **lateral parts of the cord** and consist of **myelinated B fibres**. SNS fibres may pass up or down several segments of the spinal cord before exiting which is important when looking at the effects of a spinal/epidural.

Postganglionic fibres arise from the **ganglia** located on the **sympathetic chain** and are **unmyelinated C-fibres**. Note the adrenal medulla is a modified ganglion and the outflow is from **medullary catecholamines** of adrenaline: noradrenaline 70:30.

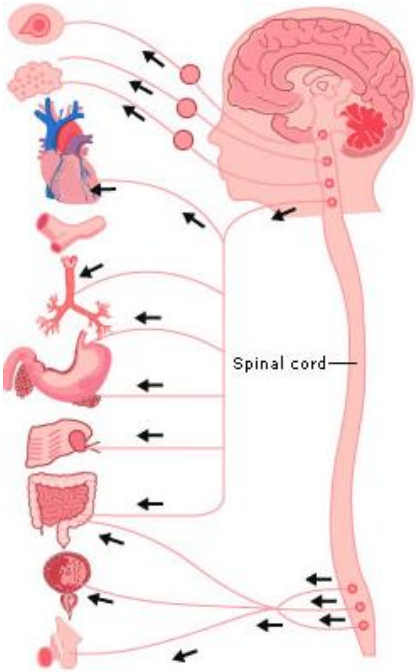


+/-	Action	Affected area
+	Dilates pupil	Eye
-	Inhibits secretion	Sweat and salivary gland
+	Accelerates heart rate	Heart
+	Increases force of cardiac contraction	Heart
-	Constricts most blood vessels	Blood vessels
+	Dilates bronchi	Lungs
-	Inhibits activity	Pancreas, liver, gall bladder
+	Stimulates glucose release	Pancreas, liver, gall bladder
-	Inhibits activity	Stomach
+	Stimulates adrenal secretion	Stomach
+	Relaxes bladder	Bladder
-	Inhibits genitals	Genitals

Parasympathetic Nervous System

Maintains internal organ function whilst at rest. Outflow is described as **craniosacral** with cranial nerves 3, 7, 9, 10 and the sacral outflow. **Preganglionic fibres** arise from the **brainstem** and are **myelinated B fibres**. The ganglia are located close to the effector organs. **Postganglionic fibres** tend to be **short**.

+/-	Affected area	Action
-	Eye	Contracts pupil
+	Sweat and salivary glands	Stimulates secretion of lacrimal and salivary glands
-	Heart	Slows heart rate
	Blood vessels	
-	Lungs	Constricts bronchi
+	Pancreas and liver	Stimulate gastric & pancreatic activity
+	Gall bladder	Stimulates gall bladder
+	Stomach	Stimulates GL motility & secretion
-	Bladder	Contracts bladder
+	Genitals	Stimulates genitals



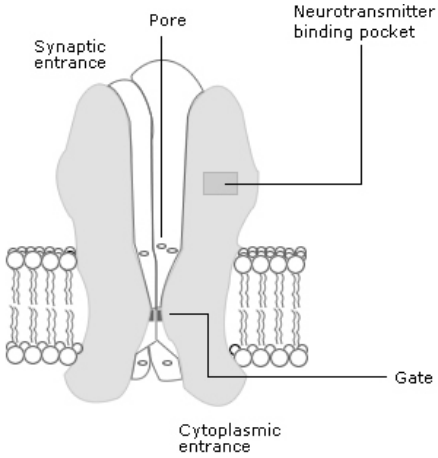
Transmitters

Acetylcholine

The receptors were originally mapped using naturally occurring alkaloids: Nicotine from the *tobacco plant* and muscarine from the hallucinogenic mushroom *amanita muscaria*. These materials have identified **muscarinic (mAchR)** and **nicotinic (nAchR)**.

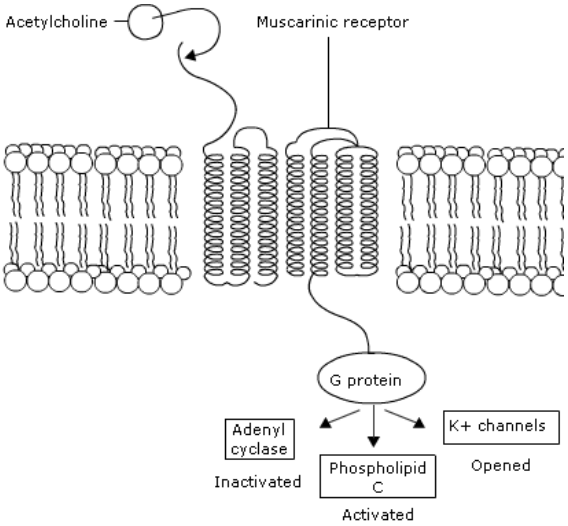
Nicotinic receptors are located in **all autonomic ganglia**.

Muscarinic receptors are located in **postganglionic synapses of the PNS** and in **sweat glands** and **skeletal muscle blood vessels** of the **SNS.**



Nicotinic Receptors

This is a **ligand binding ion channel**. There are 4 subtypes: Skeletal muscle, *CNS pain pathways, Autonomic ganglia* and CNS movement and *cognition pathways*.



Muscarinic Receptors

These are **G-protein receptors** with **5 classes** depending on the secondary messenger ($M_1 \rightarrow M_5$). There are co-transmitters that are released along-side Ach at the postganglionic synapses of the PNS with unknown function.

Noradrenaline

This is released at **SNS nerve endings** and it acts on **adrenergic receptors**:

- **Alpha (α) receptors** – divided into α1 and α2.
  - α2 receptors are presynaptic
  - Increase cellular permeability to sodium – therefore are EXCITATORY
- **Beta (β) receptors –** divided into β1 and β2.
  - Both are postsynaptic (but there is good evidence for a presynaptic β receptor
  - Increases **potassium efflux** from the cell and **chloride influx**, **hyperpolarizing** the cell. This is **INHIBITORY**. In the heart they are excitatory.

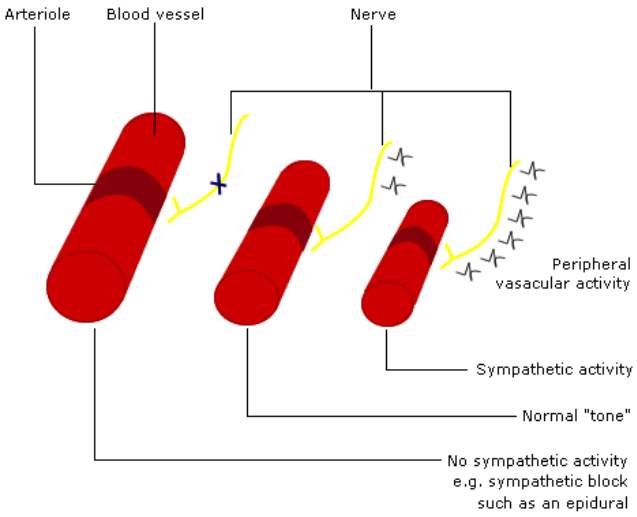
The functions of the ANS are more detailed on the module online.

SNS allows **mild constriction of pulmonary circulation** allowing more even distribution of blood flow across the 3 zones of the lung. **PNS** *has no effect on the pulmonary circulation*.

PNS has greater activity on the resting heart rate as no ANS input – intrinsic HR = 100-120.

Peripheral Vasculature

They receive **α1 sympathetic stimulation** which induces vasoconstriction. The **sympathetic tone** determines the **controlled diameter** of the vessels and provides **peripheral vascular resistance**. Although it is mediated by noradrenaline, other neuropeptides are also released – neuropeptide Y1, purines and ATP whose receptors are located on smooth muscles and therefore contribute to SNS effects.



Neurological Reflexes

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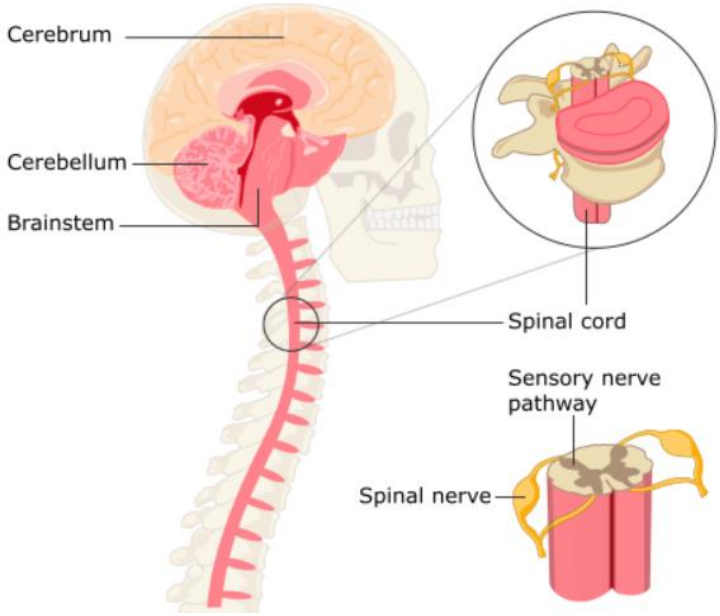
Musculoskeletal System

Provides support, stability and movement for the body. Muscles act usually in pairs across each joint with opposing actions, this is provided through a complex system of neuronal feedback and reflexes.

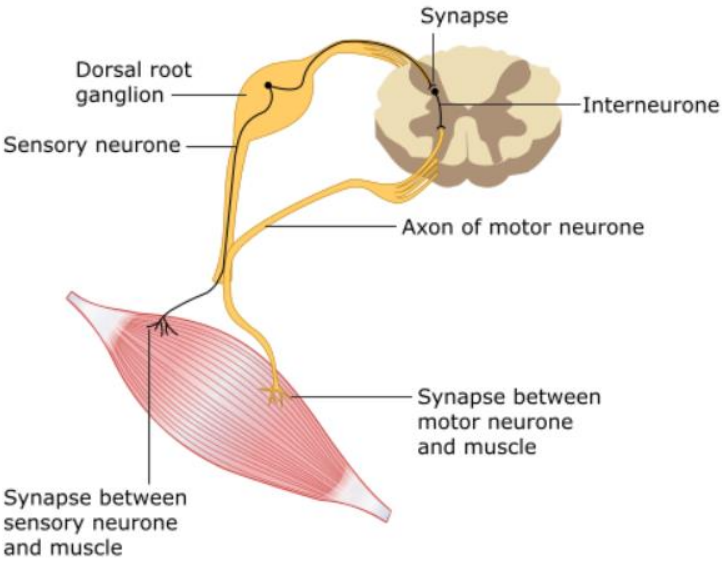
There is a **hierarchy of neuronal systems** of increasing complexity cephalad to achieve smooth coordinated movement:

1. **Cerebrum:** providing complicated motor skills
2. **Brainstem and Cerebellum:** Postural control, proprioception and maintenance of balance
3. **Spinal cord:** Integrates sensory and motor inputs and allows simple spinal reflexes.



Reflexes are controlled within the **grey matter** of the spinal cord. The main components are:

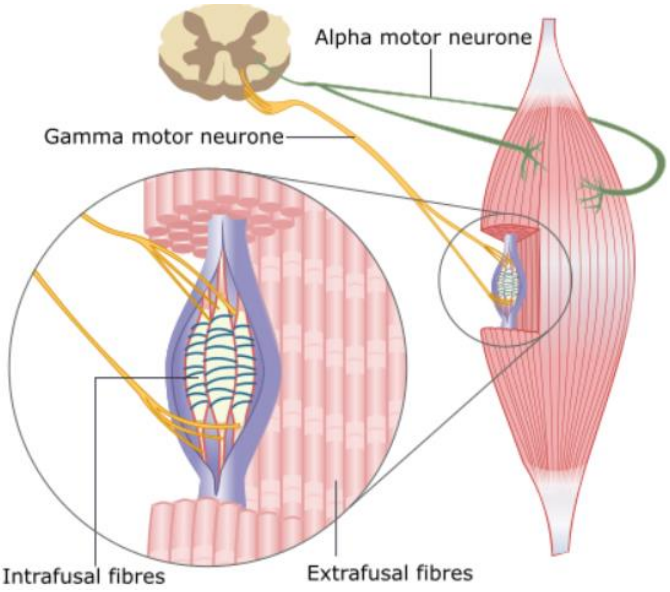
- **Sensory neurones** – Large diameter type Ia and Ib fibres. Cell body is in the dorsal root ganglion and fibres enter the spinal cord in the **dorsal horn**.
- **Interneurones** – Intermediary connection between the sensory and motor neurones. There are large number of these to allow interaction with descending tracts and other neurones enabling integration of the MSK system.
- **Motor neurones** – Large diameter alpha fibres connecting directly to the main skeletal muscle body.



*NB some sensory neurones connect directly with motor neurones (**monosynaptic**) e.g. the Patella reflex.*

Muscle Fibre Types

EXTRAFUSAL FIBRES: These are the main group making up the contractile unit. A group of fibres innervated by a single **alpha (α) motor neurone** is called the **motor unit.**

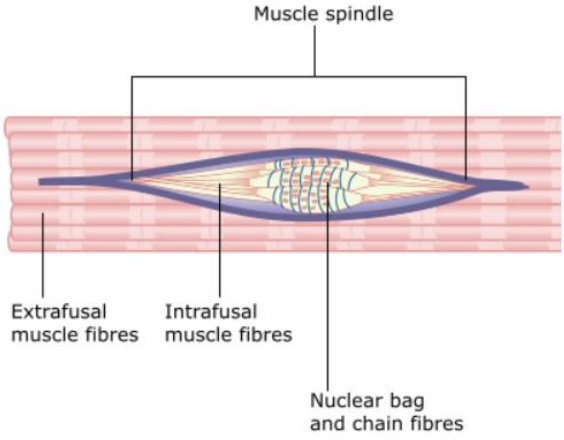


INTRAFUSAL FIBRES: These are part of the **muscle spindle** and are innervated by smaller **gamma (γ) motor neurones**

Muscle Sensory Units

MUSCLE SPINDLES: Convey information concerning **actual** and **changes in the length and tension** of muscle fibres to the CNS. They **respond to stretch** and stimulate **contraction** of the extrafusal fibres to **maintain the length of the muscle**.

GOLGI TENDON ORGANS: Senses **change in muscle tension**. Each Golgi tendon organ is innervated by a single afferent type Ib myelinated axon.

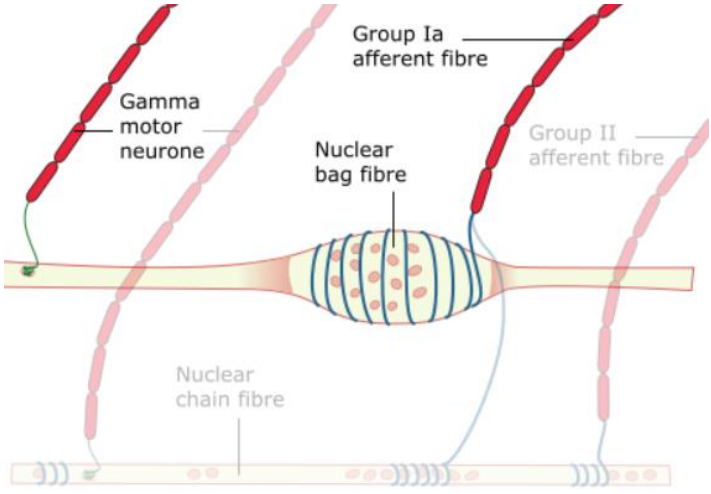


Muscle Spindles

As these signal actual and change in length of muscle fibres, they are found mainly in muscle groups providing posture and fine movement i.e. hand, foot, neck. They also help smooth voluntary movements and allow coordination of movement.

Each muscle spindle contains up to **10 intrafusal muscle fibres** enclosed in a connective tissue capsule. They lie ***in parallel*** to the extrafusal fibres and attach to them or the tendons. The 2 types of intrafusal fibres include:

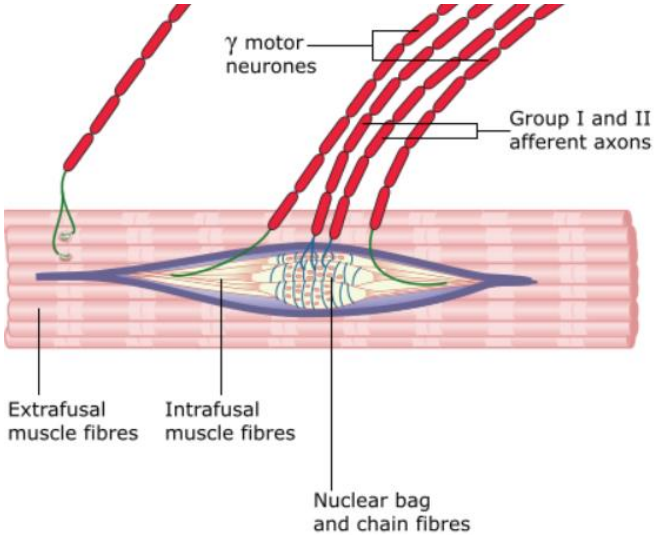
1. **Nuclear Bag Fibre**: Dilated central part involved in the dynamic and static response to stretch of the muscle. They lie in the centre of the muscle spindle
2. **Nuclear Chain Fibre**: Thinner and only involved in the static response to stretch of the muscle.



Motor Supply

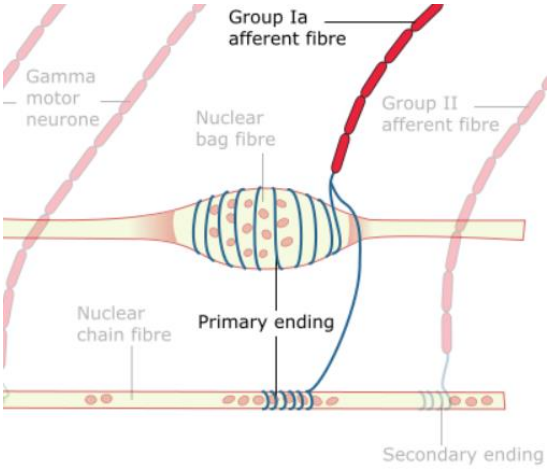
The centre of intrafusal fibres are non-contractile whilst the ends are contractile. They are supplied exclusively by **gamma (γ) motor neurones** which **regulate how sensitive the spindle is to stretch** by **tightening or relaxing the fibres** within the spindle.

For example, descending fibres of the facilitatory reticular formation causes **γ motor neurone activation.** This stretches the central portion which **increases sensory output** leading to **activation** of **spinal reflexes** and **increased muscle tone**.



Sensory Supply

Found in the central region of the muscle spindle and there are 2 types:

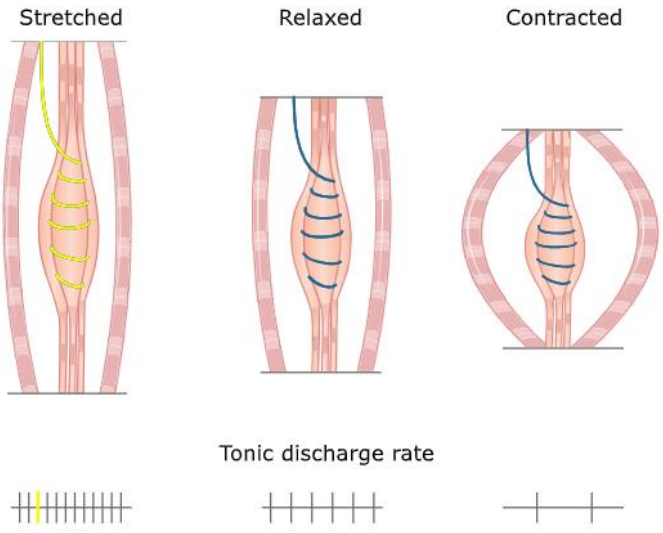


- **Primary (annulospiral) nerve endings:** Large Ia fast fibres encircling both nuclear bag and chain fibres responding to actual and rate of change in length of the intrafusal fibre.
- **Secondary nerve endings:** Smaller II fibres predominantly at the ends of nuclear chain fibres only sensitive to the actual length so contribute only to the sense of the position.

Overall Function of the Muscle Spindle

Continuously signals muscle length to the CNS. Both primary and secondary fibres discharge tonically with a rate dependent on the muscle length.

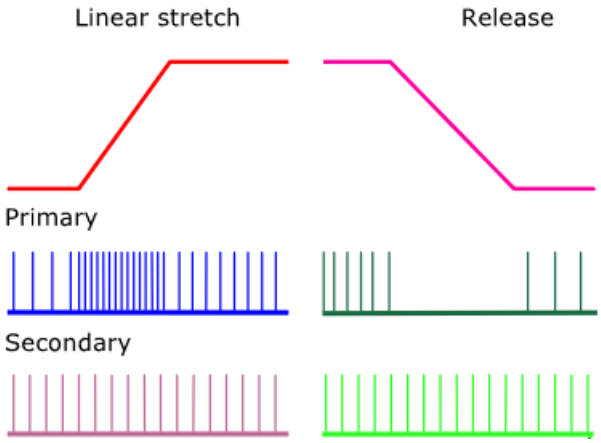
The amount of tension in the muscles depends primarily on the resting rate of discharge of a-motor neurones which itself is mainly influenced by the rate of discharge of the primary annulospiral (Ia) spindle afferents. Gamma motor neurones are also involved through their action on intrafusal muscle fibres.



DYNAMIC/PRIMARY RESPONSE:

Primary annulospiral endings increase their background rate of discharge when there is a **change in length** (increased rate of stretch) of the muscle spindle.

This triggers **contraction of extrafusal fibres** proportional to the rate of stretching allowing **reduced stretch**. On release of stretch from muscle contraction, rate of primary ending discharge is reduced.



STATIC/SECONDARY RESPONSE:

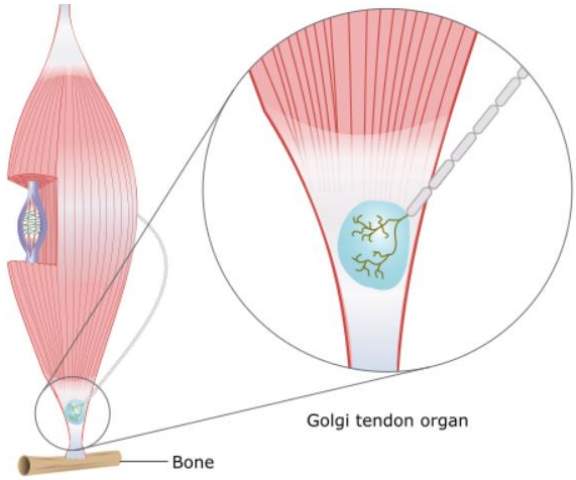
This is a weaker more sustained response to the **actual length of the muscle** and discharges as long as the new length is held. This is done mainly through the **secondary nerve endings** on nuclear chain fibres. NOTE, there is NO dynamic response.

Golgi Tendon Organ

Located in the **tendons of muscles** and are **in series** with extrafusal muscle fibres (unlike intrafusal fibres which are in parallel).

When the **tendon is stretched** (i.e. increased tension) the Golgi tendon organ is squeezed and **activated**. This **inhibits motor neurone activation** via an inhibitory interneurone in the spinal cord and relaxes the same extrafusal muscle. This aids to **protects from overstretching damage**.

This is the opposite action to muscle spindles.

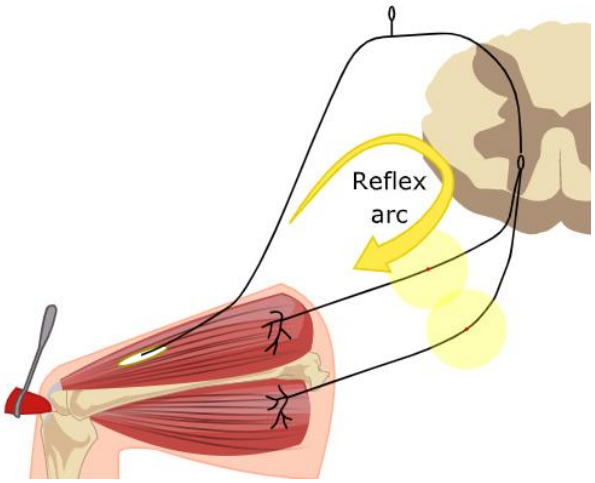


Muscle Stretch Reflex

This is a **monosynaptic reflex** in response to muscle stretching providing automatic regulation of skeletal muscle length. No interneurones/CNS influence means it is a rapid reflex over a few ms.

1. **Muscle lengthens** stretching the muscle spindle.
2. **Sensory afferents** synapse with the a-motor neurone
3. **Extrafusal muscle contraction**.

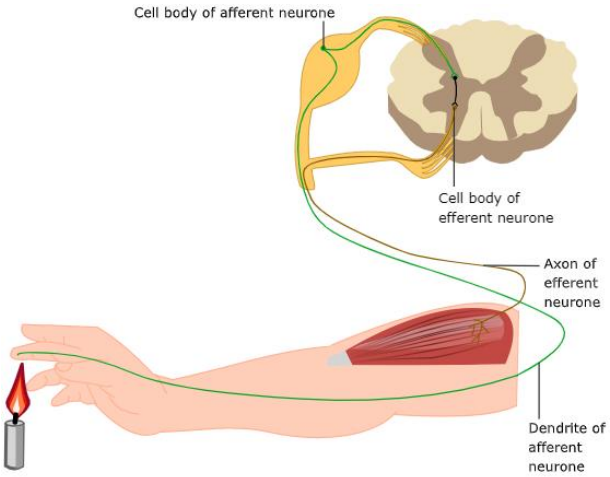
Reciprocal inhibition occurs via inhibitory interneurones allowing relaxation of the antagonistic muscle.



Nociceptive/Flexor/Withdrawal Reflex

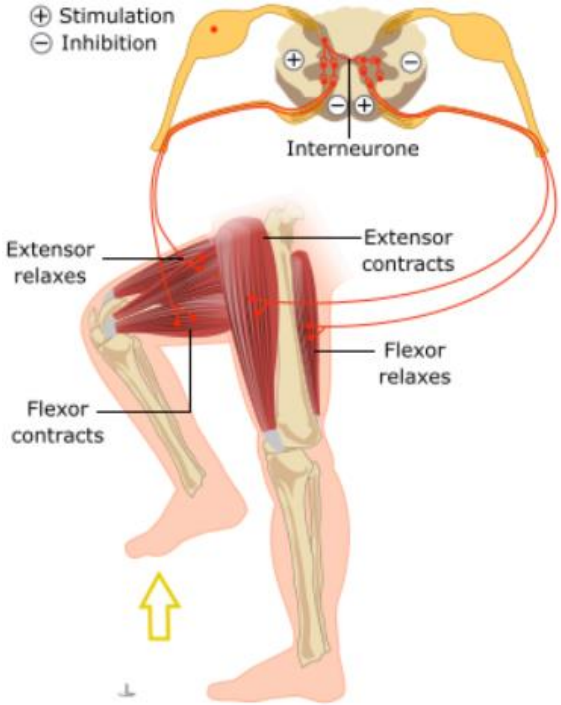
A **polysynaptic reflex** that protects the body from damaging stimuli.

1. Noxious stimulus excites the **nociceptor**
2. Primary sensory neurone synapses with an **interneurone**
3. Interneuorne synapses with an **a-motor neurone** which **excites the flexor muscle** withdrawing the limb.



Crossed Extensor Reflex

Triggered in conjunction with the flexor reflex. The spinal interneurone also connects with the **contralateral a-motor neurones** to initiate contraction and extend the limb maintaining posture and balance. The typical example is that of someone stepping on a pin:



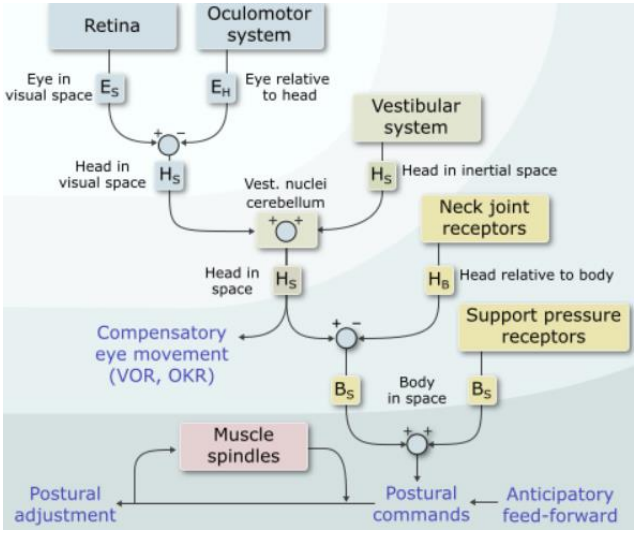
Feedback Loops

These are vital with maintaining tone and joint position and requires input from higher centres to allow the coordination of muscle groups. The interconnectivity between different centres is very complex.

Conscious movement is initiated by the motor cortex with signals running via the corticospinal tract. These synapse with interneurones to regulate activation of a-motor neurones. The cerebellum contributes to coordination, precision, and accurate timing of movement.

Hierarchical system of perception exists to provide modulation of motor function. The following diagram describes the strength of influence of each input. However, strong stimulation from one area may cause loss of balance even if the other inputs are working well.

The final focus of these sensory inputs and the corticospinal tract is the interneurones which relay instructions through activation of the a-and γ-motor neurones to initiate muscle movement and modulate spindle reactivity (muscle tone) respectively. This is why in a CVA, the central modulation is lost resulting in spastic hypertonia.



Pain Pathways and Mechanisms

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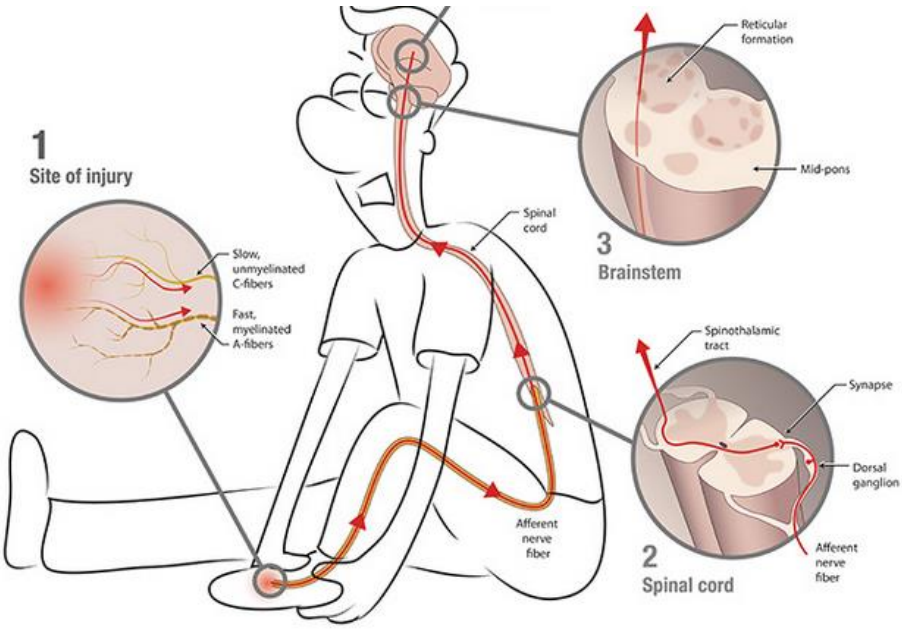
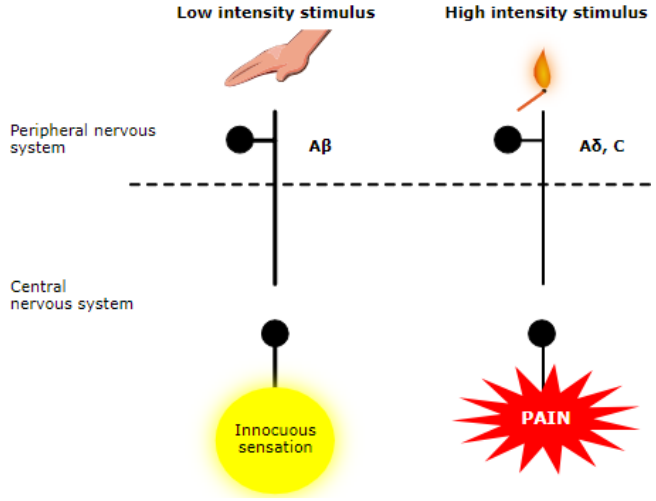
Pain: An **unpleasant *sensory*** and ***emotional* experience** which we associate with actual or potential tissue damage. In the classical description, ***Acute*** is <3 months) and ***Chronic*** is >3 months). More recently, acute pain refers to pain associated with a specific amount of trauma and chronic pain refers to pain continuing when the original injury is no longer acting. It may be **nociceptive** pain (nerves intact) or **neuropathic** pain (damage or dysfunction to any element of the nervous system). Pain is a **subjective measure**.

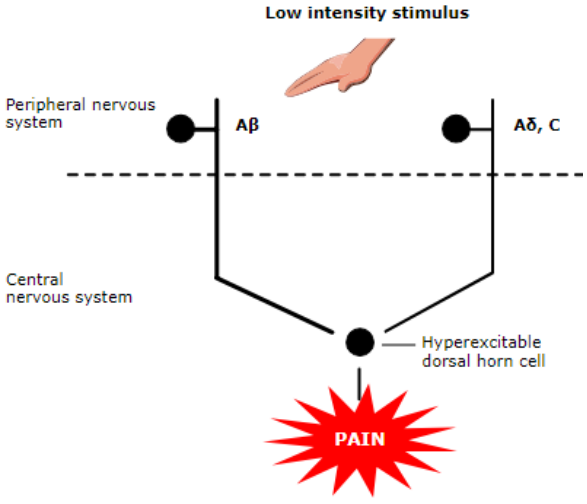


Pain is perceived when **nociceptors** are stimulated to an extent that could cause potential or actual tissue damage. This activates **mixed nerves** and the impulse enters the spinal cord via **dorsal root** and cross to the spinothalamic tract.

Physiological Pain

Physiological pain is produced with a **high intensity stimulus** and travels down **Aδ and C fibres.** Low intensity stimuli travels down Aβ fibres through a different route.





Clinical Pain

Clinical pain differs from physiological pain where even **low intensity stimuli** can stimulate Aδ and C fibres together with Aβ inducing pain.

We will now go through 4 areas that determine the differences between physiological and clinical pain.

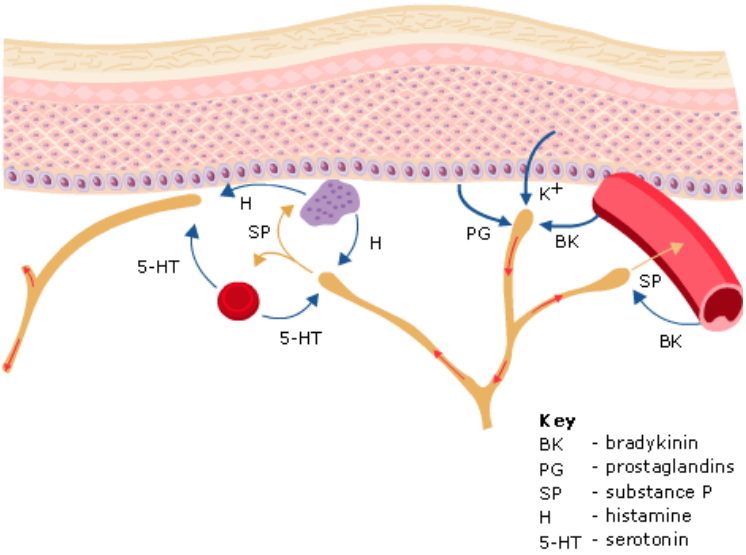
Peripheral Mechanisms

The primary **afferent nociceptors** are 2 types:

Pain fibre	Aδ myelinated	C unmyelinated
Receptor Type	Mechano-thermal	Polymodal
Conduction Velocity	0.5-2 m/s	6-30 m/s
Type of Pain	Short-lasting	Dull, burning

Peripheral Sensitisation

The process where **chemical** and **inflammatory** factors can **sensitise peripheral nociceptors** to **lower their threshold** in eliciting a painful response from a reduced stimulus. The inflammatory processes that contribute are described:

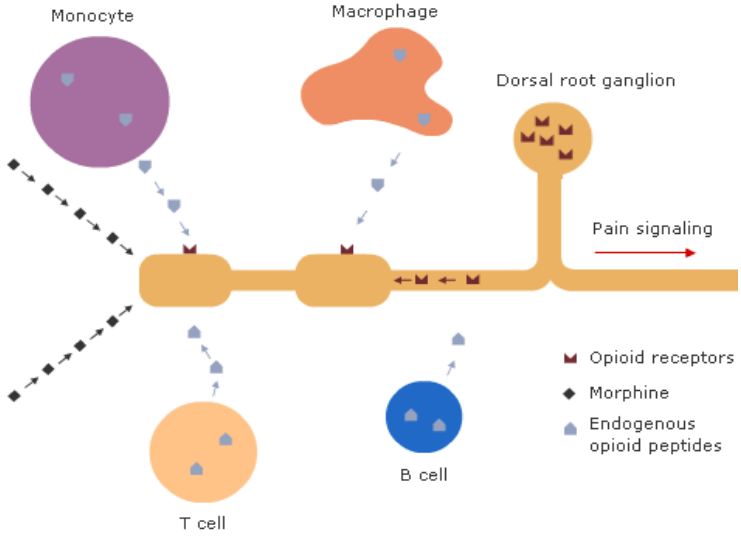


1. **Direct Activation:** Nociceptor is activated by cell damage which triggers the **release of potassium & bradykinin** and the **synthesis of prostaglandins** → increases the sensitivity of the terminal (lower threshold nociception)
2. **Secondary Activation:** The impulses **propagate to nearby terminals** which **stimulates release of substance P** → Vasodilation and further release of bradykinin and now the **release of histamine and serotonin**.
3. **Spread of Hyperalgesia:** Histamine and serotonin levels increase and subsequently **sensitising nearby nociceptors**.

Now, low intensity mechanical stimuli are perceived as painful with an increased response to thermal stimuli at the site of injury and a zone of primary hyperalgesia surrounds the injury site due to peripheral changes as described above. This is common **following surgery** or **trauma**.

Peripheral Action of Opioids

In response to **tissue damage** – there is **increased opioid receptor production** in the **dorsal root ganglion** pain fibre cell bodies. These are **passed peripherally** and are acted on by **endogenous opioids** that are **released by inflammatory cells.** Exogenous opioids i.e. morphine also act in the same way.

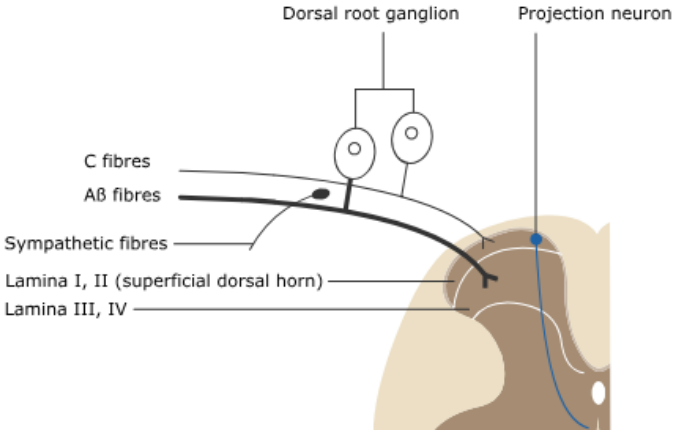


Changes following Nerve Injury

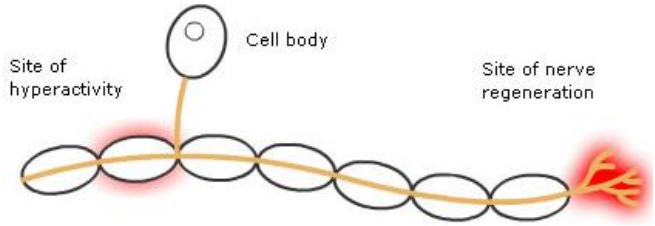
Damage of a nerve causes **ectopic firing** at the site of injury or close to the dorsal root ganglion.

Sympathetic nerve fibres sprout around the DRG.

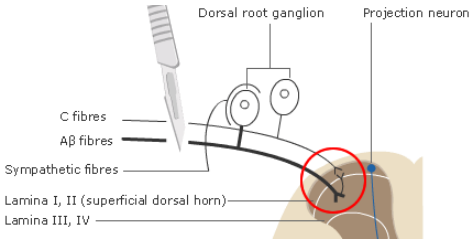
Large diameter afferent nerves sprout into the superficial dorsal horn. Dorsal horn cell bodies undergo **ectopic firing** that has lost normal afferent input.



Ectopic Discharge may therefore occur in a site of **nerve regeneration** in the periphery (regenerated nerves also have increased sensitivity). **Near a cell body in the dorsal horn** and/or at an **area of demyelination**.



Sympathetic Nervous System plays an important role in generation and maintenance of chronic pain states. Its fibres sprout around the DRG and features:



- **Allodynia** (pain to non-painful stimuli)
- **Hyperalgesia**
- **Spontaneous burning**
- Changes reflecting **atrophy of various systems** i.e. osteoporosis, hair and nail growth, swelling and abnormal sweating.

The Dorsal Horn

Where primary **afferent nerve fibres synapse with ascending afferent neurons** in the pain pathway. At this point, there are important points to consider:

- The ability for drugs to work at this point
- Process at which **central sensitisation** occurs
- **Descending modulating influences** from higher areas to modulate afferent input.

Sensory Processing Nerve Groups

There are 2 types:

Nerve	Terminates	Responds to
Nociceptive-specific	Superficial laminae (I & II)	Noxious stimuli
Wide Dynamic Range	Deeper laminae (IV & V)	Noxious & non-noxious stimuli

The wide dynamic range group will discharge at a high rate in allodynia

Neurotransmitters and Modulators

The primary afferent nerve releases a *variety* of neurotransmitters in the DRG to activate the post-synaptic cell. **Glutamate** plays a major role acting at NMDA and non-NMDA receptors i.e. AMPA. Other substances involved in nociception include:

- **Substance P**
- **Neurokinin A**
- **CRGP**

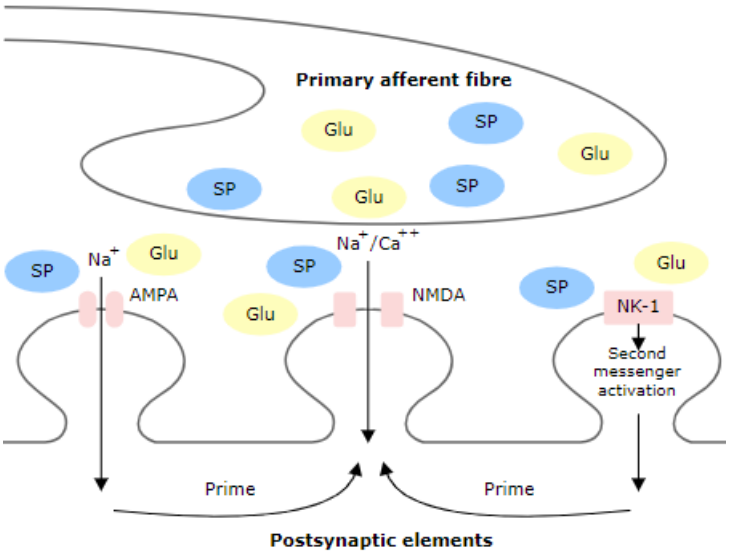
} Act at neurokinin receptors

Opioid, GABA, catecholamines and **glycine** all modulate the DRG nociceptive transmission.

Glutamate and substance P is released from afferents to activate the appropriate receptors allowing **Na^+ influx** and **secondary messenger activation**. This primes the NMDA receptor by **removing its Mg^{2+} plug** allowing **Na^+ and Ca^{2+} influx.**

NMDA receptor activation produces secondary events in the cell increasing the nociceptive responsiveness of the system.

Any sustained activation of the NMDA receptor directly or indirectly is associated with chronic pain states and is therefore of importance in development of clinical pain.



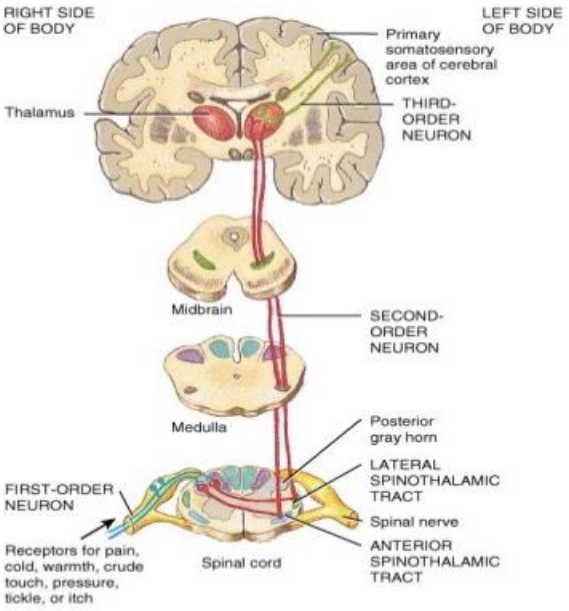
Central Sensitisation: Describes the processes that occur in the **dorsal horn** following injury. Unlike peripheral sensitisation, there is no change in response to thermal stimuli. It is also more complex with a wind up of spinal cord activity rather than a simple stimulus-response relationship and is **dependent on the NMDA receptor**. Seen as an increase in the response of dorsal horn neurons to repeated stimuli. It also causes:

- An expansion in the receptive field size of peripheral neurons
- Increase in magnitude and duration of response to above threshold stimuli
- A reduction in nerve threshold

Ascending Tracts

At the level of the **spinal cord**, the majority of nerves cross to the contralateral side before ascending in the anterolateral quadrant of the spinal cord. A few however remain ipsilateral. These second order neurons terminate in many **supraspinal structures** in the brain stem and thalamus which determines their nomenclature:

Fibres	Terminate	Involved in
Spinoreticular tract	Brain stem nuclei	Descending modulation, arousal, motor and autonomic reflexes
Spinomesencephalic tract	Midbrain and periaqueductal grey matter	Descending modulation, autonomic reflexes and the integration of responses to pain
Spinothalamic tract	Thalamus - lateral	Sensory discriminatory component of pain
	Thalamus - medial	Affective and motivational aspects of pain



CORTEX: It is seen through fMRI that **parietal** regions have involvement in **temporal and spatial features** of pain whilst the **frontal** involves the **emotional response** of pain.

Descending Modulation

The ascending passage of impulses from painful stimuli to the supraspinal structures and cortex are responsible for the powerful descending modulatory effects which act at **many levels of the pain pathway.** It may also be activated with external factures i.e. acupuncture, stress.

They arise from **multiple supraspinal structures** and the **descend in the dorsolateral funiculus** releasing a number of transmitters including **noradrenaline, serotonin** and **opioids**.

Whilst ascending pathways provide positive feedback through peripheral and central sensitisation, descending pathways can be thought of as a negative feedback system.

Visceral and Neuropathic Pain

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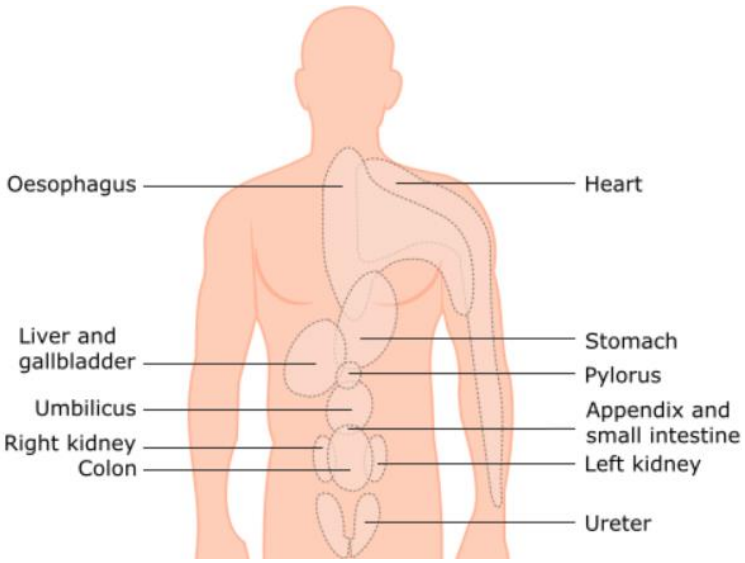
Visceral pain

Arises from internal organs and are innervated by **unmyelinated fibres** running alongside the PNS and SNS fibres.

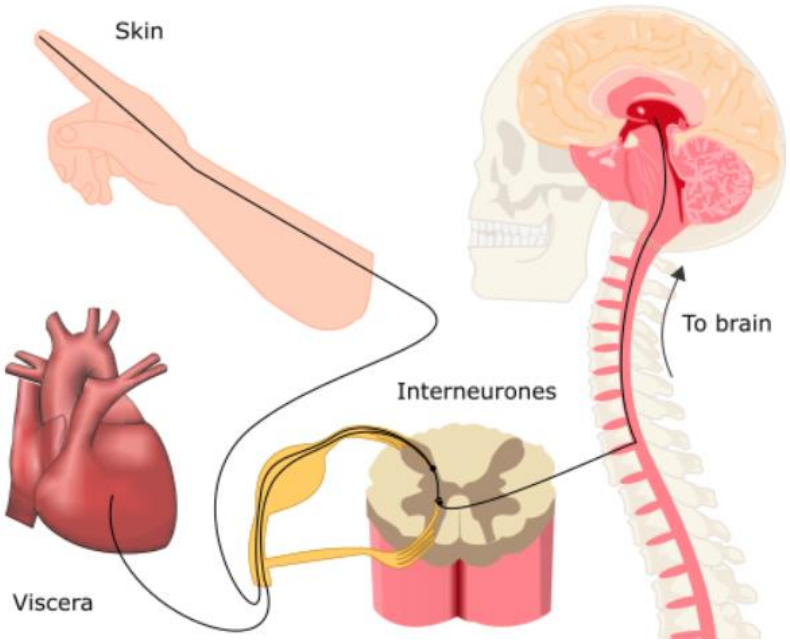
Primary visceral afferent nerves terminate primarily in **lamina I and II** of the dorsal horn of the spinal cord via the pre and paravertebral ganglia of the sympathetic trunk. There are also branches terminating in areas which regulate autonomic outflow.

There are **second order spinal afferents** which are subsequently stimulated (also from other sensory modalities) and leads to an ill-defined localisation of pain frequently perceived as being in the mid-line (*see diagram right*).



Visceral Afferent Convergence

This is when **second order neurones** receive **both visceral and somatic input**. They respond equally to activation from both sources without distinction which gives rise to **referred pain**. The location of somatic pain from visceral stimulation is usually associated with the visceral location as an embryo. For example, the heart originates in the neck and upper thorax, hence the visceral afferents enter the spinal cord along the SNS sensory nerves from segments C3 to T5.



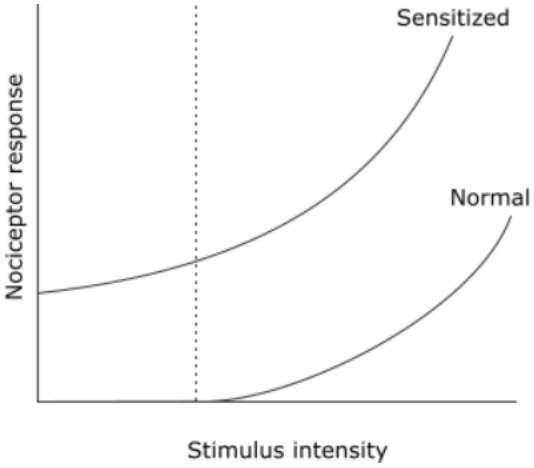
Somatic vs Visceral Pain

Somatic Pain	Visceral pain
Highly localized	Poorly localized
Highly differentiated	Poorly differentiated
Intensity proportional to tissue damage	Poor correlation between intensity and damage
	Associated with autonomic symptoms

Sensitisation

An increase in the magnitude of the response to a particular level of stimulation. Visceral hypersensitivity has 3 causes:

- **Primary visceral afferent** peripheral sensitisation
- **Ascending spinal neurone** hyperexcitability – *central sensitisation*
- **Dysregulation** of **descending pathways** that modulate nociceptive transmission.



Most afferent activity exists as reflex arcs but in sensitisation, below normal threshold stimulation may reach higher centres to cause the sensation of pain.

Central Sensitisation Syndrome (CSS)

Hyperalgesia and **allodynia** are the clinical manifestations where an inappropriately high response to a specific degree of stimulation occurs. Goth genetic polymorphisms (COMT, 5HTT, ADRB2) and environmental factors are implicated in its development. **NMDA** is thought to play a particular role in mediating persistent pain and hyperalgesia in the spinal cord.

Treatment of Visceral Pain

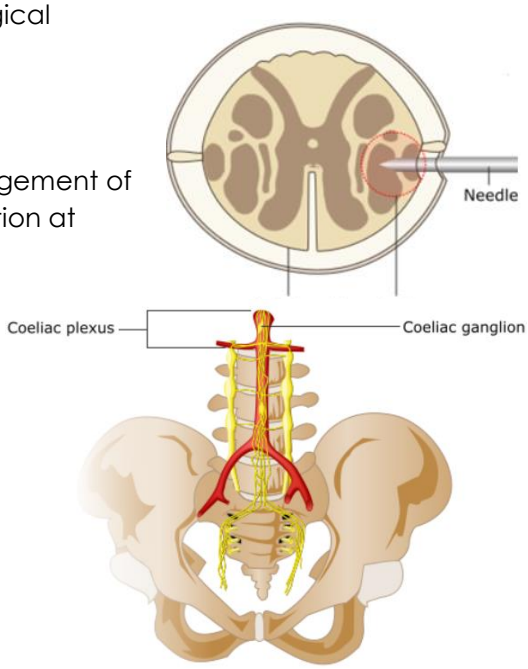
The main strategies can be divided into the following 3:

Pharmacological management: WHO analgesic ladder is used. Underlying management of cause i.e. cardiac or GORD or GI spasms is also required. With CSS (**chronic visceral pain**), antidepressant drugs i.e. TCAs, SNRIs and SSRIs work well. This is thought to be due to serotonin and noradrenaline enhancing descending pain inhibition and not due to depressive management.

Psychological management: Psychotherapy, CBT, hypnotherapy improve symptoms in patients with IBD. Making the patient aware of the psychological impact on pain is important.

Interventional management: This can be divided into 2:

1. **Percutaneous Cervical Cordotomy:** Indicated in management of severe unrelenting unilateral pain due to cancer. Ablation at C1/2 will lose pain and temperature sensation on the contralateral side below C4. Dorsal columns remain intact.
2. **Sympathetic Blocks:** Commonly involves the:
    a. Coeliac plexus located anterolaterally to L1 posterior to the pancreas. This receives visceral afferents from the greater, lesser and least splanchnic nerves and vagus nerve from the organs of the upper abdomen.
    b. Superior hypogastric plexus block

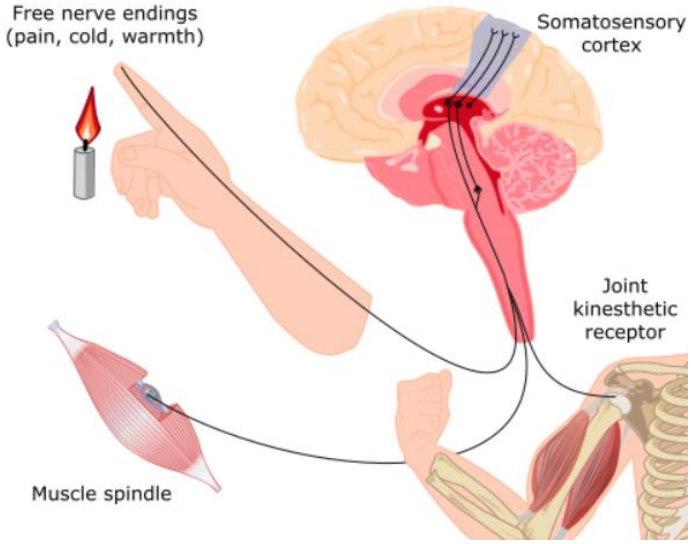


Neuropathic Pain

This is defined by the International Association for the Study of Pain (IASP) as:

*"**Pain arising as a direct consequence of a lesion or disease affecting the somatosensory system**"*

Thought of as a process of abnormal activation of the pain processing pathways which are shown in the diagram.



Symptoms

These are very diverse but can be divided into

Positive symptoms: abnormally increased sensation:

Symptom	Definition
Hyperaesthesia	Increased sensitivity to a stimulation.
Hyperalgesia	Increased response to a normally painful stimulation.
Dysaesthesia	Unpleasant sensation resulting from a stimulus which would not normally be unpleasant.
Allodynia	Painful response to a stimulus which would not normally be painful.
Paraesthesia	Abnormal sensation which is neither unpleasant nor painful: often tingling, prickling or heat sensation.
Hyperpathia	Abnormal response to a normally painful stimulation. Altered location, identification, radiation and after sensation may be present. May be a positive or negative symptom.

Negative: abnormally decreased sensation

Symptom	Definition
Hypoaesthesia	Decreased sensitivity to stimulation.
Hypoalgesia	Decreases sensitivity to normally painful stimulation.
Hyperpathia	Abnormal response to a normally painful stimulation. Altered location, identification, radiation and after sensation may be present. May be a positive or negative symptom.

Physical Signs: These may occur even when pain is not present:

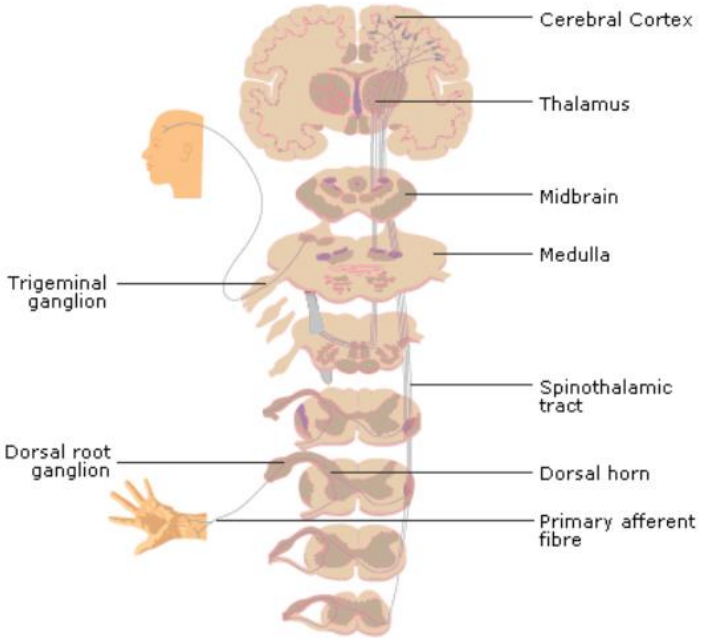
Physical sign	Examples
Tropic changes	Hair changes, skin thickening.
Vasomotor	Temperature, colour change and oedema.
Sudomotor	Sweating changes.
Musculoskeletal	Muscle wasting, osteopaenia.

Pain descriptors are usually different to somatic pain using terms such as burning, tingling, itching, pins and needles, shooting or electric shock-like.

Types of Neuropathic Pain

Central Neuropathic Pain (CNP): Lesion or disease in the CNS anywhere in the spinothalamo-cortical pathways (imaged right). Stroke, MS and Spinal cord injury are the most common causes. CNP always occurs within an area of altered sensation and spinothalamic dysfunction is almost always present.

Peripheral Neuropathic Pain: Lesion or disease in the PNS including cranial nerves, spinal nerve roots etc. The change in function occurs in the distribution of the affected nerve.



Development of Neuropathic Pain

CNP development is not well understood but many mechanisms as shown in the table may result in **disinhibition, sensitisation and neuroplastic maladaptive changes in the CNS**.

Mechanism	Example
Vascular	Central post stroke pain – infarction or haemorrhage
Traumatic	Spinal cord injury Surgical lesions of the brain or cord Compression myelopathy
Autoimmune	MS
Congenital	Syringomyelia
Neoplastic	Neoplasms of the brain or spinal cord
Metabolic	Subacute combined degeneration of the cord

Peripheral neuropathic pain is caused by damage to the peripheral nervous system through different mechanisms:

Mechanism	Examples
Metabolic	Diabetic peripheral neuropathy Thiamine deficiency
Autoimmune	Guillian-Barré syndrome
Traumatic	Phantom limb pain Avulsion of nerve roots in the brachial plexus
Ischaemic	Critical limb ischaemia
Toxins	Chemotherapy induced peripheral neuropathic pain Alcohol
Physical compression/irritation	Trigeminal neuralgia – vascular compression Intervertebral disk herniation with radicular pain

The process at which pain is generated includes the following (a few described in the previous lecture):

- **Ectopic firing**
- **Cross talk (ephatic coupling) –** Abnormal depolarization of the neighboring cell
- **Collateral sprouting –** growth of collateral fibers from sensory axons to denervated areas.
- **Sympathetic (sensory coupling) aka sympathetically mediated pain (SMP)** - increased adrenergic sensitivity of sensory neurons and proliferation of sympathetic nerve endings leading to increased SNS activation.

Common Neuropathic Pain Disorders

- **Diabetic neuropathy:** most common cause. Distal symmetrical polyneuropathy in a 'glove and stocking' distribution with sensory loss. Allodynia may occur. Experience pain as numbness and tingling, deep aching or burning pain.
- **Post-herpetic neuralgia:** Pain can persist for 12/52 after infection. Increased risk with increasing age and affects the VZV diseased dermatome.
- **Trigeminal neuralgia:** Episodic, sudden brief paroxysms of pain (seconds to few minutes) in the distribution of one of the branches of the trigeminal nerve. Some people atypically experience pain between episodes. Pain is severe and described as sharp, shock-like, shooting or electric. Mostly idiopathic (including vascular compression of the nerve) but may be caused by MS, tumors and infarction.
- **Phantom Limb Pain (PLP):** 60-80% of amputees with severe pain in 10%. Patients feel like the limb is still there and sensations of posture, movement and length may be present. Neuromas may form on cut nerve endings and SNS activation may occur. Central changes include neuroplastic reorganization of the somatosensory cortex.
- **Complex Regional Pain Syndrome** (**CRPS**) Rare and causes pain at rest, allodynia, hyperpathia and hypoaesthesia. Motor distrubance is common where the limb is held motionless and autonomic disturbance occurs in the early phase of the disease.
- In the later phase the skin may become cold with mottled blue skin. Temperature difference of more than 2^{O} between the limbs is specific for CRPS. Other sudomotor and trophic physical manifestations are common.
- Management of CRPS is difficult and responds poorly to conventional analgesics. Functional restoration using physiotherapy techniques is the cornerstone of current management. CRPS is divided into 2 types:
    - **CRPS Type 1** follows injury not directly damaging nerves
    - **CRPS type 2** follows a distinct nerve injury.

Neuropathic Pain Management

Pharmacological Management

Local Anaesthetics: Topically applied. Works best when allodynia and cutaneous hyperalgesia is present.

Capsaicin: Topically applied and depletes substance P and reduces the density of peripheral nerve fibres. Takes time to work and sometimes causing a burning sensation

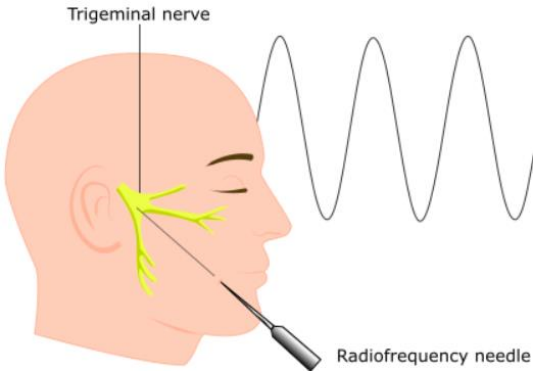
Baclofen: $GABA_B$ agonist as a skeletal muscle relaxant for spasmodic pain. Effective for trigeminal neuralgia and used during flare ups.

Ketamine: NMDA antagonist for central sensitisation. Concerns over long term use remain

AEDs: Such as **carbamazepine** acts by VG-Na^+ channel inhibition and 1^{st} line for trigeminal neuralgia. Gabapentin and pregabalin reduce Ca^{2+} currents and modulate GABA transmission.

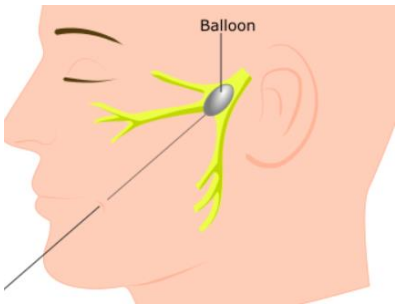
Antidepressants: SNRIs i.e. duloxetine and TCAs i.e. amitriptyline.

Opioids: Altered dose response in neuropathic pain but concerns exist over long term sequelae.



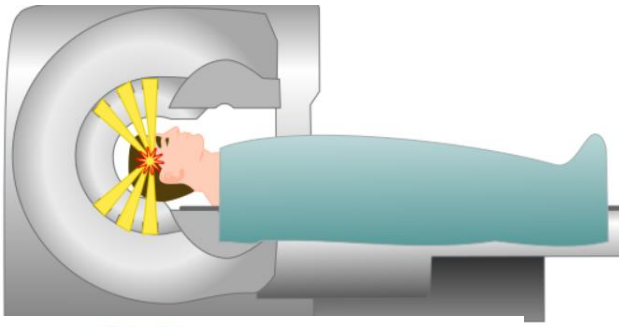
Interventional Management

Radiofrequency Ablation of the Gasserian Ganglion: Needle is inserted under fluoroscopic guidance under sedation then position confirmed by sensory stimulation. Thermal lesion created.

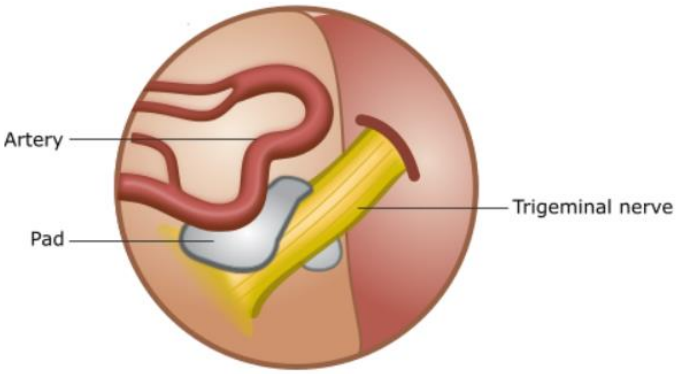


Balloon Compression Rhizolysis: Percutaneous technique where the needle is inserted via the foramen of ovale then the balloon is inflated to cause ischaemia of the Gasserian ganglion.

Both these techniques are used for trigeminal neuralgia.



Steriotactic Radiosurgery aka gamma knife: high energy radiation to the cisternal portion of the trigeminal nerve. The technique is the least invasive, but slightly less effective than open surgical or ablative procedures



Microvascular Decompression: open neurosurgical operation in which the trigeminal root is accessed through the posterior fossa. Vascular compression can be relieved by placing a Teflon patch between the vessel and nerve.

MVD is more effective than the neuroablative techniques and most likely to preserve normal sensory function of the nerve. MVD is the preferred technique for young, fit patients with ongoing severe symptoms and evidence of vascular compression

RENAL PHYSIOLOGY

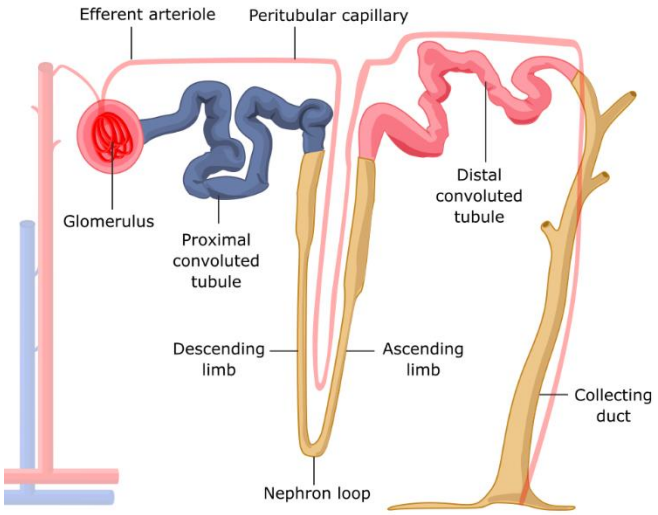
Renal Morphology, Blood Supply and Glomerular Filtration

(07b_05_01)

Nephron

Each kidney has 1-1.5million nephrons which are the functional units.

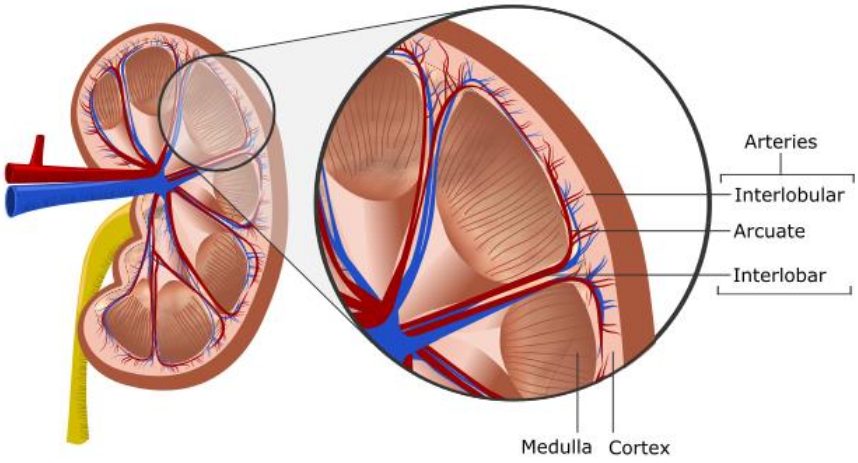
The majority are **cortical** with their glomeruli in the outer 2/3rd cortex. The loops of Henle extend into the medulla.



Blood Supply

The kidneys receive **20-25%** of **cardiac output** with the **cortex receiving 90%** of the blood and 10% to the medulla and capsule.

Of the 10% blood supply to the medulla, the **inner medulla receives 1/5th** that of the outer medulla which is important in the **concentrating ability** of the urine. It does however, make this region **most susceptible to hypoxia** with reduced renal blood flow.

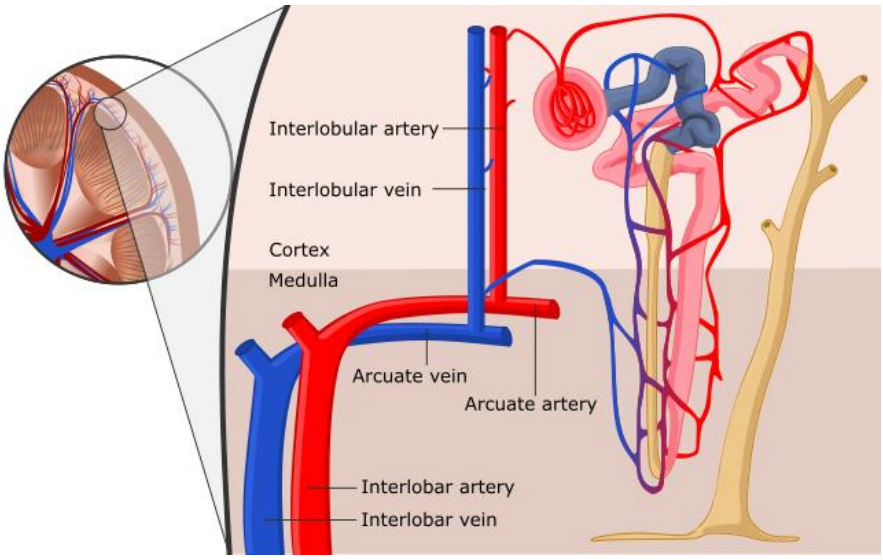


The AV oxygen difference in the cortex is only 1-2% as receives much more oxygen than it needs. In the cortex however extraction may approach 80%

Order of blood flow through vessels:

Renal a. → interlobar a.s → arcuate a.s → interlobular a.s → Afferent arteriole

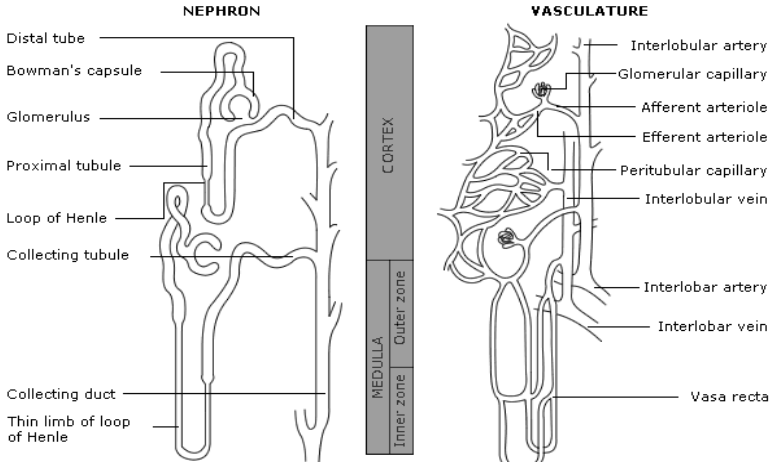
The interlobular arteries leave the arcuate arteries at right angles



Capillaries

The glomerular capillary supply is from the afferent arteriole and drainage through the efferent arteriole which then gives rise to the **peritubular capillaries**. These arrange into a network around the nephron.

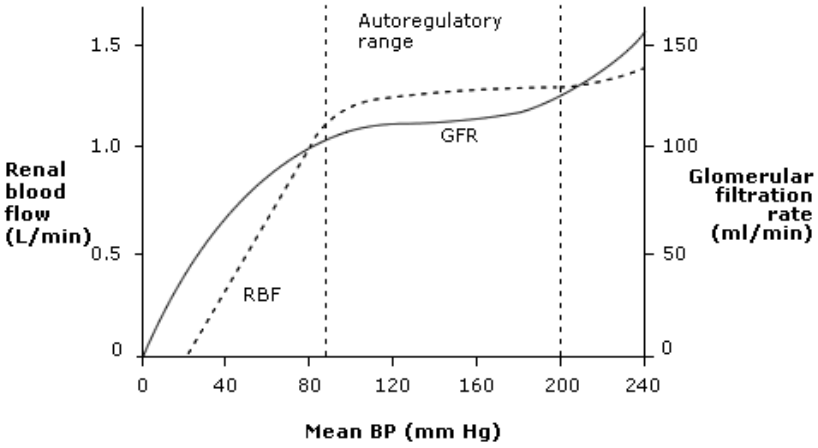
The **inner cortex efferent arterioles** also give rise to the **vasa recta**.



Functions and Regulation of Renal Blood Supply

Unlike other tissues, as well as for gas exchange, capillaries arrange in the glomerulus to allow filtration pressure for forward flow of tubular fluid.

Autoregulation of renal blood flow occurs in the kidney to allow a **constant flow** as **pressure varies**. The myogenic theory is that afferent arteriolar wall tension causes automatic smooth muscle contraction in vessel walls **between MAP 90-200 mmHg**. This is predominately brought about by varying afferent arteriolar tone.



GFR is regulated by alteration in the tone of the efferent

There are other important mechanisms to regulate blood flow:

- **SNS** induces renal vasoconstriction
- **Renal prostaglandins** attenuate the effect of the SNS
- **Angiotensin II** causes efferent arteriolar vasoconstriction which **increases filtration fraction** with a reducing GFR.

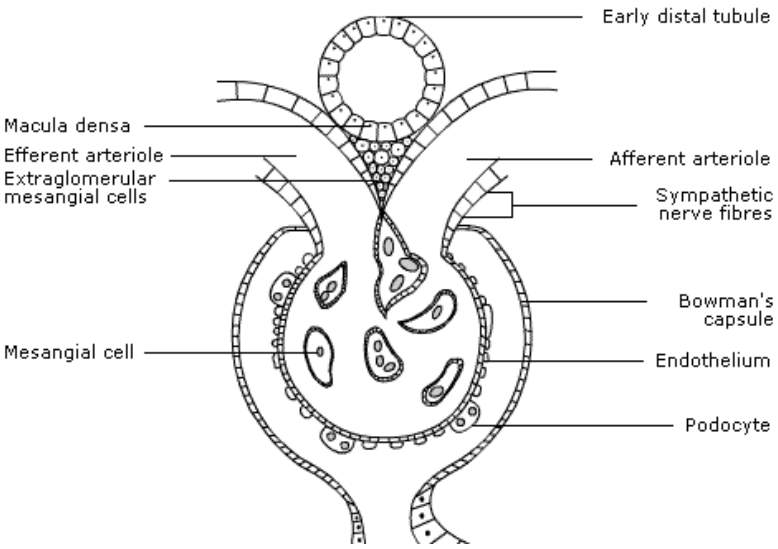
Tubulo-glomerular feedback:

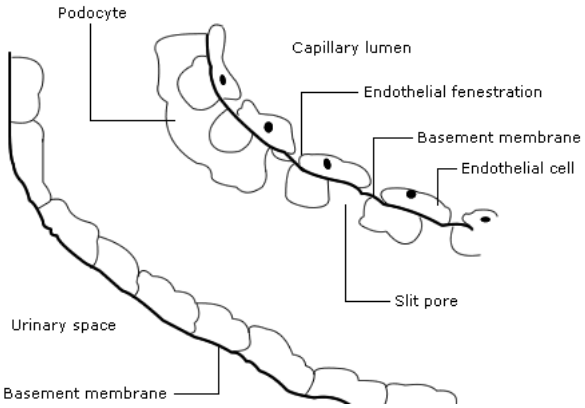
Single nephron GFR (SNGFR) is greatest in the juxtamedullary nephrons. SNGFR is influenced by the distal tubular fluid composition which is influenced by GFR. The feedback mechanism has 3 components:

1. Tubular fluid characteristic is recognised by the tubular epithelium *i.e. increased Na^+*
2. Signal is transmitted to the glomerulus *i.e. increased Adenosine and ATP*
3. An effector mechanism alters the GFR *i.e. afferent arteriolar vasoconstriction*

The Glomerulus/Renal Corpuscle

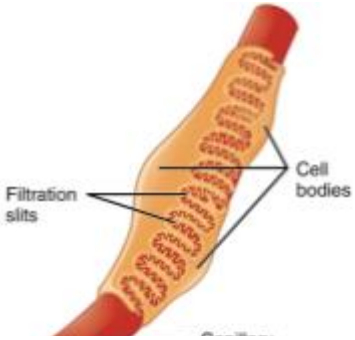
The basic structure is a capillary knot fed by the afferent and drained by the efferent arterioles. It contains **mesangial cells** for structural support and are capable of **contracting** to modify the capillary surface area available for filtration. They are also **phagocytic**.





The **basement membrane** is a **continuous layer of Type IV collagen, laminin** and **fibronectin** which are all negatively charged. It filters according to molecular size, charge and shape.

Podocytes have foot projections known as pedicels and they **interdigitate** (like a sieve) for further control of filtration:



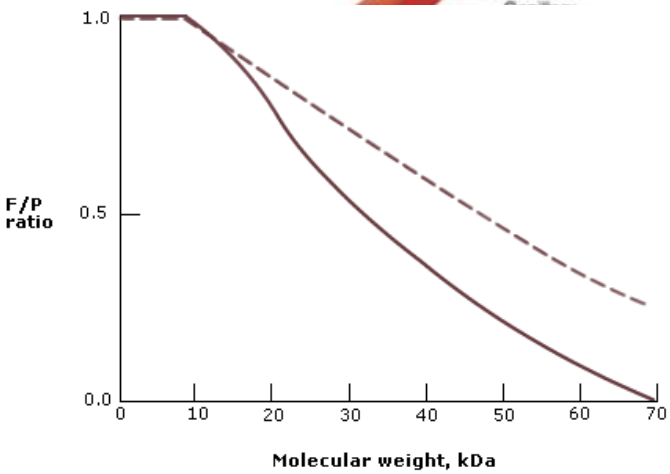
Filtration Process

GFR is around **125ml/min** or 180 L/day. The **molecular size** is the main determinant of particulate filtration. Only molecules below **7kDa** are **freely filtered** with the upper limit of 70kDa.

Due to the negative charge of the basement membrane, **negatively charged molecules** have a **reduced rate of filtration**. The dotted line shows uncharged dextran molecules being filtered.

F/P is the ratio of filtered to passed molecules.

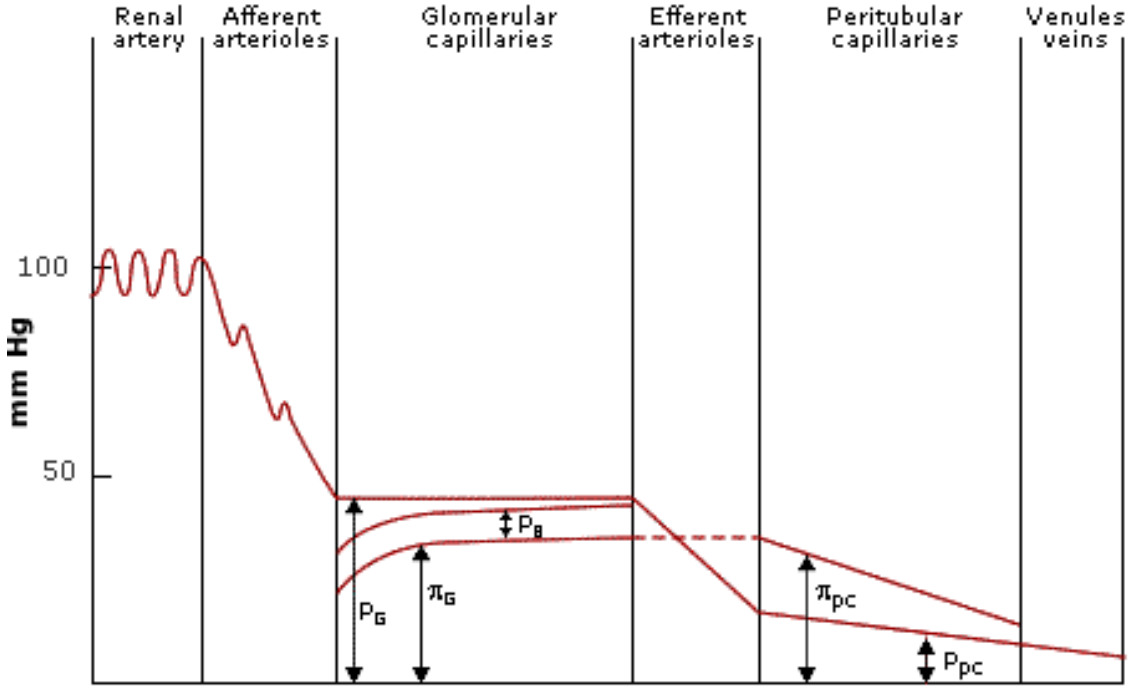
The filtrate composition will have a similar small ionic content as that of the afferent arteriole and a small amount of protein (30g/day).



Filtration Forces

The fluid movement is dependent on the balance between **hydrostatic pressure (Pc)** and **plasma oncotic pressure (π_C)**.

Compared to normal vascular beds, **pressure drops less in afferent arterioles** allowing a high Glomerular capillary pressure (**P_G**) of 45mmHg. As **bowman's capsule** is a **blind ended tube**, there is a hydrostatic pressure **(P_B)** which opposes the P_G at 10mmHg as well as an **initial** capillary oncotic pressure **(π_G)** of 25mmHg – this increases as filtration proceeds to make protein more concentrated.



The **hydrostatic pressure falls further** in **efferent arterioles** so that the peritubular capillary hydrostatic pressure **(P_{PC})** is low and reabsorption can occur. The peritubular capillary oncotic pressure **(π_{PC})** is 35mmHg and falls as more fluid is reabsorbed.

Overall: $$GFR \propto P_G - (P_B + \pi_G)$$

For conversion to real measurements, the filtration coefficient **(K_f)** is introduced which is a product of **glomerular capillary permeability** and **filtration surface area**:

$$GFR = K_f \times (P_G - (P_B + \pi_G))$$

Filtration Fraction

As the renal plasma flow is ~600ml/min and the GFR is 120ml/min, then the **filtration fraction is 20%.** Other forces than above that need to be overcome with filtration in the Bowman's capsule is **tubular fluid viscosity**, **friction of flow** and **renal interstitial pressure** to keep the tubule patent.

Tubular Transport and the Proximal Tubule

(07b_05_02)

Secretion = movement of substances from blood to tubular fluid

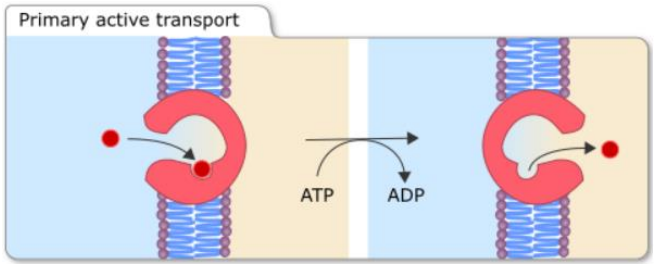
Reabsorption = movement of substances from tubular fluid to blood.

Types of Tubular Transport

Primary Active Transport

Involves the **direct coupling of ATP dephosphorylation** with a **transport process**. Basal membrane of cell = cell-capillary. Apical membrane of cell = cell-lumen. The processes requiring this in the nephron include:

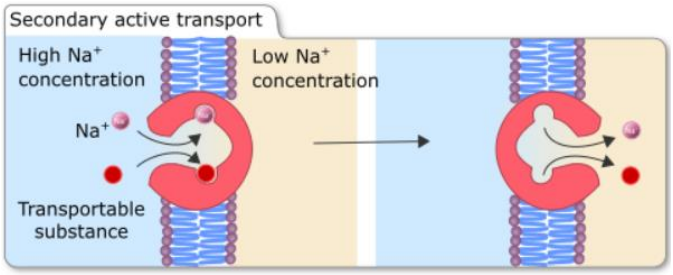
1. **Na^+/K^+ exchange** on the basal membrane
2. **Ca^{2+} reabsorption** on the basal membrane
3. **H^+ secretion** on the apical membrane
4. **H^+/K^+ exchange** on the apical membrane



Secondary Active Transport

Uses **ionic gradients** created by **ATPases** to release energy and allow transport of other solutes. This is therefore indirectly linked to ATP breakdown. The most **important ionic gradient is sodium**

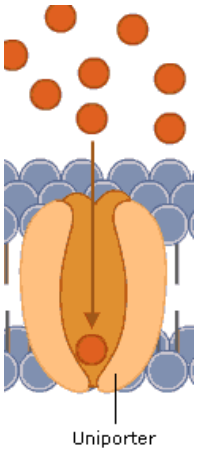
- **Cotransport** (**symport**) – movement in the same direction of Na^+ gradient (diagram)
- **Counter-transport** (**antiport**) – movement against Na^+ gradient



Ion Channels, Uniporters and Facilitated Diffusion

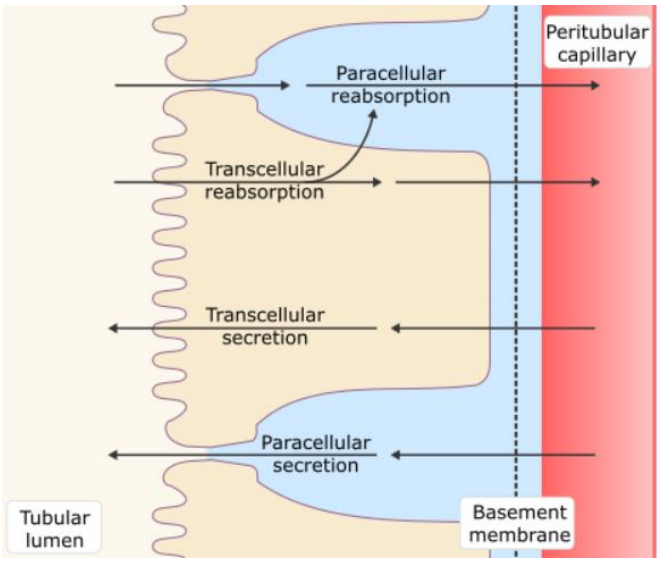
- Ion channels are proteins that exist on the *apical membranes* and include Na+, K= and Cl-.
- Uniporters transport a single substance across the membrane driven by a concentration gradient i.e. glucose

Facilitated Diffusion is the term given to describe transport via ion channels and uniporters.



Paracellular Movement

Movement occurring through the **spaces between cells** of the nephron. This may be driven by concentration, osmotic and/or electrical gradients.



Water Reabsorption

May be transcellular or paracellular. The latter may carry -ve charged ions with it and is termed '**solvent drag**'. Faster water reabsorption occurs with **aquaporins** located in the cell membrane. There are 3 types in the kidney:

- **AQP1: PCT** and ***thin descending limb*** and responsible for high permeability of water
- **AQP2:** Controlled by **ADH** in the ***apical membrane*** of the ***collecting ducts***.
- **AQP3: *Basal membrane*** of collecting ducts to allow reabsorption of water from AQP2.

AQP4 and 5 are located extra-renal.

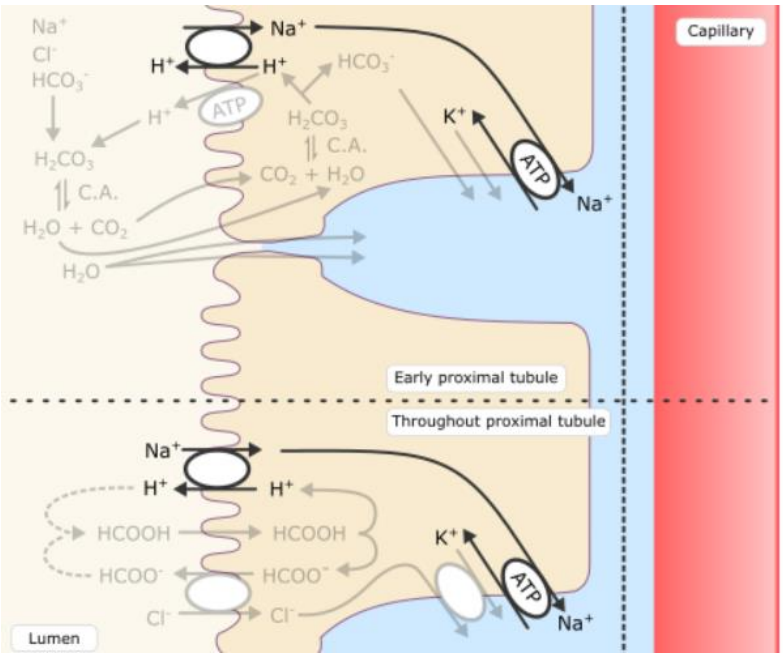
Sodium and Chloride Ion Reabsorption

In the **Proximal Tubule permeability to sodium is very high** and there is a **net reabsorption**.

Sodium entry into PCT

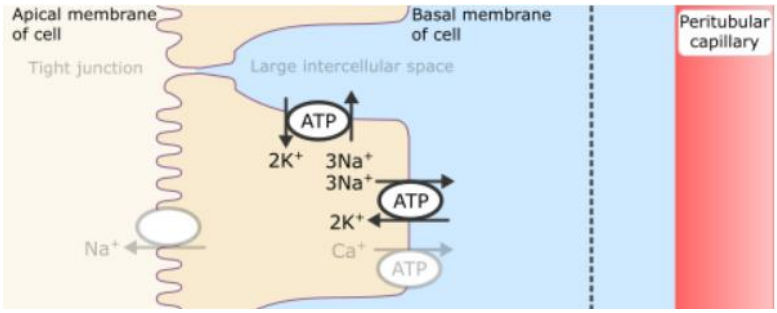
Sodium entry is driven by and **electrochemical gradient** mediated by **carrier proteins**.

- 80% is exchanged for H^+ secretion. H^+ leads to Cl^- and HCO_3^- reabsorption.



Sodium Extrusion

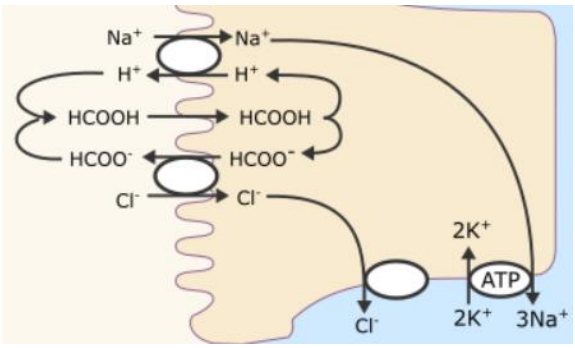
The Na^+/K^+-ATPase pump extrudes sodium against electrochemical gradients. K^+ can readily cross cell membranes and rapidly diffuse out again.



Chloride Ion Absorption: **Throughout Proximal Tubule**

The electrical gradient opposes reabsorption of chloride. As above, the reabsorption of Na^+ leads to H^+ secretion and subsequent HCO_3^- reabsorption. This **increases tubular Cl^- concentration** allowing movement **down a concentration gradient**. This occurs through antiport with either:

- Bicarbonate (HCO_3^-)
- Formate ($HCOO^-$)
- Oxalate

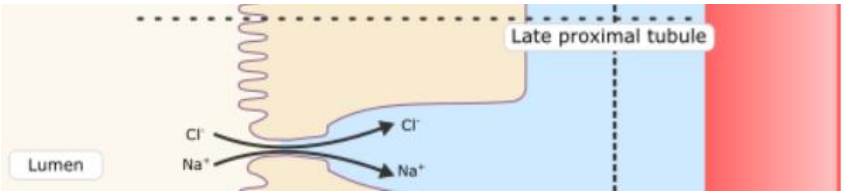


In this diagram, HCOOH (formate) is recycled either side of the membrane with dissociation in and formation out of the cell

The net result of Cl^- and Na^+ antiport systems is the reabsorption of equal amounts of Cl^- and Na^+.

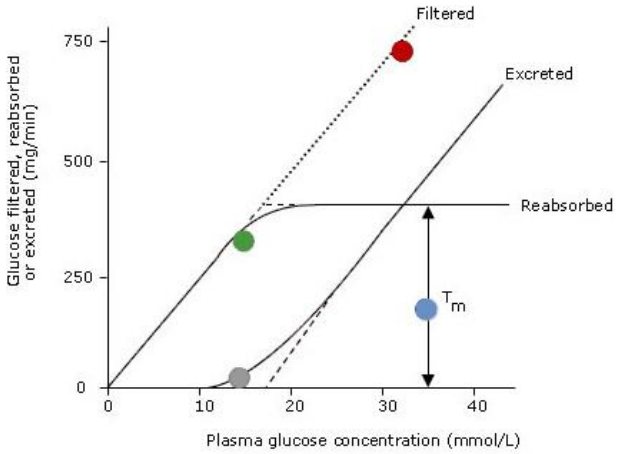
Chloride Ion Absorption: **Late Proximal Tubule**

It differs between juxtamedullary and superficial (mainly cortical) nephrons. Juxtamedullary nephrons follow the same mechanism as the above. **Superficial nephrons** have **greater permeability** to **chloride ions** than other anions and so is **reabsorbed paracellularly** once the electrical diffusion gradient has been established in the early proximal tubule from HCO_3^- reabsorption. **Sodium follows**. 20% of NaCl reabsorption occurs by this method.



Glucose Handling

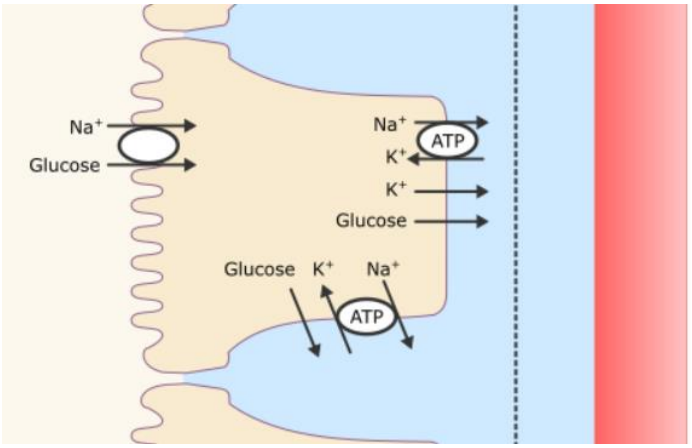
Nearly all glucose that is filtered is reabsorbed and occurs almost entirely in the proximal tubule. In normal plasma concentrations the amount filtered is directly proportional to the plasma concentration.



Glucose Reabsorption Mechanism

Entry is linked to a **secondary active transport symport mechanism with sodium** . The transport molecule is saturable. This means, above a threshold concentration further absorption is not possible and the filtered glucose is lost in the urine. This threshold concentration is around 22mmol/L

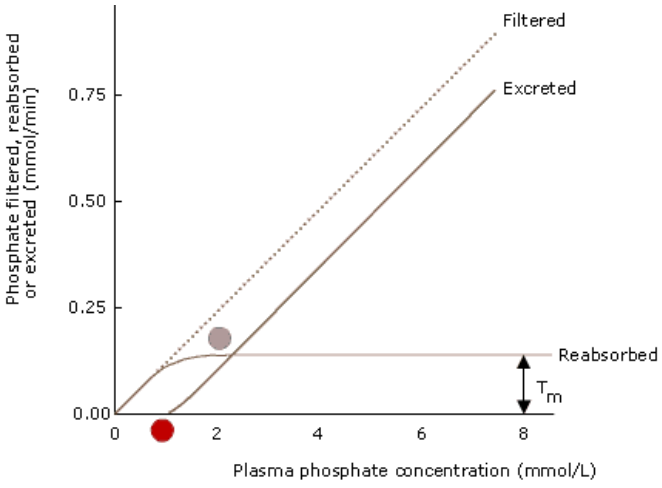
The **Tubular Maximum (Tm)** is the highest rate of transport of a particular molecule.



Other Solutes

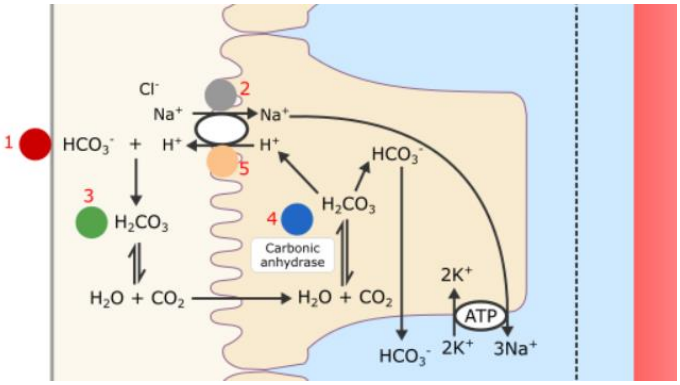
Amino Acids are filtered in the glomeruli with a very **high T_M** resulting in negligible excretion. The uptake of amino acids is by the same process as glucose (**symport with Na^+**) and there are several independent transport processes due to multiple different amino acids.

Phosphate excretion is usually <20% that which is filtered. However, above 1.2mmol/L, the increments in excretion match filtration. It is only **reabsorbed in the presence of sodium reabsorption** and the basal membrane transport mechanism is unclear. The rate of reabsorption is **regulated by Parathyroid Hormone (PTH).**



Urea: 40-50% of filtered urea is reabsorbed. This occurs in the proximal tubule by simple **diffusion** as its concentration gradient increases in the tubule. This is as an indirect effect of sodium reabsorption.

Bicarbonate: 90% of filtered bicarbonate is reabsorbed in the proximal tubule. It combines with **secreted H^+** which makes bicarbonate and dissociates into water and CO_2 in the tubule. These are reabsorbed through the apical membrane and recombines with the help of carbonic anhydrase. H^+ antiport occurs and HCO_3^- is reabsorbed through the basal membrane:



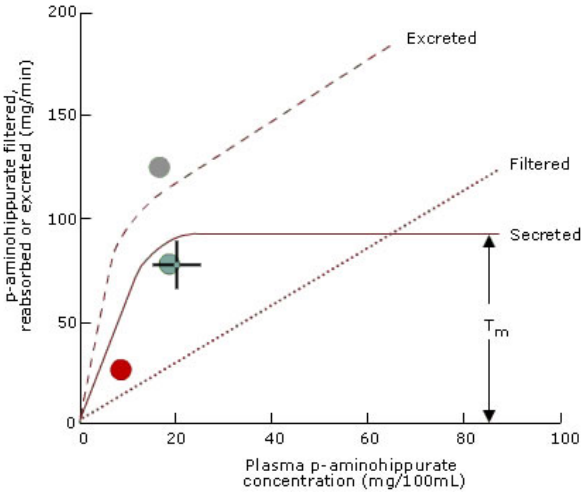
Secretion in the Proximal Tubule

Active or passive and may be:

- **Gradient-time limited** i.e. in hydrogen ion secretion or
- **T_M limited** i.e. in organic acids such as penicillin, chlorothiazide, hippurte and p-aminohippurate (PAH). Strong organic bases i.e. histamine, choline, thiamine, guanidine, creatinine. EDTA.

PAH

(For example) is filtered and secreted proportional to its concentration. Above a **plasma concentration of 10 mg/100 mL**, T_M is reached for secretion. At this point, the rate of excretion rises proportionally to the rate of filtration.



Peritubular Capillaries

The end result of Na^+, Cl^-, HCO_3^- and water handling in the proximal tubule is the entry of essentially isotonic fluid from the tubule into the lateral intercellular spaces. A proportion of **Na^+ leaks back** known as the **pump-leak system** or gradient-time limited process. Alterations in the rate of proximal tubular sodium reabsorption can be brought about by:

1. Changes in the rate of backflux into the tubule
    a) dependent on the rate of uptake into capillaries (faster the rate, the lower the rate of backflux).
2. Changes in the rate of active sodium extrusion from the cell

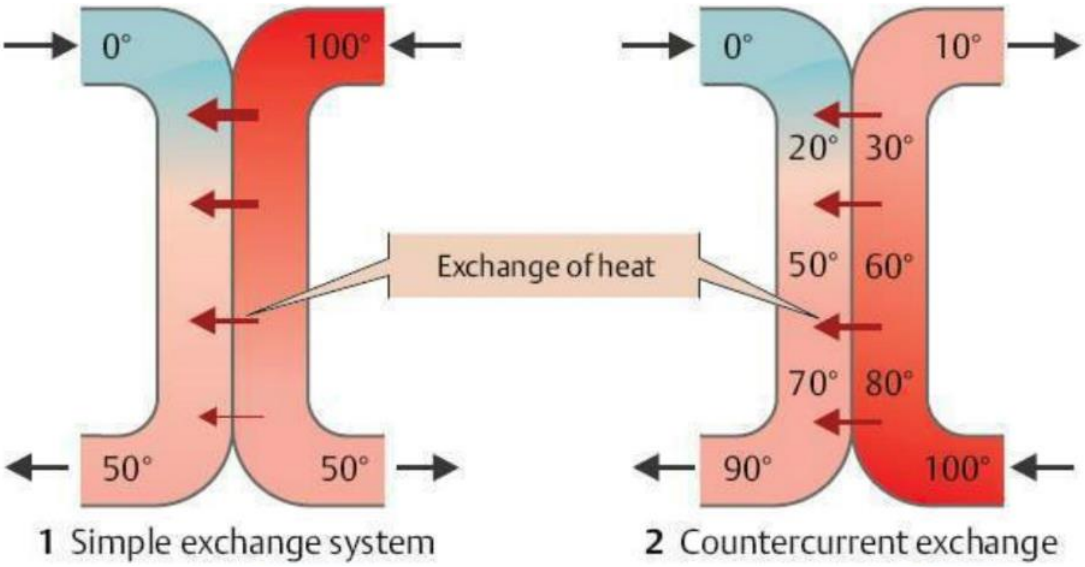
Glomerulo-tubular balance: Normally, the filtration fraction is dependent on the peritubular capillary oncotic pressure. When the GFR changes, the oncotic pressure can be adjusted to allow a fixed percentage of filtrate to be absorbed proximally.

Loop of Henle, Distal Tubule and Collecting Tubule

(07b_05_03)

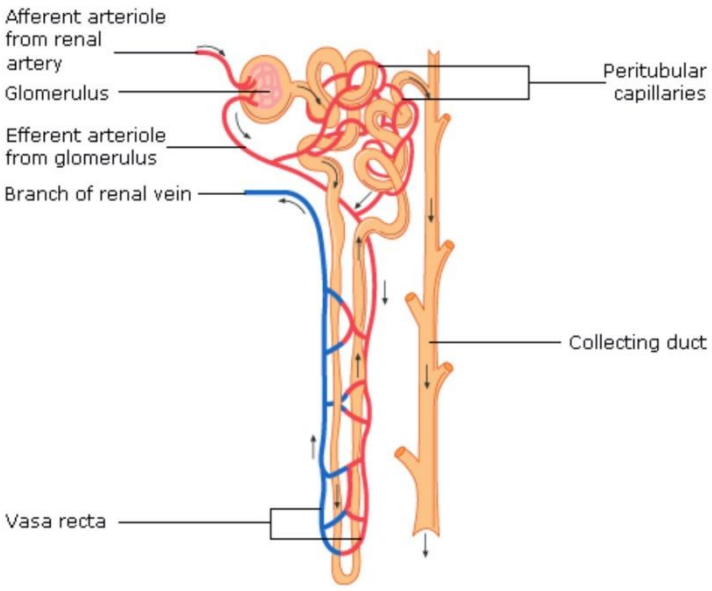
Only birds and mammals can produce concentrated hypertonic urine. This is achieved in the **Loop of Henle** and uses a **countercurrent multiplier mechanism.**

Exchange mechanisms



In the first example flow along the two vessels is in the same direction. If there is a temperature gradient this is maximal at the start- leading to rapid equilibration, at the end of the vessels there is little gradient and further temperature transfer stops. If the flow of the two vessels is reversed a smaller gradient occurs however this maintained throughout the length, there is more efficient transfer.

The structure of the nephron incorporates 2 countercurrent pairs (The Loop of Henle & the Vasa Rectae)



Loop of Henle

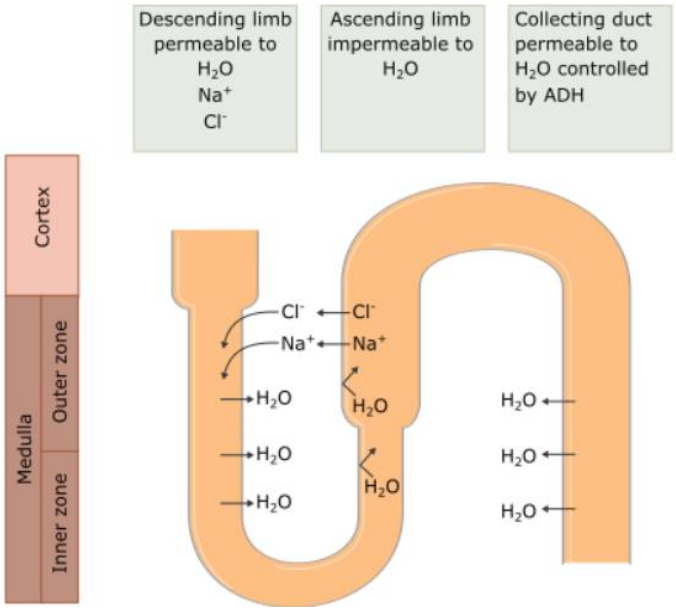
Originates in the renal cortex and passes into the medulla. Only juxtamedullary nephrons (15%) have long loops that pass deep into the medulla.

Distal Tubule and Countercurrent Mechanism

There is a small **osmotic pressure difference** between the ascending and descending limbs of the loop which is **multiplied** through the **flow** in opposite directions (countercurrent).

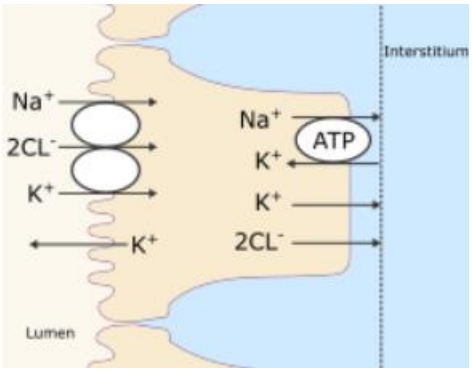
- **Thin descending limb is permeable to water and all ions.**
- **Ascending limb is impermeable to water**
  - **Thick part** only can **extrude ions**.

Therefore, the fluid in the **ascending limb osmolality** will be lower than that in the interstitial tissues.



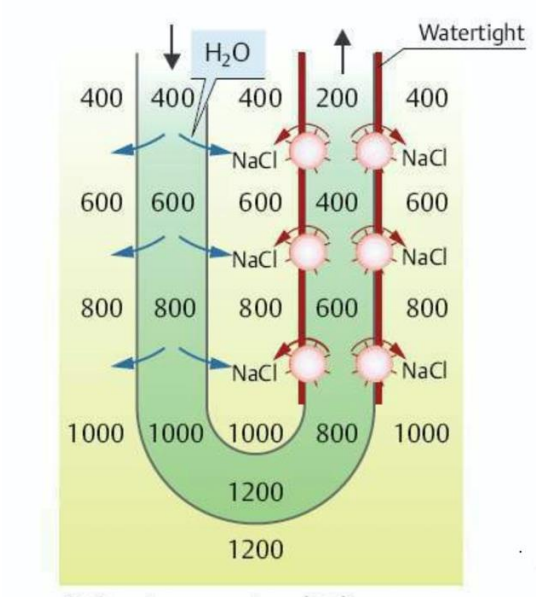
The **extrusion** of ions to interstitium occurs by tubular cells which take place in the basal membrane. The entry through the apical membrane occurs with the **$Na^+/K^+/Cl^-$ cotransporter** and is extruded through **Na^+/K^+ ATPase activity** on the basal membrane. This leads to a build-up of NaCl in the interstitium.

Cells on the ascending limb can **sustain an osmotic difference of 200mosmol/kg H_2O** through **ionic extrusion**. Fluid flux in both the Convoluted Tubule & the Vasa Rectae contribute to the function.



Establishing Medullary Hypertonicity

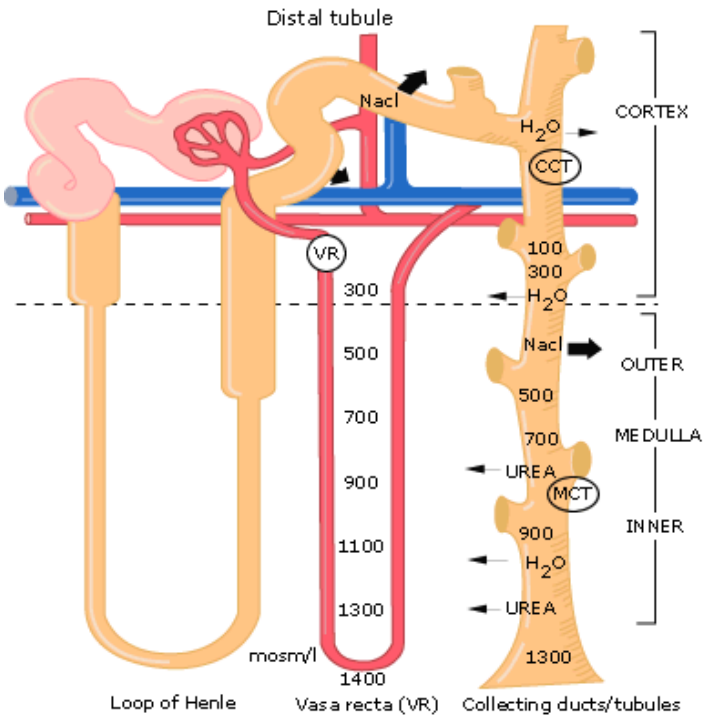
The following are theoretical steps of how this is achieved:



1. The filtrate entering the loop of Henle is **isotonic**. The proximal portion of the loop lies near the distal part of the loop (before it joins the DCT) & the Vasa Recta (before it leaves to join the renal vein).
2. As the pumps in the Ascending PCT create a 200mosmol/kg gradient at any point, the osmolality of the fluid in the ascending limb falls as the fluid approaches the DCT.
3. Tubular fluid nearing the DCT is hypotonic but the interstitial fluid nearby is slightly hypertonic, this draws water from the fluid in the descending loop.
4. As filtrate descends in the loop, water is drawn into the interstitium. The tonicity slowly rises. Some of this water passes along a concentration gradient into the ascending Vasa Recta and out of the kidney. The tonicity of the interstitial fluid is virtually the same as that of the fluid in the descending loop at that point.
5. As the interstitial tonicity rises the cells in the ascending loop maintain the 200mosmol/kg gradient.
6. This process continues until the **maximal achievable medullary tonicity** has been reached which is 1400 mosmol/kg H_2O. The hypotonic fluid entering the distal tubule is 90 mosmol/kg H_2O.

Collecting Tubules

The transition from the DCT → collecting tubules is the point where cells with low permeability to water become variably permeable to water. They have both **cortical (CCT)** and **medullary (MCT) sections** and **water permeability is increased with ADH** to increase urine osmolality.



The medullary interstitium is hypertonic and theoretically, urine can be concentrated to the same osmolality which is 1400 mosmol/kg H_2O.

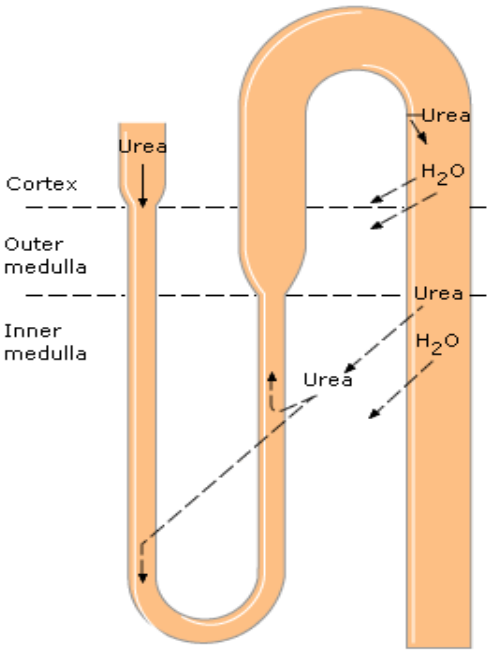
The greater the volume of fluid reabsorbed, the more dilute the medullary interstitium will become and the less concentrating ability there is. Therefore, **66% of fluid delivered to the cortical collecting tubule (CCT) is absorbed prior to arrival at the medullary collecting tubule (MCT).** Less than 5% of GFR will arrive in the MCT and the osmolality will have increased from the CCT to the MCT from 90 to 290 mosmol/kg H_2O.

Urea in the Countercurrent Mechanism

Remember that 50% of freely filtered urea is reabsorbed in the proximal tubule. Tubular concentration is increased by diffusion of urea into the descending limb of the loop of Henle. When fluid **enters the DCT and CCT**, its concentration rises from water reabsorption as there is **no permeability** to urea.

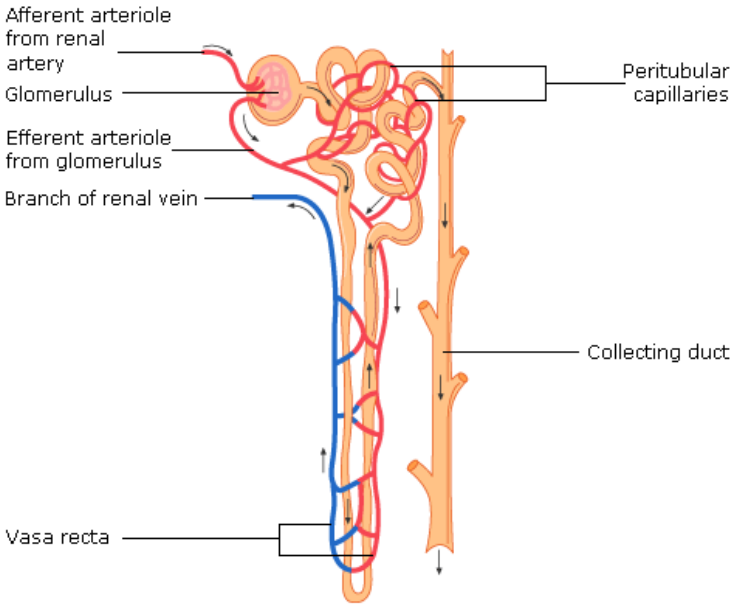
In the MCT, the **permeability to urea increases** and diffusion occurs out into the interstitium to increase its concentration necessary for diffusion into the descending limb.

ADH activates urea uniporter in the apical and basal membranes of the medullary collecting tubule cells for **facilitated diffusion** and therefore, the highest urinary osmolality occurs in the face of higher ADH levels.



Vasa Recta in the Countercurrent Mechanism

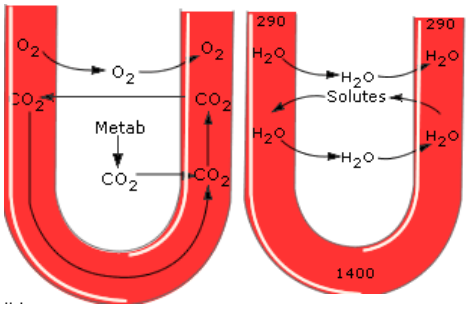
As mentioned previously, arises mainly from **efferent arterioles of juxtamedullary nephrons** and has a **hairpin arrangement** dipping deep into the medulla. They provide nutrients and remove waste products from the renal medulla **without** interfering with the solutes and medullary hypertonicity.



In the **descending limb of the vasa recta**, water is **osmotically extracted** from the blood vessel: this causes **higher osmolality and viscosity of the blood** which results in a **reduced flow rate** of blood → minimal solute washout.

Water re-enters the ascending limb of the vasa recta.

This system also undergoes O_2 and CO_2 countercurrent exchange. This system minimises washout of medullary solutes but is inefficient at O_2 delivery and CO_2 removal.



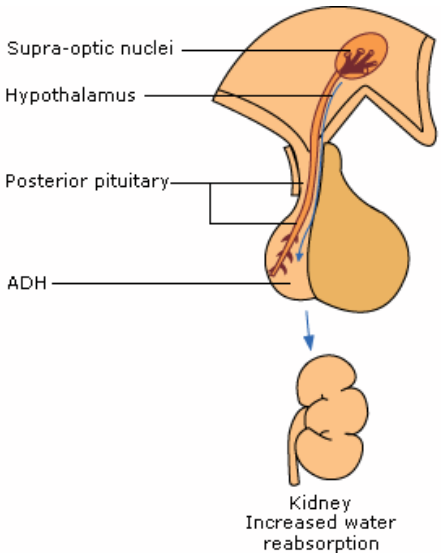
Regulation of Urine Concentration

23L/day of water enters into the DCT and CCT which is subject to reabsorption with ADH.

Synthesis and Storage of ADH

Synthesised in the **hypothalamus** in the **supra-optic nuclei** which is then transported for **storage in the neurohypophysis. Osmoreceptors** are located near the supra-optic nuclei and paraventricular nuclei which **regulates ADH release**.

Plasma osmolality of 280-290 mosmol/kg H_2O is associated with circulating ADH levels of 4 pg/ml. Extracellular sodium is the main regulator of osmolality.

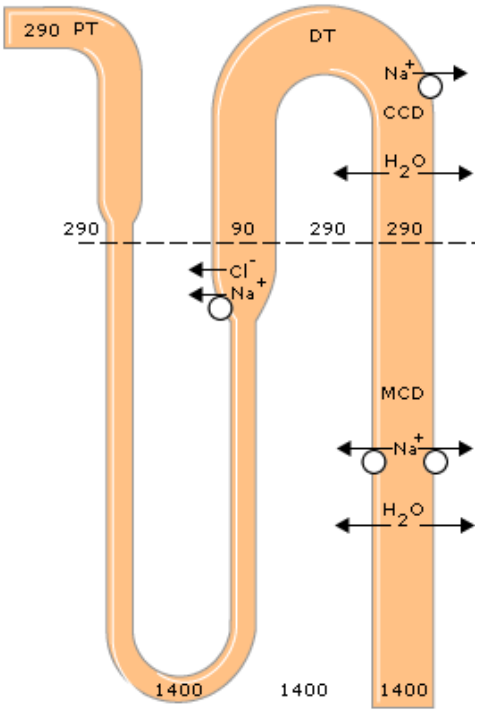


Mechanism of Action of ADH

Acts on **V2 receptors** on the basal membrane of tubular cells → G-protein receptors that **increase AQP2 water channel activation** on the **apical membrane**.

Osmolality Galore

At the end of the CCT, the tubular fluid will have matched with the interstitial fluid osmolality of 290mosmol/kg H2O. In the face of **maximum ADH production**, there will be a maximum concentration of **MCT osmolality of 1400mosmol/kg H_2O**. This will manifest as a total of 400ml urine/day.



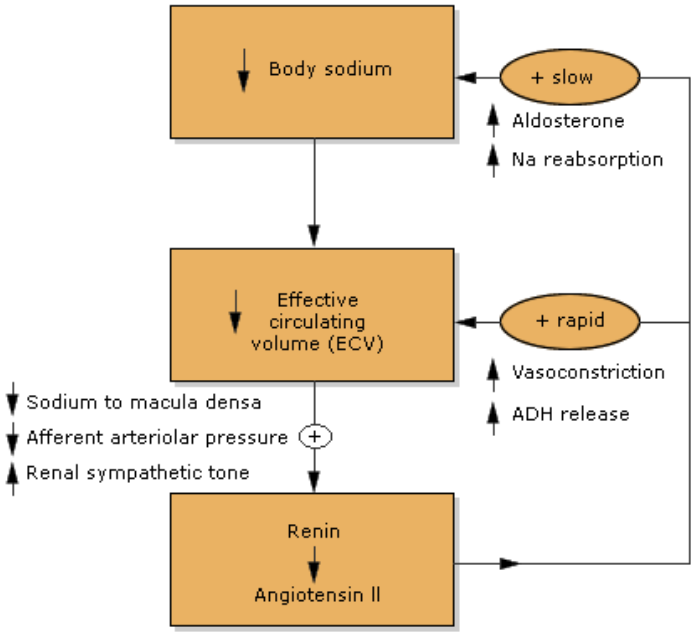
No ADH production means urine osmolalities are very low at 60mosmol/kg H_2O and **23L urine / day** can be produced. However, there is **normally a small amount of ADH present** allowing urine volumes of 1.5L/day with an osmolality between 300-800.

Regulation of Electrolyte and Acid-Base Balance

(07b_05_04)

Effective Circulating Volume

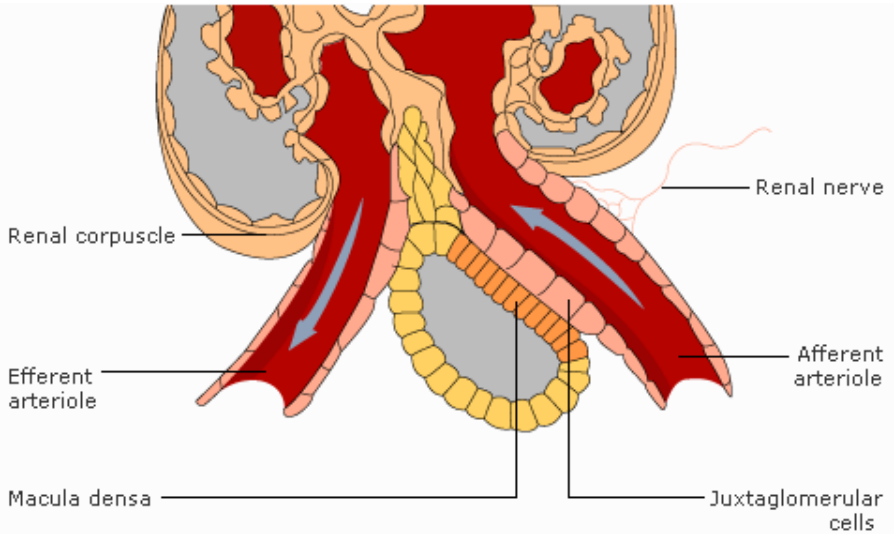
The ECV is the volume of blood that is **perfusing the tissues** and is not the intravascular volume, although not an anatomical space it roughly equates to 30% of Body Weight – in heart failure, large IV volume may still have a low ECV. This is largely **determined by body sodium content**.



Falls in BP and atrial filling, presence of **haemorrhage** and **stress** lead to **rapid responses in ADH release** to correct the abnormality. Without this response, the plasma osmolality may be maintained in the presence of low sodium (but normal concentration) reducing the ECV.

For corrections in body sodium, one requires **adequate sodium intake**. May take hours-days to occur.

Renin-Angiotensin-Aldosterone System



Juxtaglomerular Apparatus

Located at the junction between the **early distal tubule** and the **afferent and efferent arterioles**:

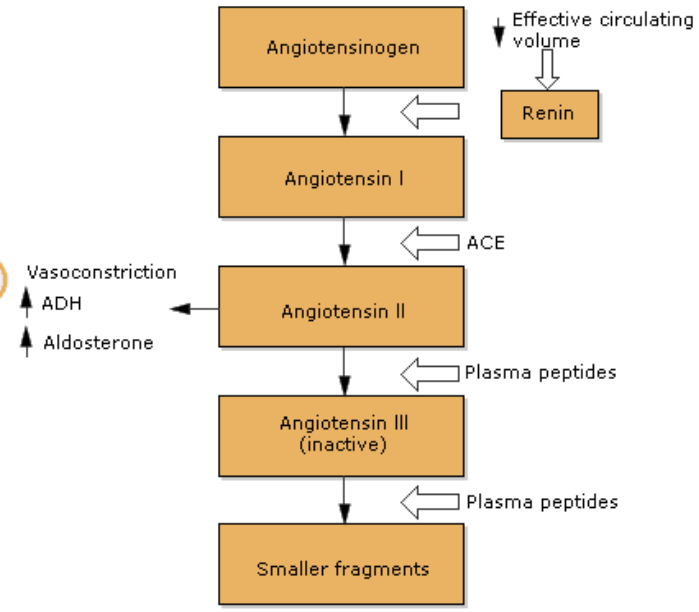
The **afferent arteriolar granular cells contain prorenin** and is in contact with the **macula densa** specialised cells. The granular cells are **innervated by the SNS** via **β-receptors** to **stimulate renin release** (converted from pro-renin prior to systemic release)

Renin release also occurs with **reduced afferent arteriolar wall tension** and is termed the **afferent arteriolar baroreceptor.** Note that the SNS activity on the afferent arteriole occurs upstream from the granular cells.

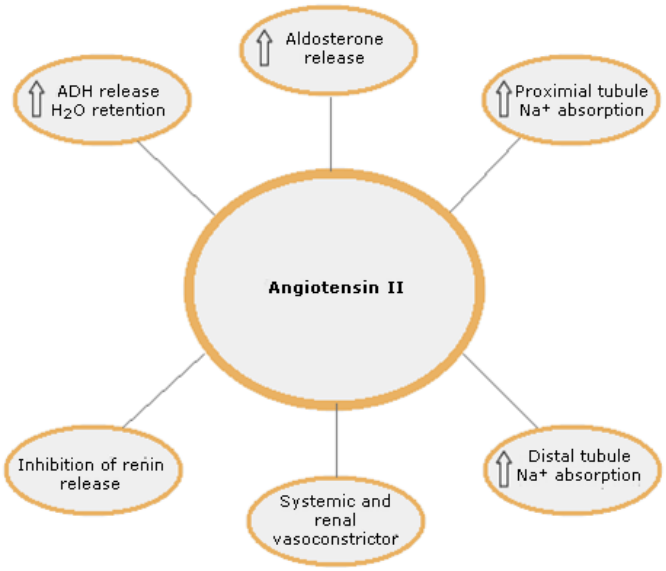
The 3rd mechanism is less understood but is where **reduced NaCl delivery to the macula densa stimulates renin release**.

Angiotensin II

Produced following cleavage process seen on right. ACE is present on endothelial cells. It is an **octapeptide**.



Actions are as follows:



Aldosterone

This is produced from the zona glomerulosa in the **adrenal cortex** and plays a ***permissive*** role in sodium absorption and excretion which means in abnormalities of aldosterone release, sodium absorption and excretion is often unimpaired as is regulated through other mechanisms and hence so is ECF volume.

- Increased renal Na^+ reabsorption primarily in the CCT.
- Increased K^+ secretion (with H^+)
- Increased ATPase synthesis and activity in tubular cells
- Increased GI and sweat gland Na^+ reabsorption

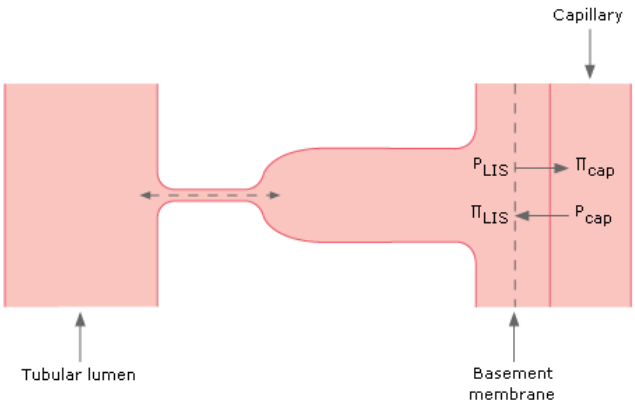
Through increased Na^+ and K^+ channels.

Proximal Tubular Sodium Reabsorption

Hydrostatic and Oncotic pressure on Sodium control

With **increased ECV**, there is a larger peritubular hydrostatic and reduced oncotic pressure which will cause **reduced capillary uptake of sodium and water in proximal tubule** causing an **increased delivery of NaCl in the distal nephron tubule** → reduced Renin release

The opposite occurs with **reduced ECV**.

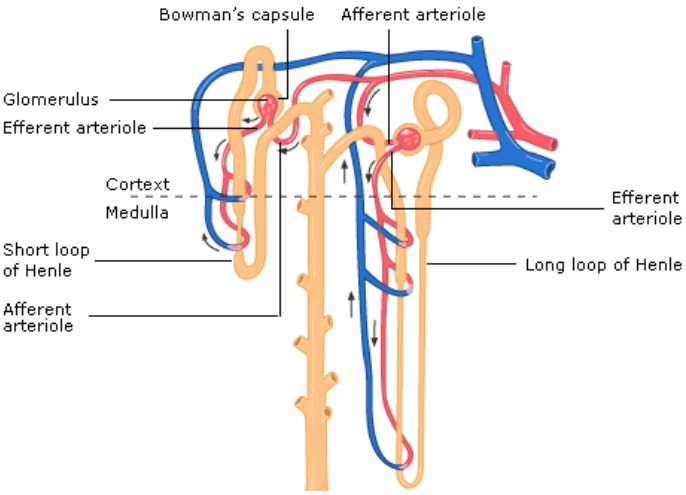


Long and Short Loops of Henle

In **long loops of Henle,** NaCl reabsorption is directly linked to NaCl delivery to the ascending limb. This appears to counteract reduced proximal tubular Na^+ reabsorption.

However, nephrons with **short loops of Henle** are not able to reabsorb all of the excess NaCl delivered from the proximal tubule.

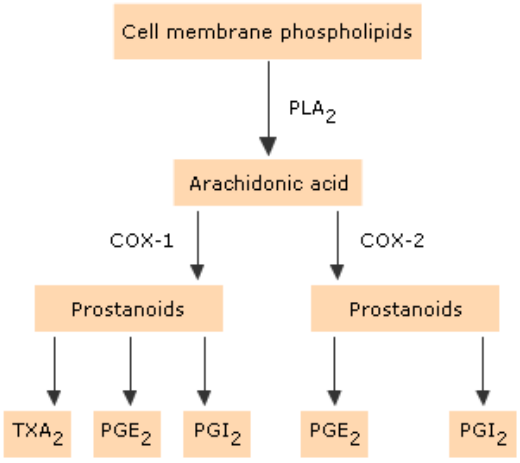
Blood flow is redistributed between these 2 populations of nephrons may play a part in responding to changes in the ECV. **SNS stimulation** results in **reduced blood flow to superficial nephrons** (short loops)



Prostaglandins

Produced from the **arachidonic acid pathway**. Arachidonic acid is stored **esterified** in membrane phospholipids which is converted by **cyclo-oxygenase** into unstable endoperoxides which give rise to the renal prostanoids. These occur in 3 sites:

1. **Cortex –** predominantly PGI_2 (prostacyclin)
2. **Medullary interstitial cells –** predominantly PGE_2
3. **Collecting duct epithelium**



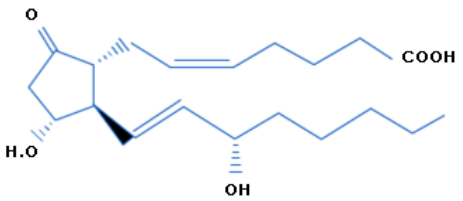
Functions

They act locally:

CORTICAL LEVEL: PGI_2 and PGE_2 are both **vasodilators** and are **produced** in large amounts when **renal perfusion is compromised.** Therefore, renal vasoconstriction and hypoxic injury is minimised. **NSAIDs inhibit COX** and therefore **reduce prostaglandin synthesis** which will be a problem in those with already existing vasoconstricting pathology/stimuli but not in normal individuals.

MEDULLARY PROSTAGLANDINS: Act on tubules to impair the action of ADH to limit the extent of Na^+ reabsorption. This may be protective as this process requires ATP → reducing energy required protects the medullary cells from excessive hypoxia.

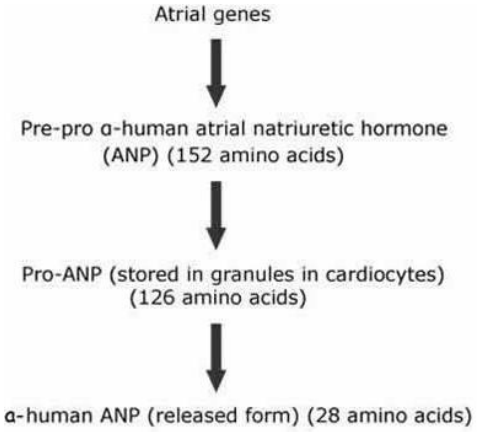
PGE_2



Atrial Natriuretic Peptide

ANP is released by **atrial** stretch (secondary to **increased ECV)** and act through **cGMP.** The kidneys themselves synthesize an ANP analogue called **urodilatin** which acts in a similar way. Acts on:

- Inner medullary collecting duct – closure of Na^+ epithelial channels and inhibition of Na^+/K^+ ATPase → Reduced Na^+ reabsorption
- Inhibition of aldosterone
- Reduced renin release
- Vasodilation of mainly afferent arterioles → increased GFR.



Other factors in regulation of sodium

Dopamine is synthesised and acts on the cells of the proximal tubule to inhibit Na^+/K^+ ATPase and decrease Na^+/H^+ antiport activity.

Kinins are vasodilatory peptides and have similar effect to prostaglandins – mechanism unclear

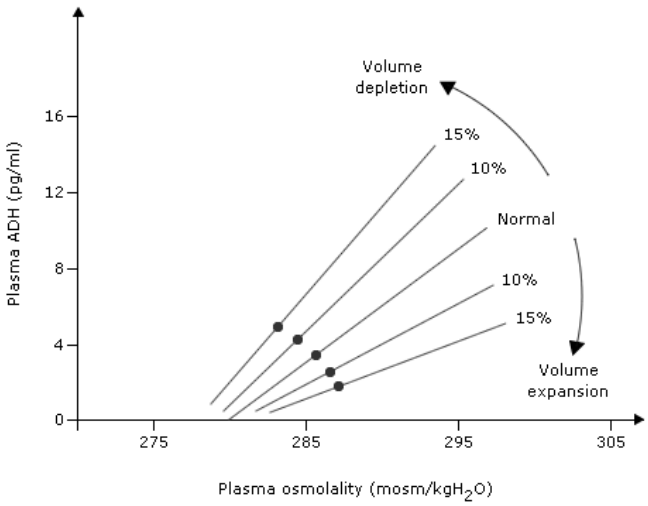
Nitric Oxide is synthesised in epithelium of afferent arterioles to cause vasodilation and increase GFR – unclear if it plays a role in Na^+ regulation.

Natriuretic hormone is unidentified in origin but increases fraction of Na^+ excreted in the urine.

Osmotic vs. Volume Regulation

Sometimes, controlling ECV is more important than osmolality i.e. in haemorrhage. Changes in ECV is detected by low pressure **atrial stretch receptors** and high pressure **arterial stretch receptors**. This leads to alterations in ADH release (greater stretch, less ADH secretion).

The relationship is on the graph as follows: the body accepts a reduced osmolality as a price for maintaining volume at a higher level than it otherwise would be. The dots represent the normal ADH plasma level for a particular blood volume.



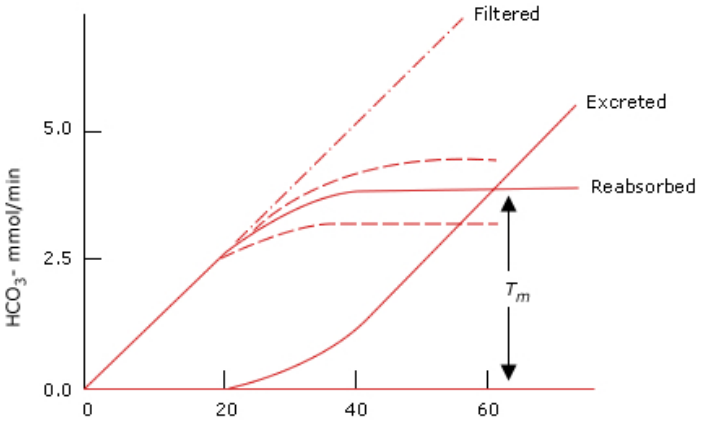
Renal Regulation of pH

(07b_05_05)

The description of buffer systems and pH with the **Henderson-Hasselbalch equation** can be found in the general physiology notes.

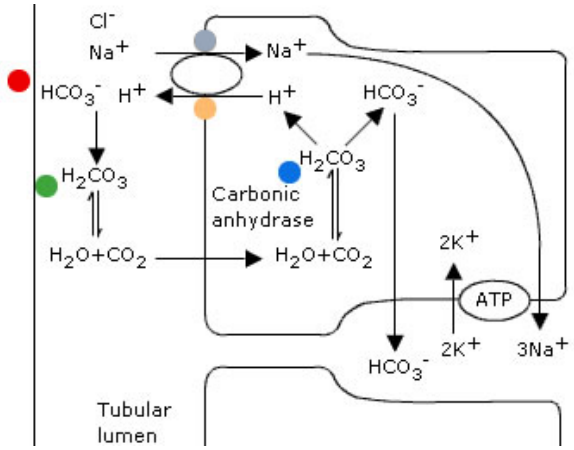
Renal Handling of Bicarbonate

Bicarbonate is **freely filtered** and therefore has a concentration of **24mmol/L** in the tubular fluid (same as plasma). This also happens to be around the **T_M (maximal tubular reabsorption of bicarbonate)**. This varies according to the **rate of H^+ secretion.**



Reabsorption of HCO_3^-

Reabsorption of HCO_3^- has been covered in the previous lecture. See the following diagram:



This occurring in the proximal tubule accounts for 90% of all HCO_3^- reabsorption. This process also occurs in the distal tubule and collecting duct but only accounts for 10% of all HCO_3^- reabsorption. This occurs with H^+/K^+ ATPases Na^+/H^+ linked transport is of less importance. Aldosterone works here.

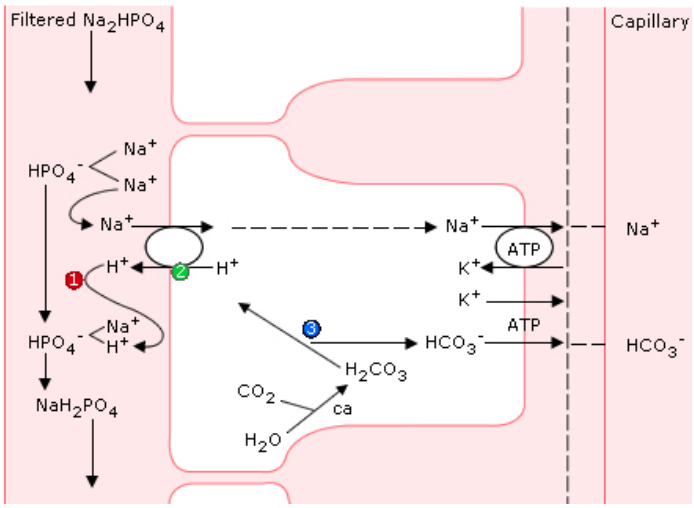
Considering that most HCO_3^- reabsorption occurs through H^+ secretion, the gradient of H^+ ions will become very large which is where **urinary buffers come into play**.

Urinary H^+ Buffering

Alkaline/Acid Phosphate

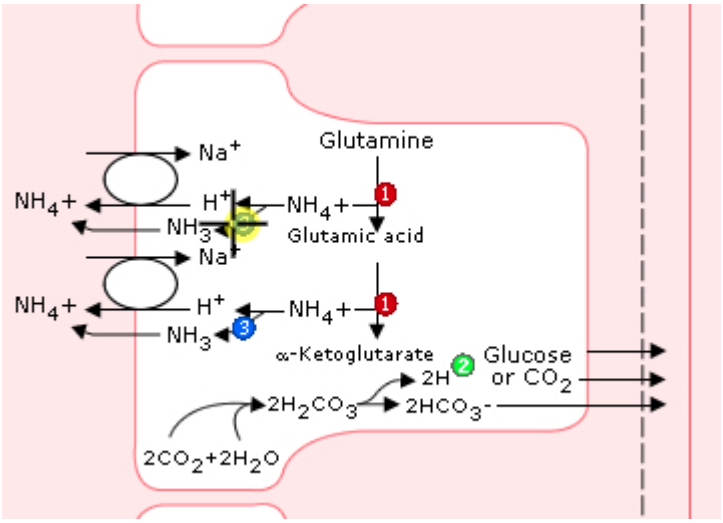
Na_2HPO_4 (alkaline phosphate) is filtered into the tubular fluid. This dissociates and one Na^+ is exchanged for a H^+ to form NaH_2PO_4 (acid phosphate) to be excreted.

The pK is 6.8. Therefore, in the plasma, the ratio of alk:acid phosphate is 4:1. This reduces in the tubular fluid from H^+ secretion and mainly occurs in the **distal tubule**.



Ammonia Secretion

The following mainly occurs in the **proximal tubular cells**.



Ammonium is formed with a-ketoglutarate (from glutamic acid) from **deamination of glutamine** (by enzymes that thrive in acidosis) which then dissociates into H^+ and NH_3.

Glutamine → 2-oxoglutarate + $2NH_4^+$

H^+ is secreted per normal exchange and ammonia diffuses through the apical membrane once again to form ammonium in the lumen. 2-oxoglutarate (a-ketoglutarate) reacts with $2H^+$ ions from hydrogen bicarbonate intracellularly to form glucose or CO_2 which allows bicarbonate to leave into the bloodstream.

A **large fraction of secreted NH_4^+ is reabsorbed by the thick ascending limb** by $Na^+/NH_4^+/2Cl^-$ cotransport across the apical membrane and accumulates in the medullary interstitium. The NH_4^+ is secreted as NH_3 by the collecting tubule cells and converted into NH_4^+in the lumen by combination with secreted H^+. NH_4^+ cannot readily diffuse back and is therefore trapped and excreted.

THRIVING IN ACIDIC ENVIRONMENTS: Due to:

1. Increased deamination of glutamic acid
2. Increased secretion of H^+ ions in collecting duct → greater collecting tubular concentration of ammonium → high rate of ammonia diffusion into tubule for further ammonium production (impermeable in collecting tubule to NH_4^+)

If NH_4^+ was not secreted, it will be **used by the liver with bicarbonate** to produce urea for excretion and hence no bicarbonate gain in plasma. Therefore, the **more the ammonium secretion** occurring in the tubules, the greater the amount of HCO_3^- will be available in the plasma.

GASTROINTESTINAL PHYSIOLOGY

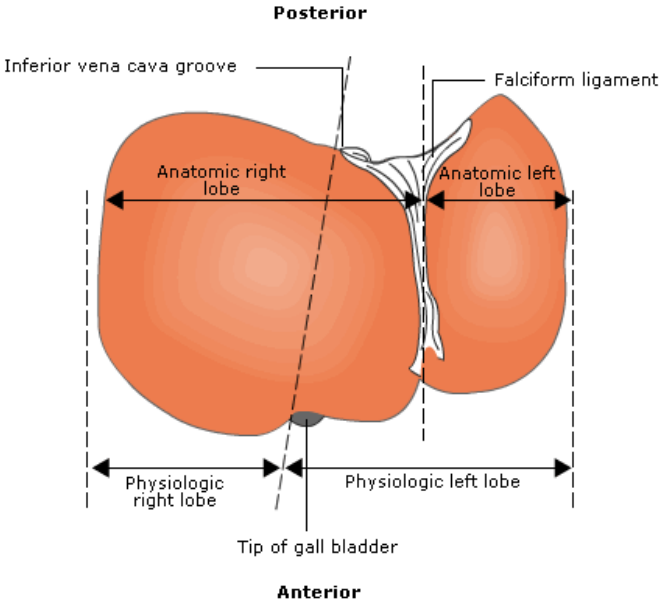
Functional Liver Anatomy and Blood Supply

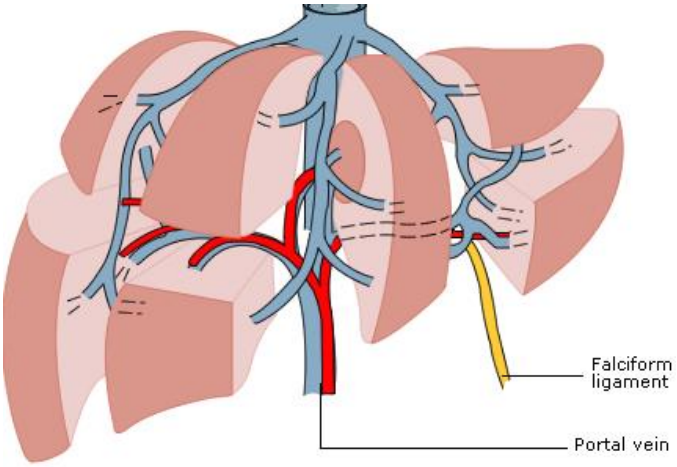
(07b_06_01)

The liver is the largest internal organ at 1.5kg. The **falciform ligament** divides the liver into anatomical right and left lobes – no functional significance.

The other dotted line from the tip of the gall bladder to the groove of the IVC separates the liver physiologically between **true right** and **true left lobes** on the basis of biliary drainage and blood supply.

It can be **further divided into 8 functional segments** based on vascular distribution and biliary drainage. 1-4 in L lobe and 5-8 in R lobe.

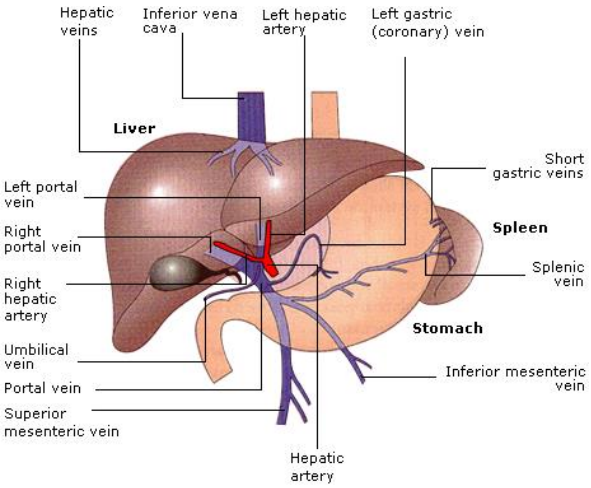




Blood Supply

It has a unique **dual blood supply** and receives a total of **25% of cardiac output** and 75% is provided via the **portal vein** draining the whole capillary system of the GIT. The remaining 25% is provided by the **hepatic artery**. However, they each provide 50% of the oxygen supply.

Porta Hepatis: Site of entry for the portal vein and hepatic artery which then divide into the R and L branches and run in parallel. It is also the exit point for the bile ducts and lymphatics. The venous drainage from the liver occurs in the hepatic veins that drain into the IVC.

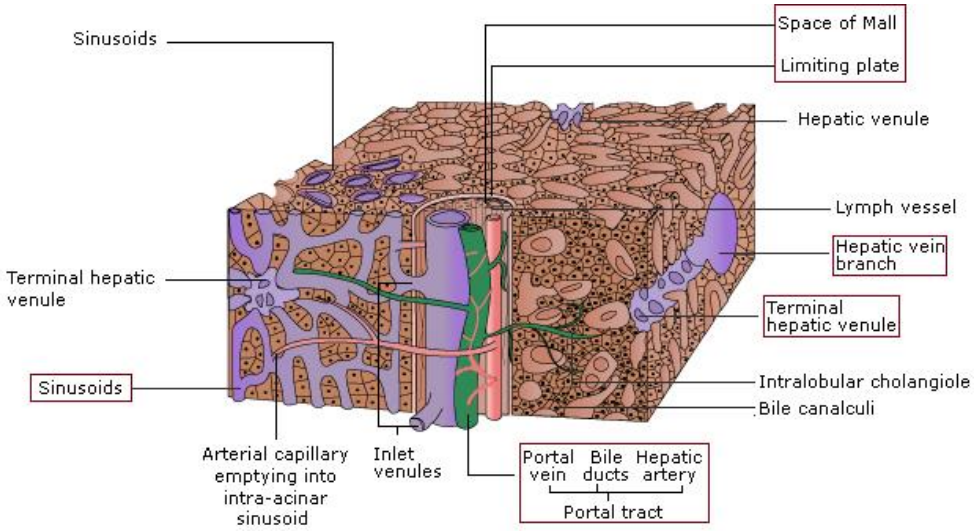


Normal blood volume in the liver is **450ml** which can increase to 500-1000ml with increased R atrial pressure. The liver can act as a **vascular reservoir** which can release 250ml systemically with volume depletion such as acute blood loss.

Microcirculation

Portal tracts (containing a bile duct, portal vein & hepatic artery) are surrounded by fluid in a periportal space known as the **Space of Mall** and is surrounded by a limiting plate which is pierced by **inlet venules** to supply:

Sinusoids are a network of vessels surrounding hepatocytes (see later).



Sinusoids drain blood into **terminal hepatic venules**.

Blood Flow Control

This only comes from the **hepatic artery**. The flow changes through the portal vein through a wide variety of stimuli but there is no evidence that the liver can direct this flow. 3 types of control:

1. **Intrinsic control**
    a. Autoregulation to MAP of 60mmHg
    b. **Hepatic arterial buffer response** (HABR):

HABR occurs when total **portal venous flow is reduced**, there is a **rapid increase** in **hepatic artery flow** and visa versa. Possible this is secondary to the **degree of adenosine clearance (**powerful vasodilator of the hepatic artery). This clearance is increased through portal flow washout of adenosine in the space of Mall (secreted into this space at a constant rate)

2. **Neural Control**
    a. Most likely negligible control as denervation has no overall effect. Nonetheless, SNS may cause vasoconstriction

3. **Humoral Control**
    a. Secretin, CCK-PZ, glucagon, prostacycline and low dose adrenaline → dilatation
    b. Noradrenaline, dopamine, angiotensin, vasopressin and high dose adrenaline → vasoconstriction of hepatic and splanchnic arteries.
    c. **Nitric oxide:** Plays a key role. Vasodilation of splanchnic circulation and increased blood flow in sinusoids.

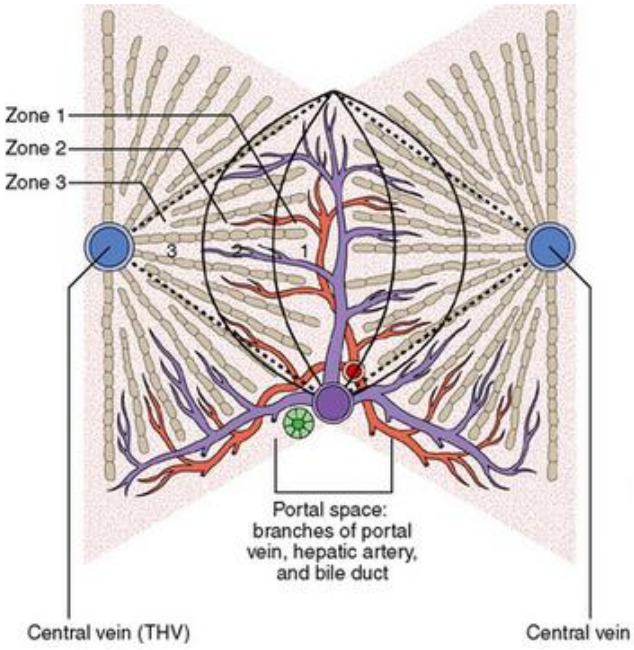
Functional Organisation

Liver Acinus

The image shown describes a **liver acinus** with the **afferent vessels** central draining into the **terminal hepatic venules**. The zones describe the distance from the afferent blood supply:

Zone 1: Hepatocytes which receive **the most oxygen and nutrients**. They therefore have an **abundance of mitochondria** and are suited for **oxidative metabolism** and **glycogen synthesis**.

Zone 3: Receives the **least amounts of oxygen and nutrients** and are therefore most prone to hypoxic injury. Optimal for **anaerobic metabolism**. They have an **abundance of smooth ER and cyt P450** allowing biotransformation of the majority of drugs, chemicals and toxins.



N.B. ***Classic lobule*** is the hexagonal arrangement with the central THV in the middle but this is not the functional unit so is not used for physiological descriptive purposes.

Sinusoids

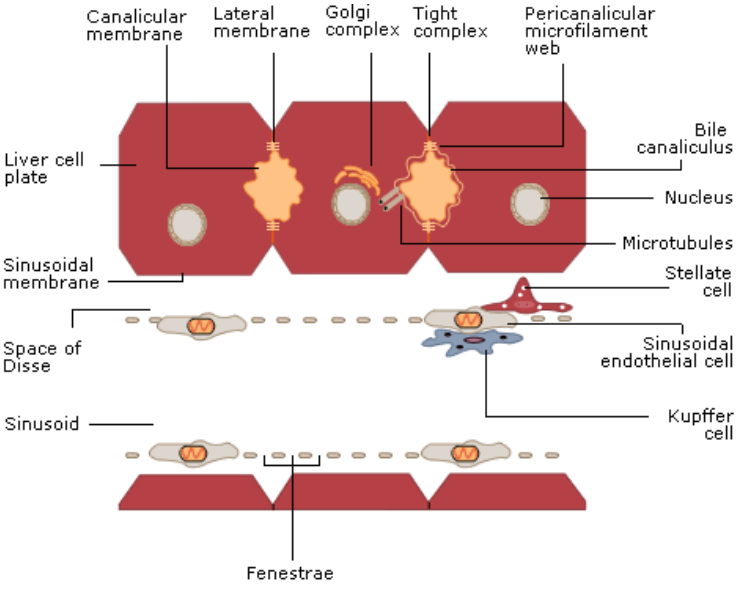
These are **specialised fenestrated capillaries**. The fenestrae are dynamic and are regulated by an actin cytoskeleton.

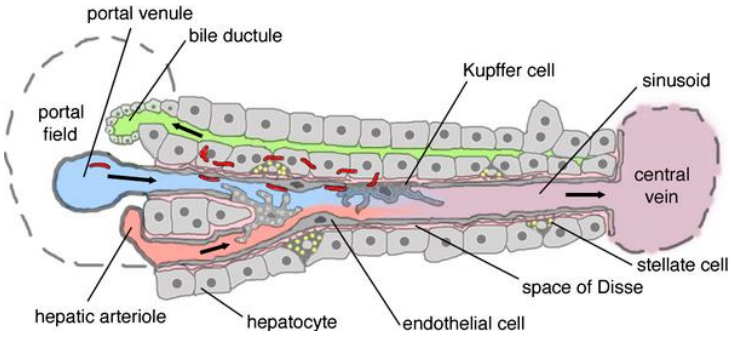
Within sinusoids exist the **Kupffer cells** which guard the entrance from the portal field.

There are also **stellate cells** that **store vitamin A** and wrap around sinusoids to contract or relax and mediate tone.

With **no basement membrane**, direct contact between plasma and the **perisinusoidal space**, known as the **Space of Disse**, can occur.

In **cirrhosis**, there is widespread defenestration and formation of basement tissue preventing normal blood flow into the Space of Disse.



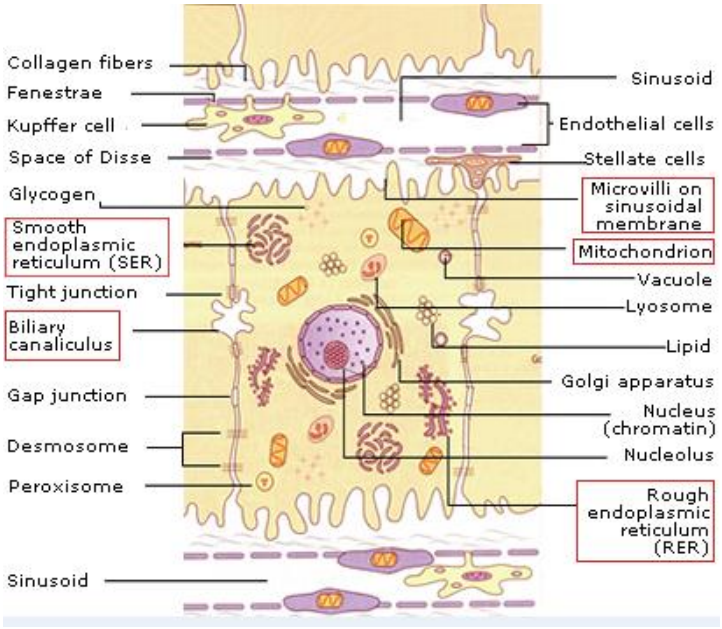


Hepatocytes

These have **microvilli** projecting into the space of Disse and increases its surface area to facilitate cellular transport. The **mitochondria** have oxidative phosphorylation occurring on the inner membrane and urea and citric acid cycles in the matrix.

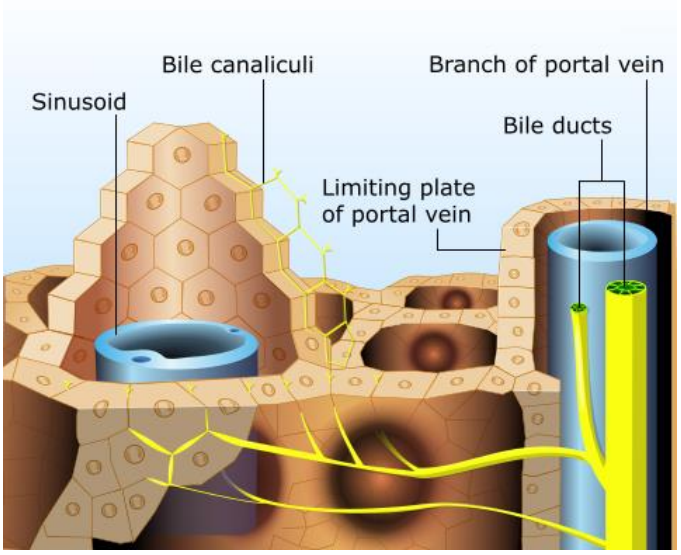
Smooth ER is where metabolism of xenobiotics and fats occur, cholesterol and bile acid synthesis and where cyt P450 is in abundance (prevalent in zone 3).

Rough ER is the site of all protein synthesis and better developed in zone 1.



Biliary Drainage

Biliary canaliculi are on the lateral surface of hepatocytes with active excretion being the main mode of transport. They form an anastomosing network in a single plate of hepatocytes to drain into the **periportal cholangioles → interlobular bile ductules** (in portal tracts) **→ intrahepatic bile ducts → right** and **left hepatic duct**.



Lymphatic Drainage

Vital as large amounts of fluid and protein permeate through the sinusoids → lymph forms in space of Disse. This fluid then collects in the space of Mall where it is collected in lymphatic vessels. Large vessels leave the porta hepatis and drain into the **thoracic duct**.

In pathology, excess interstitial fluid volumes will be moved as a transudate resulting in ascites.

Metabolic and Synthetic Functions of the Liver (part 1)

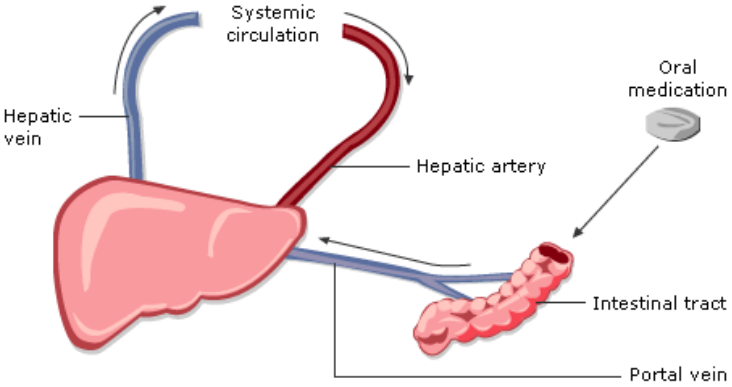
(07b_06_02)

Covering **biotransformation, bile formation and excretion, haem metabolism**

Xenobiotics: chemicals found in the organism not normally found in the organism. This accounts for most drugs.

Biotransformation (**detoxification**)

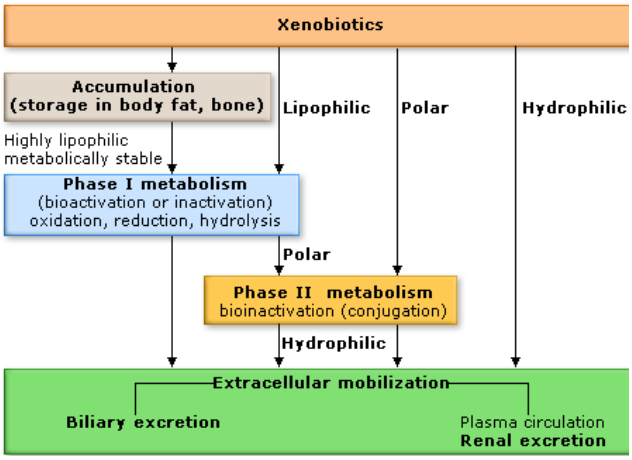
The process where metabolic products, xenobiotics and toxins convert to safe soluble substances eliminated by urine or bile. Exogenous and endogenous substances undergo biotransformation by the same process. Note, this does not only occur in the liver.



All substances in the gut pass via the portal vein to the liver for **first pass metabolism**. When the compounds in the systemic circulation is presented to the liver, this is known as **second pass metabolism**.

Phase I Metabolism

Alteration of a substance to make it polar and/or water soluble. Usually involves **reduction** or **oxidation**. This occurs through the **cytochrome P450 enzymes**. These are **haem-containing enzymes** with 57 members belonging to the CYP1, 2 and 3 gene families. Located on the **smooth ER** of hepatocytes and located next to cofactors such as phase II enzymes and electron donors. Following phase I metabolism the substance may become:



- Sufficiently water soluble and excreted
- A substrate for phase II metabolism
- Bioactivated
- Toxic requiring further metabolism

Phase II Metabolism

Synthetic reactions. Involve **conjugation** to a **hydrophilic endogenous substrate** derived from compounds used in the metabolism of amino acids and carbohydrates. Products are almost never toxic. Performed by the **transferase enzymes** to transfer a moiety from a donor molecule to a drug recipient i.e. *glucuronyl transferase*. Examples of phase II reactions include:

- **Glucuronidation** (smooth ER). Endogenous steroids, thyroxine, catecholamines and bilirubin.
- **Sulphation** (cytosol). Endogenous steroids and carbohydrates used
- **Acetylation**
- **Methylation**
- **Glutathione conjugation**
- **Glycine conjugation**

Glutathione metabolism occurs which binds highly toxic intermediates to form mercapturic acid conjugates catalysed by **glutathione-S-transferase**. This is central to paracetamol detoxification. If stores of **glutathione are depleted**, toxic metabolites **bind covalently to liver proteins → hepatic necrosis**. Stores are replenished by **cysteine containing drugs** and **dietary sulphydryl compounds**.

Bile

500-1200ml of bile is secreted by the liver/day. It consists of cholesterol, bile acids, bilirubin, phospholipids, inorganic electrolytes and biliverdin. As **bile is alkaline,** bile acids form Na^+ and K^+ salts. Bile functions to

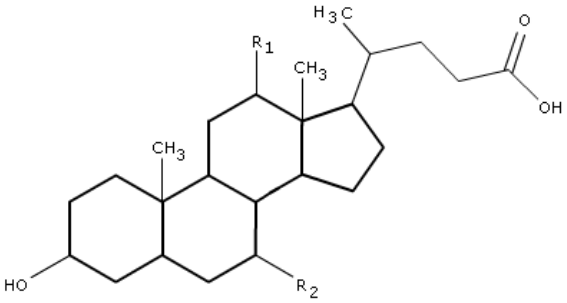
- Elimination of cholesterol, phospholipids and bilirubin
- Facilitation of fat digestion and absorption
- Absorption of fat-soluble vitamins A, D, E and K.

Without these functions, patients become rapidly catabolic.

Bile Acids

Main constituent of bile and formed **from steroids** in hepatocyte **smooth ER** to create a **water soluble acidic steroid** with **amphiphatic anion** properties (both hydro-philic/phobic properties)

- Hydrophobic portion: Cholesterol
- Hydrophilic portion: amino acid conjugate



Cholic acid	R1 = OH, R2 = OH
Chenodeoxycholic acid	R1 = H, R2 = OH
Deoxycholic acid	R1 = OH, R2 = H
Lithocholic acid	R1 = H, R2 = H

Primary bile acids are those formed in the liver (cholic acid and chenodeoxycholic acid)

Secondary bile acids are those formed in the colon following bacterial modification (deoxycholic acid and lithocholic acid).

Following their synthesis, they are **conjugated with glycine or taurine** to be secreted into the canalicular lumen which then joins to the enterohepatic circulation.

Bile Salts

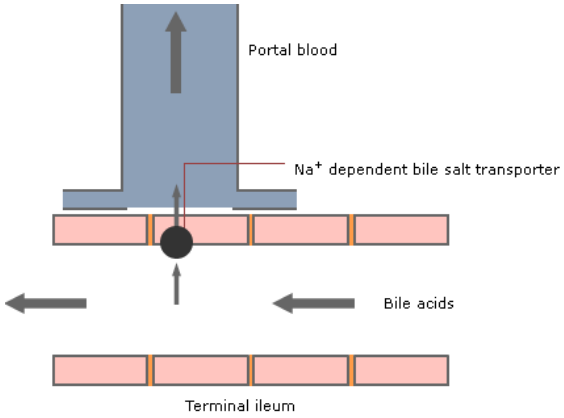
When a critical concentration is pumped into the canalicular lumen, the bile salts form **micelles** which are extremely **cytotoxic** from their **detergent like activity** so is **neutralised** by the incorporation of **cholesterol, phospholipids** and other **organic molecules**.

Bile is concentrated 5-20-fold in the gall bladder through active transport of sodium out via the gall bladder wall followed by secondary water and Cl^- reabsorption out through the gall bladder wall. The gall bladder stores 30-60ml. 25% of bile bypasses the gall bladder. Different constituents of bile in liver and gall bladder are as follows:

Component	Liver	Gallbladder
Water	97.5 g/dL	92 g/dL
Bile salts	1.1 g/dL	6 g/dL
Bilirubin	0.04 g/dL	0.3 g/dL
Cholesterol	0.1 g/dL	0.6 g/dL
Lecithin	0.04 g/dL	0.3 g/dL
Na^+	145 mmol/L	130 mmol/L
K^+	5 mmol/L	12 mmol/L
Ca^{2+}	5 mmol/L	23 mmol/L
Cl^-	100 mmol/L	25 mmol/L
HCO_3^-	28 mmol/L	10 mmol/L

Movement in the GIT

Foods with **high fat content** in the duodenum stimulate the **intestinal mucosa** to **produce cholecystokinin**. This causes **gall bladder contraction** releasing bile into the duodenum through **relaxation of the sphincter of Oddi** through **vagal stimulation**.



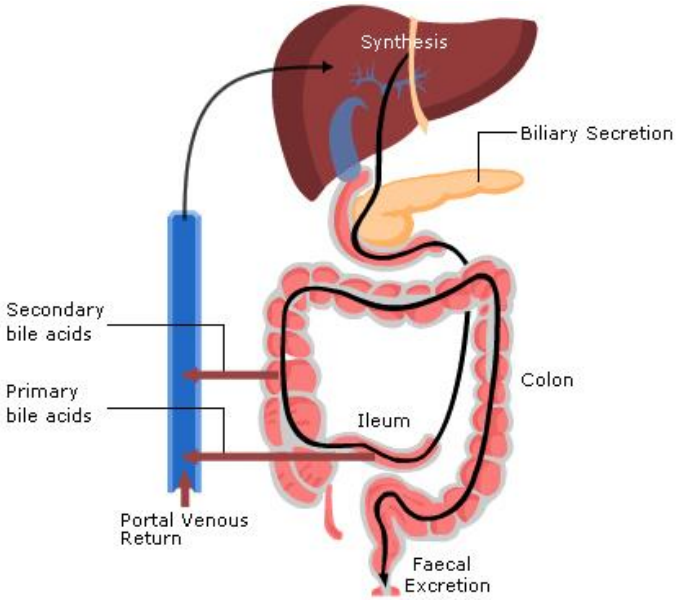
As the bile released into the duodenum is **conjugated** (and hence **polar**), they are not absorbed in the proximal intestines and so accumulate to **facilitate fat absorption**.

90% of bile acids are **absorbed** in the **terminal ileum** by **active transport.** The remainder is **deconjugated** and transformed to secondary bile salts by bacterial action. Some is reabsorbed and some lost to faeces.

Enterohepatic Circulation

The process described above with portal blood flow to the liver is the **recycling of bile salts** mostly bound to albumin. It is taken up by hepatocytes that express proteins to pump bile salts in and out of the cell. The **enterohepatic cycle** occurs about **8 times/day** and may occur twice a meal. Without it, there will be fat malabsorption.

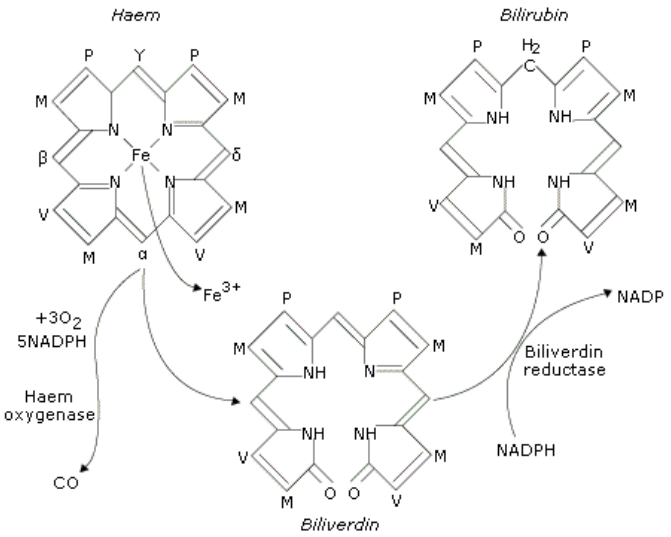
This allows efficiency and signals to the liver from the intestine about regulation of synthesis and transport rates.



Bilirubin

This is the end product of **haem metabolism**. Approximately 1-2 x 10^8 erythrocytes are destroyed per hour which equates to **6g haemoglobin/day**. **Haemoglobin → globin** and **haem** in the reticuloendothelial system (i.e. Kupffer cells and spleen). Globin is reticulized (fragmented) whilst haem:

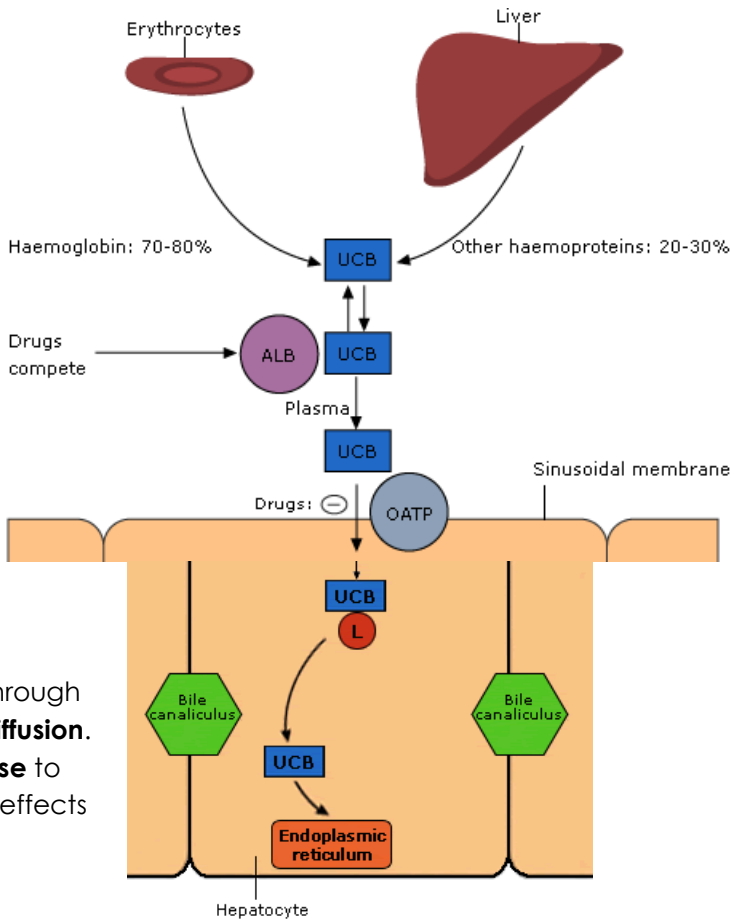
- Undergoes oxygenation → release of iron and conversion to biliverdin and CO
  - Carbon monoxide is exhaled
  - Iron enters the iron pool
  - Biliverdin → bilirubin.



70-80% of bilirubin is produced this way with the remaining produced from haemoproteins i.e. myoglobin, cytochrome and peroxidase. 250-400mg bilirubin is made / day.

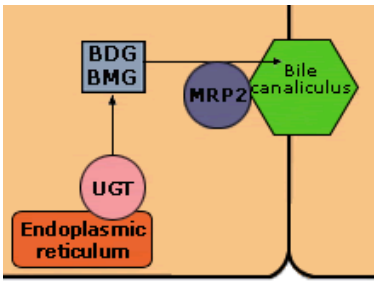
Unconjugated bilirubin (UCB) needs to be **conjugated** in the liver as is toxic to many cells so is transported with tight binding to albumin. Note some drugs can cause displacement & hypoalbuminaemia may cause toxicity. Its physiological significance is not yet clear in adults but has good antioxidant properties in neonates.

At the sinusoidal surface, UCB is taken up by hepatocytes after dissociating from albumin through the transporter protein **(OATP)** via **facilitated diffusion**. Once inside, it binds to **glutathione-S-transferase** to reduce efflux of bilirubin and prevents its toxic effects to organelles.



Conjugation

Conjugated with **glucuronic acid** in the smooth ER by the enzyme uridine diphosphoglucuronate glucuronosyl transferase (**UGT**) which has several isoforms into **bilirubin diglucuronide (BDG)**, with some **monoglucuronide (BMG).** CB travels into the bile through unidirectional transport against a concentration gradient.

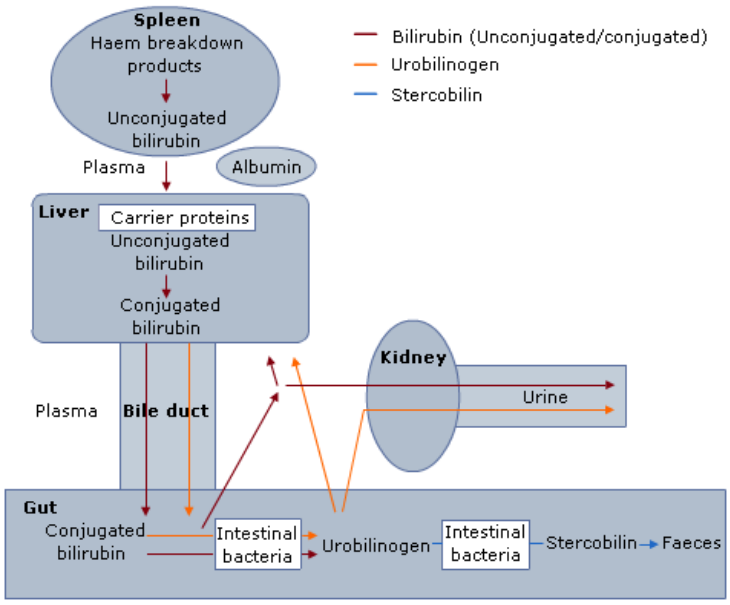


Further Facts

In the small intestine: bacteria convert **bilirubin → urobilinogen** which is reabsorbed to be **excreted in bile** and to a lesser amount via **urine.** It is initially colourless but **oxidised** to form **urobilin** which forms colour in urine and faeces. Renal excretion plays a larger role with **biliary obstruction**.

UCB can only be excreted in the urine with albuminuria. Some UCB is excreted in bile and undergoes enterohepatic circulation.

UCB:CB ratio: Normal in plasma is 25:1 (4% CB)
Haemolysis – both rise proportionally.
Obstruction/hepatocellular disease – both accumulate.



Metabolic and Synthetic Functions of the Liver (part 2)

(07b_06_03)

Overall classification of liver function is as follows:

METABOLIC FUNCTION	Biotransformation of: • Drugs • Xenobiotics and toxins • Hormones (e.g. T4 → T3)
	Bile formation and excretion
	Haem metabolism

	Intermediate metabolism of:
	• Carbohydrates
	  ○ **Includes lactate metabolism**
	• Proteins
	  ○ **Synthesis of plasma proteins and clotting factors**
	  ○ **Includes elimination of ammonia**
	• Lipids

IMMUNOLOGICAL FUNCTION	Immunological function involves: • Kupffer cells • Cytokines • Intrahepatic lymphocytes • Antibody production and transport

Those in **bold** will be covered in this session.

Lactate

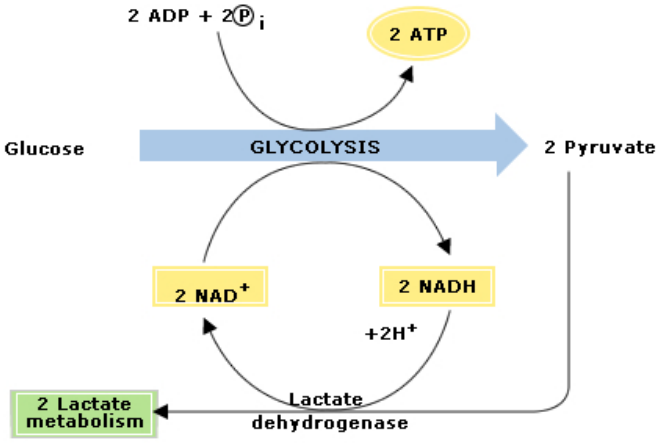
This is the end product of **glycolysis** with **pyruvic acid.** The pKa of lactic acid is 3.85 so it is more than 99% dissociated in the plasma into La^- and H^+. **Pyruvate** is the end product of glycolysis in the cytosol which then continues in the mitochondria with oxygen but will be converted to lactate in the following conditions:

- Cells without mitochondria i.e. erythrocytes
- Anaerobic conditions
- Rapid increase in metabolic rate (pyruvate production exceeds mitochondrial capacity)
- Glucose metabolism exceeds the oxidative capacity of the mitochondria – seen in inborn errors of metabolism and following catecholamine administration.

There are **3 pathways pyruvate may follow:**

1. Movement into the mitochondria to enter the **citric acid cycle**
2. **Conversion to glucose** through gluconeogenesis
3. Undergo **transamination** to **alanine** in the liver and kidneys.

Lactate dehydrogenase is the enzyme converting pyruvate to lactate located in all cells but the pancreatic islet cells. The **1st step** in lactate metabolism is the **conversion back to pyruvate**.



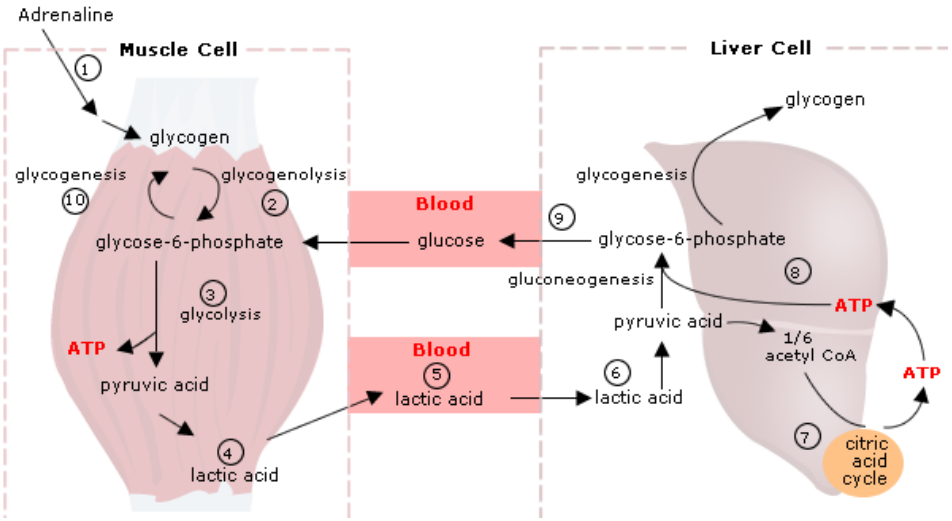
Does Lactate have uses?

Lactate is in fact useful! It is not only **produced** in anaerobic conditions but continuously under **aerobic conditions** and is **utilised** as an **energy source** (both immediate and stored) by numerous tissues distal to its production site. It is also found to be an **important intermediate in wound repair and regeneration**.

It is actively oxidised at all times – especially in exercise accounting for 75% of its removal. It is formed in white glycolytic muscle fibres and oxidised in adjacent red oxidative muscle fibres. **25%** of lactate is transported to the liver for **gluconeogenesis**:

Cori Cycle

This is the recycling of lactate into glucose – **gluconeogenesis**. It can only occur under **aerobic conditions**. The glucose will either be taken up by active muscles or undergo glycogenesis if muscle activity is ceased.



Synthetic Functions of the Liver

Plasma Proteins

Albumin is the most abundant of plasma proteins. The majority have little or no enzymatic activity but often **bind to molecules** to make them inert and convey reduced toxicity. Albumin and a-fetoprotein bind to many different ligands whilst others are specific i.e. caeruloplasmin with copper.

Albumin synthesis is regulated by changes in nutritional status, osmotic pressure, inflammation and steroids. In normal healthy adults, if it is lacking, there will be few manifestations as other proteins compensate for its absence.

Coagulation Factors

Factors II, VII, IX and **X** are produced in the liver. Prolonged PT may be due to impaired synthetic function or cholestatic syndromes impairing absorption of vitamin K for the activation of these factors. The latter is improved with IV vitamin K but not with impaired synthetic function.

Antithrombin III, **protein C** and **protein S** are important **inhibitors of haemostasis** and is also produced by the liver. However, in CLD, the overall effect is bleeding due to disproportionate deficiency in procoagulants.

Ammonia

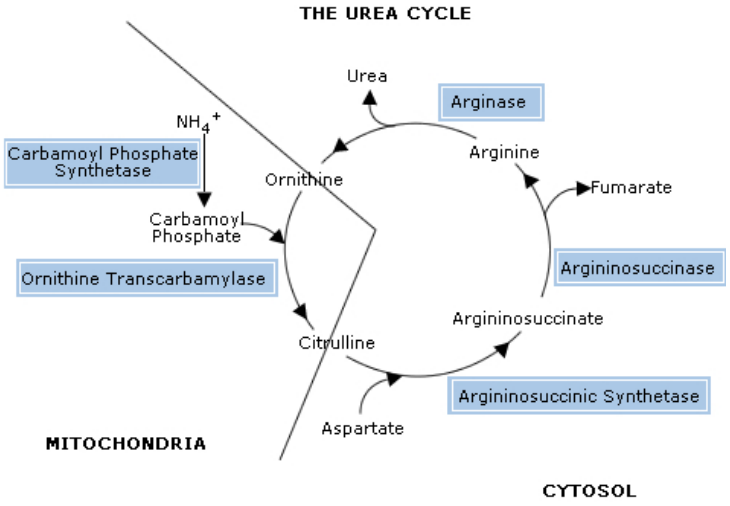
This is a major by-product of protein and nucleic acid metabolism and is produced in large quantities by **intestinal bacteria** from dietary protein and urea. Therefore, it arrives through the portal system before the systemic circulation accounting for the difference in concentration. The **liver removes most of the ammonia** to make levels <35µmol/l. Any higher and it is neurotoxic. **At physiological pH**, the bulk of NH_3 is **in its ionised form, ammonium** (NH_4^+). It is an important regulator of **nitrogen metabolism.**

In the liver, **ammonia → urea** and **glutamine**. This also allows regulation of systemic pH as the H^+ produced from ammonium during urea synthesis neutralises excess bicarbonate produced from amino acid breakdown.

Urea Cycle

Urea is electroneutral. Much of the ammonia used is derived from extra-hepatic tissues i.e. kidneys, liver, muscle and brain. 25% arrives via portal vein. **Most of the nitrogen is transported** as **amino acids** for incorporation into urea.

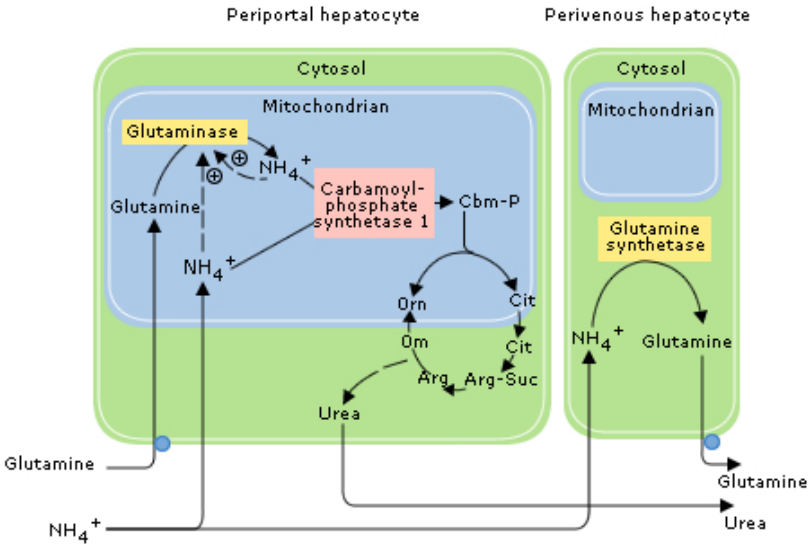
30% of the urea produced is hydrolysed into ammonia in the colon which is recycled and presented back to the liver



Carbamoyl phosphate synthetase is the key regulator of urea synthesis and is located **periportally** but minimally in zone 3 of the acinus.

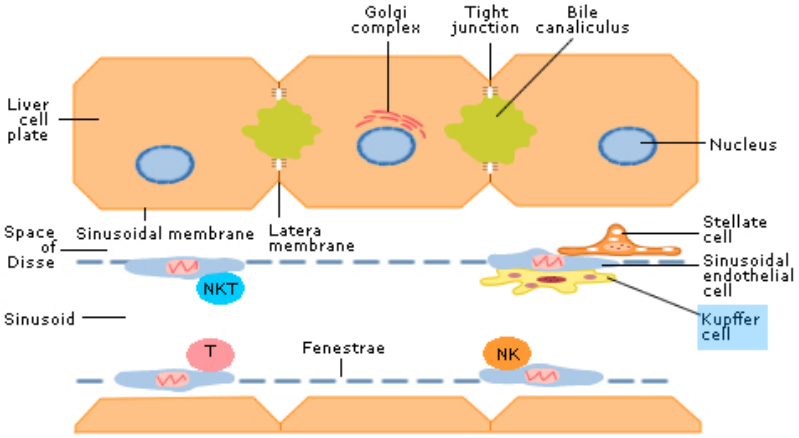
Perivenular cells (also called scavenger cells) express **glutamine synthetase** which scavenge any remaining ammonia to form **glutamine** and is a high affinity system.

Therefore, as blood flows through the acinus, the ammonia is depleted. With damage to zone 3 – there will be hyperammonaemia.



Immunology

It is a major organ of the **innate immune system** as is exposed to a large number of antigens from the GIT. The following cells are involved:



Natural Killer Cells

Cytotoxic activity and they produce large amounts of proinflammatory and antiinflammatory cytokines upon activation. Cytotoxicity is **spontaneous** and enhanced by interferon γ and β and interleukins. It also acts as a bridge to the adaptive immune system.

NKT cells are as above but share characteristics of classic T-cells. They also require a **stimulatory signal for the development of cytotoxicity**.

Kupffer Cells

Resident **hepatic macrophages** from **circulating monocytes** and specialise to function through **phagocytosis**, and **antigen processing and presentation**. Significant role in the removal of particulates, cells, toxins, infectious agents and foreign substances from the portal blood. They also are the source of **vasoactive substances**.

Once **activated,** there is a large amount of inflammatory mediator release including

- Cytokynes (e.g. TNFa, interleukins 1, 6 and 10),
- Chemokines (e.g. IL-8),
- Nitric oxide,
- Superoxide
- Prostanoids.

These can allow further activation of other Kupffer cells or the downregulation of proinflammatory mediators. They are able to phagocytose and bind complement coated pathogens.

Turnover of the whole Kupffer cell population is from 21 days to 14 months.

Humoral System

Important in the transport, function and clearance of **IgA** which is **synthesised in the bowel**. Once in the liver, it is **secreted into bile** to be recycled in the bowel.

Gastric Function

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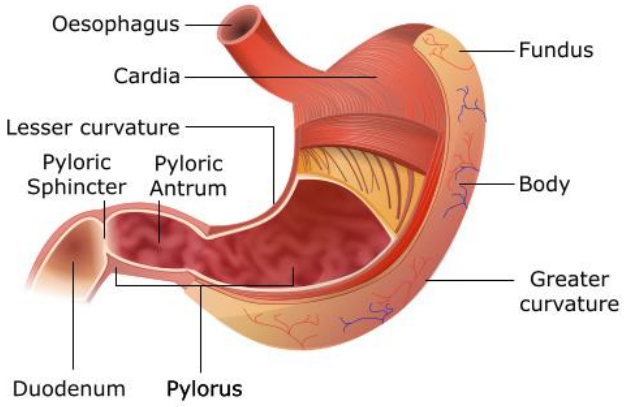
Anatomy

The **proximal stomach** (fundus and body) has thin walls and are **highly distensible** and therefore acts as a **food reservoir**.

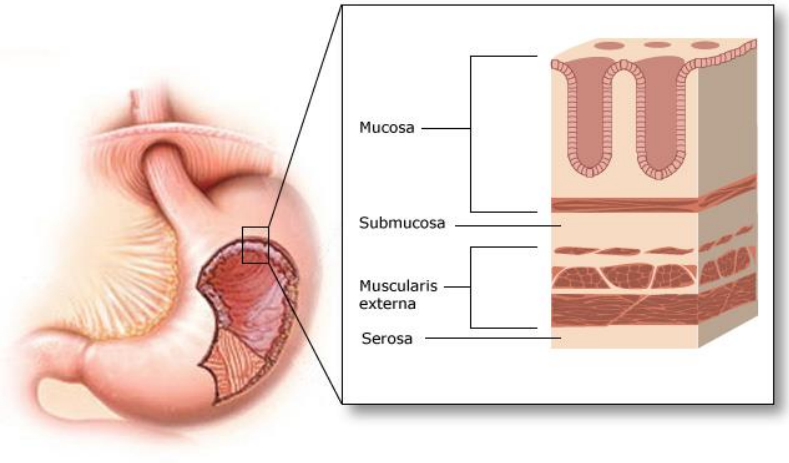
The **distal stomach** (antrum and pylorus) has **thick muscular walls** important in grinding & churning food into smaller food particles.



Layers

Layers from the outside in:

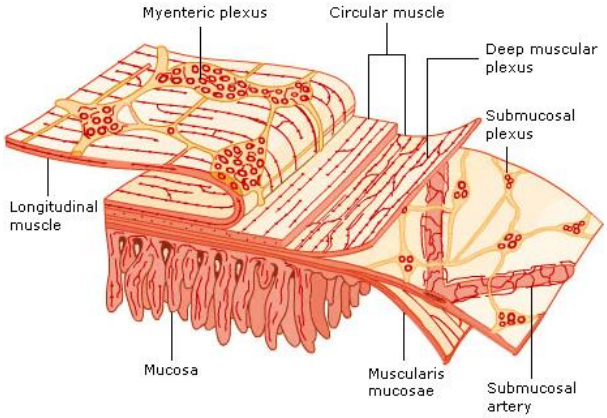
Serosa → 3 muscular layers (longitudinal, circular, oblique (only ant. and post. sides)) → **Submucosa → muscularis mucosae → mucosa** consisting of lamina propria and columnar epithelium.



The muscles have different orientations to contract in different planes in order to allow churning, grinding and kneading as well as propulsion. Rugae are irregular folds more prominent in the proximal stomach and flatten out with a full stomach.

Innervation

Parasympathetic supply is from the vagus nerve (X) which has sensory, secretomotor and motor fibres. **Sympathetic** supply is via splanchnic nerves with sensory and motor fibres (inhibition)



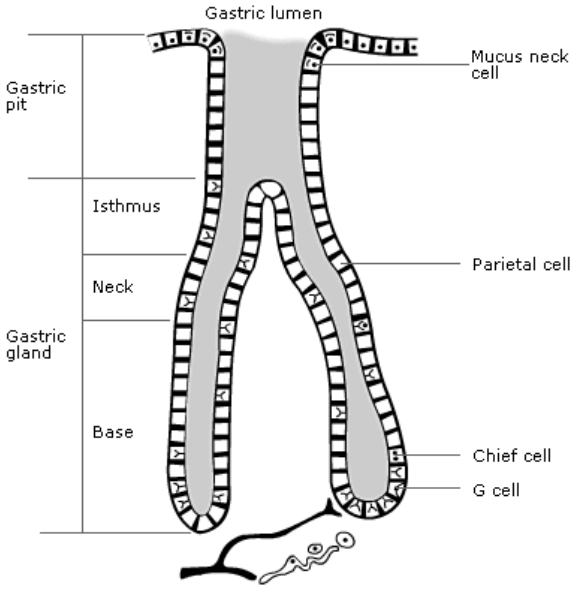
Intrinsic supply is from 2 plexi:

- **Myenteric (Auerbach's plexus)** situated between longitudinal and circular muscle layers regulating intestinal motility and sphincter function
- **Submucosal (Meissner's plexus)** within the submucosa and responds to and regulates epithelial cell and submucosal blood vessels.

There are multiple connections between the intrinsic and extrinsic innervation with many hormones involved.

Mucosa

Different cells exist within the **columnar epithelium** of the gastric mucosa. There are 100 gastric pits per mm^2 of epithelium. Each pit has 2-3 tubular shaped glands containing several types of secretory cells which convey secretory fluid to the lumen → **protective alkaline fluid containing mucus.**



- **Mucus neck cells** – at the opening of the gastric pits which secretes **mucus** (different from surface epithelium)
- **Chief Cells** – found at the base of the gastric glands mainly in the body of the stomach secreting **pepsinogen**. They have extensive rough ER and secretory granules fit for purpose
- **Parietal (oxyntic) cells –** pyramidal in shape found mainly in the fundus and body secreting **HCl** and **intrinsic factor**. They have numerous mitochondria.
- **G cells –** enteroendocrine cells found mainly in the antrum that secrete **gastrin**
- **D cells –** enteroendocrine cells that produce **somatostatin** in response to gastric acidity.

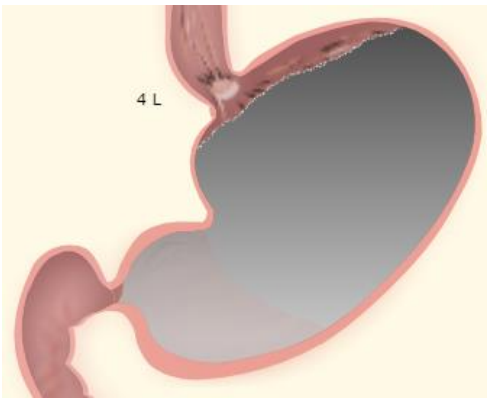
The Functions of the Stomach

These will all be taken in turn but as an overview includes:

1. Temporary storage of food input
2. Mechanical digestion to smaller food particles
3. Chemical digestion with protein breakdown
4. Intrinsic factor secretion for B12 absorption
5. Regulation of output of chyme into duodenum

Temporary Storage

Occurs in the **fundus** and the **body** with distensible thin walls when the stomach content exceeds 1L and holding up to 4L. This occurs through **vagal response to stretch** in:

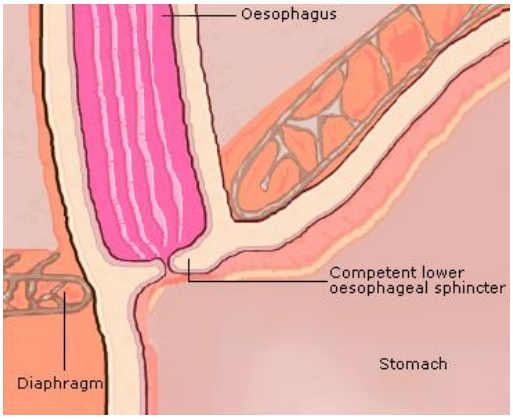


1. The oesophagus allowing a receptive relaxation
2. Through presence of **food in the proximal stomach – adaptive relaxation**

Neurotransmitters are **VIP** (vasoactive intestinal polypeptide) and **NO**. The contractile properties are different in fed and fasted states.

Lower Oesophageal Sphincter

This is physiological and cannot be defined anatomically. It is formed from **circular muscle contraction** of the lower 2-4cm of the **oesophagus** and closes at a pressure of **15-25mmHg above gastric pressure**. It is aided by the acute angle where the oesophagus joins the stomach. It has constant tone until relaxation from **vagal** input with VIP and NO through oesophageal stretch.



Tone is affected by many things. Anatomically, the acute oesophageal-gastric junctional angle and passage of the oesophagus through crura of the diaphragm are factors.

Other factors are included in this table:

- **Reflux**: Breaching of LOS
- **Regurgitation:** Past the upper OS and contaminates the pharynx (***passive***)
- **Aspiration:** Contents contaminate the lungs with reduced consciousness.

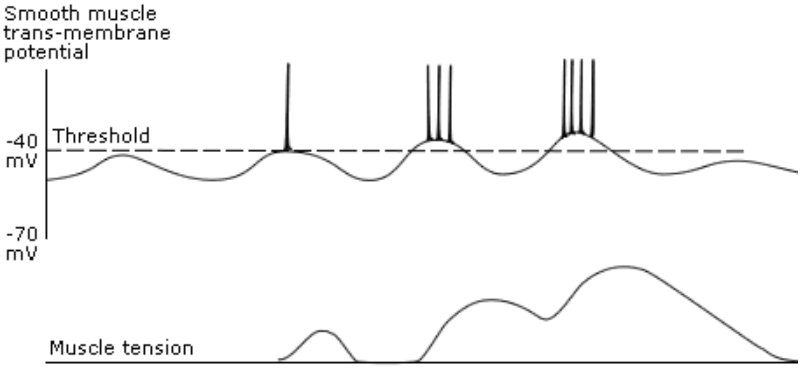
Factors that increase LOS tone 'Good influences' as reflux is less likely	Factors that decrease LOS tone 'Bad influences' as reflux is more likely
Gastrin	Swallowing
Cholinergic stimulation	Antimuscarinics e.g. atropine, glycopyrrolate
Metoclopramide, prochlorperazine	Dopamine
	Alcohol
Cyclizine	Opioids
Suxamethonium	Thiopentone
	Oestrogen, progesterone

Mechanical Breakdown of Food

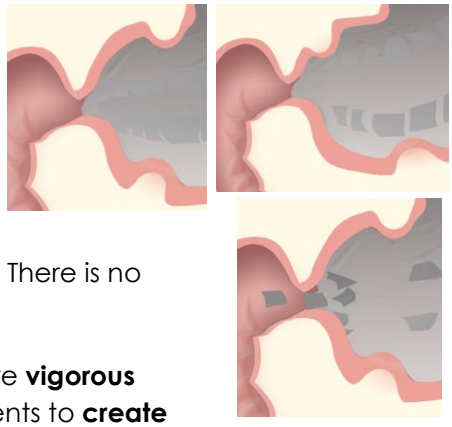
Cellular Level

The electrical potential of smooth muscle cells varies at a **baseline electrical rhythm** (**BER**) between -40mV and -70mV. Originates in the **longitudinal muscles of the greater curve**. Once depolarisation occurs, electrical activity is able to be spread to neighbouring cells due to numerous gap junctions – **syncytium**.

It is as a result of **intrinsic excitability of nerves**. **Frequency** is constant but changed with neural and humoral factors. The **intensity of contraction** is determined by the **frequency of spike potentials** (AP at threshold) increasing with **vagal stimulation** (increased amplitude) and **gastrin**.



The **SNS** makes smooth muscle more **hyperpolarised** making spike and burst activity less likely to occur. Secretin inhibits muscle activity.



Fed State – **Hopper and Mill**

Triggered by the sight, smell, ingestion of food and gastric distension. **Hopper effect** describes the exertion of a steady pressure in the proximal stomach through **sustained tonic contractions** up to 6mins long. This gradually moves food distally. There is no *slow wave activity*.

The grinding of distal stomach contents is the **Mill effect.** There are **vigorous contractions** to **mix and knead the gastric juices with food** contents to **create chyme**. The antrum and pylorus contract simultaneously to grind against a closed pyloric sphincter (normally relaxed). Only **small food particles <1mm** can pass into the duodenum. An eddy of retropulsion allows fragmentation of larger food particles.

Fasted State

Migratory motor complexes (**MMC**) are regulated coordinated contractions that start in the antrum every 90-120mins and progress along the small bowel at 6-8cm/min. This allows **removal of indigestible contents** such as vegetable fibre.

Stomach Contents

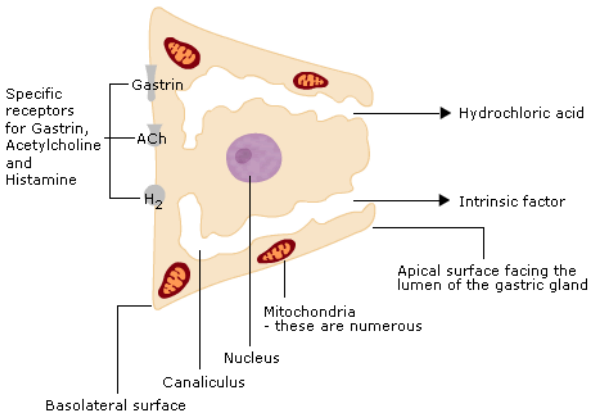
Volume and pH determined by oral intake, gastric juice and gastric emptying. It has a pH of 1-1.5 and produces about 2L/day. A summary of the cells and the product secreted is shown:

Cells	Secretion produced	Type of secretion
Parietal	Hydrochloric acid, Intrinsic factor	Constituents of gastric juice
Chief	Pepsinogen	Constituent of gastric juice
Mucus	Mucus	Constituent of gastric juice
G	Gastrin	A hormone
D	Somatostatin	A hormone

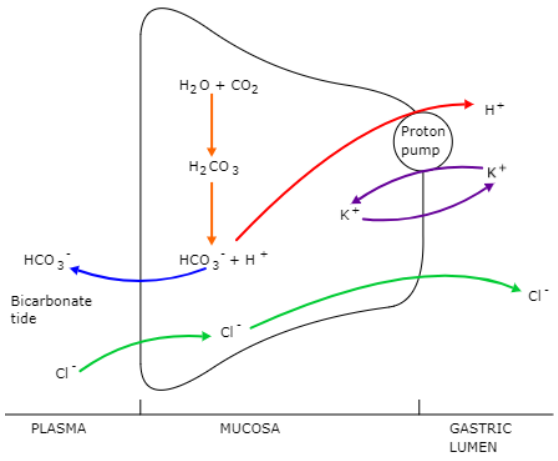
Hydrochloric Acid

Secreted by **apical surface** of **parietal cells**. HCl secretion is stimulated by the activation of the basolateral membrane receptors as shown in the diagram. **Gastric acid allows**:

- Breakdown of protein
- Activation of pepsinogen → pepsin and conditions for its optimal activity
- Improves solubility of Ca^{2+} & iron
- Kills pathogenic micro-organisms



1. H2O and CO2 form bicarb and H+
2. H+ is secreted out in exchange with K+ in through the H+/K+ ATPase (cAMP levels determine its activity)
3. HCO3- is exchanged with Cl- at the basolateral membrane
4. Cl- leaves with K+ across the apical membrane



5. Further exchange of H+ out and K+ into the cell occurs with the proton pump.

Therefore, the **basolateral membrane** secretes an **alkaline tide**.

Intrinsic Factor

Glycoprotein that is secreted by the **parietal cells** in response to the same stimuli for HCl. It **binds to vitamin B12** to **prevent its enzymatic destruction** during its passage to the terminal ileum where it is absorbed as a complex. Deficiency leads to a **megaloblastic anaemia**.

Following a gastrectomy, anaemia is more likely to result from iron deficiency (see above functions of HCl) rather than B12 deficiency.

Pepsin

This is a **proteolytic enzyme** which **hydrolyses peptide bonds** in the protein molecule producing polypeptides and amino acids. It is stored as inactive pepsinogen in membrane bound zymogen granules in **chief cells**. Pepsinogen→pepsin occurs in the acidic environment of the gut lumen with maximal activity at pH<3. Pepsinogen release is stimulated through:

- **Vagal stimulation**
- **Acid pH**
- **Gastrin**
- **Secretin (from duodenum)**
- **β stimulation.**

Mucus

Prevents autodigestion from acid, proteolysis and mechanical stresses. Secreted by **mucus neck cells** at the opening to the gastric pits and **surface epithelial cells** to form a layer up to 200µm thick. The latter secretes a **bicarbonate rich (less viscous) fluid** to keep the mucosa at a neutral pH whilst having **tight junctions** to create an overall mucus barrier. Mucus production is stimulated by vagus, gastrin and prostaglandins E_2 and I_2 (locally produced).

Peptic ulcers are caused when mucosal resistance is reduced or increased acid production and are associated with H *Pylori* production found in the deep mucus layer (neutral pH) and induces gastrin production.

Gastrin

Produced by **G cells** found in the gastric glands of the **stomach antrum** and **duodenum**. It is a hormone separated from gastric juice released in response to:

- Vagal stimulation
- Antral or duodenal stimulation and
- Presence of amino acids or peptides.

Inhibited by gastric acidity and secretin. It **stimulates** the **release of enzymes** and **mucus, increases gastric** and **gut motility** and **regulates mucosal growth**.

Phases of Secretion

1. **Cephalic phase** accounts for 30% of secretion. Stimulated when food is **anticipated by sight or smell** and is initiated by **vagus efferents**
2. **Gastric phase** accounts for 60% of secretion when food reaches the stomach through **gastric distension** to initiate a vagal reflex. **Antral distension, amino acids** and **peptides** stimulate the release of **gastrin.** This is self-limited when a low pH prevents its further release.
3. **Intestinal phase** accounts for 10% of secretions when chyme enters the duodenum. **Gastrin** is secreted from **duodenal distension** and **amino acids** and **peptides.** This is short lived from the release of somatostatin from duodenal S-cells.

The timing and mechanisms of these phases overlap.

Absorption

Absorption in the stomach is minimal as the pH is so low. However, the pKa of aspirin is low at 3.5 so is mainly unionised in the stomach and can be absorbed. Alcohol is lipid soluble so is absorbed in significant amounts.

Gastric Emptying

Can only occur when the **antrum pressure > pyloric sphincter.** Antral pressure increases with stomach distension (from vagal stimulation) so emptying occurs at a rate proportional to **gastric volume**. Smaller particles occurs faster than solids. It can be measured through **US, scintigraphy**, **paracetamol absorption** (as it is only absorbed in the duodenum) & applied **potential tomography**.

Multiple factors can influence gastric emptying:

Factors increasing gastric emptying	Factors delaying gastric emptying
Stomach distension	Duodenal distension (by vagal inhibition)
Cholinergic stimulation Anticholinesterases	Antimuscarinics, e.g. Atropine, glycopyrrolate
Stomach content: liquid or smaller particles	Duodenal chyme high in H^+. Fat, protein or osmolality
	Sympathetic stimulation, pain, anxiety, stress
Prokinetic drugs, e.g. metoclopramide,	Opioids
	Alcohol

Duodenal neural and hormonal mechanisms allow negative feedback known as the ***entero-gastric reflex*** to allow prolonged absorption time in the duodenum.

Composition of chyme – if too acidic, **secretin is released** which inhibits smooth muscle. If too fatty, **cholecystokinin (CCK)** is released for gallbladder contraction and also reduces gastric emptying. Hypertonic chyme is detected by osmoreceptors in the duodenum reducing gastric emptying.

Physiology of Nausea and Vomiting

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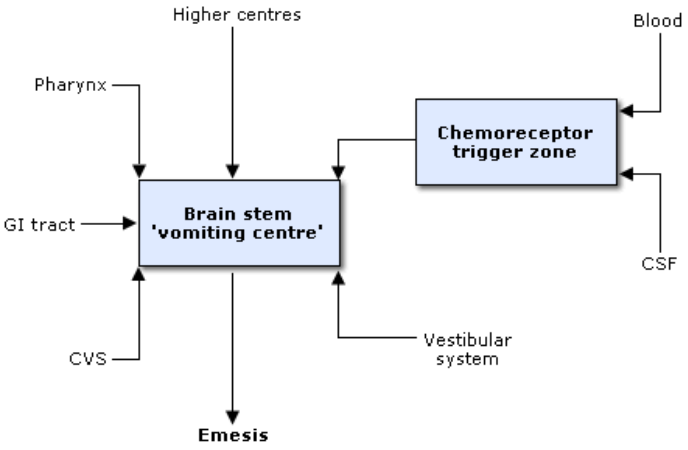
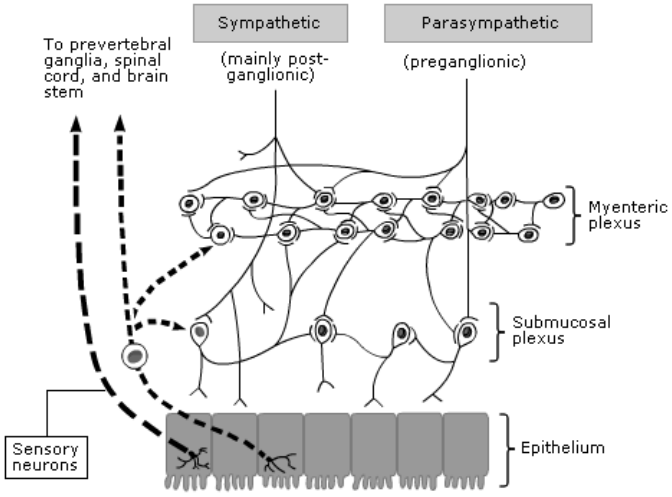
Overview

Vomiting is coordinated through the brainstem which receives multiple afferent signals.

Much of our knowledge in this area remain poorly understood.





GI Tract Motility

It is a hollow viscus consisting of **circular**, **oblique** and **longitudinal muscles** with serosa, submucosa and mucosa. Its function is dependent on the interaction between the ANS and the intrinsic myenteric and submucosal nervous plexi. Movements can be either **peristaltic** or **mixing** and is stimulated by an intraluminal food bolus.

Normal Swallow Reflex

Afferent: Trigeminal (V), Glossopharyngeal (IX) and Vagus (X) nerves → **swallow centre**. **Stages**:

1. It starts as a **voluntary** propulsion of food to the pharynx at the back of the tongue which **initiates** stage 2:
2. **Involuntary wave of contraction** towards the stomach from sensory receptors located on the tonsillar pillars sending impulses to the brainstem. The **soft palate** is **pulled up** to isolate the posterior nares. The **palatopharyngeal folds** are **pulled medially** to form a sagittal slit before entering the posterior pharynx.
3. **Larynx** is **drawn upward and anteriorly** by the neck muscles and the epiglottis is pulled over the vocal cords with **glottic closure** as well as widening of the **upper oesophageal sphincter** sometimes known as the pharyngo-oesophageal sphincter.
4. **Pharyngeal stage:** Pharynx **contracts** in a **peristaltic wave** forcing food into the oesophagus. It is initiated by the presence of a food bolus in the oropharynx and **coordinated by swallowing centre** in the **reticular substance** of the **medulla**. It is constant and lasts 1-2s.

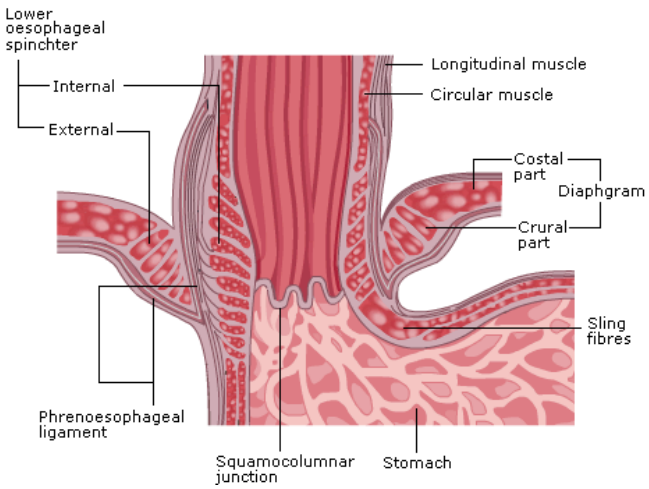
The swallowing centre **inhibits** the **respiratory centre** preventing respiration during swallowing.

Barrier Pressure

Oesophageal barrier pressure (OBP) is the lower oesophageal sphincter pressure (LOSP) – gastric pressure (GP). This is usually 15-30mmHg

OBP = LOSP – GP

The LOS provides 90% of the basal pressure – its afferents → spinal and vagal to CNS and synapse in the **nucleus tractus solitarius** (close to the CNX nucleus). NO, ATP, VIP and CO all aid LOS relaxation during swallowing and belching.



Decreased OBP

These are the factors that reduce barrier pressure:

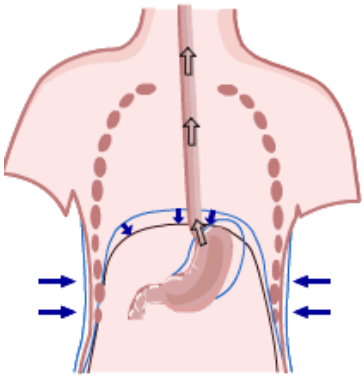
- LMA (Laryngeal Mask Airway) (7%)
- Cricoid pressure in the awake patient
- Volatile anaesthesia (5% at 2.5 MAC)
- N_2O
- Thiopental

Propofol, remifentanil and NMBs (except pancuronium and Vecuronium which increase barrier pressure) have a negligible effect.

Nausea and Vomiting

33% of elective patients will experience nausea or vomiting without prophylaxis.

Vomiting requires complex **active** coordination of muscles to expel gastric contents back through the mouth. It cannot be localised to one area of the brainstem. In contrast, **regurgitation** is a **passive** process.



Vomiting Reflex

This can be split into 2 phases:

1. **Pre-ejection phase:** Gastric relaxation with retrograde peristalsis to the stomach
2. **Ejection phase:** Crura relax whilst abdominal and diaphragmatic muscles contract. Oesophagus undergoes retrograde peristalsis and upper oesophageal sphincter relaxes.

Airway protection is essential and whilst the stomach undergoes retrograde peristalses, the following occur:

- **Deep inspiratory breath** precedes elevation of hyoid bone and larynx which opens the upper oesophageal region
- **Closure of glottis** and **elevation of soft palate** to close the posterior nasal space.

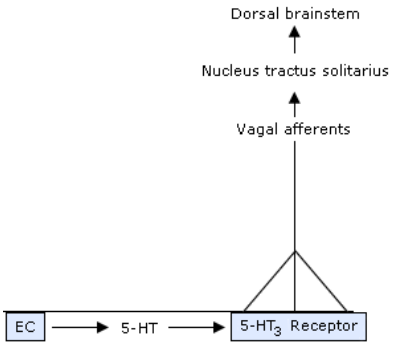
Nausea

Animal systems have serious limitations in the study of nausea. There are a few notable differences in nausea and vomiting:

- 5-HT_3 antagonists effectively treat vomiting but 50% still complain of nausea in chemotherapy patients (vomiting is therefore easier to treat)
- Raised ICP induces vomiting without nausea; as well as radiotherapy and pregnancy
- Although vagal afferents are responsible for N&V initiation, bilateral vagotomies have shown patients to still experience nausea suggesting a possible spinal pathway
- Nausea sensation requires above brainstem conscious sensation formations.

Stimulation of Vomiting

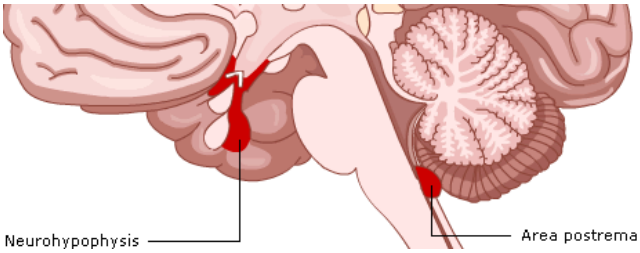
5 main pathways:



1. Toxic materials in gut lumen

Mediated by **enterochromaffin cells** which releases substances such as 5-HT in response to toxins. **5-HT_3 receptors** are **coupled to Na^+ channels** to allow initiation of **vagal afferents**. 5-HT_3 receptors are also found in the **NTS** and **chemoreceptor trigger zone**.

2. Toxic Substances in the blood



This is stimulated by the **chemoreceptor trigger zone** aka **area postrema** and is 1 of 7 **circumventricular organs** in the CNS. It has a rich capillary network and **fenestrated endothelium** so is highly permeable to blood substances. As well as 5-HT_3 receptors, it has some **dopamine receptors** and **neurokinin 1 receptors** (response to substance P). Propofol is the only TIVA that is not implicated in N&V stimuli. All volatiles are implicated.

3. Gut or Visceral Pathology

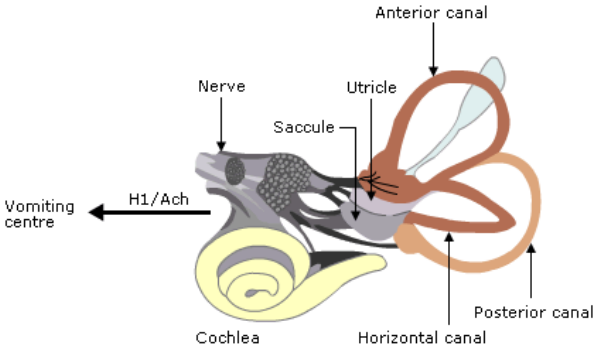
This mechanism is not well understood but include **vagal afferents** such as in **MI or renal failure**. **Pulmonary vagal afferents** however **inhibit the emetic reflex**. It may also be **mechanical** such as with gastric outlet obstruction in pyloric stenosis.

4. Central Nervous System Stimuli

These are **not usually preceded by nausea or retching** and the mechanism is **poorly understood**. It may be induced by raised ICP, brain trauma, fear or just anticipation

5. Vestibular System Disturbance

Motion sickness and **Meniere's disease** are both common causes of N&V. It is thought that as these are **risk factors for PONV**, **vestibulocochlear disturbances modulate** the **sensitivity of brainstem** to **emetogenic stimuli**. Both **Ach** and Histamine **(H1)** receptors are implicated in transmission of impulses to the vomiting centre.



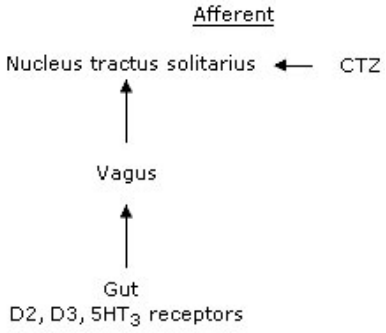
Dopamine Receptors

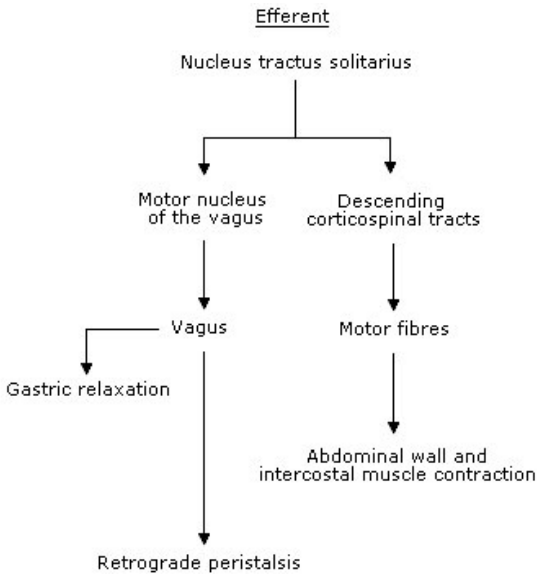
D1 (and **D5**)**: Stimulation** of **adenylate cyclase**

D2-like receptors (D2, D3 & D4) all **inhibit adenylate cyclase**. They are located in **NTS** and **area postrema**. Animal studies have suggested that D1, D4 and D5 are *not* involved in N&V.

D2 is clearly **linked with N&V** and D3 may also evoke vomiting or facilitate through D2 activation. Overall receptors involved include **D2/3**, **H1**, **M3/M5**, **5-HT$_3$** and **NK1**.

Overview of Pathways in N&V





PONV

There are multiple risk factors for its development:

- **Patient specific risk factors:**
  - Female sex
  - Non-smoking status
  - History of PONV/motion sickness
- **Anaesthetic risk factors:**
  - Use of volatiles for 0-2 h
  - N_2O
  - Use of intraoperative and postoperative opioids
- **Surgical risk factors:**
  - Duration of surgery
  - Type of surgery (laparoscopy, ENT, neurosurgery, breast, strabismus, pelvic, laparotomy, plastic surgery)

With **children**, PONV **increases with age** and its incidence is twice that of adults when >3yo whilst rare in <2yo. Gender differences are seen after puberty.

Metabolic Pathways

(07b_06_07)

Metabolism is the series of complex chemical reactions to modify absorbed products of digestion. Controlled by enzymes and hormones. This is done for the following reasons:

- Extract chemical energy
- Produce heat
- Synthesise new molecules for maintenance and growth
- Detoxify waste products and prepare for excretion

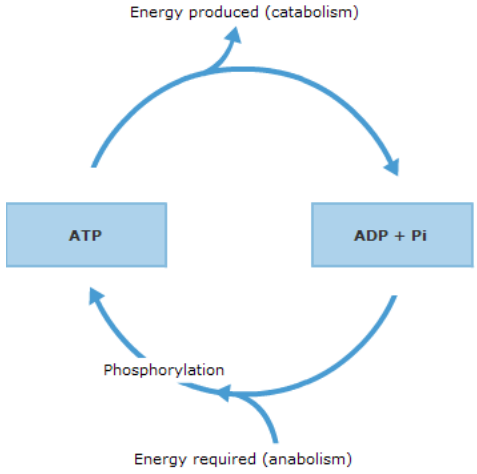
Types of Metabolic Reactions

EXERGONIC: Energy releasing reactions including catabolism, oxidation and energy released from breaking of chemical bonds

ENDERGONIC: Energy requiring reactions i.e. anabolism (formation of higher energy compounds i.e. ATP), reduction and maintenance of acid-base balance.

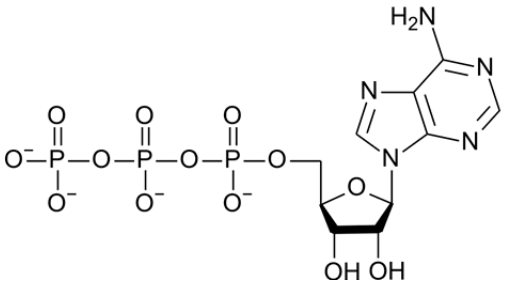
Total energy expenditure = Work done + Heat produced + Energy stored

Basal Metabolic Rate (**BMR**): This is the **net amount of energy liberated** by a **starved human body** at **complete rest** and **comfortable temperature**. It is influenced by many factors but in an average young adult is about **70–100 kcal/h**.



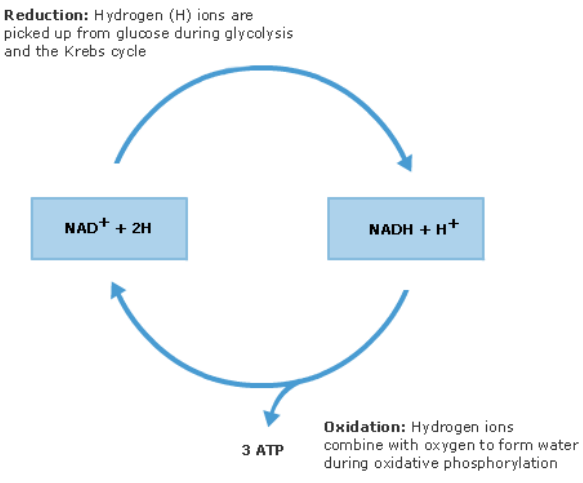
Adenosine Triphosphate (ATP)

Energy released with **hydrolysis** to ADP from the Pi bond. The supply of ATP to keep metabolic pathways in balance is key as they are usually reversible according to the phosphorylation state of ATP/ADP.



NAD+ and FADH

These store energy through **reduction** and addition of hydrogen ion during glycolysis and the citric acid cycle. They undergo **oxidative phosphorylation** in the mitochondria in order to generate large amounts of ATP.

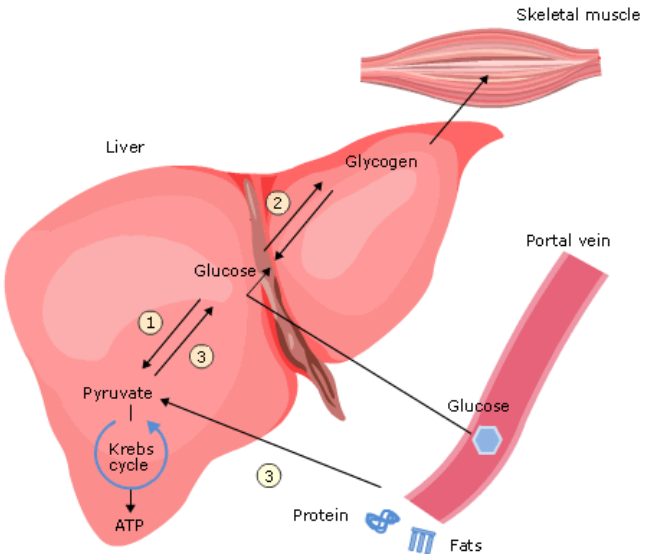


Sources of Fuel

CARBOHYDRATES: Broken down initially by salivary and pancreatic amylases to **oligosaccharides** which are then broken down further into monosaccharides (6-carbon compounds) by intestinal **maltase, lactase** and **sucrase**. These are absorbed by active transport. *NB Only glucose, fructose and galactose can be broken down whilst cellulose for example is indigestible*.

Carbohydrates can either undergo **glycolysis (1)**, **glycogenolysis** and **glycogenesis (2)** or **gluconeogenesis (3)**

Daily requirement: 5-10g/kg/day



PROTEINS: Initially broken down by **pepsin** and **trypsin** in the stomach and **chymotrypsin** in the small intestines to amino acids and di-/tripeptides.

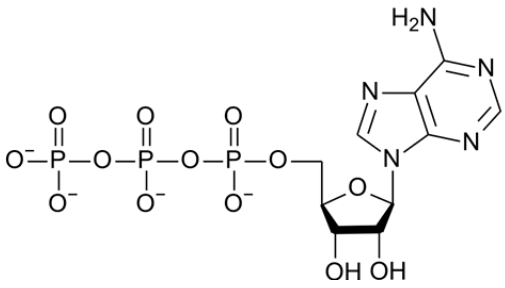
Daily requirement: 0.5-1g/kg/day

FATS: Click here to link to lymphatic system notes for further information on fat absorption

Daily requirement: 1-2g/kg/day

Cellular Metabolism

ATP is the high energy compound most used for cellular processes and is produced from catabolism of carbs, fat and protein. 3 phases in aerobic metabolism:



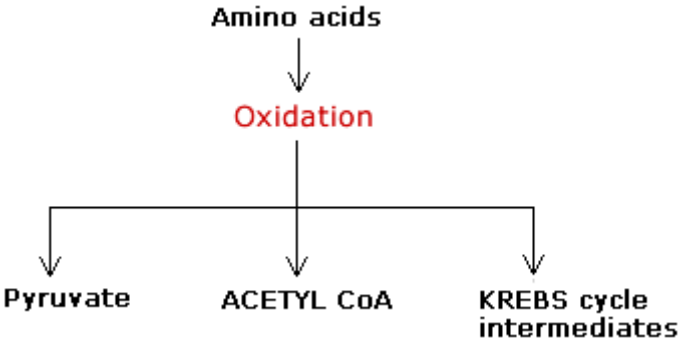
- **Phase 1:** production of two carbon compounds (acetyl CoA)
- **Phase 2:** Citric Acid Cycle
- **Phase 3:** Electron Transport chain

PHASE 1

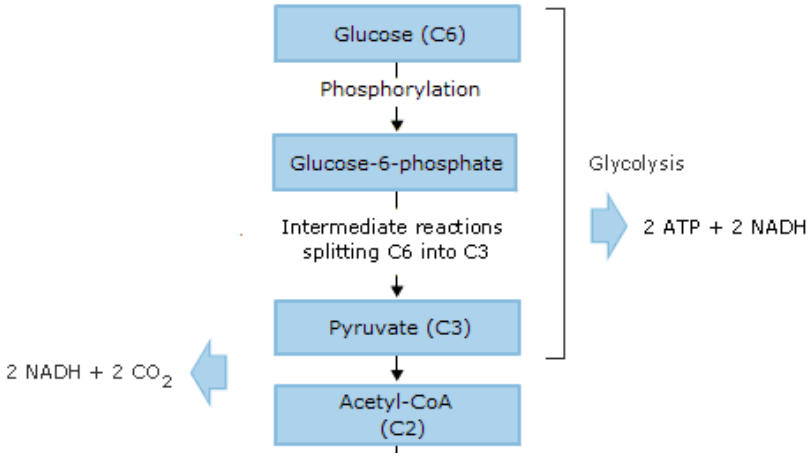
Small components of metabolic fuels are initially processed to produce 2 carbon compounds for phase 2 reactions:

FREE FATTY ACIDS: Undergo **β-oxidation** to produce **Acetyl CoA via ketogenesis.** *NB, only glycerol can be converted into pyruvate*.

AMINO ACIDS:



GLUCOSE:

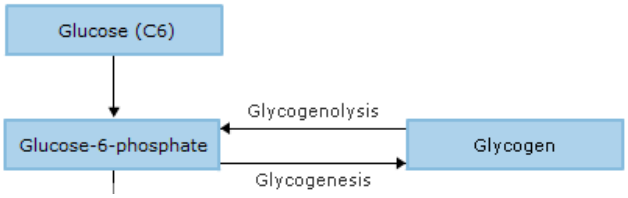


Glycolysis occurs in the **cytoplasm** and is the process where pyruvate is made from glucose. The first step is glucose → glucose-6-phosphate by the enzyme **glucokinase**.

ONLY under **aerobic conditions, pyruvate** is transferred to the **mitochondria** to undergo **oxidative decarboxylation to acetyl CoA**

***Anaerobic conditions*:** NADH Is not able to dissipate its stored energy to oxygen so is transferred **directly to pyruvate** resulting in the **formation of lactate.**

Glycogenesis: **Glucose molecules are activated by phosphorylation** and added to the glycogen chains using **glycogen synthetase**. Highly efficient storage mechanism controlled by hormones.

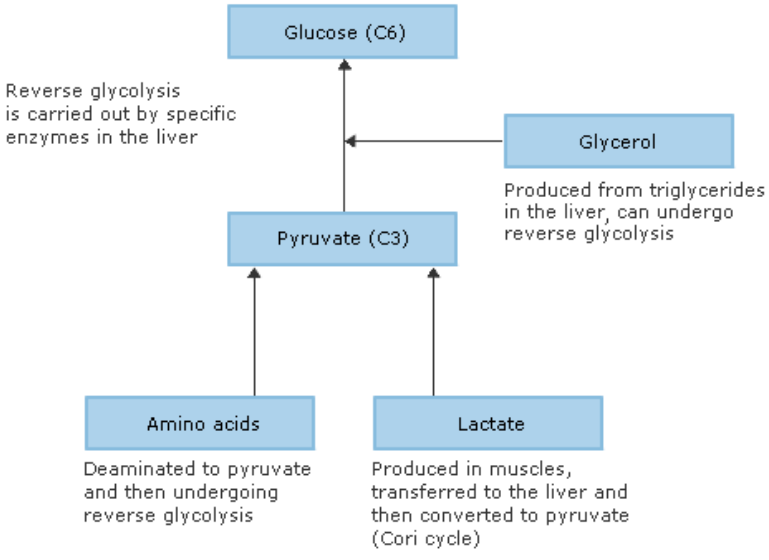


Glycogenolysis: Requires a debranching enzyme to split off the glucose from glycogen.

Glycogen is stored in skeletal muscle: liver in the ratio 3:1.

Gluconeogenesis

Generation of glucose from substrates i.e. **lactate** and **pyruvate** in tissues needing it as an energy source i.e. the brain. Therefore, amino acids can be converted into glucose via pyruvate and fats via glycerol. This occurs in the **liver** as a **'reverse glycolysis'** reaction.



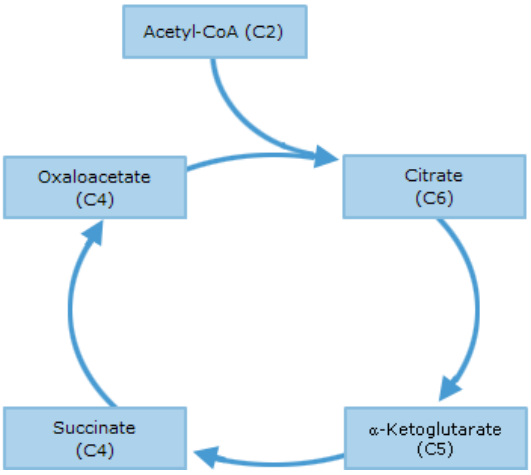
PHASE 2 – Citric Acid Cycle

Acetyl CoA enters the **Citric Acid Cycle** aka the Krebs Cycle.

It combines with 4C **oxaloacetate** to produce 6C **citrate** following which, there are a series of intermediary compounds formed by **decarboxylation**. The last compound produced is oxaloacetate – hence a re-cycle. Compounds created:

- 2 ATP
- 6 $NADH_2^+$
- 2 $FADH_2$
- 4 CO_2

Product of 1 glucose molecule or x2 cycles of the Citric Acid Cycle (x2 Acetyl CoA per glucose molecule)



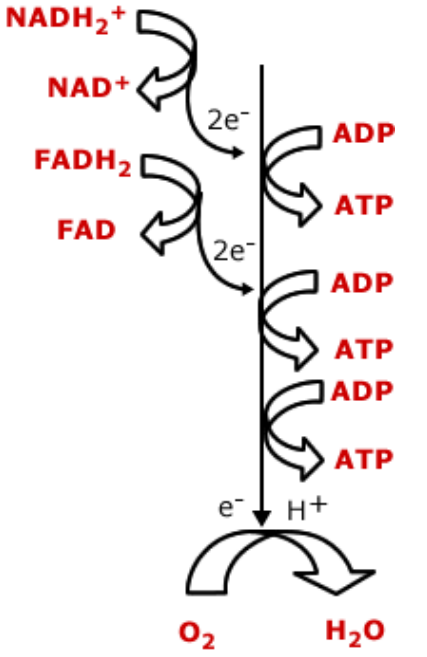
PHASE 3 – Electron Transport Chain – OXIDATIVE PHOSPHORYLATION

Oxidisation of the reduced molecules which releases ***electrons*** and ***energy***. This occurs on the **inner surface of the inner mitochondrial membrane.** The energy is utilised for **oxidative phosphorylation** of **ADP → ATP**.

- ***$NADH_2^+$*** enters at the beginning of the chain → converts 3 molecules of ADP
- ***$FADH_2$*** enters further down the chain → converts 2 molecules of ADP

Electrons are passed through a **series of cytochromes** until at the end of the chain, **oxygen** is the final electron acceptor in the chain and combines with hydrogen ions to produce water. Without the presence of oxygen, phase 3 is unable to commence.

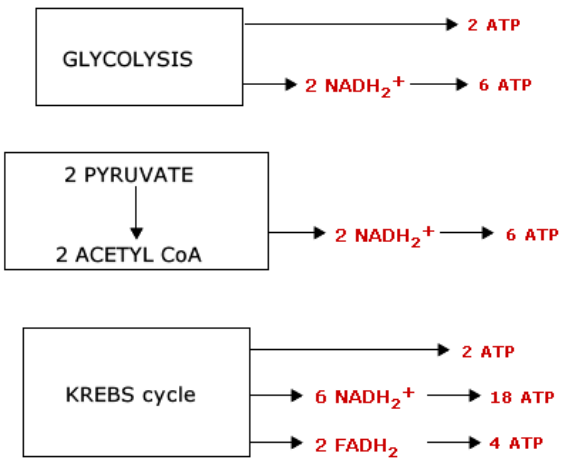
Cyanide works on the final cytochrome a3 → unable to undergo oxidative phosphorylation.



Summary

All three phases of metabolism is performed in aerobic respiration. A total of **38 ATP** is produced per molecule of glucose.

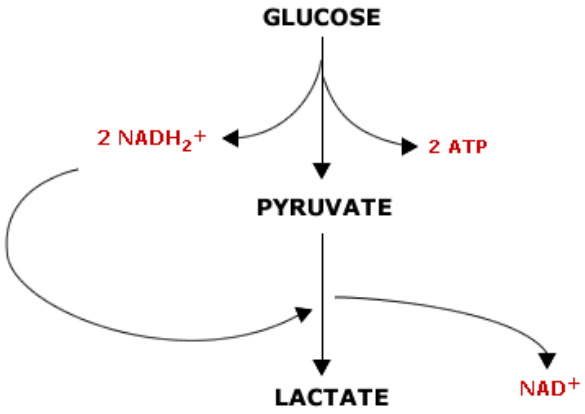
$6\ O_2 + C_6H_{12}O_6 = 6\ CO_2 + 6\ H_2O + 38\ ATP$



Anaerobic metabolism is the absence of oxygen and therefore:

1. Electron transfer chain stops
2. NADH+ and FAD is not re-formed so the Citric Acid Cycle ceases

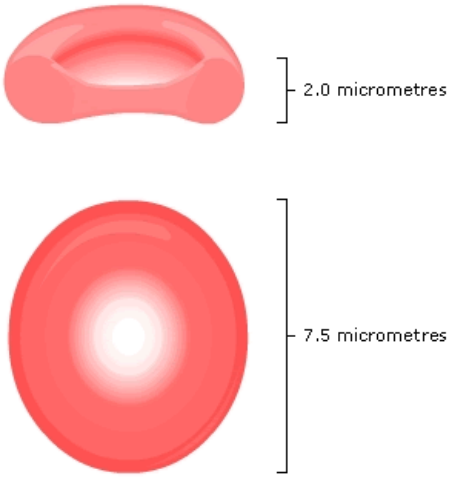
Only glycolysis is available and the $NADH_2^+$ is used to convert pyruvate to lactate. Therefore, **2 ATP** is produced per molecule of glucose.



HAEMATOLOGY AND IMMUNOLOGY

Red Blood Cells

(07b_07_01)

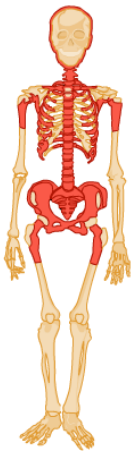


Erythrocytes

The mature red blood cell is an **anucleate biconcave disc** to increase flexibility through the narrow capillary lumen and to increase its surface area: volume ratio allowing more efficient gas exchange. The average survival is 120 days.

The main function is for **respiratory gas transport** and is reflected by the fact that **95% of the protein is haemoglobin** and the remainder being enzymes that are required for maintenance of Hb.

ATP is **anaerobically generated** to avoid consumption of the oxygen transported by the Hb.



Erythropoiesis

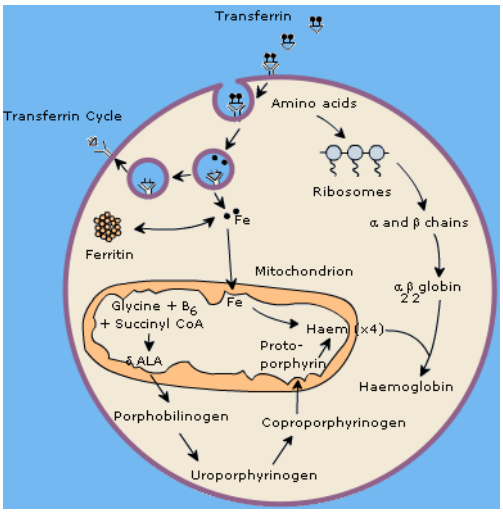
Red marrow is the site of red cell production and occupies all bones at birth. At 5yo, it is gradually replaced by fat until in adult, marrow is limited to the **axial skeleton, girdles, skull** and **epiphyses of the humerus and femur**. Red cells require many essential nutrients: Fe, B12, Folate, Pyridoxine, Riboflavin, Vitamin E and copper.

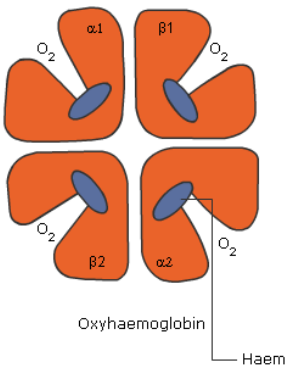
***Erythropoietin*:** A hormone that controls erythropoiesis and 90% is produced in the kidneys and 10% in the liver in response to reduced oxygen tension in the blood. The balance is made between tissue hypoxia and blood viscosity.

Haemoglobin Turnover

***SYNTHESIS*:** Occurs in **mitochondria** of RBC where:

1. **Protoporphyrin** is synthesized from the condensation of glycine and succinyl coenzyme A.
2. Protoporphyrin **combines with iron** in the ferrous state (Fe2+) **to form haem**.
3. **Globin chains are formed** in the ribosomes





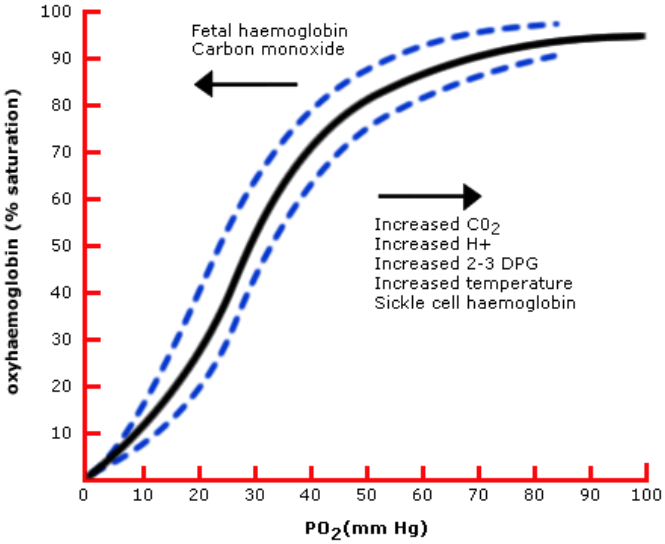
4 of the above will produce 1 haemoglobin molecule. Crosslinks between globin chains are **non-covalent salt links** with **a1β1 and a2β2 contacts** for stabilisation and **a1β2 and a2β1 interaction** located close to the haem group to undergo conformational change with oxygenation. When **oxygen is unloaded**, the **β chains are pulled apart** where 2,3-DPG can insert.

Recap

Three types of haemoglobin:

1. **HbA**: $\alpha_2 \beta_2$ – 96-98%
2. **HbA$_2$**: $\alpha_2 \delta_2$ – 1.5-3.2%
3. **HbF**: $\alpha_2 \gamma_2$ – 0.5-0.8%

The genes for the globin chains occur in two clusters ζ and α on chromosome 16 and ε, γ, δ and β on chromosome 11. The α gene is duplicated. Combinations are determined according to the stage of life or the individual.



Haemoglobin Disorders

Occur with either production of abnormal globin chain or reduced rate of synthesis of normal haemoglobin:

SICKLE CELL DISEASE

Caused by a **single base mutation (A→T)** in the β globin gene on chromosome 11 where **valine (abnormal) is substituted for glutamic acid (normal)** at position 6 of the β globin chain.

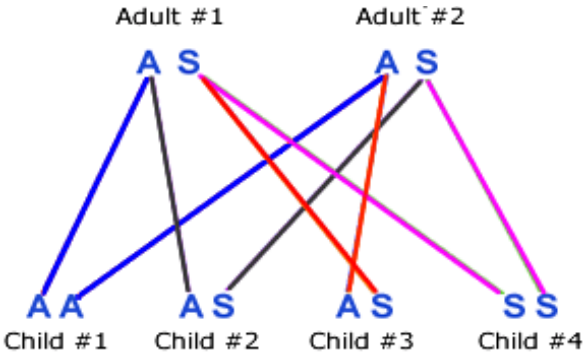
Genetics

Autosomal recessive disease.

Homozygote disease HbSS: both β globin genes are abnormal which results in **sickle cell anaemia** The P_{50} is higher than normal and the oxy-haemoglobin dissociation curve is shifted to the right.

Heterozygote (trait) HbAS: clinically much less severe with no increase in perioperative morbidity and mortality. Other haemoglobins inhibit the degree of sickling to varying degrees. From most to least:

HbS, HbC, HbD, HbA then HbF



Geography: Common in Africa, parts of Asia, the Arabian Peninsula and Southern Europe. This is due to its advantage in protecting against malaria.

Clinical Features

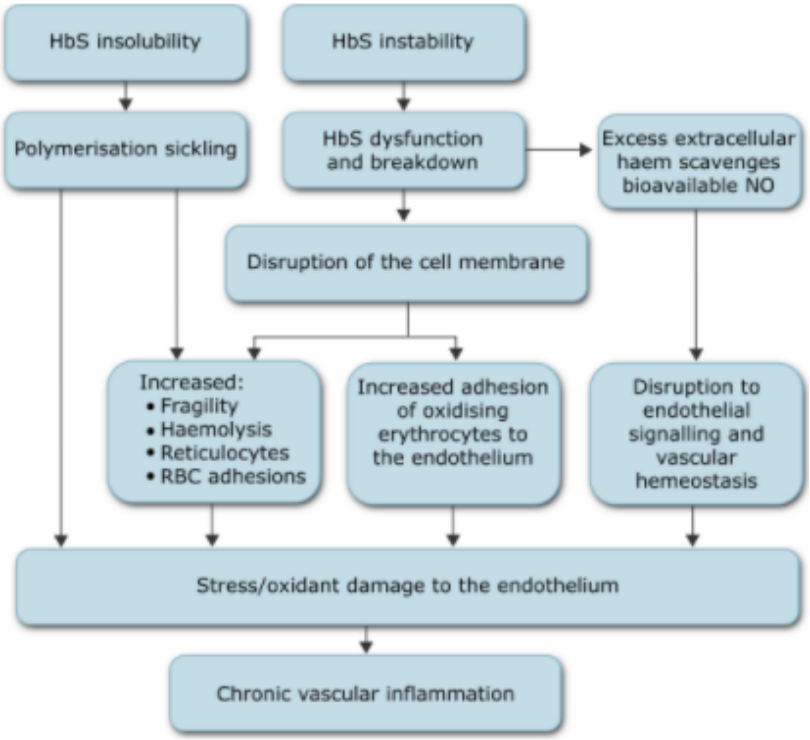
The main features are a haemolytic anaemia. Crises may include visceral/sequestration, aplastic, haemolytic or vasoocclusive.

Vasoocclusive crises **(VOC)** are the most frequent cause of morbidity and mortality although there is no obvious precipitant but can be associated with infection, acidosis, dehydration and deoxygenation. Theories of triggering are below. Examples of features may include:

1. Bony pain
2. Acute chest syndrome
3. Stroke
4. Acute abdomen
5. Splenic infarction

HbS instability occurs as iron is not as well shielded as normal HbA and can react with free radicals (as iron is highly reactive). This leads to **oxidant damage to the cell membrane** increasing the adhesion of the iron in the erythrocytes to the vascular endothelium → increased shear and oxidant stress of endothelium → **vascular inflammation**.

Note that disruption of cell membrane also causes increased fragility… (see diagram). **Reticulocytes express more adhesive proteins than erythrocytes** worsening the vascular inflammation.

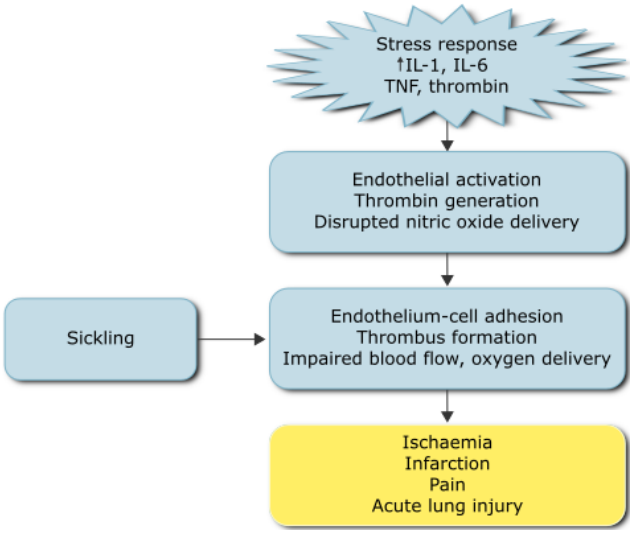


Nitric Oxide Dysfunction: Extracellular haem compounds are in excess from increased breakdown of unstable HbS. They **bind avidly to free NO** and **changes vascular homeostasis** (anti-thrombogenic and vasodilatory compound). Haem scavenging by NO may play a key role in vascular inflammation but its pathophysiology is not well understood…follows last 2 points of flow diagram.

Role of Sickling: Not the main pathophysiology but still contributes to chronic vascular inflammation through the **direct effect** of stress inducing attachment of sickle cells to the endothelium and the **indirect effect of haemolysis which increases reticulocytosis**.

Vascular inflammation plays the dominant role in pathophysiology of VOC. Previous thought that VOC may be induced by increased sickling due to insolubility of deoxygenated HbS → flow impairment.

Changes in endothelial regulation of flow and homeostasis induce VOC rather than acute fluctuations in sickling. **Endothelial activation** occurs through infection and surgical stress through release of **inflammatory mediators** by immune cells → increased **adhesion molecule expression** → binding of activated white cells to the endothelium, fibrin and platelet deposition, and the increased attachment of sickle erythrocytes. Also, see NO dysfunction above.



SUMMARY: Note that sickling does NOT trigger VOC, rather it is a secondary, exacerbating event

Haemolytic Crises manifesting as a fall in Hb and a rise in reticulocytes and bilirubin → may trigger a VOC.

Visceral Sequestration Crises occurs mainly in **infants** and massive **pooling of RBC** occur in the spleen → abdominal pain, splenomegaly, anaemia, hypotension.

Aplastic Crises occurs as a result of infection with **parvovirus** or through **folate deficiency** causing a sudden drop in Hb and reticulocytes.

Other Clinical Manifestations

System	Complication
Respiratory	Progressive restrictive lung impairment
Neurological	Delayed growth/development
Renal	Nephropathies/chronic renal failure
Gastrointestinal	Bilirubin gallstones, dyspepsia, viral hepatitis (complication transfusion)
Orthopaedic	Osteonecrosis/osteomyelitis/dactylitis
Vascular	Leg ulcers/proliferative retinopathy
Immunological	Spleen enlarged in childhood, reduced in adults as a result of infarcts/increased susceptibility to infections, haemolytic transfusion reactions secondary to alloimmunisation

Diagnosis

Haemoglobin electrophoresis is required for an exact diagnosis. A **sickle solubility test** detects the presence of HbS but provides no information about other haemoglobins.

***Blood film*:** sickle cells, target cells, Howell-Jolly bodies if HbSS. HbAS will be normal.

***FBC*:** Anaemia – Hb will be lower than expected for symptoms of anaemia as there is a R shift to the oxygen dissociation curve and therefore, oxygen is delivered to the tissues more readily.

Management

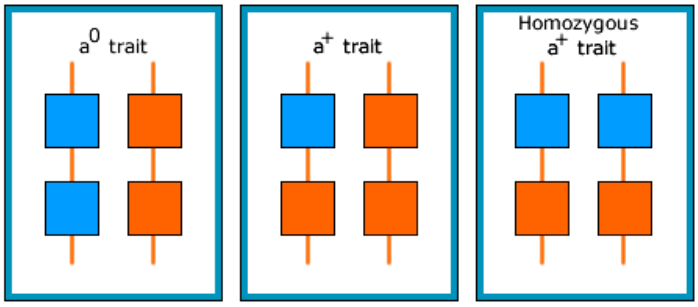
- **Splenic Dysfunction:** Pneumococcal/Haemophilus Influenza B/Meningococcus vaccinations and penicillin prophylaxis. Folic acid supplements from young age
- **Severe disease:** Hydroxyurea which stimulates HbF production and reduces proportion of HbS.
- **Allogenic bone marrow transplantation** in patients <16yo is potentially curative

THALASSAEMIAS

Abnormal ratios of α and β chains are produced (rather than equal quantities). With excess of globin chains comes precipitation resulting in **haemolysis** and **anaemia.**

α-Thalassaemia

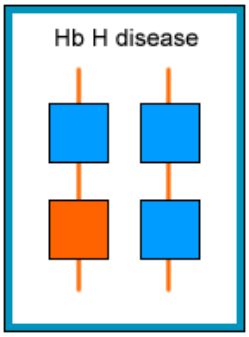
Seen in people from the Mediterranean, Central Africa and Southeast Asia. Occurs from **DELETIONS** of 1 or all 4 of the alpha genes on chromosome 16. Varies in clinical severity according to number of deletions:



α-thalassaemia *TRAITS*: Results from loss of 1 or 2 genes and named accordingly:

Not usually associated with anaemia though there is a hypochromic, microcytic picture with a raised RBC count.

Normal HbA2 level and haemoglobin electrophoresis is normal. Globin chain synthesis can show a reduced α:β ratio, but a diagnosis by molecular analysis is preferred.



HbH Disease: Describes 3 α gene deletions which results in a production of haemoglobin with β4 (HbH) which can be detected with electrophoresis.

It causes a moderately severe anaemia (Hb 7-11 g/dl, microcytic, hypochromic anaemia and splenomegaly with normal development) not usually requiring transfusion

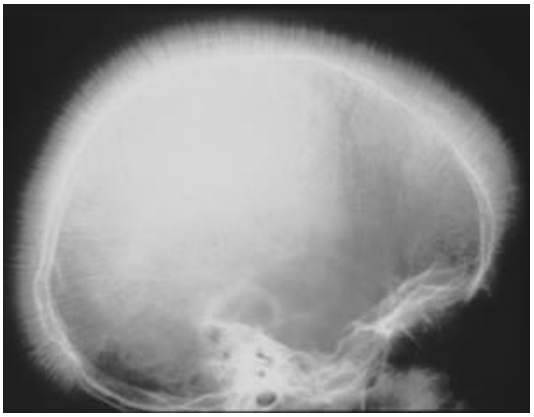
Hydrops Fetalis (α0 α0/α0 α0): loss of all 4 genes is incompatible with life → death in utero

β-Thalassaemia

Seen in populations from the Mediterranean, the Indian subcontinent or Southeast Asia. Results from **gene mutation** causing:

- **β-Thalassaemia minor/trait:** Reduced production of β chains (β+) – causes a mild anaemia that can resemble IDA.
- **β-Thalassaemia major:** Absent production of β chains (β0) – becomes apparent between 3-6 months of age when the switch to HbA occurs. The excess α chains combine with whatever β, δ or γ chains are present, forming **excess HbA2** and **HbF**. This is a severe transfusion dependent anaemia.

Complications occur in the absence of transfusion.



- **Bone marrow hyperplasia** leads to thalassaemic facies and cortex thinning → increased susceptibility to fractures. Bone marrow expands into cortical bone with XR showing a 'hair on end' appearance.
- **Hepatosplenomegaly** as a result of excessive red cell destruction and extramedullary haemopoiesis.

With transfusion, there is normal development for the first decade but then iron overload becomes clinically apparent.

β-Thalassaemia Intermedia refers to a patient, who is able to maintain a haemoglobin level in the 7-10 g/dl range without taking blood transfusions. This is still a mutation of both β genes but one may be milder. They may still present with bone deformities and extramedullary haemopoiesis. It may remain silent until periods of stress i.e. pregnancy.

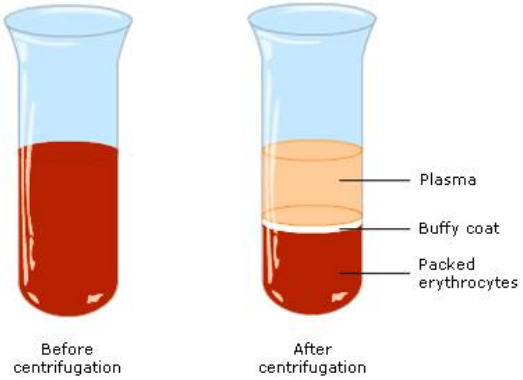
Transfusion Physiology

(07b_07_02)

The ABO grouping of blood was discovered in 1930 which had been the basis of **compatibility**.

Whole Blood

It is collected from donors into citrate anticoagulant solution and through centrifugal force, is separated into component parts. The white 'buffy' coat contains WBC, platelets and plasma.



Cellular elements include RBC, platelets and WBC. This is always **leucodepleted** prior to use and **viral antibody screened**.

Plasma is collected to make **FFP** which can be further processed to produce cryoprecipitate through slow thawing which is rich in factors VIII and fibrinogen.

Storage

RBC survival **correlates** with levels of ATP. Utilisation of ATP reduces 40-fold if stored at 4°C. Additive solutions are used to prolong RBC viability during storage. UK uses **SAG-M:**

- **Saline**
- **Adenine:** increases levels of ATP but increases rate of depletion of 2,3-DPG levels.
- **Dextrose:** Prolongs cellular viability
- **Mannitol:** osmotic stabilizer reducing cellular haemolysis.

As 2,3-DPG levels fall, the haemoglobin transfused results in a left shift of the dissociation curve but this resolves after 24h.

Transfusion Preparations

Whole blood is rarely used for transfusion except in the military as it doesn't allow for optimisation of donated blood components. However, most hospitals have Major Transfusion Packs that approaches the constituents of whole blood.

Packed RBCs

Usually 200ml of concentrated RBC solution mixed with 100ml of SAG-M → haematocrit of 50-70%. Transfusion of 4ml/kg body weight increases Hb by approximately 10g/l. *Shelf-life = 35 days*.

Platelets

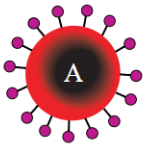
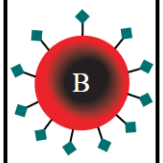
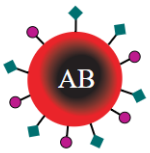
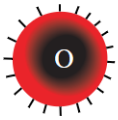
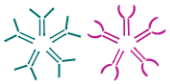
1 unit is usually 150-300ml which comes from a **pool of 4-5 donors**. Platelets have to be **stored at 22°C** in order to preserve its function which deteriorates rapidly below 18°C. As there is a risk of bacterial proliferation if stored any longer the *Shelf-life = 3 days*.

Fresh Frozen Plasma (FFP)

It has a volume of 200-300ml consisting of plasma harvested from a **single donation**. The plasma is **rapidly frozen** to -25°C to preserve the labile coagulation factors. *n*. However **once thawed**, needs to be used within 6 hours to prevent coagulation factors to lose their function.

ABO Blood Classification

Over 250 RBC antigens have been recognised in humans but the one with the greatest clinical significance is the ABO and Rhesus D groups. ABO is inherited in a Mendelian dominant manner.

	Group A	Group B	Group AB	Group O
Red blood cell type				
Antibodies in Plasma	 Anti-B	 Anti-A	None	 Anti-A and Anti-B
Antigens in Red Blood Cell	 A antigen	 B antigen	 A and B antigens	None

If transfusion occurs with mismatching of antigens on transfused RBC and recipient antibodies, there is a potentially life threatening **haemolytic transfusion reaction**.

Antibodies to ABO antigens are also known as **agglutinins**.

Group O = universal donors

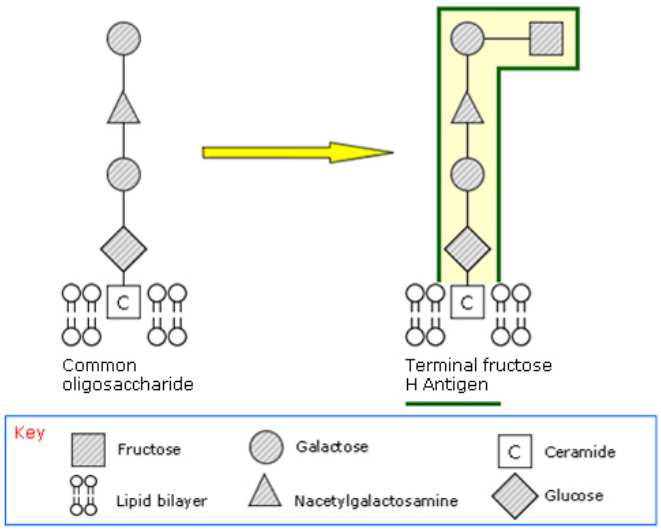
GroupAB = universal recipients

Blood Typing

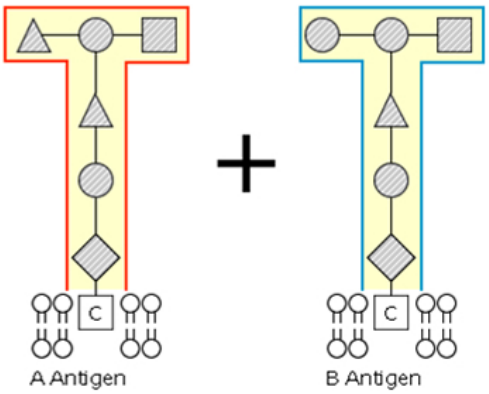
Unknown blood sample is mixed with **known anti-sera** with known agglutinins. Agglutination is seen if there is a reaction. The same principle is used to determine rhesus (Rh) status.

H Antigens

The ABO system of antigens are also present on many other tissues within the body. The **basic building block of ABO antigens** is an **oligosaccharide** known as the **H antigen** found in all blood types. The H gene codes for a fructose transferase which places fructose at the end of the oligosaccharide, creating the H antigen. How this is further modified from the individuals genetic code determines the different ABO antigens:



- **Group A** → N-acetyl galactose amine
- **Group B** → terminal galactose
- **Group AB** → have both transferases
- **Group O** → no active terminal transferase gene: Persistence of H antigen.



Rhesus Antigens

Named after the Rhesus monkey in whom it was first described. Unlike ABO, they are **confined** to the surface of RBCs and composed of surface proteins. Rhesus D is the most antigenic and therefore most clinically significant.

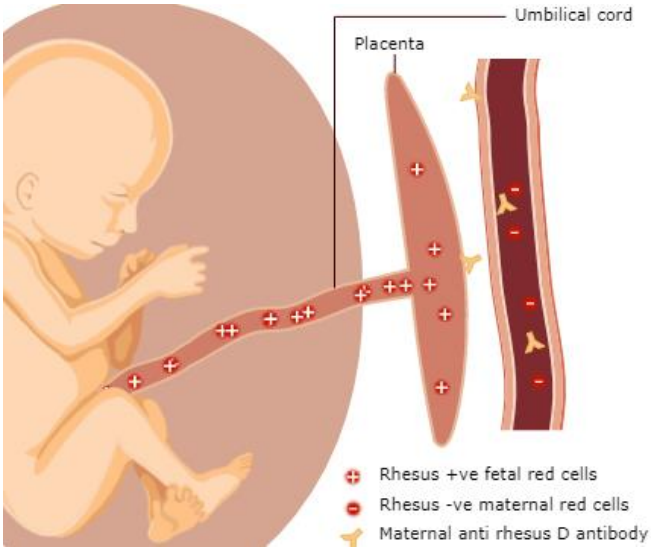
- **Caucasians**: 85% Rhesus D positive, 15% Rhesus D negative
- **Asians**: 99% of Asians are Rhesus D positive

Again unlike the ABO system, Rhesus D negative individuals DO NOT have rhesus D antibodies but only develop following exposure to rhesus D positive blood through foetal blood or transfusion.

Foetal Haemolysis → Haemolytic Disease of the Newborn

In pregnancy, if a **rhesus negative mother** is **exposed to rhesus positive foetal blood**, e.g. during birth, she will produce **IgG rhesus D antibodies.** In a **subsequent pregnancy,** these **antibodies** will **cross the placenta** (unlike ABO IgM) and cause **haemolysis of the newborn if it is also Rh positive**. Therefore, at risk mothers are immunised with anti-D antibodies to prevent sensitisation and protect later Rh D +ve pregnancies.

This will also occur in transfusion of Rh D +ve blood to Rh D -ve recipients.



Cross-Matching Blood

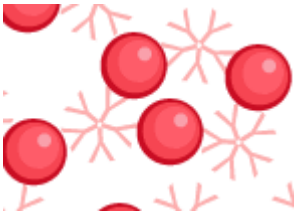
Allows direct compatibility of a donor to a recipient. The recipient's plasma is mixed with donor red cells and is checked for agglutination, if there is none, they are deemed compatible. This process **checks for incompatibility** with **non-ABO/Rh D antigens** such as anti Kell, Duffy and Kidd (a few of many). In emergency situations, this may not be possible and group specific blood (ABO and Rh D classified) can be issued.

Transfusion Complications

(07b_07_03)

Complications are classified into **immediate** (within 24h), **early** (within days) and **late** (within weeks).

IMMEDIATE COMPLICATIONS



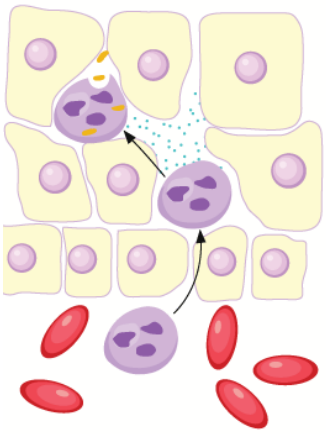
Acute Haemolytic Reaction: Severe **antibody** mediated reaction which is secondary usually to **ABO compatibility**. **Complement proteins** then lyse transfused RBC releasing Hb and cell stroma to the plasma and also cause **cytokine release** with platelet activation and mast cell degranulation → severe reactions including:

- Fever
- Anaphylaxis
- Dyspnoea
- DIC
- Renal Failure
- Hypo/hypertension
- Haemolytic anaemia

Severity of the reaction is proportional to the recipient's antibody titre at the time of exposure

Non-Haemolytic Febrile Transfusion Reactions (NHFRs): Common and usually mild and is caused by **recipient's antibodies** reacting **against donor leucocyte antigens**. Pyrogens subsequently release including IL-1, IL-6 and TNF-a. Most commonly occur in multiparous/ multi-transfused patients. Symptoms: pyrexia shivering general discomfort

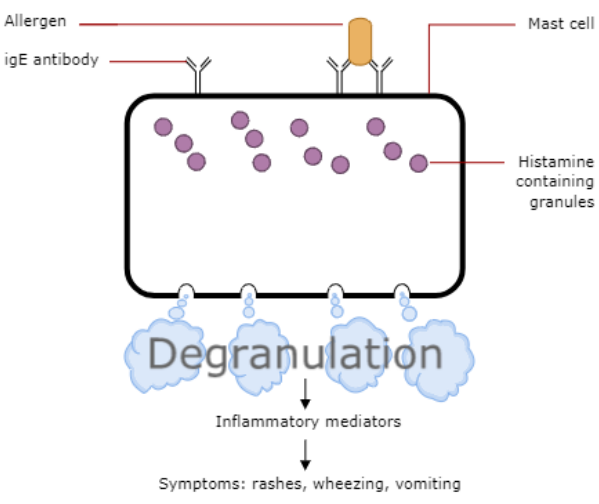
NB these symptoms are common to the early signs of acute haemolytic reactions.



Transfusion Related Acute Lung Injury (TRALI): Occurs within **6 hours** of transfusion. Caused by a reaction between **donor antibodies** and **recipient leucocyte antigens**. They lodge **in pulmonary vasculature** and cause damage to the endothelium. Most commonly occurs with multiparous women donors. The patient ends with a **non-cardiogenic pulmonary oedema.** It is the most common cause of morbidity and mortality following transfusion.

Symptoms: *Dyspnoea* *Hypoxia* *Non-productive cough*

CXR reveals a bilateral diffuse opacity.



Allergic Reaction: This is **IgE mediated** against **foreign proteins in transfused product** → leads to mast cell degranulation and histamine release.

Anaphylactic reactions occur in **hereditary IgA deficient patients** as **anti-IgA antibodies** are present which **react against those in the donor plasma**. RBC can be used from donors that are washed of residual plasma in saline for these patients.

Bacterial Contamination: Severe acute reaction with rapid onset of pyrexia, hypotension, rigors and collapse mimicking that of acute haemolytic reactions and is more likely in platelet transfusions (stored at 22°C). The pack can be gram stained to confirm diagnosis.

Transfusion Associated Circulatory Overload (TACO): This is commonly associated with massive blood transfusion and is a consequence of acute L ventricular failure. The derangement of electrolytes will cause an imbalance and may lead to shock coma or exacerbated heart failure.

EARLY COMPLICATIONS

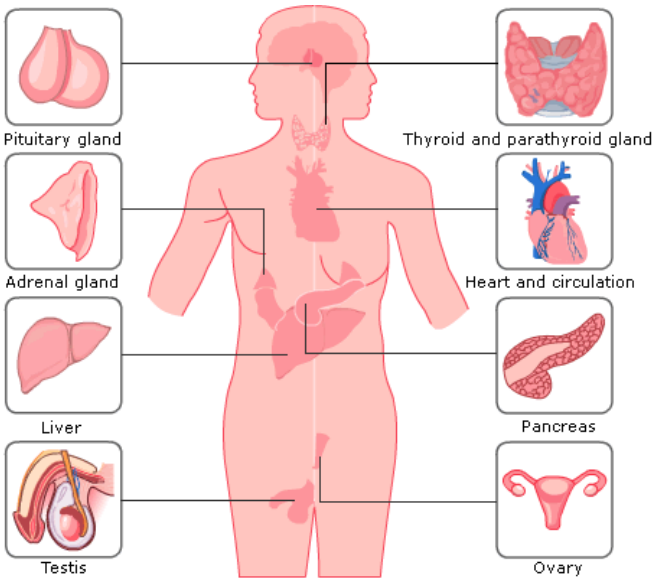
Delayed Transfusion Reactions (DTRs): Occur >24 hours (typically 5-10 days) following transfusion. Caused by non-**ABO mismatching** (mainly Kidd and Rh) and is caused by **raised IgG antibody titres** produced from previous exposure to the antigen (previous pregnancy/ prev transfusion) resulting in a **secondary immune response**. They are not bound by complement, rather they undergo **extravascular haemolysis** in the reticuloendothelial system. This is less severe:

- Falling haemoglobin concentration
- An unexpectedly small rise in haemoglobin
- Jaundice
- Fever

Immune Sensitisation: Transfusion of blood products lead to the development of antibodies in the recipient against the proteins found in the allogenic blood product → immune reaction on repeated exposure. Haemolytic disease of the newborn is an example.

LATE COMPLICATIONS

Iron Overload: 250mg of Iron is transfused per unit and this may **exceed iron elimination pathways**. It is unclear when this is clinically significant but it is likely to be about 12-20 units of transfused RBCs. Iron overload can damage many organs:



Blood-borne Infections: Donors undergo a screening questionnaire with high risk patients excluded from giving blood. The blood then undergoes routine testing (in the UK) for Hep B, Hep C, HIV, Human T-cell leukaemia virus, syphilis and vCJD. Those received blood prior to 1980 are excluded from donating as there is a risk of vCJD (prion) transmission.

NB there is still a possibility of false negative results so the risk remains. Incidence:

Blood-borne infection	Incidence
Hepatitis B	Estimated risk of an infected blood product entering the blood is **2.2 per million donations**
Hepatitis C	Estimated risk of an infected blood product entering the blood is **0.05 per million donations**
HIV	Estimated risk of an infected blood product entering the blood supply is **0.22 per million donations**
Human T-cell leukaemia virus	**No cases** identified in the UK arising from transfusion **since routine leukodepletion was introduced in 1999**
Syphilis (Treponema pallidum)	There have been **no reports** of syphilis transmission from blood transfusion **in recent years**
Cytomegalovirus	Can cause morbidity in CMV-negative immunocompromised patients. No cases identified
Malaria	**5 cases** secondary to transfusion **in the UK in the past 25** years. Donor with potential exposure triggers a screening test

Massive Blood Transfusion

This is defined as the **replacement of circulating blood (70ml/kg) with transfused blood within 24h.** This may result in the following abnormalities (along with the above aforementioned):

Coagulopathy: There is a large consumption of clotting factors and the transfusion of RBC results in a dilutional coagulopathy. FFP and platelets should be considered.

Hypothermia: RBC should be warmed before infusion wherever possible. Rapid infusions of cold blood and may induce hypothermia which can cause:

- Exacerbation of the coagulopathy.
- Reduction of hepatic metabolism which shifts the dissociation curve to the left
- Shivering → increased oxygen demand and consumption

Electrolyte Derangement:

- **Hyperkalaemia:** Particularly if RBC are towards the end of their shelf life as potassium leaks across the membrane of stored cells.
- **Hypocalcaemia:** Residual citrate used as anticoagulant (mainly in FFP and platelets) binds to calcium reducing its ionised concentration after metabolism in the liver → worsened coagulation and muscular contraction
- **Acid-base imbalance:** Lactate in stored blood (rapidly metabolised). Bicarbonate as a product of liver metabolism of citrate may result in alkalosis. In practice acidosis results from inadequate resuscitation and tissue hypoxia.

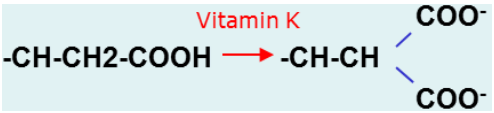
Haemostasis and Coagulation

(07b_07_04)

All clotting factors are produced in the liver apart from vWF and Tissue factor made in the endothelium/platelets and smooth muscle cells/fibroblasts respectively.

Vitamin K

Serine protease coagulation factors II (prothrombin), VII, IX and X are dependent on vitamin K **carboxylation** for function. These factors are manufactured with an **enzyme active site** and a **long tail of glutamic acid molecules** and are non-functional in this state.



Vitamin K acts as a co-factor to convert glutamic acid to **negatively charged γ-carboxyglutamic acid**.

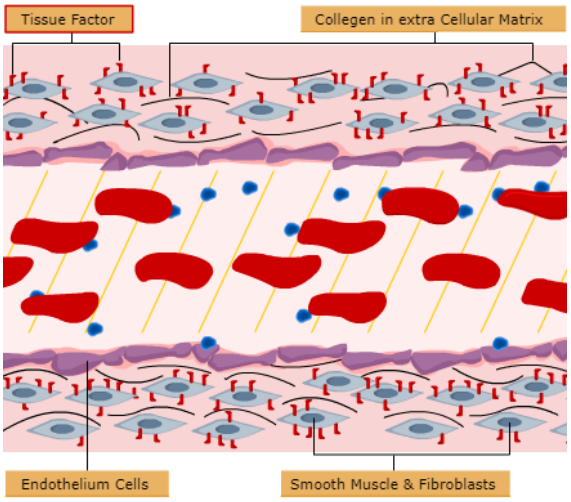
This negatively charged tail can now be bound to the negatively charged activated platelet through **electrostatic bridges of calcium ions** and can now take part in haemostasis.

Normal Haemostasis

In normal blood flow, endothelium is intact and inhibits haemostasis as tissue factor and collagen (activators of coagulation) are not exposed to platelets and clotting factors

Von Willebrand Factor (**VWF**) is also found mainly subendothelial bound to collagen but some is released into plasma and **in low shear**, is tightly curled up in its ***inactive form*** and cannot bind to platelets.

Shear forces are greatest in small blood vessels and in bleeding → VWF stretches out and exposes binding sites that spontaneously bind to inactivated platelets.

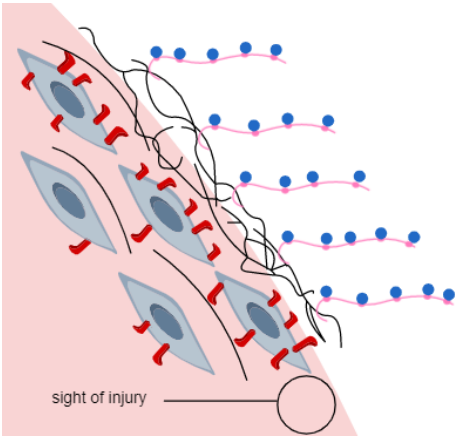


Damaged Blood Vessels – Primary Haemostasis

In **high shear environments**, microvascular bleeding may occur which leads to:

- VWF unravels, exposing platelet binding sites and platelets start to be trapped by VWF through **glycoprotein GP1b** on the platelet surface. They slowly begin to adhere to the vessel wall.
- Factor VII from plasma binds to **tissue factor** on the surface of smooth muscle cells and fibroblasts and activated to VIIa (see below).

These processes occur simultaneously and dependent on each other

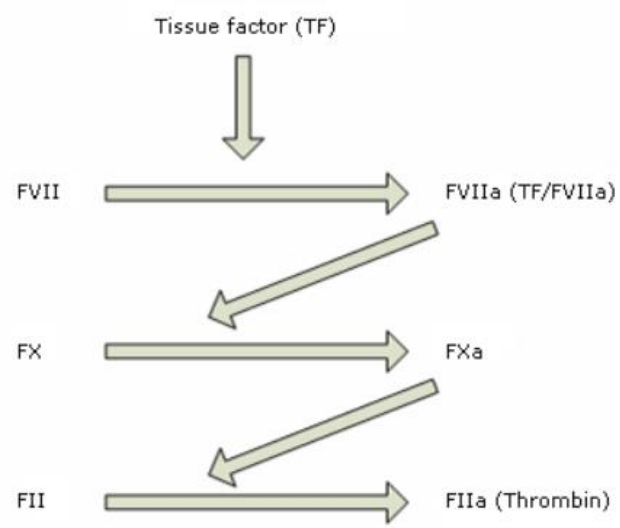


Coagulation Factor Activation

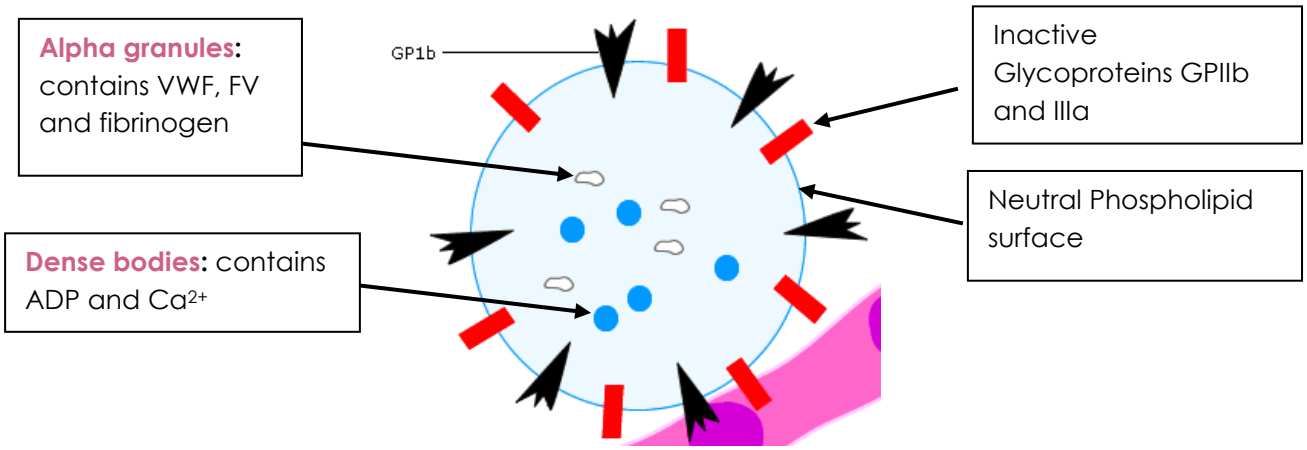
The **extrinsic pathway** is activated through exposed **tissue factor** activating FVIIa which leads to **thrombin production**. Note that at this stage, although thrombin is produced, there is **insufficient amounts to cleave fibrinogen to fibrin** and clot blood but there is enough to:

1. **Activate platelets**
2. **FV→ FVa**
3. **FVIII →FVIIIa**
4. **FXI → FXIa (intrinsic pathway)**
5. **FXIII → FXIIIa**

Thrombin (factor IIa) is inactivated by the plasma protein **TFPI (Tissue factor pathway inhibitor)**.



Platelets



Activation of Platelets

Activated via:

1. **Thrombin pathway (TF/FVIIa)** as mentioned above
2. **Direct binding to collagen** via GP1a
3. **ADP** released from other platelets
4. **VWF binding** via GPIb.

Response of activated platelets include:

- **Degranulation of dense bodies** → ADP and Ca^{2+}
- **Degranulation of alpha granules** → VWF, FV and fibrinogen release
- Translocation of phospholipids outside the platelets → **-ve charged membrane**
- **Activation of GPIIbIIIa**

Platelet aggregation commences through binding to VWF, Cross-linkage through activated GPIIbIIIa with VWF, Ca^{2+} and Fibrinogen. This platelet plug is unstable.

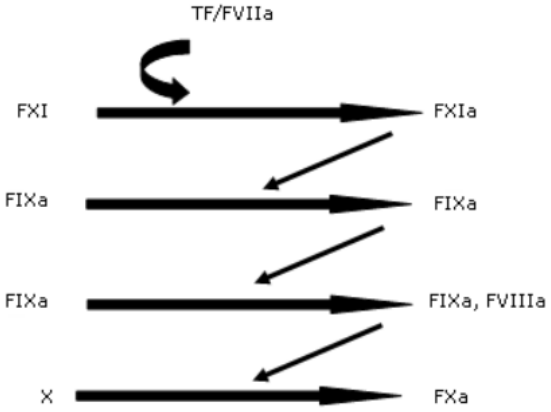
Secondary Haemostasis

Occurs on the surface of activated platelets in the platelet plug and as heard before, starts with small scale thrombin production (initiation phase) which:

1. **Activate platelets**
2. **FV→ FVa**
3. **FVIII →FVIIIa**
4. **FXI → FXIa (intrinsic pathway)**
5. **FXIII → FXIIIa**

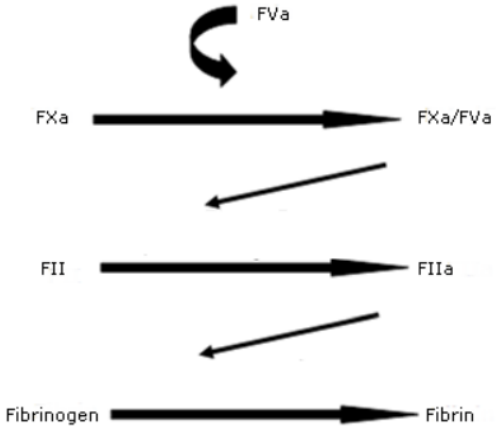
Coagulation initiation

TF/FVIIa activates XI → Xia which follows down through IXa to X but FX is unable to be cleaved as its active site is not held in a stable position. However, **FVIIIa is a cofactor** to FIXa and the active site is now held in the right position for cleaving.



Fibrin Clot Formation

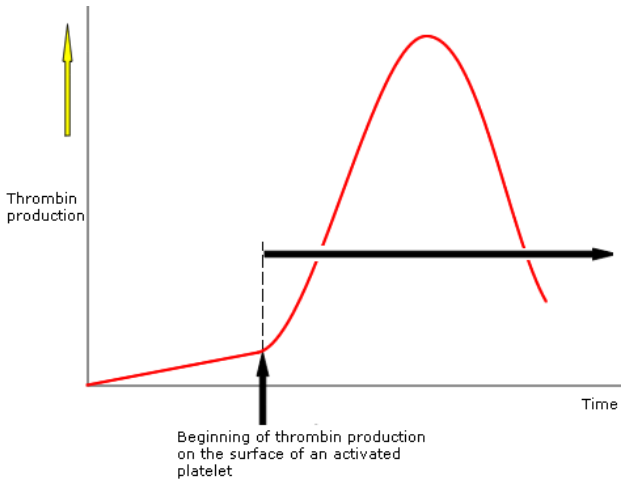
FVa is another cofactor to Xa which helps cleave prothrombin and now, **large scale amounts of thrombin are produced** which can now create **fibrin** through cleaving of fibrinogen.



Thrombin Generation

The above reaction results in a huge increase in thrombin generation – aka **thrombin burst** through enzyme amplification. The 2 phases of thrombin generation are:

1. **Initiation phase** which occurs on the surface of fibroblasts with TF and acts via the ***extrinsic pathway*** and leads to the above.
2. **Propagation phase** (Black arrow) via the ***intrinsic pathway*** where there is a thrombin burst via the FIXa/FVIIIa complex



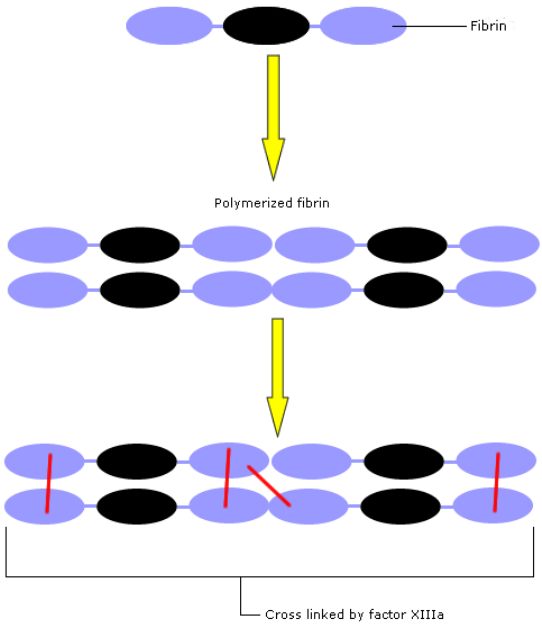
Fibrin Production

There are 3 phases involved in fibrin production:

Phase 1: Thrombin burst cleaves the end of fibrinogen → fibrin.

Phase 2: Spontaneous polymerisation of fibrin into a fibrin clot:

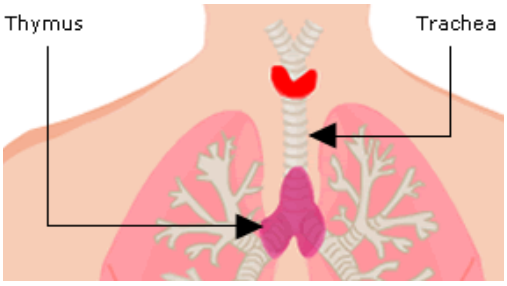
Phase 3: Cross-links occur through **FXIIIa** which further **stabilises the fibrin clot and platelet plug.**



Immunity

(07b_07_05)

All the organs of the immune system are known as **lymphoid organs** concerned with growth, development and deployment of immune cells.



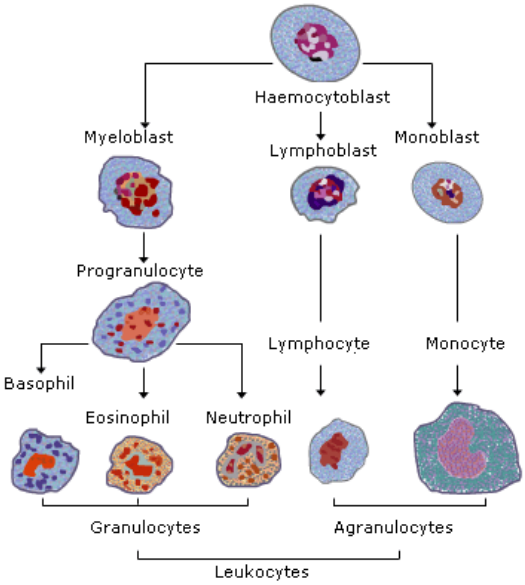
Thymus

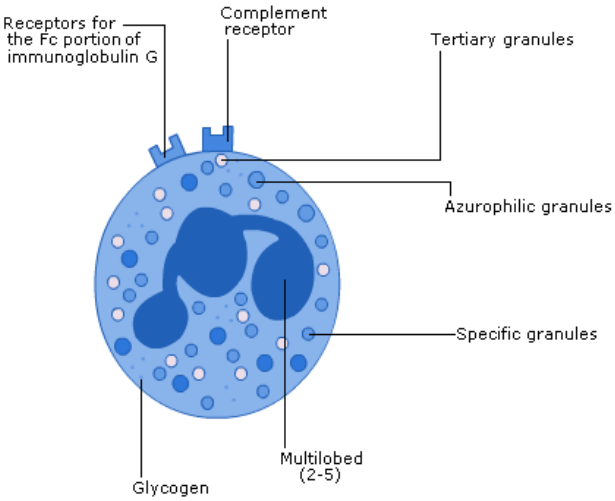
Bilobed, located in upper anterior thorax behind the sternum which enlarges in childhood and atrophies in puberty. Of central importance in **T-cell maturation**.

Leukocytes

AKA WBCs defend the body against infection and foreign materials. Their development comes from a common precursor haematopoietic stem cell in the bone marrow. Leukocytes are sometimes classified into:

- **Granulocytes:** have differently stained granules in their cytoplasm when viewed under light microscopy which are membrane bound enzymes.
- **Agranulocytes:** Absence of granules in the cytoplasm including macrophages, monocytes and lymphocytes.





Neutrophils

The most abundant WBC. Their nucleus is **multi-lobed** connected by thin strands of chromatin. The half-life of an inactivated neutrophil is 4-10h and on activation and migration to tissues, they survive for 1-2 days.

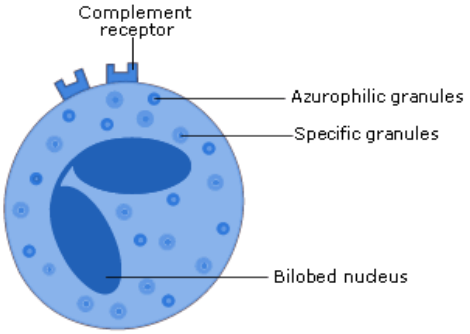
They **migrate** to the site of inflammation through **chemotaxis**.

Basophils

Leased common granulocyte and has a **bilobed nucleus** with large cytoplasmic granules. They degranulate to release **preformed mediators** (histamine, proteoglycans and proteolytic enzymes) and secrete leukotrienes and cytokines – importantly IL-4 and IL-13.

Eosinophils

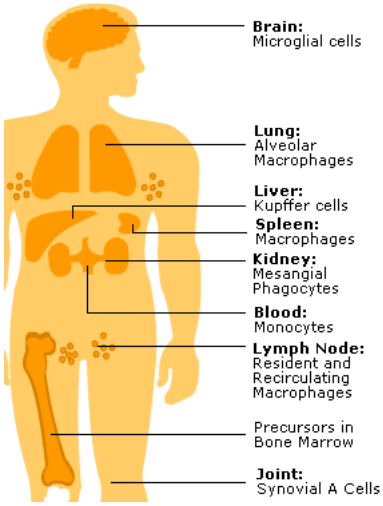
Appear brick red when stained with eosin. They are circulating and also found in GIT, ovary, uterus, spleen and lymph nodes. They persist for 6-12h in the circulation and survive in tissue for 2-3 days in the absence of stimulation. Important in combating **parasites** and control **allergic mediated reactions**. Their granules contain many chemical mediators i.e. histamine and proteins.



Monocytes

Large nucleus and many internal vesicles to process foreign material. Divided into **normal** and **inflammatory cells**. Monocytes are created in the BM from **monoblast** precursors, circulate in the blood for 1-3 days and then migrated to other tissues to become **macrophages**. Some tissues have a specific name for the macrophages.

They function by **phagocytosis → triggering of complement pathway**.

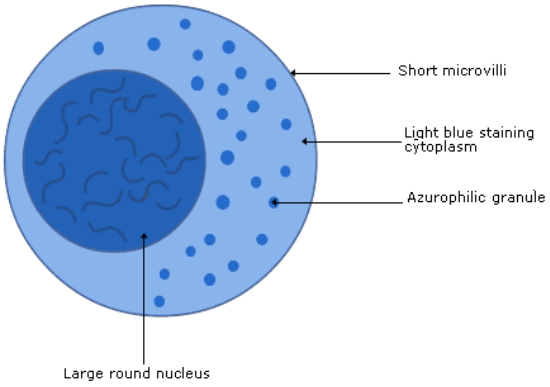


Lymphocytes

Rounded cells with a **unilobular nucleus** and are either small (T- and B-cells) or large and granular i.e. NK cells. Their formation occurs from a common lymphoid progenitor in the bone marrow.

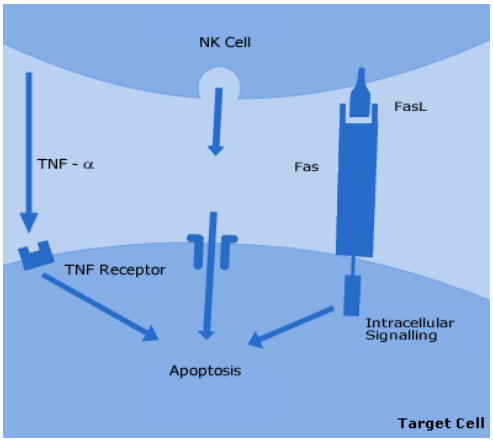
- **B-cells** remain in the **Bone marrow** to mature
- **T-cells** migrate to the **Thymus** to mature.

Following maturation, they enter the circulation/ peripheral lymphoid organs to act in **adaptive** immunity by creating effector, memory and helper lymphocytes.



Natural Killer Cells

Composes a major part of the **innate immune system.** They do not need to recognise a specific antigen to destroy the cell. Native cells express an **inhibitory signal** avoiding targeting by the NK cell. Without it, NK cells release ***perforins*** and ***granzymes*** to create a hole in the target cell. Produces **TNF-α** which acts on **target T cells** and **FasL** interacts with **Fas** of target T cell causing **apoptosis.**



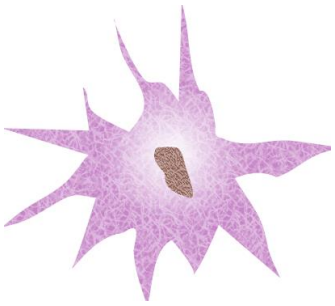
B-Cell

Involved in the **humoral immune response**. They are produced and matured in the bone marrow. Their principle function is to make **antibodies against soluble antigens**.

The B-cell receptor recognises the antigen in the **native form** (cf. T-cells which recognise it in its processed form as a peptide) and is activated with the help of **Th cells (**mainly Th2) and further differentiates into a plasma cell with a short half life of 2-3 days which secrete antibodies. 10% become life-long **memory B-cells**.

Dendritic Cells

They have many spine-like projections (hence the name). They are **phagocytes in tissues** in contact with the external environment i.e. skin, nose, lungs, stomach and intestines. Key cell in the **adaptive immune system** and they **present antigens to T-cells**.

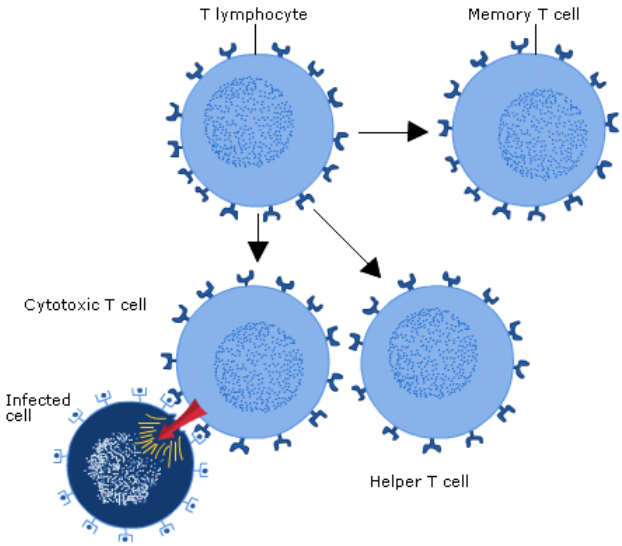


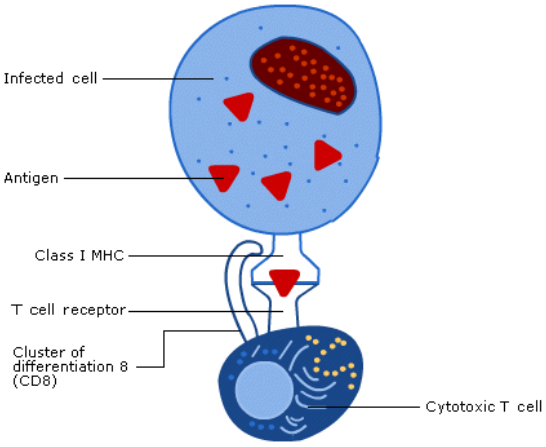
T-Cells

Contribute to **cell mediated immunity**. There are T-cells in existing stages of differentiation:

- **Naïve cells** – not yet entered circulation but are in lymphatics
- **Memory cells** – previously exposed to antigen and activate on re-exposure to antigen
- **Effector Cells** – Activated cells

T lymphocytes help in the elimination of tumour cells, foreign grafts, intracellular pathogens and infected cells





Cytotoxic T-Cells

Differentiate from naïve CTLs into effector cells when activated. These travel the body to search cells with the unique **MHC class I** with **CD8** receptor. They release perforin and granulysin which are cytotoxins that form pores in the target cells. Some CD8 T cells remain to be memory cells to improve subsequent effector response to the presented antigen but the majority die and are cleared by phagocytes.

T-Helper Cells

They express **T-cell receptors** and **CD4** to bind to antigens presented on **MHC class II** molecules. These are divided into Th1 and Th2 cells. They are involved in the following functions through the **release of cytokines**:

- Activation and growth of cytotoxic T cells
- Maximisation of bactericidal activity of phagocytes
- Promote B-cell activity

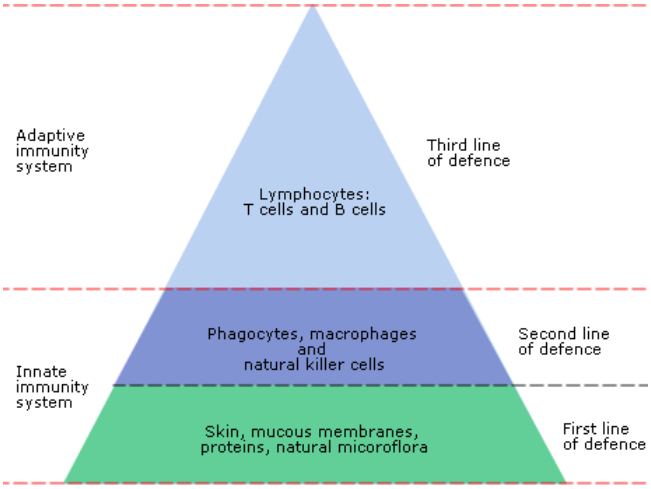
Note they do not have any cytotoxic or phagocytic activity.

INNATE or ***non-specific***	**ADAPTIVE** or ***specific***
Response is non-specific	Pathogen and antigen specific response
Exposure leads to immediate maximal response	Lag time between exposure and maximal response
Cell-mediated and humoral components	Cell-mediated and humoral components
No immunological memory	Immunological memory develops
Present in nearly all forms of life	Present in only some vertebrates

Innate Immune System

No long lasting/protective immunity to the host. There are 4 components to this system:

1. Barriers
2. Cells
3. Serum Proteins
4. Complement System



1. BARRIERS

Physical, mechanical or chemical barriers. The largest **physical barrier** is the skin. **Mechanical barriers** include lungs → coughing and sneezing; tears and urine → flushing action of foreign objects. **Chemical barriers** include low pH in stomach and lysozyme in tears which cleaves peptidoglycan in the cell wall.

2. CELLS

Phagocytes, **Natural Killer (NK) cells** and **degranulating cells** (mast cells and basophils).

Phagocytes engulf and destroy pathogens through phagocytosis, formation of phagosome, secretion of ROS and hydrolytic enzymes into the phagosome to destroy the pathogen. This consumption of O_2 during generation of ROS is called the ***respiratory burst***.

Natural Killer Cells are kept inactive through **inhibitory NK receptors**. These recognise the **MHC class I** presented by native cells. If it is not recognised, → ***activation of NK cell*** and cytotoxic effects commence. It is also activated through cytokines such as IL-12 separate from MHC I expression.

Macrophage Activation occurs in 2 stages:

- **Stage 1:** Primed where they have reduced proliferative capacity but increased MHC II expression and oxygen consumption.
- **Stage 2:** Response to secondary stimuli to become fully activated and is defined by their ability to kill intracellular parasites, cause tumour cell lysis and secrete inflammatory mediators

3. SERUM PROTEINS

Acute Phase Proteins (APP) are produced by the liver in response to injury/infection/trauma. These include CRP and serum amyloid A. These induce ***fever, leucocytosis*** and ***thrombocytosis***.

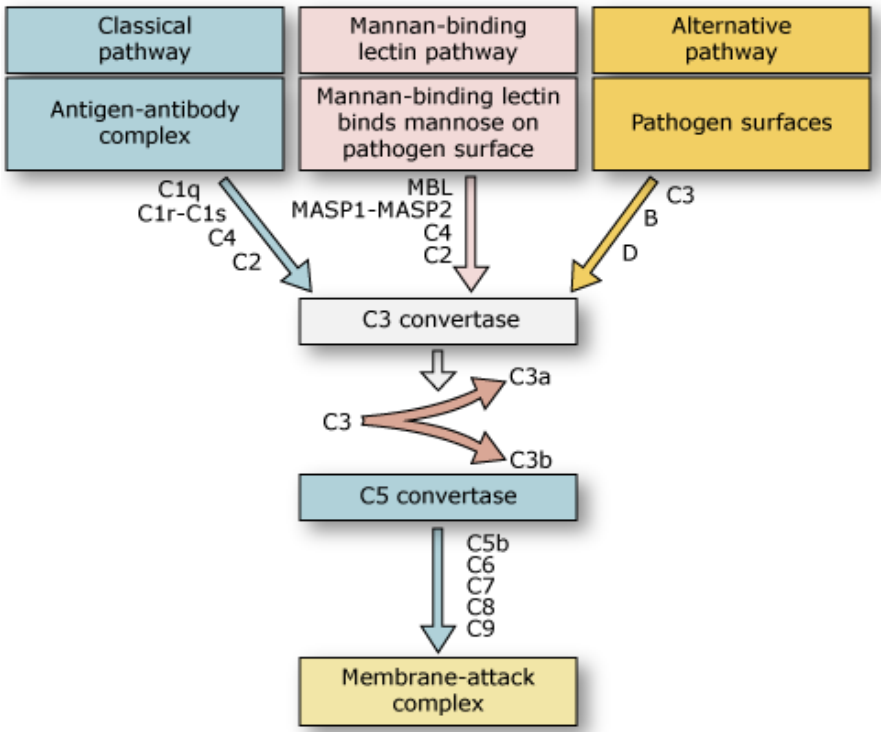
- **Secreted Agents:** Lysozyme as an example
- **Antimocrobial agents:** Lactoferrin (enzyme system of 20proenzymes which enhances phagocytosis and cell lysis) and CRP (activates NK cells and complement cascade)
- **Mannan-binding Lectin** (**MBL**): plasma protein → pattern recognition of carbohydrates on the surface of a large number of pathogenic micro-organisms → ***complement system***
- **Proteins produced by innate immune cells:**

IFNα	Produced by virally infected cells
IFNβ	These interferons induce genes to destroy viral DNA and also induce MHC class I expression
IFNγ	These activate NK cells and macrophages

4. COMPLEMENT SYSTEM

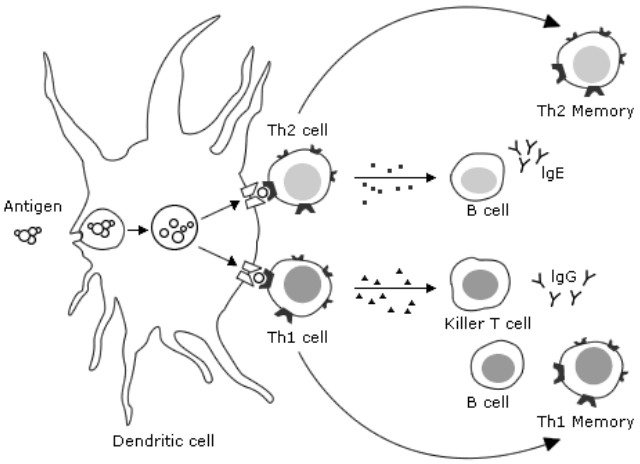
Biochemical cascade which stimulates proteins to ***release cytokines*** and **initiate an amplifying cascade of further cleavages**. The end result of this activation cascade is massive amplification of the response and activation of the cell-killing **membrane attack complex** via C3b.

C1 *esterase inhibitor* is decreased in hereditary angioedema.



Adaptive Immunity

Refers to antigen specific immune response. Some cells are known as **antigen presenting cells (APC)** such as dendritic cells and B & T-cells work together to mount the response as well as recruitment of innate immune cells. Either **cell-mediated** or **humoral**.



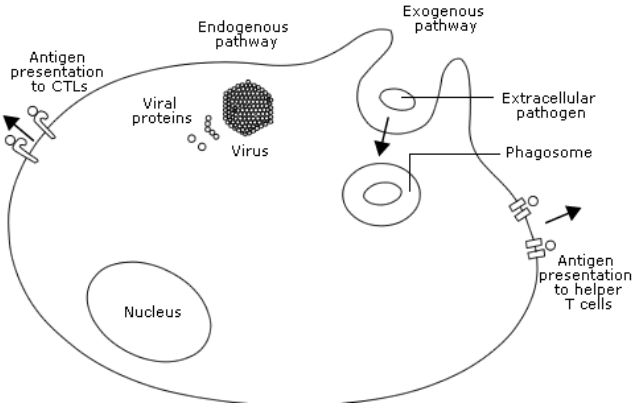
CELL MEDIATED IMMUNITY (CMI)

As well as involving the activation of T-cells, it allows NK cell and macrophage activation and allows the elimination of intracellular pathogens and infected cells, tumour cells and foreign grafts.

Antigen Presentation

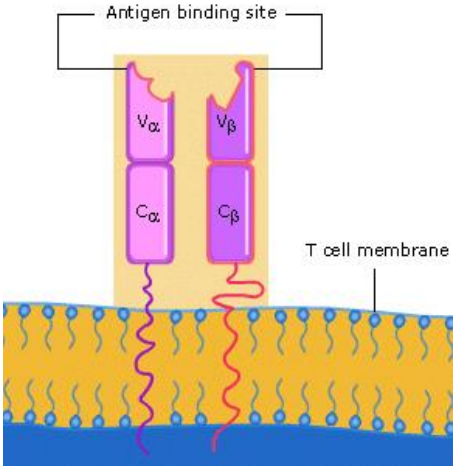
Antigens may be proteins, polysaccharides, lypoproteins and glycolipids and are either endogenous or exogenous:

- **Endogenous antigens** are proteins that are encoded by viral genes that have infected the cell. These are fragmented and displayed on **MHC class I** for activation of **CD8+ cytotoxic T-cells.**
- **Exogenous antigens** are taken up by APCs by endocytosis. The endosome/phagosome fuse with a lysosome for fragmentation and are presented on **MHC class II** for activation of **CD4+ helper T-cells**.

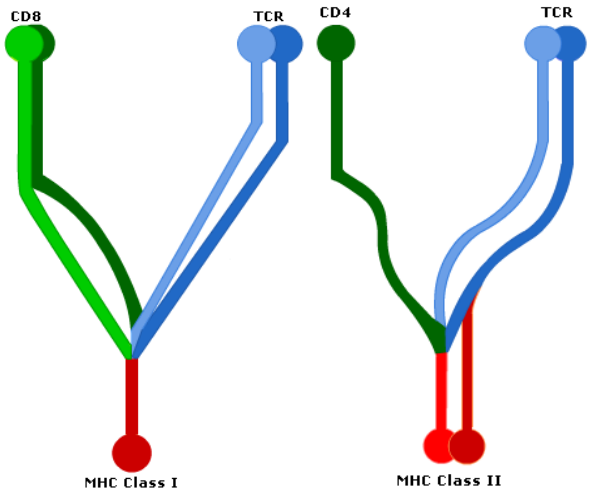


T-cell Receptors (TCR)

Responsible for **recognition of antigens bound to MHC** molecules. They have a unique binding site to the antigen known as the **epitope** and are made before the cell ever encounters an antigen. There are 2 types of TCR:

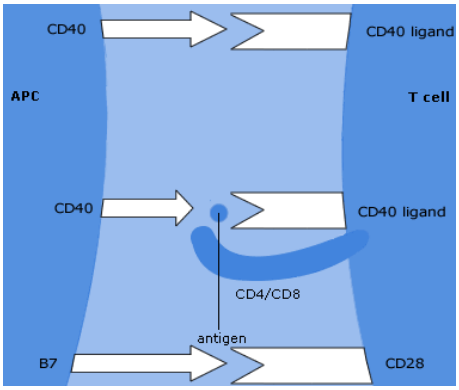


	TCR Type 1	TCR Type 2
Cytokines	IFN-γ and TNF	IL-4, IL-5, IL-6, IL-10 and IL-13
Immune System Involved	**Cellular immunity** - increases macrophage efficiency and proliferation of CD8+ T cells	**Humoral immune system**. Stimulates B cell proliferation and increases antibody production
Other Functions	Increases IL-12 production. IFN-γ inhibits production of cytokines such as IL-4 (an important cytokine associated the Type 2 response)	IL-4 acts on helper T cells to promote the production of Th2 cytokines, IL-10 inhibits cytokines, including IL-2 and IFN-γ in helper T cells and IL-12 in dendritic cells and macrophages



Major Histocompatibility Complex

These are glycoproteins expressed on the surface of **almost all vertebrate cells** and are responsible for the compatibility/incompatibility of tissues from genetically different individuals.



T-cell Activation

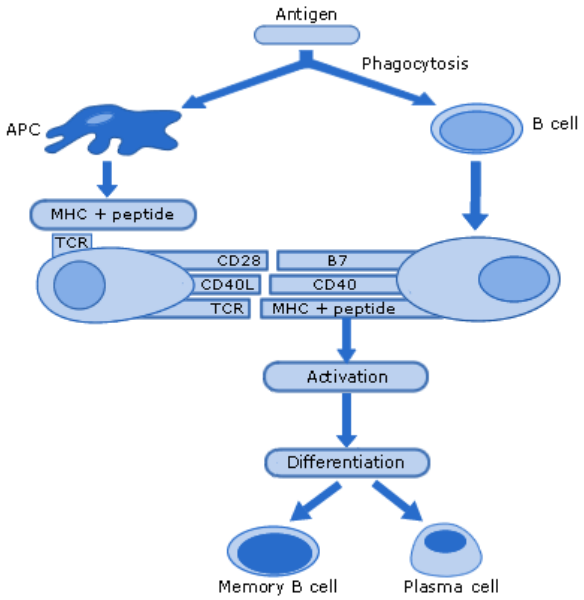
Once an antigen is presented from B cells or any other APC, the **TCR** and **CD28** bind to the **MHC** and **B7 family members** of the APC.

HUMORAL IMMUNITY

B-cells are either activated dependently or independently of T-cells. Activation leads to antibody production against the pathogen:

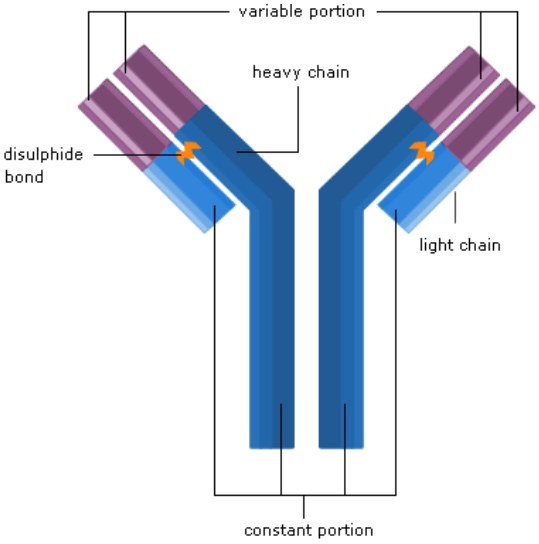
T-cell dependent: B-cell ingests a pathogen to display its fragments on **MHC class II** molecules. This is detected by the T cell which secretes cytokines to allow B-cell maturation and proliferation.

T-cell independent: May be seen in pneumococcus infection where the humoral response is brought on by antibodies efficient at eliminating the pathogen.



Once the antibody/antigen complex is created, the pathogenic antigen can be cleared through:

1. **Activation of complement**
2. **Antibody dependent T-cell mediated cytotoxicity (ADCC)**
3. **Neutralisation of bacterial toxins and viruses through blockage of surface receptors**
4. **IgA mediated mucosal immunity**



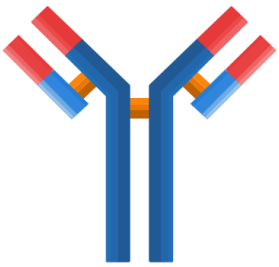
Immunoglobulin Structure

Consist of **2 heavy** and **2 light chains** linked by disulphide bridges.

- **Heavy chain**: 1 variable and 3 constant domains and **determines the Ig class**
- **Light chain**: 1 variable and 1 constant domain

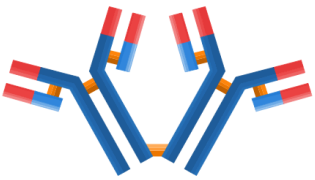
Class switching occurs when the constant region of the heavy chain changes but the variable stays the same and causes a change in class i.e. IgG→IgM. There are 5 classes

1. IgA
2. IgG
3. IgM
4. IgE
5. IgD

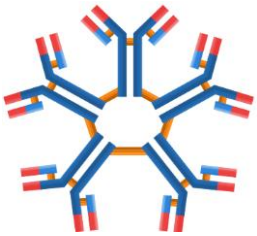


IgG, IgD and **IgE:** They are all similar in appearance and coat the microorganisms speeding up their uptake by cells of the immune system.

- **IgG** (4 types) is the main Ig in the blood; the only Ab that can cross the placenta.
- **IgD** (1 type) is almost exclusively found on the B-cell membrane
- **IgE** (2 types) in trace amounts and is key in the type 1 hypersensitivity reaction with basophil and mast cell activation and in helminth infestation.



IgA (2 types) and concentrate in **body fluids** and in **mucosal areas** to guard the entrance to the body.



IgM remain in the bloodstream as a starshaped combination and are very effective at killing bacteria prior to sufficient IgG production.

Immunological Memory

Some B and T cells develop to become memory cells to mount a strong response on re-exposure of the antigen they initially encountered. This can be **passive** (short-term) or **active** (long-term) memory.

	PASSIVE	ACTIVE
Features	Preformed immunoglobulins transferred to individual	Adaptive immune develops only on contact with antigen
	Antibodies available immediately	Immunity system takes time to develop the response
	Immunity remains only for the lifespan of the antibody (between a few days and several months)	Long lived immunity
Example	Snake bite treatment with antivenom Maternal IgG in new-borns	Vaccination with oral polio

Autoimmune Disease

Where the body exerts an immune response on self. It is usually caused by **autoantibodies** that attach to cells directly or that form complexes that deposit on organ membranes.

The **Human Leukocyte Antigen (HLA)** haplotype is the most common cause of genetic aetiology. I.e. HLA-DR4 in rheumatoid arthritis.

INFECTION: Note **molecular mimicry** may occur when infective micro-organsims generate an immune response against self. The infection may sometimes cause inappropriate MHC II upregulation. Non-specific activation of T and B-cells may also occur.

Autoimmune Condition	Clinical Presentation
Ankylosing Spondylitis	Chronic, painful, progressive inflammatory arthritis
Aplastic Anaemia	Autoimmune attack on the bone marrow causing anaemia
Graves' Disease	Caused by anti-thyroid antibodies causing hyperthyroidism
Systemic Lupus Erythematosus	Autoantibodies are produced against DNA, blood cells and clotting factors resulting in arthritis, rashes and glomerulonephritis
Rheumatoid Arthritis	Primarily affects the joints and is due to the production of antibodies (rheumatoid factor)
Goodpasture's Syndrome	Characterised by lung haemorrhage

Immune Deficiency

Primary immune deficiencies are genetic disorders and the majority are diagnosed in early life.

Secondary immune deficiency is the result of a disease or ageing i.e. malnutrition, HIV, cancer, chemotherapy

Allergy and Inflammatory Response

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Hypersensitivity Reactions

Defined as undesirable reactions that are produced by the normal immune system causing **inflammation**. These immune responses can be mounted against:

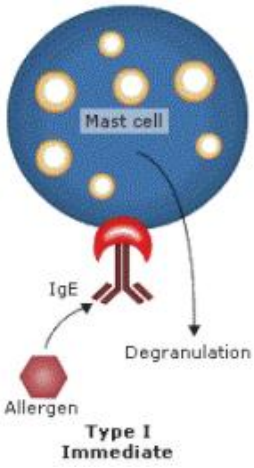
- *Harmless foreign antigens* (allergy, contact hypersensitivity)
- *Autoantigens* (autoimmune diseases)
- *Alloantigens* (serum sickness, transfusion reactions, graft rejection)
- *Infectious agents* (that are not cleared and lead to chronic immune mediated damage)

Gell and Coombs Classification

- **Type I: Immediate Hypersensitivity**
- **Type II: Antibody-dependent Cytotoxicity**
- **Type III: Immune Complex Mediated**
- **Type IV: Delayed Cell Mediated**

TYPE I: Immediate Hypersensitivity

Mediated by IgE which plays an important role in allergy and anaphylaxis. It binds to mast cells & basophils through their FceR1 receptor.

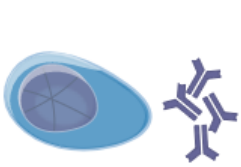


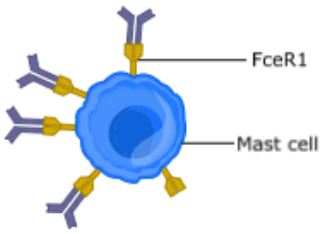
Initial Antigen Exposure

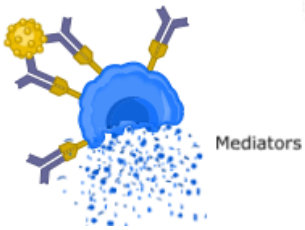
B-cell activation occurs to create plasma cells regulated by Th2 cells which secrete IgE. IgE binds with high affinity to mast cells & basophils.

Repeat Antigen exposure

The antigen cross-links the IgE bound on Mast Cells/Basophils to degranulate to release mediators.







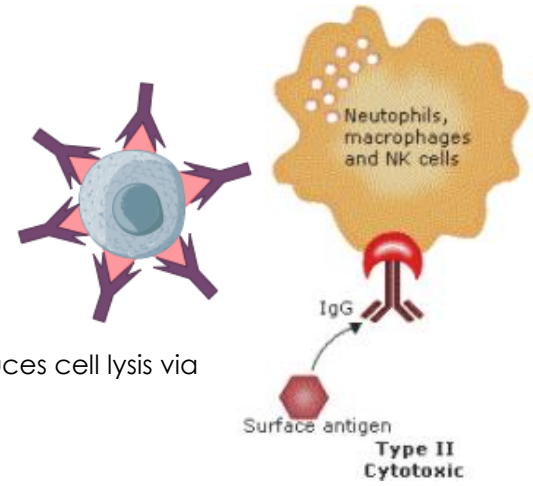
Mediators released by mast cells may be pre-formed or newly formed each producing various effects as chemo-attractants, activators and spasmogens.

Clinical Features

Takes 15-30mins to manifest from antigen exposure and may cause

- Conjunctivitis
- Rhinitis
- Shock
- Urticaria/eczema
- Bronchospasm
- Gastroenteritis
- Cardiovascular collapse

TYPE II: Antibody-dependent Cytotoxicity



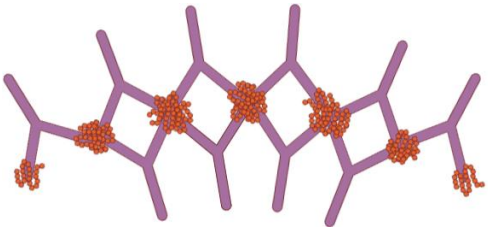
This is usually **organ specific** and the clinical presentation depends on the target tissue. Mediated through **free serum antibodies IgG** and **IgM** which attaches to the cell surface antigen and kills the cell through **Fc dependent mechanisms:** Occurs within minutes-hours.

1. **Activates complement** via classical pathway. This induces cell lysis via MAC.
2. **Phagocytosis** via phagocyte binding to Fc.
3. **ADCC** via NK cell binding to Fc.

Organ-specific autoimmune diseases

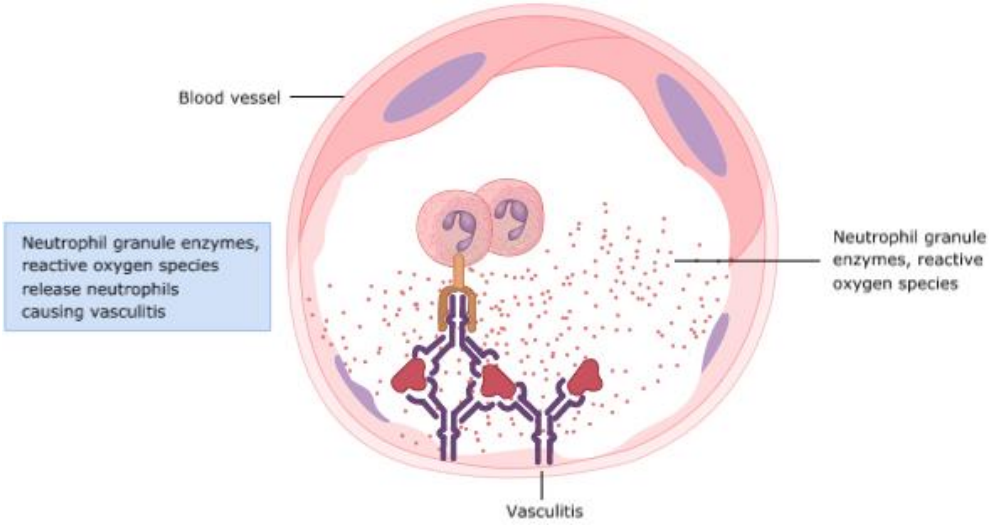
- **Myasthenia gravis** (Acetylcholine R Ab)
- **Glomerulonephritis (Goodpastures)** (Anti-glomerular basement membrane [GBM] Ab)
- **Pemphigus vulgaris** (Ab - epithelial cell cement)
- **Haemolytic anaemia** and **incompatible blood transfusions**
- **Idiopathic Thrombocytopenia Purpura**: destruction of platelets
- **Rheumatic Fever** – heart and joint antibodies.

TYPE III: Immune Complex Mediated



This involves the formation of **antigen-antibody complexes** with **IgG/IgM** and soluble antigens in the circulation. These are normally broken up by complement and transported to the spleen for phagocytosis. However, in disease, these are **deposited in a tissue → complement activation → cell recruitment** (macrophages and PMNs) & clotting cascade activation → tissue damage (vaculitis) i.e. systemic lupus erythematosus (SLE).

The site of deposition is dependent primarily on the localization of the antigen i.e. serum sickness, SLE, aspergillosis, rheumatoid arthritis, lupus nephritis.



TYPE IV: Delayed cell mediated

This is a **T-cell mediated response** with no role of antibodies: therefore, passive transfer by serum requires transfer of the antigen specific T-cells also (unlike Type I, II and III hypersensitivities). The **induction of response** i.e. via macrophages may take up to 2-3 days.

Chronic graft rejection
GVHD (Graft vs. Host Disease)
Coeliac disease
Contact i.e. nickel
Tuberculosis
Tuberculoid leprosy

→ TH1, IFN-γ mediated

Asthma
Rhinitis
Eczema

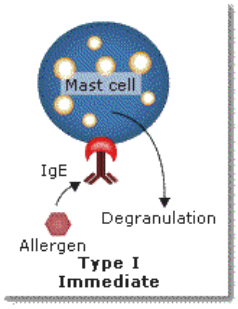
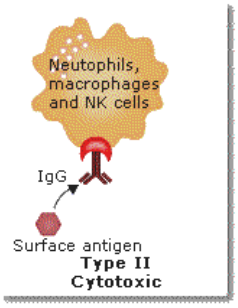
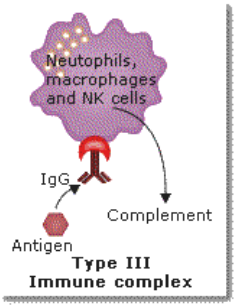
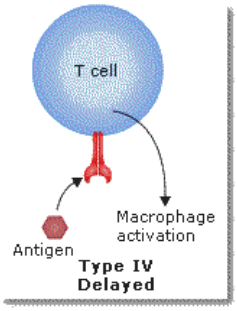
→ TH2, IL-4/5/13 mediated

This mechanism is induced by a persistent/transient antigen. TNF-a is the mediator that induces tissue damage.

Other diseases include:

- **TB/Wegener's/pneumonitis**
- **T1DM**

Summary

				
Antibody	Immunoglobulin E (IgE)	Immunoglobulin G (IgG) Immunoglobulin M (IgM)	Immunoglobulin G (IgG) Immunoglobulin M (IgM)	None cell mediated
Antigen	Exogenous	Cell surface	Soluble	Tissues and organs
Response time	15-30 minutes	Minutes to hours	3-8 hours	48-72 days
Appearence	Weal and flare	Lysis and necrosis	Erythema and oedema	Erythema and induration
Examples	Allergy	Goodpasture syndrome	Systemic lupus erythematosus	Tuberculin test

Acute Inflammatory Response

Occurs in response to irritation or injury characterised by redness, swelling, heat and pain. The response to i.e. a cut and local invasion of pathogens is as follows:

1. **Release of chemical mediator**
2. **Vasodilation** and **increase in blood flow** with **increased vascular permeability**
3. **Diapedesis of macrophages and neutrophils** (squeezing through vessel walls)
4. Antimicrobial chemicals and clotting proteins result in swelling → pressure on nerve endings and subsequent pain.
5. Blood clot formation and further phagocytosis of bacteria → pus formation
6. Damaged tissue is repaired and clot is absorbed/falls as a scab.

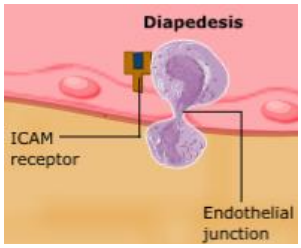
Chemical Mediators

- **Cytokines** released as effector protein helping adaptive immune response and promotion of inflammation, growth and differentiation of leukocytes
- **Fibrinolytic system** to help clot formation and angiogenesis.
- **Kinin production** → pain and increased vascular permeability
- **Complement system**
- **Arachidonic Acid cascade**

Leucocyte Migration

This is through complex signalling molecules expressed on the endothelium and occur in 3 stages:

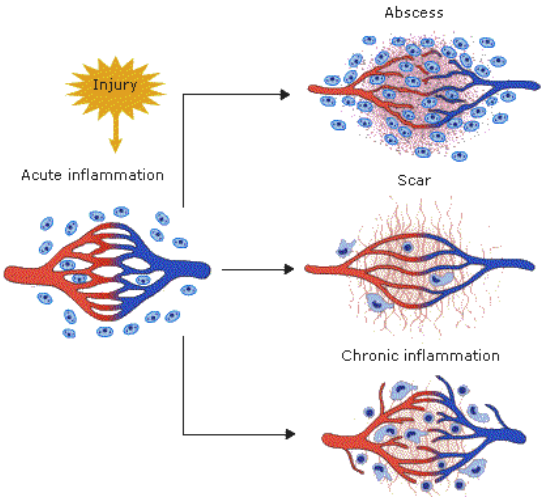
1. **Margination**: Slow-down in circulation, through weak interactions between selectins (act as brakes). Adhesion to vessel wall via **Integrins** and **ICAMs** (Inter-cellular adhesion molecules)
2. **Diapedesis**: Trans-endothermal migration of neutrophils – active process between or across endothelial cells.
3. **Chemotaxis**: ECM binding (via integrins) to prevent loss and chemo-attractants allow migration along a chemotactic gradient to source of inflammation.



Outcomes

If the inflammatory response does not terminate as per normal, there are 3 possible outcomes:

- **Abscess formation** – Cavity containing pus, (dead white blood cells and bacteria with general debris from destroyed cells)
- **Formation of a collagenous scar** – Filling with fibroblasts, collagen and new endothelial cells
- **Chronic inflammation** - If the injurious agent persists. Characterized by continued presence of macrophages in the injured tissues, which release toxins (including ROS). This process lasting days to years, may lead to the formation of a chronic wound.



ENDOCRINOLOGY

Hormonal, Metabolic and Inflammatory Responses to Surgery

(07b_08_01)

The **stress response** refers to the series of hormonal, inflammatory, metabolic and psychological changes, which occur in response to trauma or surgery.

- ***Physiological Changes*:** Includes substrate mobilization, muscle protein loss and sodium and water retention.
- ***Psychological Changes*:** the cause of postoperative malaise and fatigue is uncertain but many psychological factors may play a role in its development.

Metabolic Response

Multi-organ failure is considered **protective** and therefore **functional** through reduced cellular metabolism → increased survival of important cells. This results from acute phase changes in hormones and inflammatory mediators. Reducing these effects may reduce postoperative morbidity and expedite recovery. Factors inducing the **metabolic response** include:

- **Preoperative**
  - Personality and preoperative mental status
  - Anxiety and fear
  - Dehydration
  - Partial starvation
- **Perioperative**
  - Haemorrhage
  - Hypothermia
- **Postoperative**
  - Immobilization
  - Infection
  - Hypoxia
  - Alterations in normal diurnal rhythms

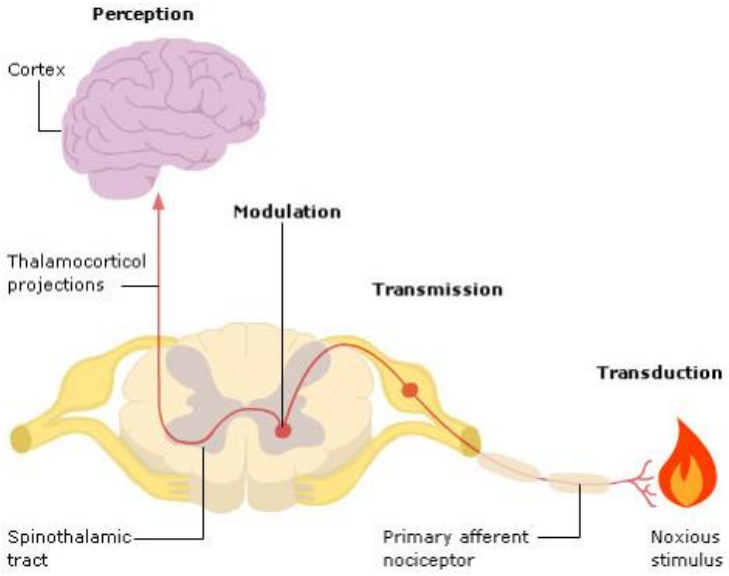
Hormonal Response

The **hypothalamic-pituitary axis** and **SNS** are activated by neuronal input and cytokine release from site of injury. The **pituitary response** is the increased secretion from anterior pituitary of:

- **ACTH**
- **GH**
- **Prolactin**
- **β endorphin**

And from the posterior pituitary:

- **AVP**



As a result, there will be:

- **Cortisol**, **aldosteron**e and **catecholamine** secretion from the adrenals,
- **Hyperglycaemia** from excess GH,
- **Catabolic hormone release – glucagon** from the pancreas
- **Water retention** and **reduced urine output** from kidney reabsorption. **Renin** is increased.

The magnitude of response is linked to the severity of the trauma. Normal feedback mechanisms are not as effective in these circumstances and **anabolic hormone secretion**, such as insulin, oestrogen, T3 and testosterone, **is reduced**.

Sympathoadrenal response: Hypothalamic **activation of SNS** results in the increased **catecholamine secretion** and well recognised CV effects of HTN, tachycardia and hyperglycaemia.

Inflammatory Response

Cytokines include IL, IFN and TNF and are all of low molecular weight (<80kDa). They can exert **paracrine** (local) or **endocrine** (systemic) effects. In surgery the most important is:

- **IL-6:** Increases 2-4h after start of surgery with peak values at 12-24h. Increasing amounts with increasing severity of tissue damage. Therefore, in laparoscopic surgery, values are much less than open surgery.

Cortisol levels increase as **ACTH secretion** from the anterior pituitary is **stimulated by IL-6**. Cortisol provides negative feedback to genetic expression of cytokines and therefore helps to minimise the inflammatory response to surgery.

Acute phase response is the increase in acute phase proteins i.e. CRP, complement, fibrinogen, amyloid A & P and ceruloplasmin in **response to IL-6** and other cytokines. They **promote haemostasis, limit tissue damage** and enhance **repair and regeneration**. They also cause **fever, granulocytosis** and **lymphocyte differentiation** to help with the immune response and for wound healing and repair. Their synthesis is at the expense of other proteins i.e. albumin and transferrin.

Psychological Changes

Feelings of malaise may last for several months post-operatively known as **post-operative fatigue**. Its physiological cause is completely unknown. Psychologically, there is a link to motivation and association with depression. For example, in major abdominal surgery, it is rarely life-enhancing but is life threatening which may reflect on the patient.

Modification of the Stress Response

Intravenous Induction Agents: Etomidate is used in patients with limited CV reserve and **inhibits cortisol** and **aldosterone production** (through 11β-hydroxylase step) for 8h after pelvic surgery to **obtund the stress response.** However, there are no adverse effects on these patients hypothesising that only resting values were necessary for normal outcome after surgery. **Diazepam** and **midazolam** also decrease the cortisol response to peripheral surgery and major upper abdominal surgery.

Volatile Anaesthetic Agents: Little effect on the HPA axis. No change in response between 1.2-2.1 MAC of halothane.

High Dose Opioid Anaesthesia: Has a profound **suppression of the HPA axis** and reduces the response of cortisol to surgery. **Fentanyl 50 µg/kg** abolished the cortisol response in pelvic surgery.

Regional Anaesthesia: Complete afferent blockade (somatic and ANS) is required to **supress ANS stimulation** i.e. with pelvic T4-S5 block. This does not occur with upper abdominal regional blocks. However, this does not suppress the cytokine response occurring as a result of tissue trauma.

Epidural/spinal anaesthetic techniques have not been shown to improve outcomes in major surgery.

Post-operative epidurals however, have been shown to provide better pain relief and shorten the intubation time of ICU post-op patients of specific procedures and reduce DVT risk. In high risk major abdominal surgery patients, there is no difference in morbid outcomes.

Following GI surgery, improved pain control, a shorter duration of postoperative ileus and fewer pulmonary complications, but did not affect the incidence of anastomotic leakage, intraoperative blood loss, transfusion requirements, risk of thromboembolism or cardiac morbidity.

NSAIDs: Diclofenac is associated with **lower IL-6** and higher IL-10 concentrations and a lower white cell count. If beneficial effects are sought, they must be used **24h preoperatively**

Minimal Access Surgery: The classic neuroendocrine response (increases in cortisol, glucose and catecholamines) are not significantly altered in laparoscopic techniques i.e. lap chole instead of open but they cause a **reduced acute phase response** and **preserved immune function**. **Il-6** and **CRP** are reduced.

- ***Malignant disease***: no change in survival and disease control of colorectal cancer but improves morbidity, hospital stay, tumour recurrence and cancer related survival. Uncertain mechanism but suggested due to less tumour manipulation and better immune function.

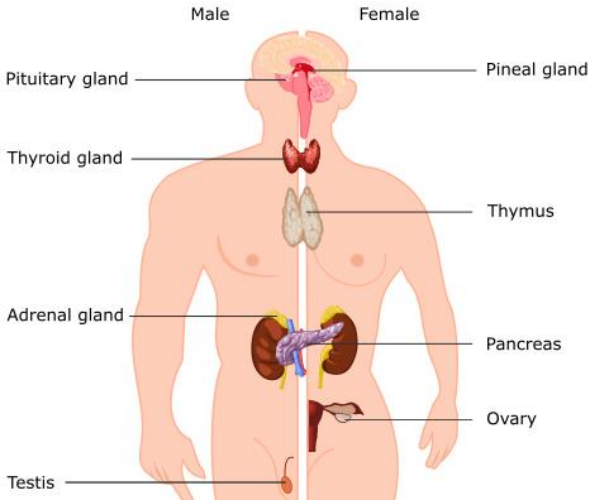
Physiology of Hormones

(07b_08_02)

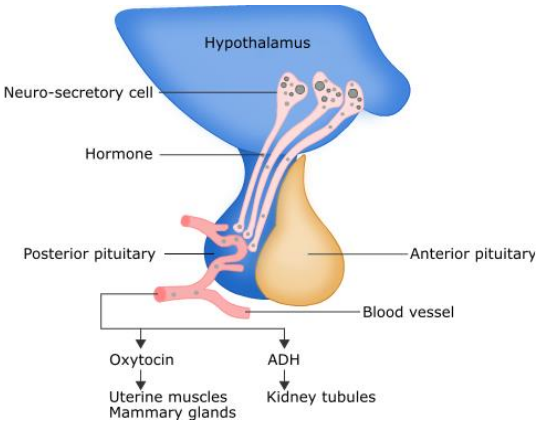
The endocrine and ANS are linked. Whilst the ANS acts mainly in response to factors outside the body, the endocrine system is more focused on the internal environment and long-term regulation of functions.

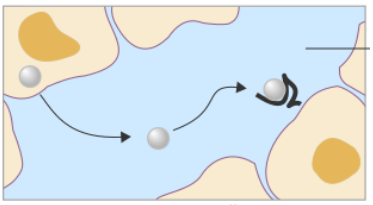
Endocrine function: Transmission of a molecular signal via the blood stream to induce a biological action on a cell/tissue.

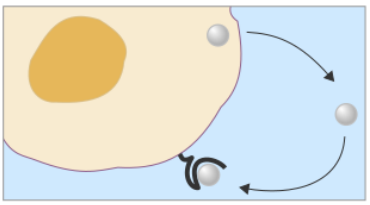
The endocrine system is a network of anatomically and embryologically discreet glands which are usually ductless and specialised in function. However, other organs may also release hormones i.e. ANP from heart.



Neurocrine System: Hormones are released from the **posterior pituitary** and **hypothalamus** in response to **nerve cells**. i.e. oxytocin, ADH.




Paracrine signalling


Autocrine signalling

Paracrine and Autocrine Systems

Paracrine: excretion of hormones to ECF to exert a **local effect** on cells within the **same organ/tissue.**

Autocrine: Excretion of chemical from a cell to affect the activity of the same cell. Hormones are therefore defined as a chemical produced which effects any target cell.

Chemistry of Hormones

There are 3 distinct chemical groups of hormones:

1. **Proteins and peptides**
2. **Amines** – derived from tyrosine and have an alpha amino group on a benzine ring. These include catecholamines and thyroid hormones
3. **Steroids** – cholesterol is the common precursor and includes adrenal cortex and sex hormones and vitamin D.

Hormones are usually released in active form but may require further modification in tissues.

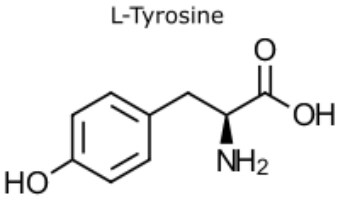
Proteins and Peptides

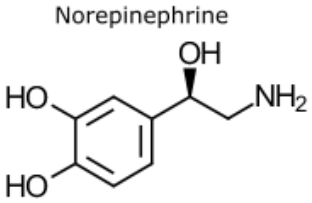
These are the largest hormone group with each containing 3-200 amino acids. Some have glycoprotein sides. Initially produced by the rough ER as large pre-hormones and then processed and packaged by the Golgi apparatus and smooth ER. Active product is stored in vesicles rendering it inactive.

They are secreted through **exocytosis** triggered by **increased cytosolic Ca^{2+}**. They usually travel unbound so are water soluble and have **specific membrane receptors** as are not lipophilic.

Amines

Catecholamines and thyroid hormones with an **alpha amino group** on a **benzene ring**.



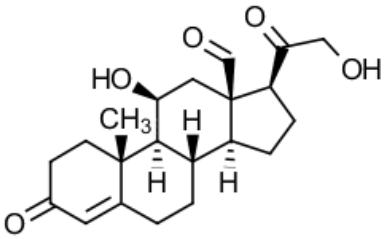


Catecholamines (Ad, NA and Dopamine) are produced from the adrenal medulla. Ad is produced from NA using the enzyme **'phenylethanolamine N–methyltransferase'** which is only present in the adrenal medulla so adrenaline is produced in greater amounts. They are stored in vesicles to be released with SNS stimulation taking <1min. **50% bound to albumin**.

Thyroid hormones T3 and **T4** are stored in the **cytoplasm bound with thyroglobulin** (a protein). Once split, they diffuse out of the cells and bind to **thyroid binding protein**. There is little free T3 or T4 as they are largely protein bound (99%) and with the free fraction in balance with the bound portion, the active free portion remains stable over longer periods.

Steroids

Cholesterol is the common precursor and all retain the **basic 4 ring structure** with physiological differences due to side chain modifications (aldosterone right). Their production requires a large number of enzymes that exist within different organelles for completion with specific completed hormones existing due to the enzymes the specific cell contains. They are **highly lipophilic** and diffuse readily out into the ECF. There is no intracellular storage of these hormones but only of the precursor cholesterol.



Endocrine Activity and its Control

Endocrine control is concerned with **homeostasis.** Broadly, activity is dependent on 3 factors:

1. **Rate of production and release of active hormone**
2. Rate of its removal
3. Sensitivity of the end organ

Factor 1 is the most important. In general, **compounds with high plasma binding** have **long durations of action** and vice versa

Integrated Systems

There are 3 systems control the rate of production and release, and thus the activity, of hormones:

1. **Negative feedback**
   a. Activity of a hormone applies negative feedback to the initial (trophic) hormone that controls its release. i.e. T4 → -ve to TRH and TSH.
2. **Positive feedback**
   a. Less common where secretion of a hormone is enhanced by an increase in the target substrate i.e. LH → oestrogen → +ve to LH
3. **Neuronal activity**
   a. The response is usually consequent upon external rather than internal influences. Close integration of ANS and endocrine system i.e. catecholamine release.

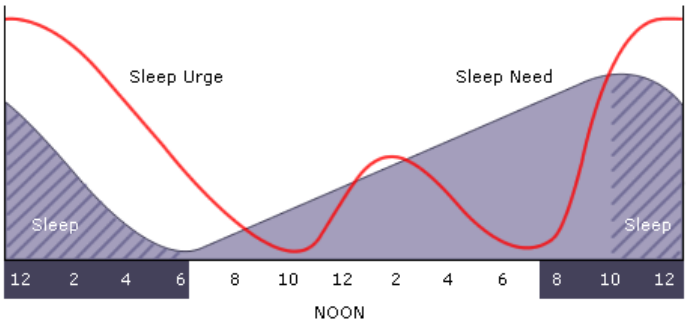
Trophic hormone: controls the activity of another hormone

Cyclical Variations

Some hormones have rhythmical release patterns. This may be genetically coded and is poorly understood. As well as puberty, menstruation, the following is an example:

Circadian Rhythm: Appears to be controlled by the **suprachiasmatic nucleus** of the **hypothalamus**. This intrinsic clock cycles over 24h. The external stimulus of the light/dark continually adjusts this clock.

Melatonin increases by up to 10-fold at night and induces normal sleep. Receptors in the retina signal to the **pineal gland** which controls melatonin secretion.



Growth hormone and ACTH have distinct 24 hour rhythmicity.

Seasonal Affective Disorder is thought to relate to low or reduced light levels with associated release of the complex pro-hormone POMC that is cleaved into ACTH, β-Endorphin & α-Melanocyte Stimulating Hormone.

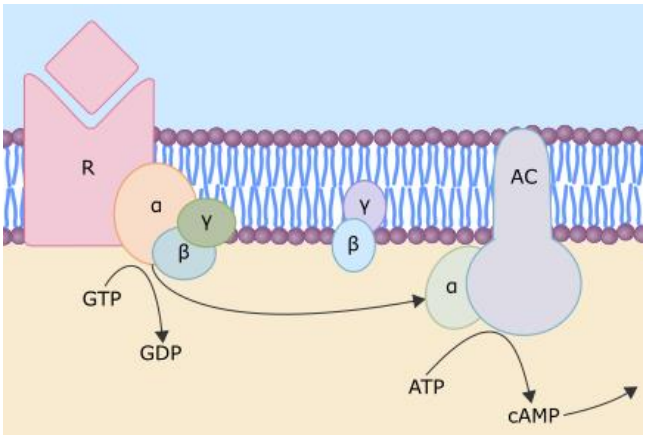
MOA of Hormones

Receptors are usually of **high affinity** and **specificity** as hormones circulate in low concentrations. The mechanisms may include:

- **Cell membrane receptors** and then via a 2nd messenger system i.e. G-protein linked
- **Gated ion channels** allowing ion passage with binding of hormone to receptor
- **Second messenger systems** describe a cascade of reactions for ultimate activation of an intracellular protein.

G-Protein Linked Receptor

A **single long peptide chain** with **7 transmembrane domains** and is coupled to a 3-subunit guanine binding (G) protein for **GDP→GTP** and **separation of G-protein** for **dissociation** and **initiation of intracellular response** through the **alpha subunit**. This may be through an enzyme or ionic channels.



Second Messenger: Most common is **adenyl cyclase** for phosphorylation of proteins. Switched off through the enzyme **phosphodiesterase**. Others include:

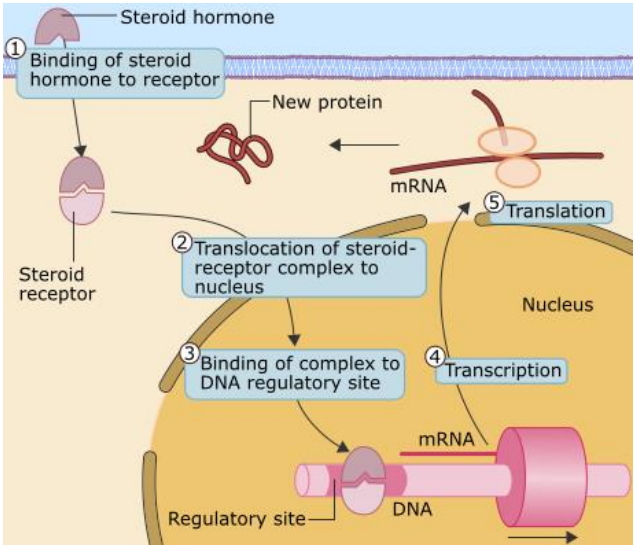
- Phospholipase C → protein kinase activation and Ca^{2+} activation
- Ca^{2+} binding → activation of calmodulin

Tyrosine Kinase receptors bind insulin and ILG factors which directly couple intracellular kinases.

Intracellular Receptors

Steroids and thyroid hormones cross the cell membrane and act on nucleic proteins. Steroid receptors are usually cytosolic and binds to cell specific DNA.

Thyroid hormone receptor is located in the nucleus already bound to target DNA (inhibiting transcription). Thyroid hormones have the **longest latency** of all hormones of about 2 days and also the longest duration of action.



Hypothalamic and Pituitary Function

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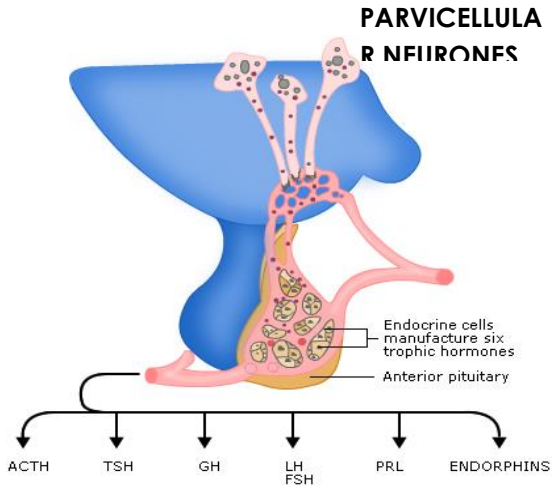
The hypothalamus and pituitary gland are 2 separate structures. The pituitary gland consists of the:

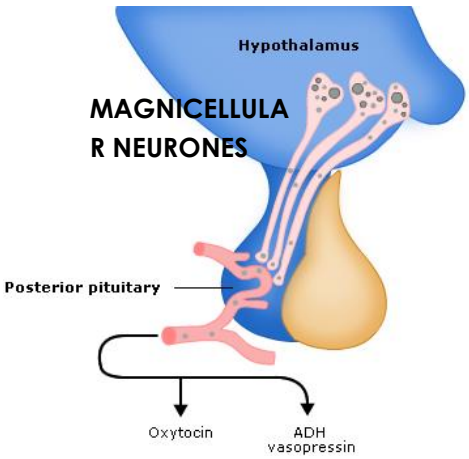
- **Anterior pituitary** formed of endocrine tissue
- **Posterior pituitary** of neuronal tissue.



Pituitary Gland

Anterior Pituitary develops as a pouch in the oral cavity embryologically and becomes separated from its origin by the sphenoid bone and sits on top of the S**ella Turcica**. The pouch is obliterated and only separates the anterior from the posterior pituitary. It secreted **6 trophic hormones**.

These are under control of specific stimulatory and inhibitory hormones produced from terminal axons in the hypothalamic median eminence and carried down the **portal veins from the capillary network**.



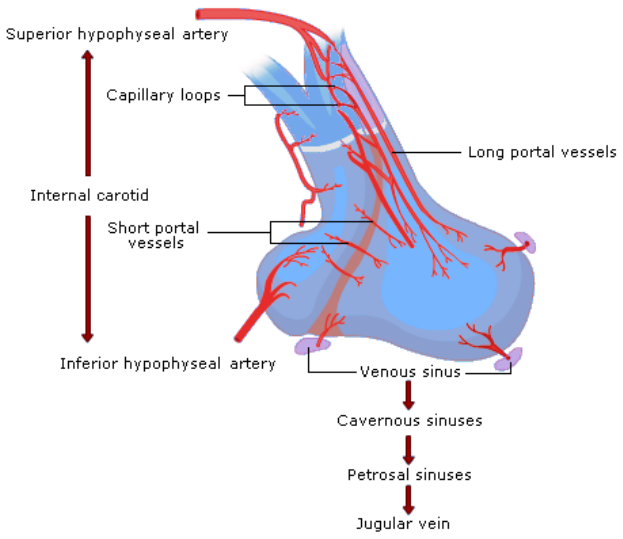
Posterior Pituitary is derived from a downward projection of neural tissue from the floor of the 3rd ventricle. Its stalk attaches to the **median eminence of the hypothalamus**. Oxytocin and ADH are released from the posterior pituitary.

These are manufactured in **hypothalamic cell bodies** and pass down the axons to the terminal points in the posterior pituitary. They are released on stimulation to the capillary plexus.

Blood Supply

Superior hypophyseal artery supplies blood to the median eminence and stalk. A capillary plexus invests this area and forms **long portal veins** to the anterior pituitary forming another plexus.

Inferior hypophyseal artery supplies the posterior pituitary. Some **short portal vessels** supply the anterior pituitary but the majority passes into **venous sinuses**.



Hypothalamic Function

The hypothalamus receives inputs from all parts of the CNS, has specialised receptors to measure physical properties and influenced by feedback arms. Key functions include:

1. Autonomic nervous system function
2. Temperature regulation
3. Regulation of food intake
4. Regulation of water intake
5. As the key regulator of the pituitary gland

Posterior Pituitary Hormones

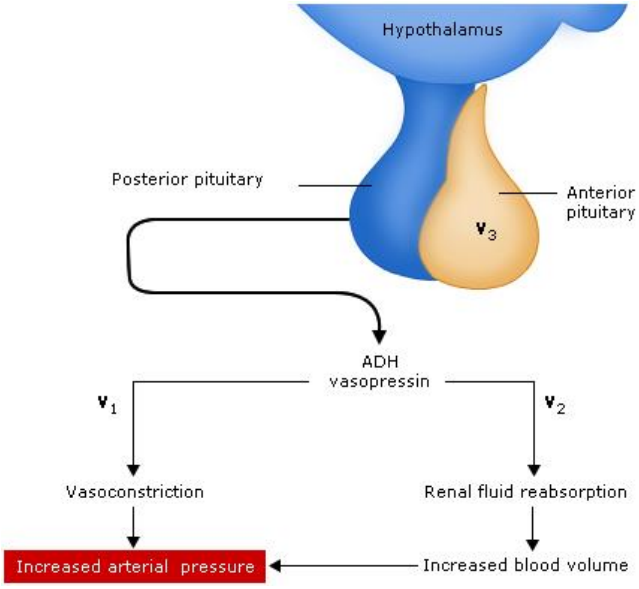
Antidiuretic Hormone (ADH)

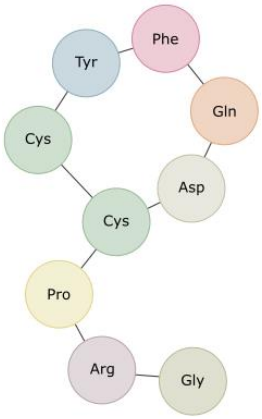
The primary target are cells in the distal convoluted tubules and collecting ducts. Their receptors are all **G-protein linked** and 3 have been identified:

- **V1:** Smooth muscle and visceral organs for **vasoconstriction**
- **V2:** Renal fluid reabsorption
- **V3:** ACTH secreting cells in anterior pituitary

V2 stimulates **adenylyl cyclase** and increases cAMP

V1 and **V3** receptors activates **phospholipase C** to build up intracellular Ca^{2+}.





ADH is **synthesised** in the **supraoptic hypothalamic nuclei** and released through axon terminals via **magnocellular neurons** in the posterior pituitary. Each molecule consists of **9 peptides**.

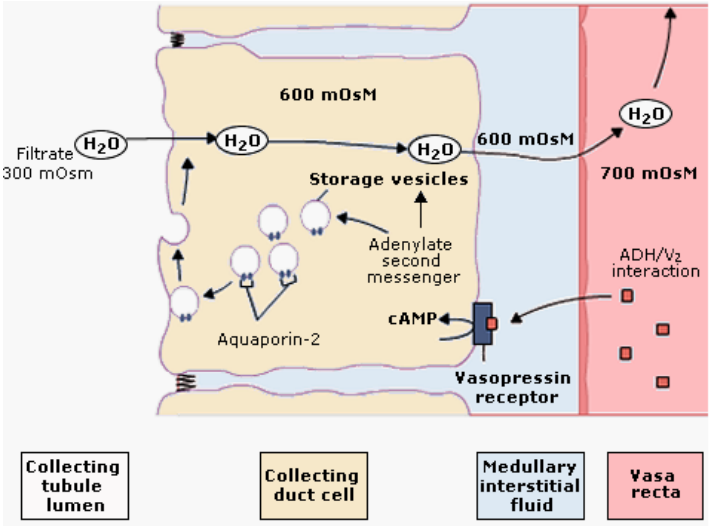
It is **released in response to many factors including: increased plasma osmolality; low blood volumes, low BP**, standing, reduced CO, stress, N&V, various drugs, Angiotensin, nicotine.

Inhibited by increased blood volume and hence ANP, a-agonists and ethanol.

ADH has a short T½ <5mins and remains unbound in plasma.

Mechanism of Action

90% of filtered water is reabsorbed in the PCT and loop of Henle through **AQP1**. The majority of the remaining filtered **water** (~16 litres) is **reabsorbed in the distal tubule and collecting duct**. This occurs through:



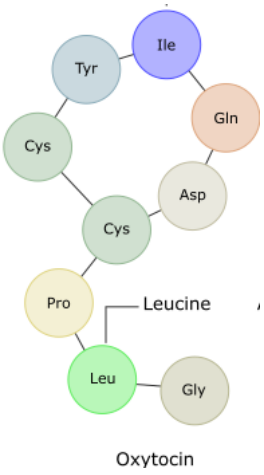
ADH/V2 interaction → increasing adenylyl cyclase and induces **AQP2 water channels** to be inserted into the **apical membrane** to allow water to pass through. AQP3 and 4 are present in the capillary border.

Dissociation of ADH leads to rapid removal of AQP2 channels from the membrane back onto cytoplasmic vesicles.

Under **pathological conditions** i.e. haemorrhage and in supraphysiological concentrations, ADH can be a **potent vasoconstrictor** specifically in coronary and splanchnic beds.

Oxytocin

A 9-peptide hormone of very similar structure to ADH largely derived from **paraventricular hypothalamic nuclei**. Its primary role is for **milk ejection** in response to nursing, in contractions in the late stages of labour and thought to have a role in sexual arousal.



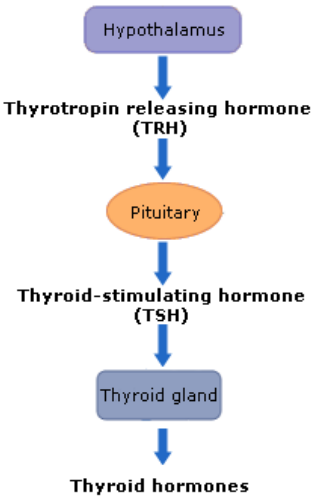
Oxytocin

The only difference in structure with ADH is the amino acid 3 being isoleucine instead of phenylalanine and amino acid 8 being leucine instead of arginine.

Anterior Pituitary Hormones

Thyroid Stimulating Hormone (TSH)

TSH aka **thyrotropic hormone** is released in response to **TRH from the hypothalamus** released by **parvicellular neurons** which arise in hypothalamic nuclei and terminate in the median eminence. *TSH release* is **inhibited by thyroid hormones** T3 (and to a lesser extent T4). Considering TRH is released steadily, the T3 feedback seems to prevent TSH release. TSH releasing cells account for 5% of the total cells in the hypothalamus.

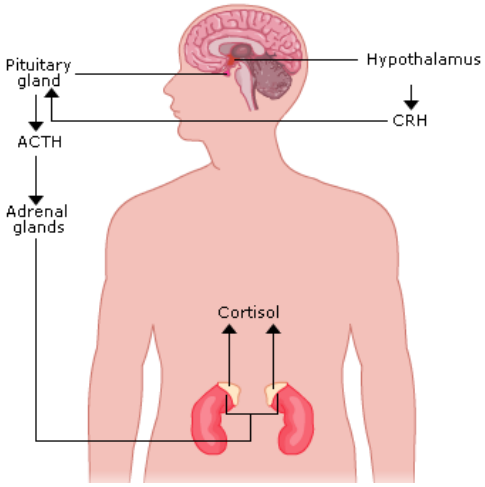


TSH acts on the **thyroid gland** to promote hyperplasia and hypertrophy. It also stimulates all aspects of function including iodine uptake and T3, T4 synthesis and release. **Physiological changes** induced by up or down regulation have a **time lag** of days due to the **nucleic action** of thyroid hormones.

There are multiple other factors that influence TRH and TSH release. Fasting reduces them, cold environment increases them. Somatostatin, dopamine, cortisol and GH inhibit TSH.

Adrenocorticotrophic Hormone (ACTH)

This is a large peptide molecule cleaved from **ProOpioMelanoCortin (POMC)** which also produces β-lipotrophin. Further cleavage results in **β-endorphin** (acts on opioid receptors) and α, and β and γ **melanocyte stimulating hormone** (**MSH**) (acts on **melanocortin** receptors (MCR)).



Control of Secretion

There are multiple factors but the main mediator is **corticotrophin releasing hormone (CRH)** from the hypothalamus. Sometimes ADH can induce its release. 10% of pituitary cells are called **corticotrophs** and are stimulated by **CRH** via the g-protein **CRH-1 receptor**.

ACTH and Cortisol

There is a **diurnal pattern** with its peak in the early hours. This is lost if the variation in light/dark cycles are interrupted (unconsciousness/blindness). **Release of cortisol** is **pulsatile** induced through ACTH (T½ ~15mins) binding to MC2R in the **adrenal cortex**. This causes:

- Hyperplasia of steroid producing cells
- Increased production and release of steroid hormones and cortisol.
- Stimulation of melanocytes along with MSH via MC1 receptor.

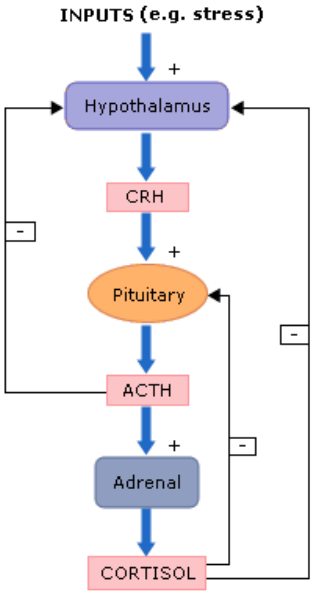
There is a negative feedback to the release of ACTH. High concentrations of ACTH are produced from low cortisol levels.

ACTH/Cortisol Axis Effects

Associated with a **physiological stress response** and concerned with preservation. Rises are seen in:

- **Hypoglycaemia**
- **Anaesthesia** and **major surgery** and **trauma**
- **Critical illness**

This stress response in high physiological levels will abolish the negative feedback of cortisol on ACTH release:



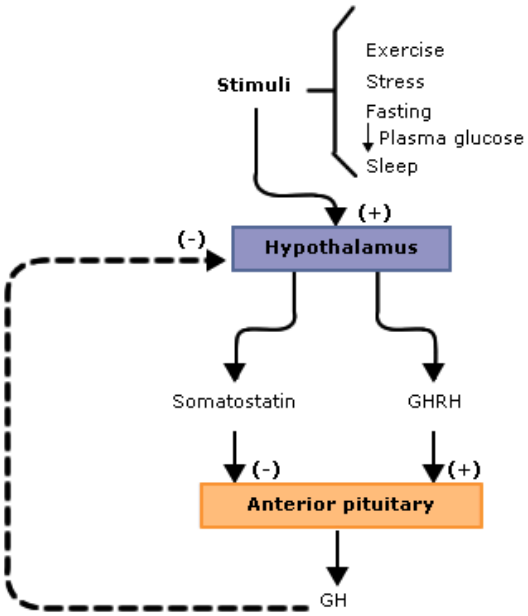
Growth Hormone (GH)

Aka **somatotrophin**. T½ ~20mins and **highly protein bound**. Its function is complex with roles in **growth, maturation** and in **substrate metabolism.** They account for **50% of anterior pituitary cells** known as **somatotrophs** which are acidophilic. 5% of its store is released daily (300-500µg). GH receptors are located throughout the body cells.

Control of Secretion

Synthesis and release of growth hormone exhibits **2-hour periodicity** and a **major nocturnal peak** is controlled by:

- **Growth Hormone Releasing Hormone (GHRH),** thyroid hormone and cortisol is stimulatory
- **Somatostatin** is inhibitory



Also reduced glucose levels and fall in free fatty acids stimulate release. Conversely a rise in amino acids is also stimulatory during normal day-to-day life. Also, androgens, oestrogens, dopamine, acetylcholine, 5HT and alpha agonists stimulate release.

Major negative factors are a rise in glucose or free fatty acids and beta agonists, as well as negative feedback through GH itself and somatomedins - mediators of some GH actions.

GH and Somatotropin Levels

Absolute GH levels increase throughout childhood and peaks during puberty and correlates with final height consistent with its role in somatic growth and maturation. This stabilises in adults and then declines in old age. Levels are tested through **insulin induced hypoglycaemia** which causes increase in levels 10-fold.

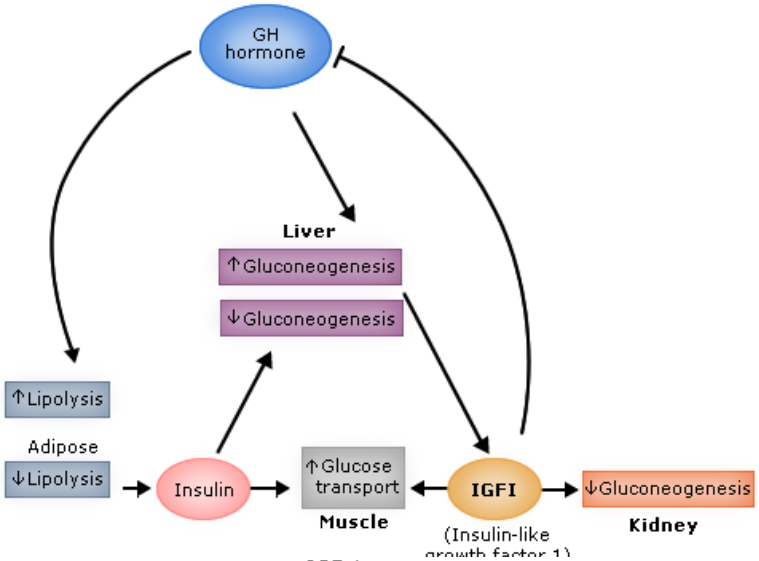
Anabolic Actions

Growth and maturation: Excess GH → **Gigantism**; Insufficient GH in early life → **Dwarfism**

- Promoting **protein synthesis**.
- **Epiphyseal activity** and **bone mineralization** are enhanced resulting in **lengthening of long bone**.
- **Hypertrophy and hyperplasia** are seen throughout the **visceral organs**, endocrine glands, skeletal and cardiac muscle, skin and connective tissue. Organ function is also enhanced.
- Also **increases ECF** through **inhibition of ANP** resulting in **salt and water retention**.

More acutely, metabolic effects over hours can occur in result from GH:

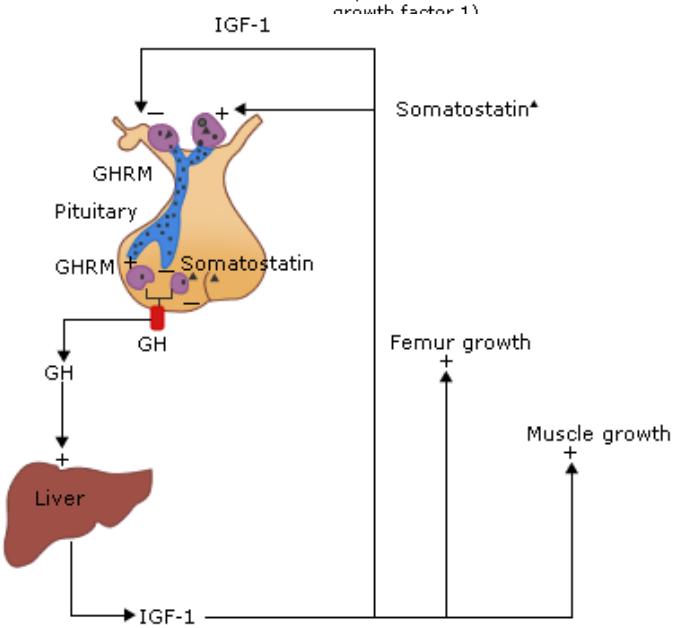
- **Lipolysis** and **ketogenesis**
- **Reduced insulin sensitivity** in liver and skeletal muscle so are antagonistic to carbohydrate and fat metabolism actions of insulin.
- **Gluconeogenesis, glycogenolysis** in the liver and hence increased glucose output.
- **Increased amino acid cellular uptake** to build protein
- In prolonged fasting, may **reduce protein breakdown**.



GH and Somatomedins

GH works through production of **somatomedins** or **insulin like growth factors (IGF)** and are likely to be the most important proteins produced by GH. They are responsible for **regulation of growth** and **tissue differentiation**. **GH** induces **local paracrine activity** of IGFs whilst the **liver** is primarily responsible for the circulating **production** of endocrine IGFs which are **highly protein bound**.

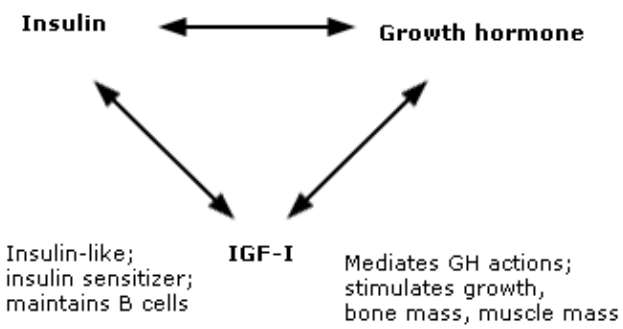
Their levels are constant throughout the day unlike the pulsatile nature of GH due to intracellular modulation.



GH, Insulin and IGF

GH induces **insulin release** but at high levels is antagonistic to its actions and increases glucose release from the liver and reduces skeletal muscle uptake of glucose. Hydrolysis of fats are enhanced and ketoacid production increased. **Insulin levels increase in response** and therefore GH is likely to be diabetogenic.

IGF levels are **low in T1DM** and are similar in action of insulin. This causes a large increase in GH probably due to loss of negative feedback. Insulin in the portal vein is thought to be essential for normal liver sensitivity to GH.

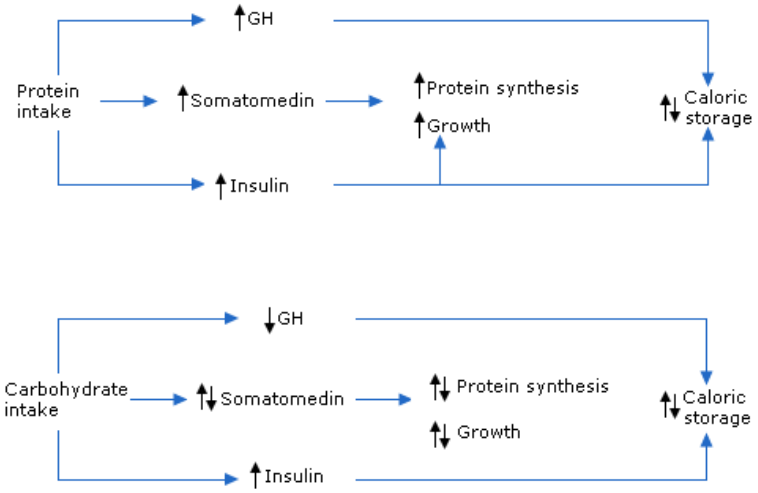


GH, Insulin, Protein and Carbohydrates

Protein load **stimulates GH** and **insulin release** → increased amino acid uptake, protein synthesis and inhibition of breakdown (anabolic)

CHO and fat disposal by insulin is antagonised by GH which prevents hypoglycaemia. CHO excess leads to a fall in GH → reduced protein anabolism.

Therefore, **insulin promotes** disposal of excess CHO to allow GH levels to rise and inhibit unnecessary protein breakdown.



In **prolonged fast low levels of insulin** (and hence IGF) and **high levels of GH** cause an initial negative nitrogen balance. Therefore, other adaptive mechanisms i.e. fall in T3, BMR, IGF allow a fall in energy requirements and change in source of energy. The CNS can increase its utilisation of FFAs. **GH mediated protein synthesis is ultimately inhibited** and catabolism takes place to maintain plasma glucose (essential for the CNS). FFAs and ketones replace glucose in all other tissues. Death from starvation is ultimately due to protein malnutrition.

Prolactin

Synthesised in **lactotrophs**, they are structurally similar to GH and **increases in response to oestrogen** and **TRH** and **decreases with dopamine**. There are many other stimuli and inhibitory factors. Main actions are for **development and growth of milk producing tissue** and the **synthesis of milk** in the breast. Among the other actions are suppression of ovarian luteal cell proliferation.

Gonadotrophins

LH and **FSH** targets the **testes** and **ovaries** important in the reproductive role of both sexes. Their release is controlled by **GnRH** with ratios dependent on the sex hormone environment. Their interactions with the menstrual cycle is very complex.

Adrenal Hormones

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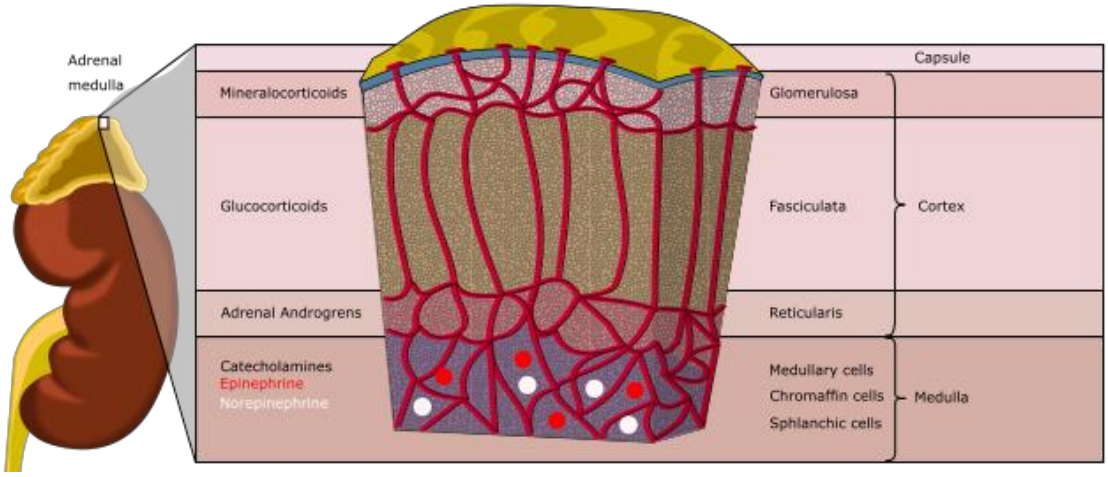
Located above the kidneys, they weigh about 2.5g each and secrete hormones that regulate the body to maintain **homeostasis during stress**.

Anatomy

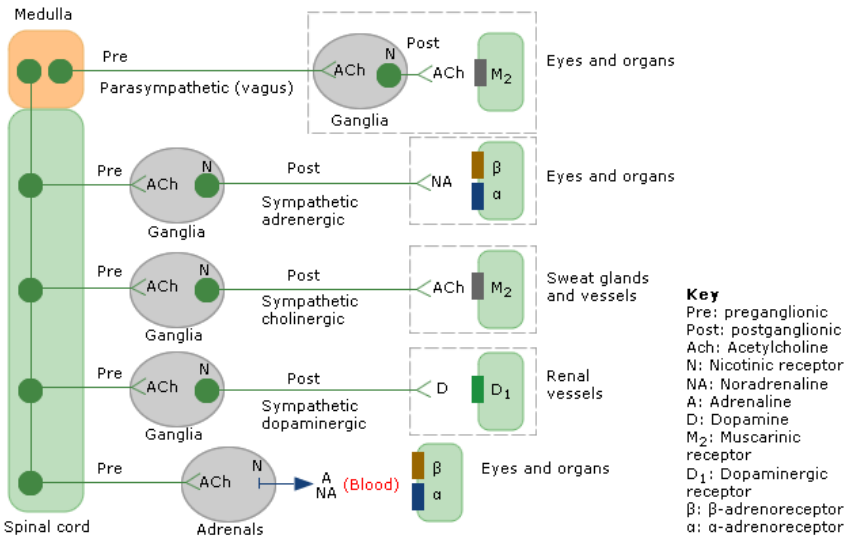
There are 2 distinct parts:



1. **Outer Cortex** derived from mesodermal tissue and responsible for the production of **steroid-like hormones**
2. **Inner Medulla** (10% of gland volume) derived from neural crest **neurectoderm** and is comprised of **chromaffin cells**. Regarded as a specialised **autonomic ganglion** secreting catecholamines in response to SNS stimulation

Adrenal Medulla

Integral to the overall stress response and fight-or-flight response of the SNS. It acts as a **postganglionic neurone** but secretes **mainly adrenaline** (80%) at **0.2µg/kg/min** as a hormone. ~20% of secretions are **noradrenaline 0.05µg/kg/min**; These are similar to the rates of infusion we use in critical care. Small amounts of secretions are dopamine with other chemicals i.e. ATP – functions of which are largely unknown.



The diagram shows the route of the ANS spinal nerves and as one can see, the preganglionic fibre of the SNS – instead of synapsing at the paraspinal SNS chain, it travels direct to the adrenal medulla.

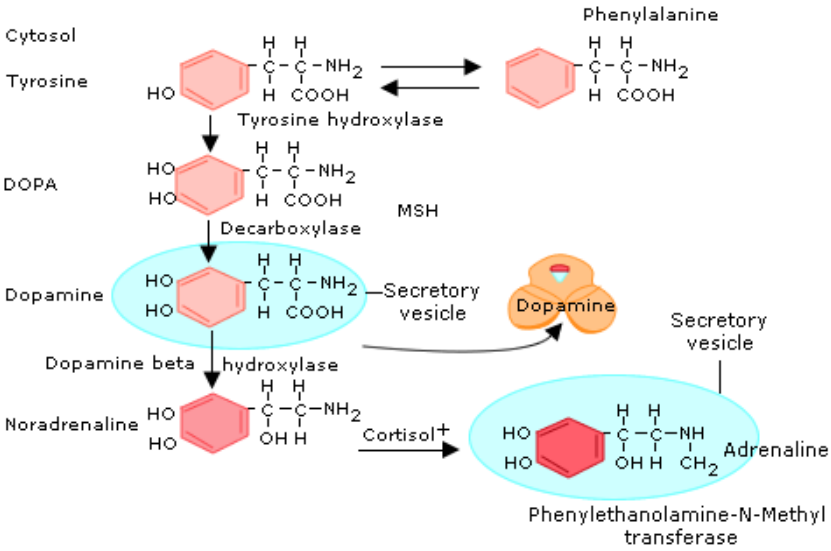
Catecholamines

All are derived from **tyrosine** or *phenylalanine* and is taken up by the **phaeochromocytes** of the adrenal medulla and converted to Ad and NA via series of reactions. For Ad to be made, L-DOPA → Dopamine → Noradrenaline steps must be taken.

Tyrosine hydroxylase is the initiating enzyme occurring in the cytosol and is the **rate limiting step** in the pathway **inhibited by NA**.

β-hydroxylation of dopamine to produce noradrenaline occurs once dopamine has been taken up into **secretory vesicles** and the enzyme is membrane-bound.

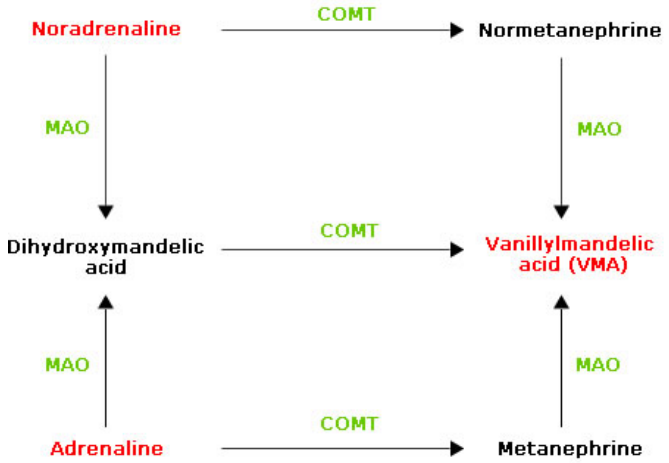
Phenylethanolamine-N-Methyl-Transferase is only present in the adrenal medulla and is responsible for conversion of NA to Ad in the cytosol after NA leaves the vesicle. Ad is actively transported back into the vesicle. The **vesicular transport** is highly dynamic with constant vesicular leak and rapid re-uptake by monoamine transporters.



Metabolism

Large amounts of **monoamine oxidase (MAO)** and **catechol-o-methyl transferase (COMT)** rapidly converts NA and Ad to their metabolites. COMT is more predominant in the **chromaffin cells** and performs the following conversions. Metanephrines diffuse into the circulation.

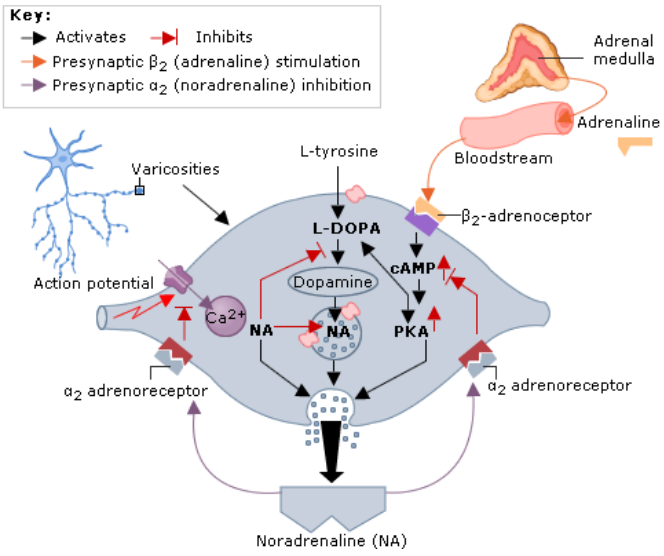
Up to 90% of catecholamines are lost in this way and the T½ of storage is between 8-12h.



Their T½ in the circulation is 30-90s by primary degradation through **COMT** in liver, kidneys and target organs. In synapses, NA is mainly degraded by MAO.

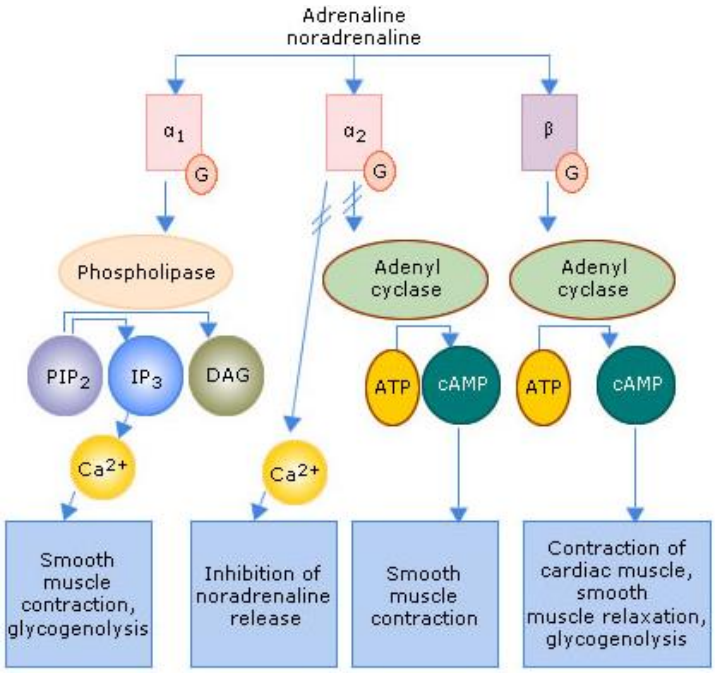
VMA is the main excretory product, 90% of which comes from the liver. Small amounts of catecholamines are conjugated with sulphate and glucuronide.

SNS stimulation and Ach receptor binding to the medulla results in **increased Ca^{2+} channels** and influx → **exocytosis** of the storage vesicles and release into the circulation. The catecholamines accelerate the metabolic rate and oxygen consumption. Their action depends on the dominating receptor at a cellular level and although affinity between NA and Ad to different receptors exist, the effect is still the same at the cellular level.



Adrenergic Receptors

All part of the G-protein linked receptor family but differ according to the secondary messenger system.



Alpha (α) Receptors

α_1 receptors generally interact with G_q class receptors and activate **phospholipase C** to cleave PIP_2 into:

- DAG activates protein kinase C
- IP_3 initiates endoplasmic reticulum Ca^{2+} release → smooth muscle contraction.

Effects: Include but not limited to:

1. Contraction of vascular and bronchial smooth muscle.
2. Increased chronotropy and inotropy. Liver gluconeogenesis.

α_2 receptors are more heterogenous but principally are **G_i class** where some may **inhibit adenyl cyclase** whilst others **open K^+ channels** or activate **phospholipase**. They are presynaptic. And **reduce SNS outflow**. Some however, also stimulate smooth muscle contraction to increase BP.

β receptors have 3 subtypes but all are **G_s class** and **activate adenylyl cyclase** with adrenaline having higher affinity at these receptors.

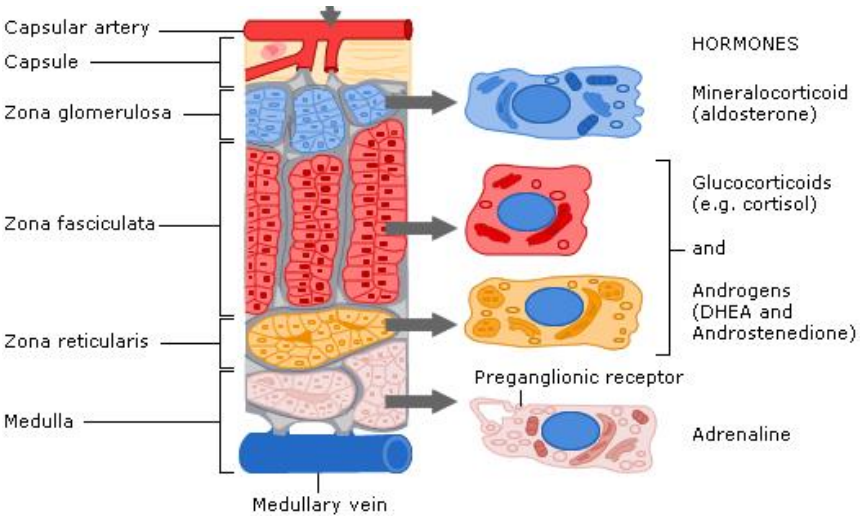
Metabolic effects include mobilization of energy stores, glycogenolysis and lipolysis, with β_3 receptors important in the later.

α_1 receptors and β_1 receptors stimulate, and α_2 receptors and β_2 receptors inhibit.

Adrenal Cortex

Blood flow is **centripetal** derived from **suprarenal arteries** and blood flows through the 3 zones, into the medulla and drains into veins entering the systemic circulation via the renal veins.

If the medulla is exposed to high glucocorticoid levels, catecholamine production is facilitated.

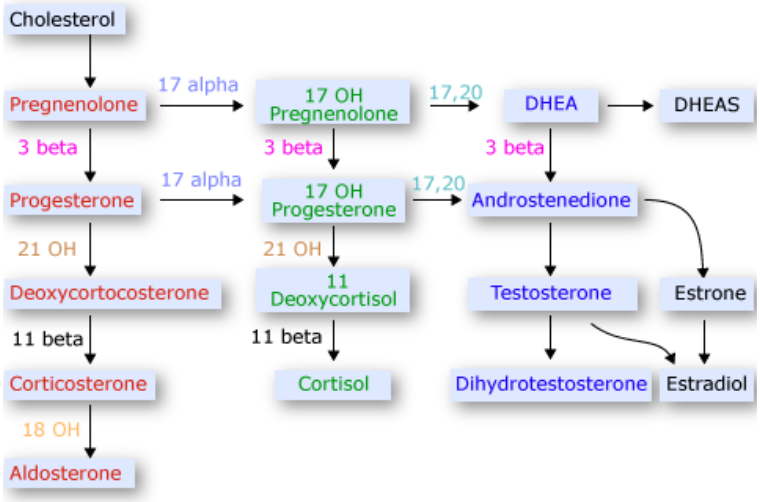


At foetal growth, the principle steroid produced is DHEA-S with smaller production of cortisol. The zones appear inside→ out in embryology without formation of the medulla which forms over 18monts post-natal and the Zona reticularis which develops between 3-8years.

Biosynthesis of Steroid Hormones

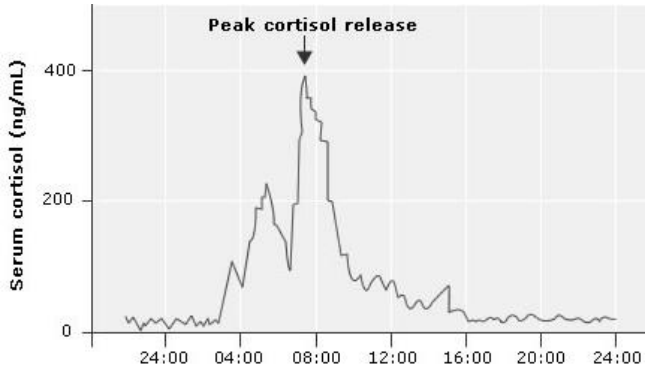
All derive from **cholesterol**. Its **side chains are removed** in the mitochondria which is the **rate limiting step**. This produces **Pregnenolone** which is the last common precursor and is a 21-carbon molecule. The final product depends on the enzymes in the steroid specific cell.

Cortisol requires the presence of 17a hydroxylation. **Corticosterone** is also produced but in smaller amounts and lacks OH at position 17. It has **weak glucocorticoid and mineralocorticoid effects** and used more as a precursor for aldosterone production.



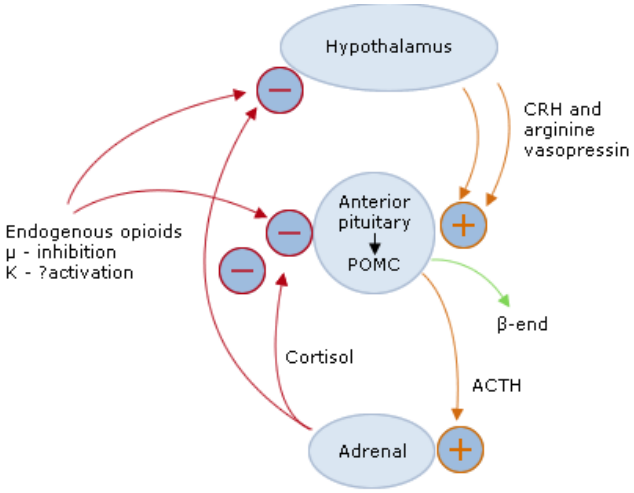
Glucocorticoids

Synthesis is mentioned above. Cortisol is a **stress hormone** whose baseline secretion is **pulsatile** at 1-2/hour with a **circadian rhythm** with levels peaking in the morning dependent on the sleep/wake and dark/light cycles.



Control of Cortisol Secretion

CRH → ACTH which directly stimulates production and release of Cortisol via G-protein receptors. Cortisol when released inhibits ACTH in negative feedback. Its control is much more complex with multiple factors causing it. CRF-ACTH release is governed by the circadian clock located in the **suprachiasmatic nucleus** of the anterior hypothalamus. The **adrenal cortex** may have an **intrinsic clock** controlling the sensitivity of adrenal glands to ACTH stimulation.



ACTH causes:

1. Release of cholesterol from lipid store
2. Facilitates cholesterol transport to mitochondria (for the rate limiting step).

Prolonged ACTH release over days results in stimulation of all steps of the biosynthetic pathway. Chronic release (weeks-months) causes adrenal hyperplasia.

Transport

~20mg cortisol produced/day and **highly protein bound** in plasma to Cortisol-Binding protein (**CBP)** (60-80%) and albumin (15-35%). **5% is unbound** and is the **active form**. Unbound, T½ = 2h. Bound, = stable active concentration.

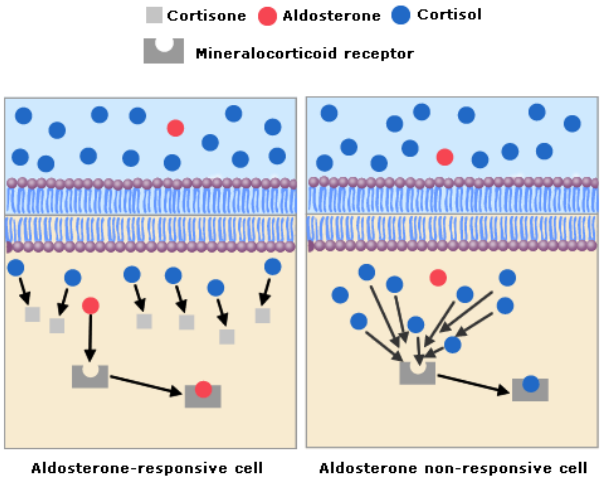
Enters into the cell **via cell membrane** as highly lipophilic and metabolised in the liver and kidneys. Metabolites are ultimately excreted in the urine.

Cellular MOA

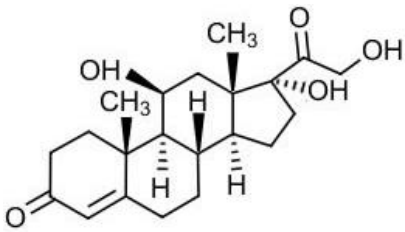
GR1 and **GR2** receptors bind cortisol in the nucleus (some exist in the cytosol). The complex binds to **nucleic acid proteins** to regulate gene transcription. Proteins produced may be tissue specific.

GR1 = mineralocorticoid activity.

Paradoxically **GR1 receptors bind cortisol with 10x higher affinity** and is produced in **amounts 100x greater than aldosterone.** Aldosterone effects on mineralocorticoid cells are differentiated through an enzyme to convert cortisol → cortisone. Cortisone does not bind to GR1.



Actions of Cortisol



It is mainly a **catabolic hormone** and has major action on intermediary metabolism. Its aim is to provide substrate for organ support.

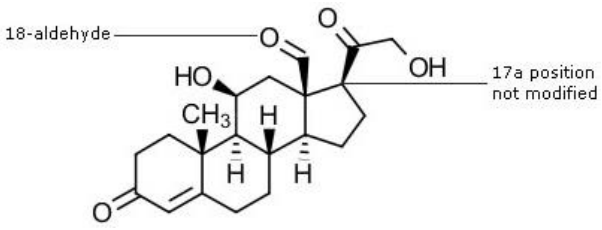
- Anti-inflammatory because it reduces lymphocyte activity and capillary permeability
- Immunosuppressive in high doses (pharmacological)
- Essential for normal cardiac and renal function
- Permissive in maintenance of blood pressure
- Required for normal smooth muscle response to catecholamines
- Required for the formation of surfactant in foetal lung

Through the liver, skeletal muscle and adipose tissue, the production of glucose increases to provide higher serum concentrations – opposes insulin and facilitates glucagon.

Mineralocorticoids

Aldosterone is the principle mineralocorticoid produced in the **zona glomerulosa**. Its production and release is **regulated by angiotensin II** (as well as ACTH) This is controlled by **renin** which is an enzyme responsible for the **rate limiting step** of conversion of Angiotensinogen → Angiotensin I. This has been covered in renal physiology.

Aldosterone is a 21-carbon steroid similar in structure to cortisol except has the absence of OH at position 17. The mineralocorticoid activity derives from the 18-aldehyde group, replacing a methyl group.



Synthesis and Transport

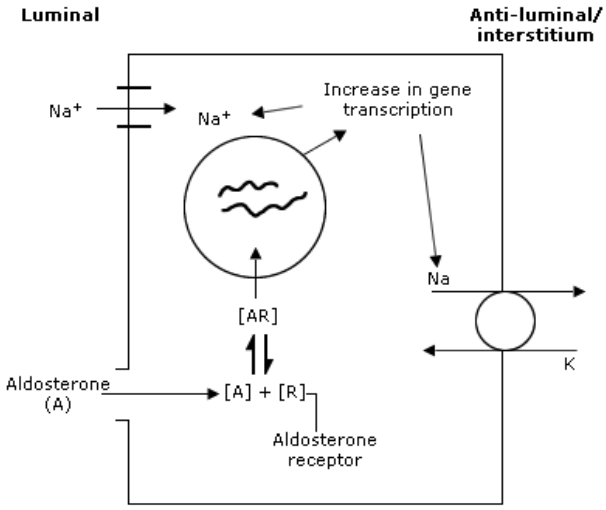
Angiotensin II increases the rate limiting step of the biosynthetic pathway (pregnenolone). High K^+ concentration independently stimulates aldosterone production. The only known inhibitor is ANP through inhibition of aldosterone and renin release.

Levels are 100x lower than cortisol, 60% is bound to albumin with no specific binding protein. Therefore, T½ is much less at 15mins.

Aldosterone Actions

GR1 receptors are mainly found monolayer epithelial cells with the main target in the **DCT** but others found in the colon and parotid gland. It causes excretion of K^+ (lost with H^+) and Na^+ is retained:

- **Stimulation** of **basolateral** membrane **Na^+/K^+ ATPases**.
- **Opening of luminal channels** to allow passive movement of Na^+ ions.



Other actions include the CVS where it increases catecholamine responsiveness, increases production of fibrous tissue and attenuates cardiac hypertrophy. Overall the compliance reduces of cardiac and vascular tissue and may result in chronic HTN and reduced cardiac function.

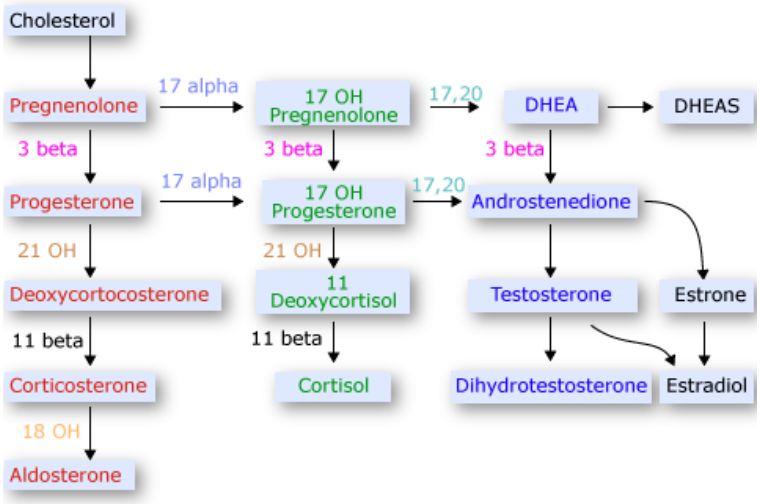
In the CNS, receptors are located in the hippocampus and cerebellum and they regulate blood pressure and thirst-salt intake.

Actions are delayed over hours due to genomic mediation but some rapid actions have been observed with unknown mechanisms.

Androgens

These are 19-carbon compounds and produced mainly in the **zona reticularis** and partially in the fasciculata. ACTH again controls this process.

DHEA (dehydroepiandrosterone) and DHEAS (its sulphate) are the 2 major androgens. DHEA reflects the circadian rhythm of cortisol but is more stable as has a longer T½ as is **highly albumin bound** (90%). They are regarded as weak precursors of **dihydrotestosterone** and **17 β-oestadiol**.



DHEAS exists in 100x higher free plasma-levels than DHEA.

Effects

Production and secretion rises rapidly at >8yo to **predate gonadal production of sex hormones**. Peak levels remain at form puberty until ~30yo, after this, they decline.

Androgens stimulate growth of pubic and axillary hair through unknown methods. **Inactivated** through conjugation in the **liver and kidneys** or **metabolized to potent androgens or oestrogens**:

- **Males**: testosterone is mainly produced in the **testes** but may also occur in hair follicles, sweat glands and adipose tissue.
- **Females**: peripheral production predominates, oestrogens are synthesized in **adipose tissue**.

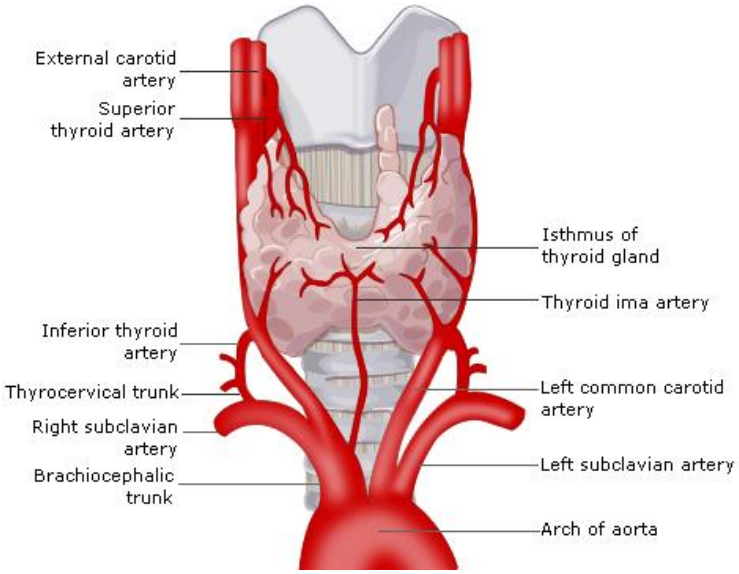
Androgen-specific receptors are located in the cytoplasm of target cells in which the complex regulates **DNA transcription.** Androgens of adrenal origin appear important in the pre-pubertal years in both sexes and an excess may result in premature puberty as well as masculinisation. They appear of little importance in adult men but adult women may predispose to hirsutism, acne, baldness, infertility and, rarely, virilization.

Thyroid and Parathyroid Hormone and Calcium Homeostasis

(07b_08_07)

Anatomy

Macroscopically the thyroid is made of **2 lobes** linked by the **isthmus**. Lying inferior to thyroid and cricoid cartilages and is mobile when swallowing. It weighs about 10-20g.



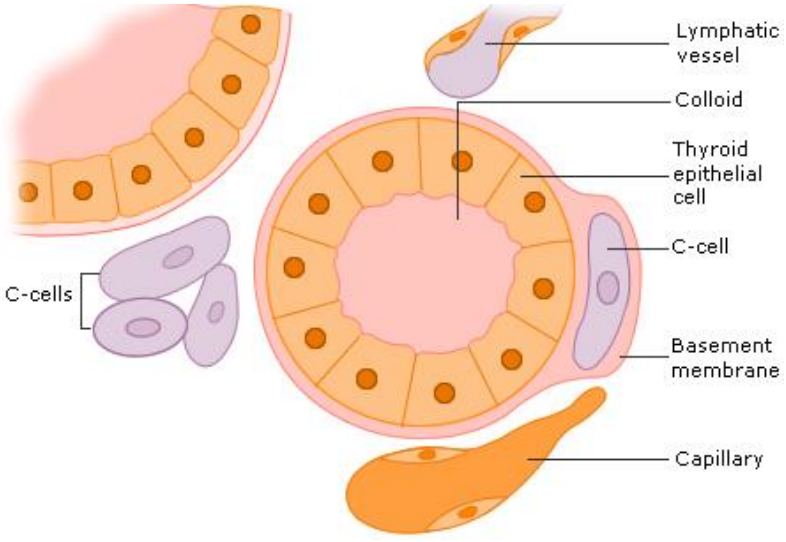
ANS innervation is from the **sup laryngeal** and **recurrent laryngeal nerves**.

Blood supply is from the:

- **Sup thyroid a**. via ext. carotid a.
- **Inf thyroid a**. via thyrocervical trunk
- Thyroid ima a. via subclavian a. (only sometimes).

Venous drainage is through the superior thyroid v. → int. jugular v. and the inferior thyroid v. → L brachiocephalic v.

Microscopically, it is made up of **spherical follicles** each surrounded by a basement membrane, has a ring of epithelial cells surrounding **thyroglobulin colloid**.



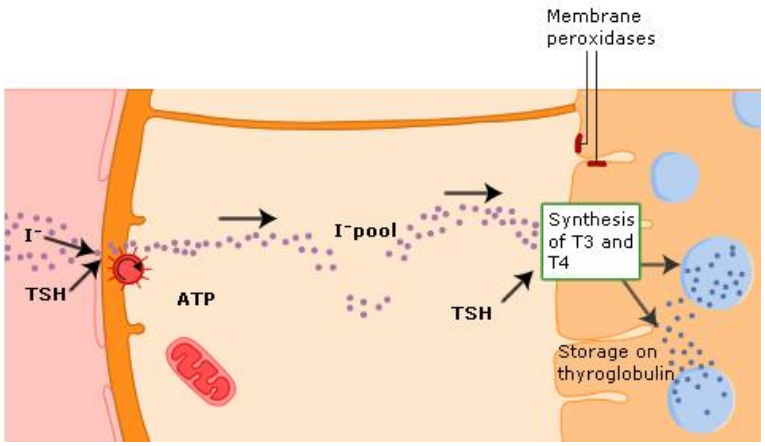
Epithelial cells are the site where thyroglobulin is manufactured and is stored in the colloid. T4 (thyroxine) and T3 (triiodothyronine) are both assembled in the colloid and transported to the capillary via endocytosis through the epithelial cells.

Therefore, as **thyroid activity increases** the **epithelial cells become more pronounced** and almost become columnar in appearance.

C-cells aka **parafollicular cells** function to **produce calcitonin.**

Thyroid Hormone Synthesis

Thyroglobulin is rich in **tyrosine residues** and synthesised on **ribosomes** from peptides. They are packaged into vesicles in the Golgi apparatus for exocytosis to the colloid.



TSH causes increased *active uptake* of **Iodide ions** to transport to the colloid where there are **membrane peroxidases** to **oxidise iodide** into **iodine**.

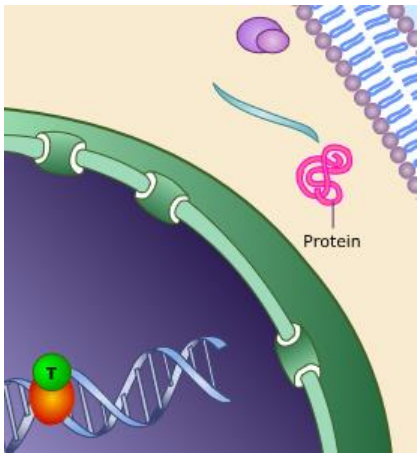
Iodination of tyrosine residues on the thyroglobulin occurs to produce **monoiodotyrosine (MIT)** and **diiodotyrosine (DIT)** which further couple with **thyroid peroxidase** to:

- MIT+DIT = **triiodothyronine (T3)**
- DIT+DIT = **tetraiodothyronine (T4)**

T3 & T4 remain attached to the thyroglobulin molecule for storage in colloid and when needed for release, are moved into the **thyroid epithelial cells by pinocytosis** and enter lysosomal vesicles to **cleave off T3 and T4 from thyroglobulin.** Following this, T3 & T4 can be released into the blood stream. Mediated by **TSH binding to G-protein receptors** on thyroid epithelial cells. Bound readily to globulin or albumin.

Thyroid Action

T3 and T4 **enter the target cell via membrane transporter proteins** and enter the nucleus to **bind to its Intranuclear receptor**, leading to a **conformational change in the receptor**.



Hormone/receptor complex **promotes transcription** by binding to the **promoter region** of the thyroid responsive sequences of the DNA.

T3 is **3x more potent** than T4. Therefore, T4 is converted into T3 in the liver and kidneys. T4 also undergoes peripheral **deiodination** to create **reverse T3 (rT3)** which is metabolically inactive. Fasting increases the ratio of rT3:T3.

Thyroid Function Tests

- Euthryoidism = Normal TSH **and** normal Free T4 or T3
- Hyperthyroidism = Suppressed TSH
- Hypothyroidism = Elevated TSH

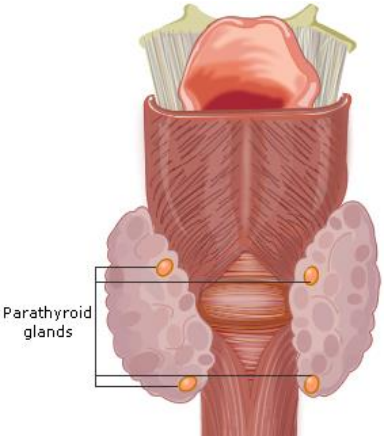
In **subclinical disease, f**ree T4 or T3 levels are still within normal ranges. **Clinical disease** shows abnormal free T4 or T3 levels.

Amiodarone can also make interpretation of TFTs difficult because it interferes with both the synthesis of TSH and the uptake of iodine by the thyroid gland.

Calcium Homeostasis

Calcium is important in muscular contraction, clotting, bone formation and in second messenger systems. Bone has large stores of calcium so dietary intake usually results in majority excretion in faeces and urine.

Plasma concentration is maintained at **2.25-2.65mmol/L** and half is available in the free (ionised) form and 40% protein bound and 10% bound in complexes.



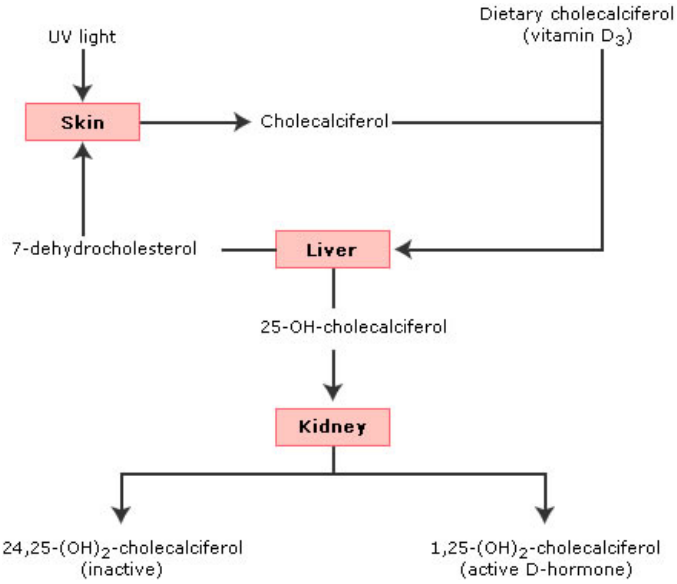
Parathyroid Hormone (PTH)

Produced by parathyroid glands attached to the thyroid lobes. It undergoes negative feedback in hypercalcaemic states. Its actions include:

1. **BONE: Increased osteoclast:osteoblast activity**
2. **KIDNEYS:**
   a. **Increased Ca^{2+} reabsorption**
   b. **Decreased phosphate reabsorption**
   c. **Increased production of D hormone**
3. **GUT:** Indirectly through Ca^{2+} reabsorption in the gut by D hormone

1,25-dihydroxycholecalciferol

7-dehydrocholesterol is stored in the skin and converted into cholecalciferol via UV light. If absent, dietary supplements are required (hence the name **vitamin** D) – *vitamins cannot be biochemically synthesised.*



Hydroxylation occurs in the 25-position in the liver and final **1-hydroxylation** occurs under the influence of **PTH** in the kidneys to make **active D-hormone** (1,25-dihydroxyxholecalciferol). This acts on **intracellular receptors** to:

- **GUT: Increased Ca^{2+} reabsorption**
- **BONE: Increased demineralisation**
- **KIDNEYS: Increased Ca^{2+} AND PO_4^{3-} reabsorption** (hence active D hormone production is also stimulated by hypophosphataemia).

Calcitonin

Acts on the bone to **inhibit osteoclast activity** to increase Ca^{2+} uptake into bone together with PO_4^{3-}. It is released by parafollicular C-cells. Used in the treatment of severe hypercalcaemia (>3mmol/L).

PREGNANCY

Physiology of Pregnancy and Labour

(07b_09_01 AND 07b_09_02)

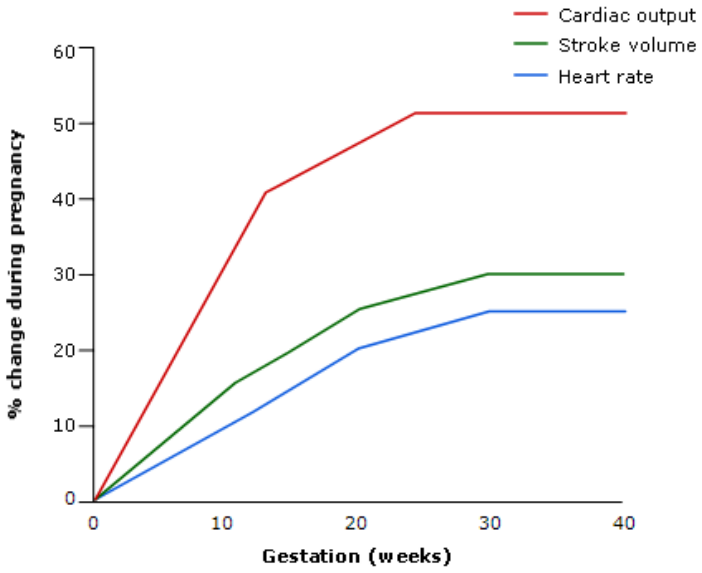
The majority of physiological changes in pregnancy are associated with the **effects of oestrogen** and **progesterone**.

Cardiovascular System

Cardiac Output

There is an overall **increased cardiac output** through pregnancy reaching 50% higher by the end of the 2nd trimester and remains till delivery. This is achieved through an **increase in SV** by 30% **and HR** by 25% @ term.

During labour, CO increases by 45% and in the 3rd stage of labour (following birth of baby and prior to placental delivery), it reaches up to 80% above 3rd trimester levels partly due to **uteroplacental transfusion**



Systemic Vascular Resistance

Decreases during pregnancy due to the **low resistance vascular bed** (intervillous space) and the **vasodilatory effects of progesterone**, **oestrogens** and **prostacyclin**.

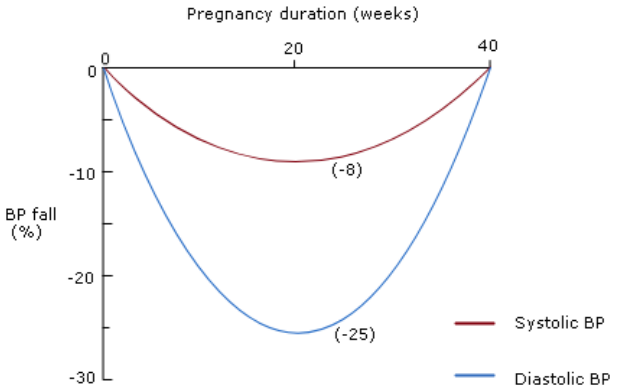
- **Pregnant:** SVR is 979 dyn.s/cm^5
- **Non-pregnant:** SVR is 1700 dyn.s/cm^5

Blood Pressure and Flow

Blood pressure falls in normal pregnancy – diastolic BP to a greater extent. 8% of mothers can have a fall in BP of 30-50%.

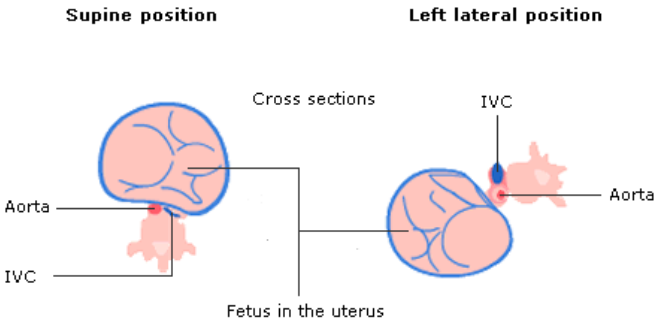
Blood flow distribution changes with **12% perfusing the placenta**. In the meanwhile, flow to:

- Kidneys and skin is increased
- Liver and brain unchanged.



Aortocaval Compression

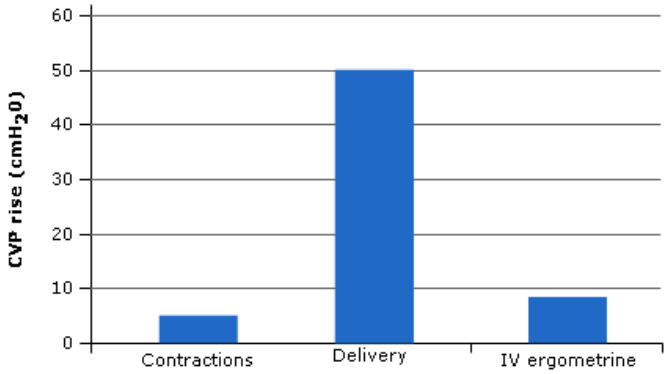
The gravid uterus from **13/40** may cause **inferior vena cava** and **aortic compression**. The severity depends in the BP, positioning and gestation. Maximal effects are seen at 36-38/40 and declines after due to foetal head descent. Sympathetic block exacerbates the effects of compression.



***Supine Hypotension Syndrome*:** Occurs when the aortocaval compression effect remains following movement from supine to the L lateral position. In the supine position, 8% of pregnant women experience a large drop in BP and can ultimately lead to **maternal shock** and **foetal asphyxia** and **bradycardia**. Women without symptoms still have compression but have effective collaterals in the epidural plexus and don't see the drop in venous return

Venous Pressure

Remains normal in pregnancy unless there is marked IVC compression. The venous pressure **rises** to different levels during different **stages of labour:**

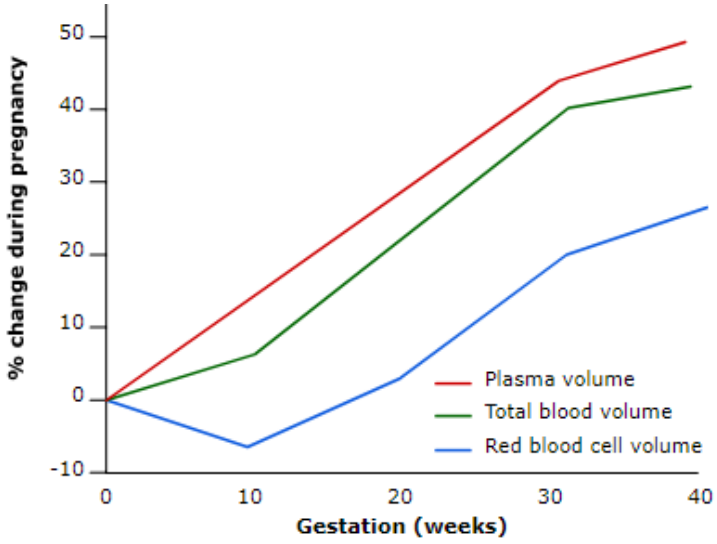


Haematology in Pregnancy

Blood Volume

Plasma volume, RBC volume and **Total blood volume** all **increase** during pregnancy.

Plasma volume rises up to 50% by term and a further 1 litre 24h post-partum. Returns to normal 6 days post-partum. This volume is mediated through **oestrogen** and **progesterone** on the **Renin-angiotensin-aldosterone system**.



Red blood cell volume initially **decreases** till 8/40, rises to normal levels by 16/40 then increases by 30% by term mediated through **EPO**. As this is to a lesser extent than plasma volume, **haematocrit** and **Hb decreases**.

White Cell Count increases up to $15x10^9$ during labour due to polymorphonuclear cells.

Coagulation

There is an overall **increased platelet turnover**, **clotting** and **fibrinolysis**. There is no overall change in platelet count so there is probably an increase in platelet production to compensate for increased consumption

With increased fibrinolysis, there is also a reduction in bleeding time, PT and APTT.
FDP = Fibrinogen Degradation Products

Increased	Decreased	Unchanged
I	**XI**	II
II	**XIII**	V
V	**Antithrombin III**	Platelets
VII		
VIII		
IX		
X		
XII		
FDP		
Plasminogen		

Plasma Proteins

Albumin decreases but **globulin and fibrinogen increases.** Overall, total protein drops to 65-70g/L. This causes:

- Reduced total colloid osmotic pressure
- Altered drug binding capacity
- Reduced plasma psuedocholinesterase by 25%
- Increased ESR and blood viscosity

Respiratory System

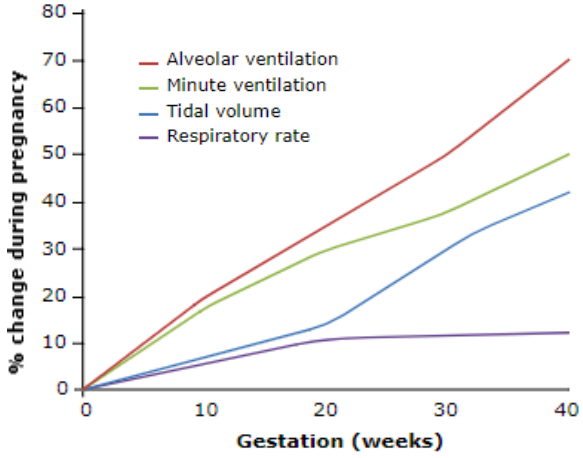
Anatomical Changes

This begins early in the 1st trimester. There is **upper airway capillary engorgement** and **tissue oedema** which can lead to epistaxis, vocal changes and nasal obstruction. This can lead to **difficulty airway management**. **Rib flaring** leads to **increased thoracic cage circumference** by 5-7cm. Later in pregnancy, the gravid uterus pushes the diaphragm superiorly.

Lung Mechanics and Volumes

Inspiration becomes mainly **diaphragmatic** as there is rib flaring reducing chest wall movement. **Bronchial smooth muscle relaxes** reducing airway resistance but **lung compliance remains**. FEV1 and FEV1:FVC is unchanged. The following describe the changes (**all increases**) that occur:

- Alveolar Ventilation by 70%
- V_T by 45%
- RR by 10%
- Minute ventilation by 50% due to increased V_T.



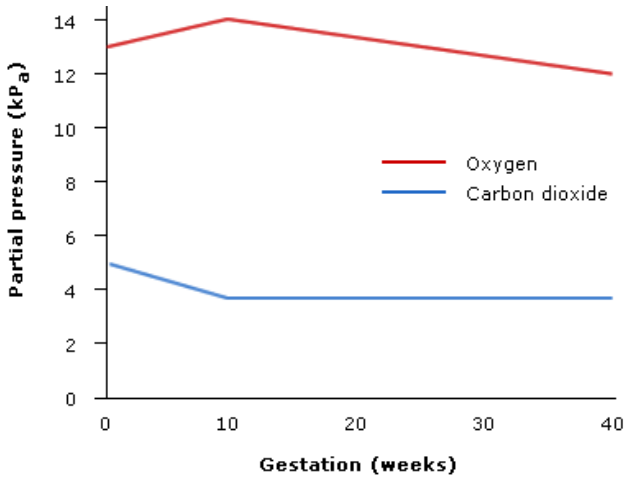
FRC reduces by 20-30% at term due to reduced RV. **Closing capacity encroaches on FRC** and leads to V/Q mismatching and may lead to hypoxia. These are thought to be a central effect from increased progesterone.

Blood Gases

Alveolar hyperventilation causes a **fall in $PaCO_2$** to around 3.7-4.2kPa and as compensation, the **plasma bicarbonate reduces** to 18-21mmol/L – this is not complete so pH increases by 0.04.

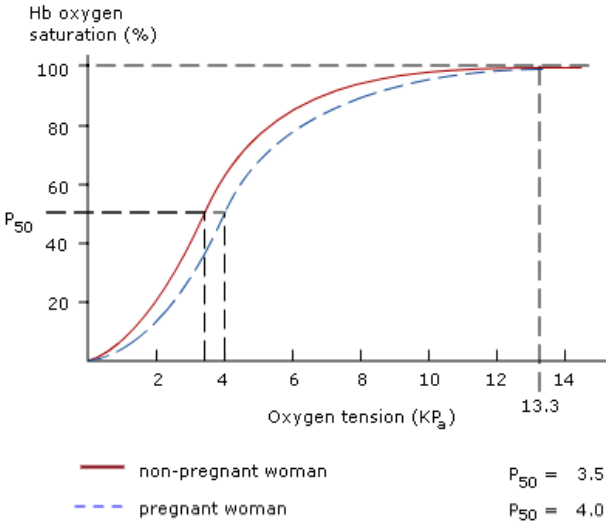
PaO_2 is slightly higher than in non-pregnant women due to lower $PaCO_2$ levels.

Towards term, O_2 consumption and CO_2 production increases by 60%. Oxygen consumption is no longer compensated fully by increased cardiac output and oxygen delivery and PO_2 slowly declines. This is enhanced in the supine position due to aortocaval compression and dependent airway closure.



Oxyhaemoglobin Dissociation Curve

Although $PaCO_2$ decreases tend to shift the curve to the left, there is actually an **overall right shift** due to a **30% rise in 2,3-DPG.**



Pulmonary Circulation

Pulmonary Vascular Resistance reduces at term to 78 dyn.s/cm^5 from 119 dyn.s/cm5. Pulmonary blood flow therefore increases and in a healthy parturient, this will not lead to an increase in pressure in the pulmonary artery, capillaries or R ventricle.

Gastrointestinal System

Barrier Pressure

The **barrier pressure is significantly reduced** in pregnancy. Remember:

BARRIER PRESSURE = LOS PRESSURE – INTRAGASTRIC PRESSURE

This occurs due to an altered stomach position pushing the gastric part of the oesophagus into the thorax lowering LOS pressure. Progesterone also acts as a relaxant. The gravid uterus increases intragastric pressure which is further increased in the Lithotomy < Trendelenburg positions.

The LOS pressure tends to return to normal 48h post-partum.

Heartburn occurs in 80% of pregnancies and can commence at <20/40.

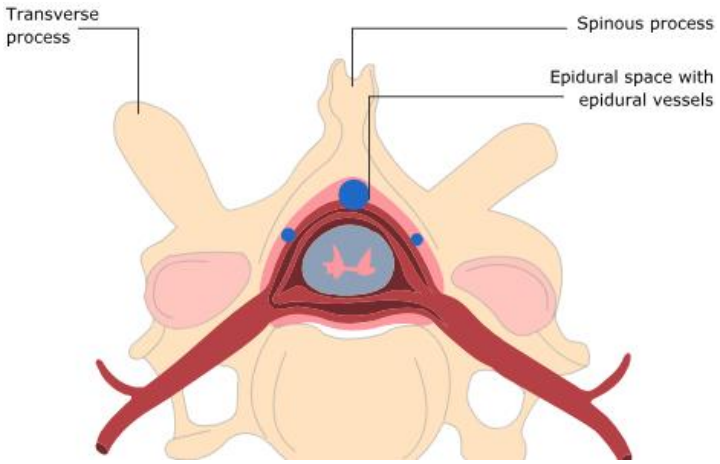
Gastric emptying ONLY slows **during labour** and enhanced with opioids. As with an ***increased gastric secretion*** and ***reduced gastric pH***. Therefore, the pregnant woman is at **higher risk of aspiration under GA**.

Central Nervous System

Epidural Space

Aortocaval compression results in engorgement of epidural veins **reducing the volume of the epidural space**. Therefore, **solutions will spread more rapidly** if injected into it.

The pressure in the epidural space is **positive** (as opposed to negative in the non-pregnant patient) and during contractions, this may rise to 8cm H_2O, while during expulsion it can rise to 60 cm H_2O.



Subarachnoid Space

CSF pressure is increased again due to aortocaval compression with baseline between contractions in labour at 28 cmH_2O and rises in the second stage of labour to 70cmH_2O.

Sympathetic Nervous System

Increases through pregnancy and is maximal at term and its effect is largely on the **venous capacitance** of the **lower limbs** to help **counteract IVC compression**. Therefore, **sympathetic block** may result in a much **larger drop in BP** than in non-pregnant patients.

Drugs and the CNS

The overall **reduction in LA doses for spinal/epidural anaesthesia** is due to:

1. Reduced epidural and subarachnoid space volumes
2. Increased nerve fibre sensitivity to local anaesthetics
3. Reduced PaCO2 leads to reduced buffering capacity and LA remain free bases for longer

MAC is reduced by 40% likely due to increased progesterone levels.

β-endorphin levels are **increased** throughout pregnancy and during labour and delivery.

Endocrine System

Thyroid gland increases in size and vascularity to develop a goitre with increased iodine uptake. **Thyroid binding globulin doubles** so therefore, the free T3 and T4 levels remain constant so the mother remains euthyroid.

Adrenal gland remains constant size but **corticosteroids increase** by up to **x5 by term** due to increased production. The T½ of cortisol is increased due to decreased clearance.

Pituitary gland increases in weight. The low-pressure portal system supplies the pituitary rather than systemic arteries and makes the anterior pituitary **sensitive to changes in blood pressure**. **Sheehan's syndrome** may develop with peripartum hypovolaemia (ischaemic pituitary).

Pancreatic islets of Langerhans and **β cell numbers** increase. There is **increased insulin production** and **receptor sites**. There is however some resistance to insulin from pregnancy hormones causing larger concentration of glucose following a meal allowing better placental glucose transfer.

Renal System

Renal plasma flow increases by 50% and GFR to 150ml/min. Therefore, **urea and creatinine decline**. There is also a **reduction in tubular reabsorption** so **glycosuria** and **proteinuria occur.** Also, drugs that are renally excreted may have to be given in larger doses than normal.

RAS together with **progesterone** lead to **Na^+ and water retention** → conservation of potassium and **reduced plasma osmolality**. **Progesterone** causes smooth muscle relaxation → **urinary stasis** → increased **UTI risk**.

Hepatic System

ALP is produced by the **placenta** and hence plasma concentrations **increase 3-fold**. Protein synthesis is reduced → **reduced plasma cholinesterase** → increased duration of NMB (rarely clinically significant). **Gall stone risk increases** in pregnancy due to **progesterone mediated reduction in cholecystokinin (CCK) release** →reduced contractile response.

Musculoskeletal System

Ligamentous relaxation due to placental production of the hormone **relaxin** → widened pubic symphysis and increased joint mobility. **Lumbar lordosis** results from the gravid uterus → **lower back pain**. **Increased MSH** causes hyperpigmentation of the face, neck and abdominal midline **(linea nigra)**.

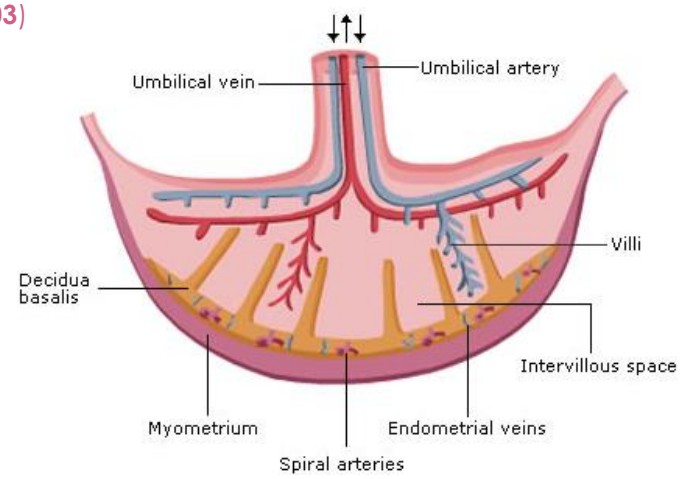
Weight Gain

Increases by 10-12kg due to increased body water and fat, the foetus, the placenta, amniotic fluid and uterine enlargement and breast enlargement.

Functions of the Placenta

(07b_09_03)

The placenta is critical in foetal development and is composed of foetal tissue (villi) that lie in maternal vascular spaces (intervillous spaces). **Blood supply** is from maternal **spiral arteries** derived from the **uterine artery** and venous drainage from intervillous spaces to the uterine veins. **2 umbilical arteries** carry **deoxygenated blood** away from the foetus and **oxygenated blood** travels to the foetus by **1 umbilical vein.**



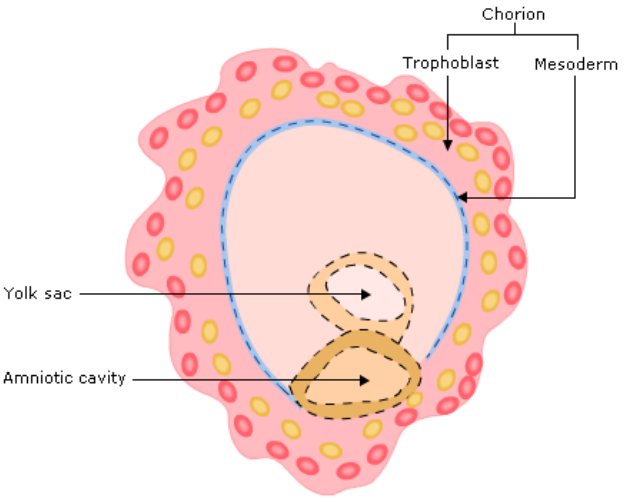
Placental Development

Chorion and Decidua

Foetal part of placenta derives from the **chorion** (made from trophoblast and mesoderm) which is part of the developing ovum – the **blastocyst**.

Maternal part of placenta arises from the **decidua** which is part of the **endometrium**.

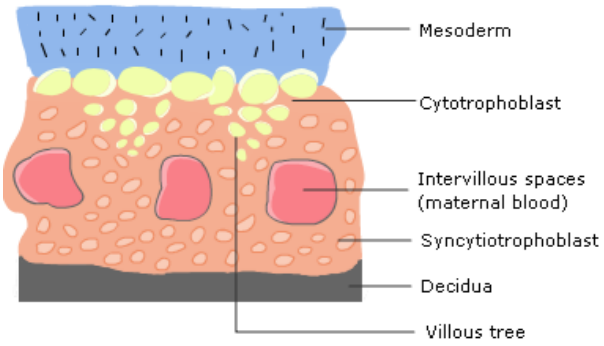
The **trophoblast** (part of the chorion) then **invades the decidua**. Since the **decidua basalis** has the spiral arteries, this invasion allows close contact of foetal tissue to maternal blood.

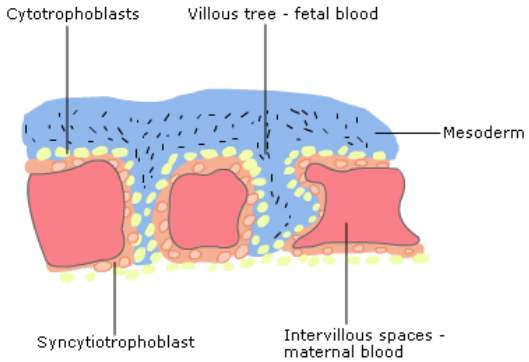


Trophoblast, Mesoderm and Villous Tree

The trophoblast **differentiates** into 2 layers:

1. **Inner cytotrophoblast** which extends to the outer syncytiotrophoblasts to form the villous tree
2. **Outer syncytiotrophoblasts** which form the intervillous space and allow maternal blood from the spiral arteries to come into close contact with foetal blood in the **villous tree**



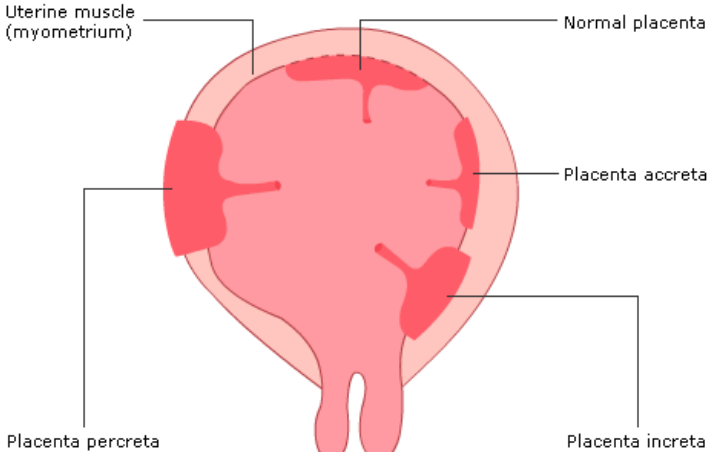


The maternal and foetal circulation are separated by the 2 layers mentioned above. The **mesoderm** forms columns within the cytotrophoblasts to form the villous tree.

Abnormalities of the Placenta

If the chorionic tissues invade through the decidua into the muscle or beyond, 3 conditions may occur:

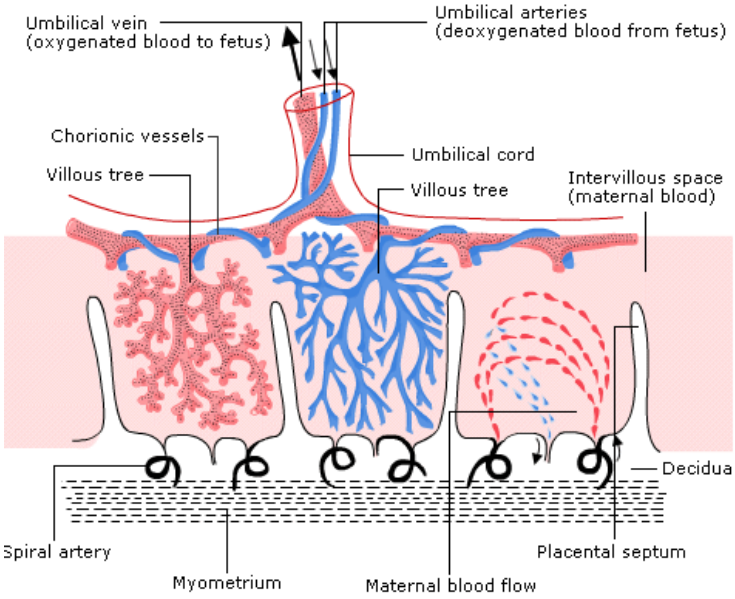
1. **Placenta Accreta**: chorionic tissue reaches the uterine muscle and there is no plane of cleavage between the placenta and its attachment to the uterus
2. **Placenta Increta**: chorionic tissue penetrates the uterine muscle
3. **Placenta Percreta**: Invades through the whole muscle and reaches the serosa or invades surrounding organs.



Placental Vascular System

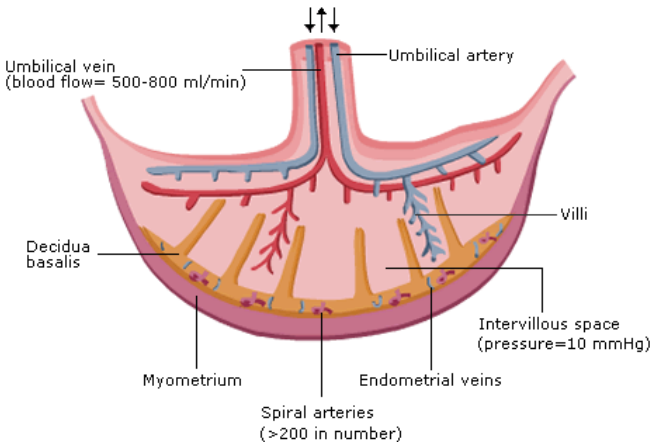
The trophoblastic invasion around the distal end of the maternal spiral arteries allow **reduced vasoconstriction** – this starts at 10/40 and complete by 16/40. **Failure** of trophoblastic invasion → **increased vascular resistance** in the **placental bed** → **IUGR** (Intrauterine growth retardation) and is one of the findings in **pre-eclampsia**.

As mentioned above, umbilical arteries and veins transfer blood to the foetus. They divide into the **chorionic arteries and veins** at the **placental side**. The 2 arteries arise from foetal internal iliac a's.



The **maternal** placental vascular system is **low pressure** (10mmHg). Up to 200 spiral arteries feed into the placenta and has a total uteroplacental blood flow (**UBF**) between 500-800ml/min at term. The weight is 500g. Average diameter is 20cm and thickness of 3cm.

$$UBF = \frac{\text{uterine arterial pressure - uterine venous pressure}}{\text{uterine vascular resistance}}$$



UBF is reduced by **increased uterine venous pressure** (i.e. contractions), **maternal hypotension** and **increased vascular resistance**.

Functions of the Placenta

The placenta is a vital link between the maternal and foetal circulation and allows:

- **Transport using several mechanisms for:**
  - **Delivery:** Oxygen and nutrients
  - **Removal:** Carbon dioxide and waste products
- **Secretion of hormones**
- **Barrier to prevent foetal infection**

The placenta modifies the foetal immune system to prevent rejection (mechanism is not well understood). During pregnancy, there is a reduction of cell-mediated immunity and activity of T-cytotoxic cells, increased neutrophils and reduced Immunoglobulin G (IgG). IgG is the only immunoglobulin that can transfer across the placenta

Transport of Respiratory Gases

This occurs in the **intervillous spaces**. The placenta uses up 30% of the oxygen delivered to it as it is metabolically active.

Oxygen transfer is dependent on:

- High maternal:foetal oxygen concentration gradient
- **Double Bohr effect:**
  - **Foetus**: *CO_2 transfer to mother → ↑foetal blood pH → ↑O_2 affinity*
  - **Maternal**: *CO_2 from foetus → ↓ maternal blood pH → ↓ O_2 affinity*.
- High HbF concentration (left shift of oxygen dissociation curve)
- Good placental perfusion and functional status

Carbon dioxide transfer occurs through simple diffusion in its dissolved form (8%), HCO_3 (62%) and Carbamino Hb (30%). This is enhanced by the **Haldane effect** where a rise or fall in O_2 tension leads to decreased or increased affinity for CO_2 respectively:

- **Foetus**: *O_2 uptake by foetal Hb → ↑foetal CO_2 release*
- **Maternal**: *↓ O_2 in maternal blood → ↑ CO_2 uptake.*

Passive Transfer of Drugs

Occurs through **passive diffusion** of **non-ionized lipophilic molecules** through the cell membrane. The rate of transfer is affected by a number of factors included in Fick's law:

$$Q/T = \frac{K\,A\,(C_m - C_f)}{D}$$

Q/T - rate of diffusion

K - a diffusion constant of the drug which depends on the physicochemical properties such as molecular weight, lipid solubility and degree of ionization

A - surface area available for transfer

C_m - free drug concentration in maternal blood

C_f - free drug concentration in fetal blood

D - thickness of the membrane

As well as Fick's law, maternal-foetal concentration gradient and placental blood flow impact the rate and ability of transfer.

Opioids: Most are able to cross the placenta as described:

- **Pethidine**: crosses the placenta freely. Maximum foetal uptake is at 2-3 h after intramuscular injection
- **Morphine**: although less lipid soluble, it crosses the placenta freely. It is weakly bound to plasma protein.
- **Fentanyl**: although it is highly protein bound, it is highly lipid soluble and crosses the placenta readily
- **Alfentanil:** readily crosses the placenta
- **Remifentanil:** readily crosses the placenta

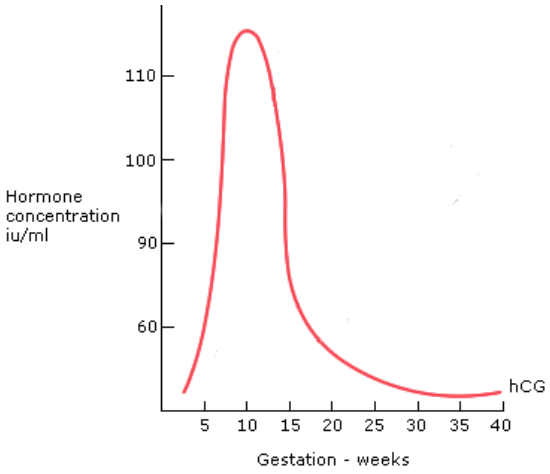
Local anaesthetics: They cross by simple diffusion as have a **low MW** and **high lipid solubility** at normal pH. Bupivacaine and etidocaine are highly protein bound so have reduced placental transfer compared with lidocaine and mepivacaine which have low protein binding.

Anaesthetic drugs:

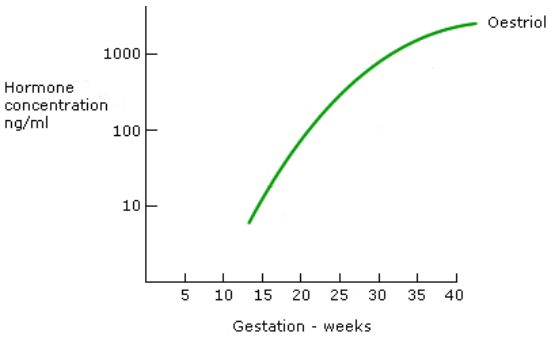
- **All volatile agents are highly lipid soluble** and have a **low MW** which facilitate rapid transfer across the placenta.
- **Induction agents** i.e. **thiopentone**, **propofol** and **ketamine** rapidly cross the placenta
- **NMBs** are fully ionised so *DO NOT* cross the placenta
- **Benzodiazepines** readily cross the placenta.

Endocrine Function of the Placenta

Hormones secreted by the placenta include:



1. **Human chorionic gonadotrophin (hCG):** rises rapidly in early pregnancy. Responsible for stimulating the corpus luteum to secrete progesterone to maintain the viability of the pregnancy. Peak is at 8-10/40. Late pregnancy function is unclear.
2. **Progesterone:** Secreted by corpus luteum until the eighth week. After 8/40 it is secreted by the placenta
3. **Human placental lactogen (hPL):** increases during pregnancy. Its functions include increased lipolysis, increased gluconeogenesis and anti-insulin effect
4. **Oestrogens:** 4 types (oestrone, oestradiol, oestriol and oestetrol) are secreted in incremental amounts. They stimulate uterine expansion to accommodate the growing foetus.
5. **TSH:** euthyroid overall due to increased Thyroid binding globulin
6. **Prostaglandins**
7. **Hypothalamic inhibitory and excitatory factors**



Maternofoetal Circulation Changes at Birth

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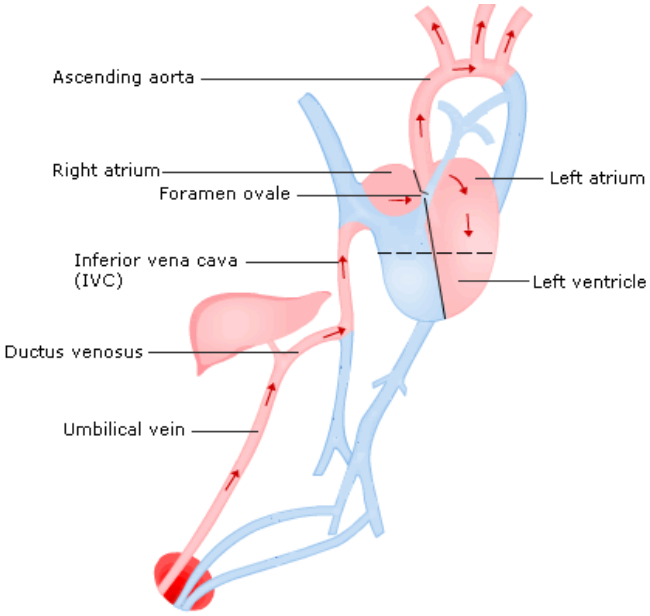
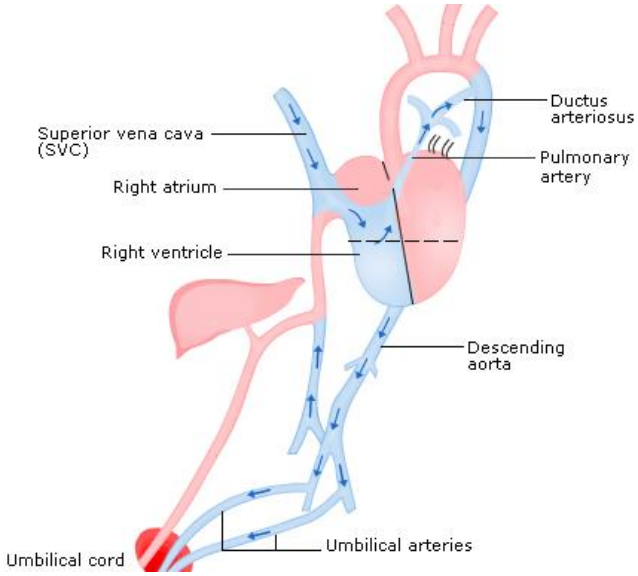
Foetal Circulation

Oxygenated blood from the placenta flows in the umbilical vein (sats 80%) through the **ductus venosus** to reach the **inferior vena cava** and enters the **R atrium**.

Subsequently, the anatomy of the R atrium as a foetus favours blood to travel through the **foramen ovale** to the **L atrium → L ventricle → ascending aorta** (sats 62%) supplying the upper body – brain and heart.



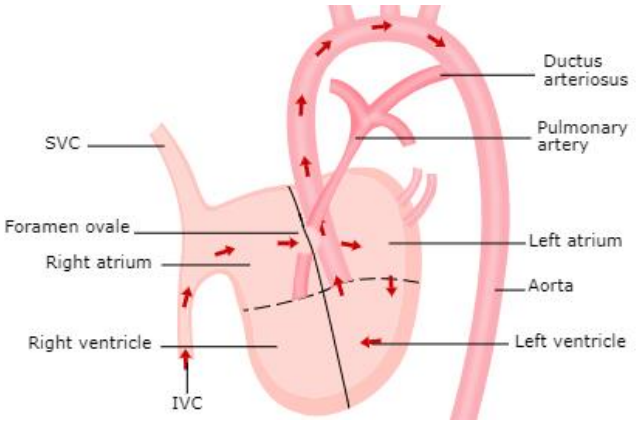


Deoxygenated blood returns to the heart from the upper part of the body via the **SVC → R atrium**. The anatomic orientation favours blood from the SVC into the R atrium to go into the **R ventricle**. The blood then goes to the **pulmonary artery** (sats 60%) but as the resistance is high, only 10% of CO from R ventricle goes to the lungs. The rest of the blood flows **via ductus arteriosus** to enter the **descending aorta** (sats 55%) to return to the placenta.

Transitional Circulation at Birth

Foramen Ovale

When blood in the pulmonary a increases after the 1st breath, pulmonary venous blood returns to and **increases L atrial pressure**. When it exceeds the R atrial pressure, the foramen ovale closes.



Ductus Arteriosus

This is kept patent in the foetal period by levels of both PGE_1 and PGE_2. Immediately after birth, **levels of both PGE_2 and the EP4** (prostaglandin specific) **receptors reduce** and **exposure to oxygenated blood** causes the **ductus arteriosus to close within <24h** to become the **ligamentum arteriosum**. Therefore, NSAIDs can induce closure of a patent DA and prostaglandins may inhibit it. Patent DA will cause a L to R shunt and pulmonary HTN.

Following closure of the DA and FO, it may take a few hours until a fully unidirectional circulation is established.

Abnormalities in the Neonatal Circulation

Persistent pulmonary HTN of the newborn may occur if the physiological changes at birth do not fully take place or if the neonate is exposed to:

- Hypoxia
- Hypothermia
- Acidosis
- Hypovolaemia

These can trigger reversal of the adult circulation back to foetal circulation and cause a **Right to Left shunt**.

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Listed below are the original authors of the online modules:

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Functions of the Placenta	Mary Mushambi
Maternofoetal Circulation Changes at Birth	Mary Mushambi

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