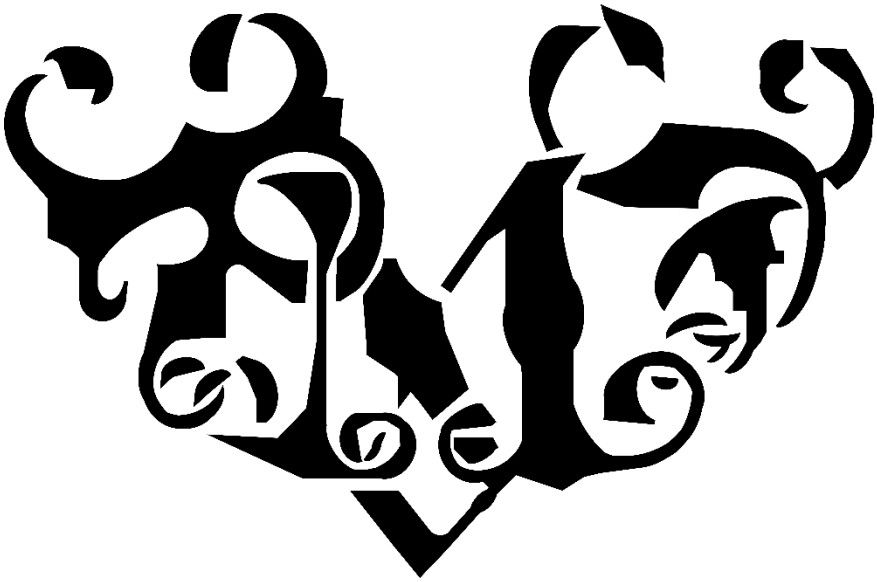
Royal Medical Society

Year 1 Revision Guide



Semester 1:

The Fundamentals of Medicine

2016; revised 2017

Royal Medical Society Year 1 Fundamentals of Medicine Module Revision Guide

This guide is intended as an aid to understanding the material in the Year 1 Fundamentals of Medicine module. The content has been condensed greatly to make revision more efficient, but explained as fully as possible to promote comprehension, which is invaluable in the type of exam questions asked. This is by no means a comprehensive handbook to the module, but simply another format in which to cover the content. The exact facts covered in lectures can change every year but every effort has been made to include the most recent information covered.

Simple diagrams have been used to make concepts as clear as possible. Certain topics require greater explanation of concepts and hence will take more of a paragraph format - processing this information into your own tables or flow charts where appropriate is a great way of revising the content.

Some tips for the OSCA exam:

- The exam is multiple choice in the format of "single best answer"
- Understanding the information properly will allow application of it in exam questions, especially as the focus is often on comprehension of the basic principles of a topic
- A very common question is "Which of the following statements is true/false?" which requires you to know disparate facts; hence, learning seemingly random pieces of information is also useful
- Anything in the lectures and tutorials can be examined, but lecturers will often indicate what is necessary for the exam and what is just included to aid your understanding
- It is often easy to narrow down answers to two or three options, which is a helpful way of approaching questions of which you are unsure
- You are not penalised for incorrect answers, so attempt every question
- Some anatomy may also be examined in these papers

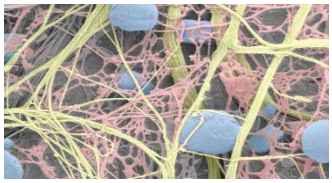
I hope this guide will be useful to you and any feedback would be greatly appreciated.

Hannah Patterson

Royal Medical Society Library and Museum Convenor 2016-2017

Topic 1 - Body Basics

Image credit: University of Rochester



LEARNING OUTCOMES

- concept of cells, tissues and organs
- names, appearances and main functions of principal cell organelles plus cytoskeleton
- how cell-cell interactions/junctions maintain tissue integrity
- epithelial structure and polarity, and the features and functions of different epithelial types
- structures and functions of secretory cells and glandular tissues
- structures and functions of connective tissues, and of the extracellular matrix and associated cells

Cells and organelles

Cells are the basic structural and functional units of an organism. Each one is composed of a cell (or "plasma") membrane enclosing the cytoplasm which surrounds the organelles, each of which is tasked with biochemical processes. Not all organelles are present in all cells; the cell's composition varies according to its function. Many body cells are bathed in extracellular fluid (filling the intercellular space).

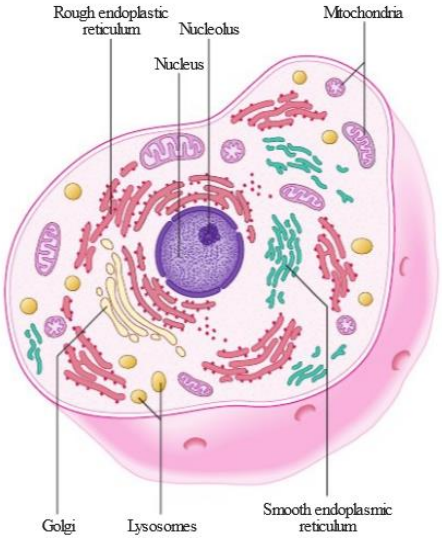
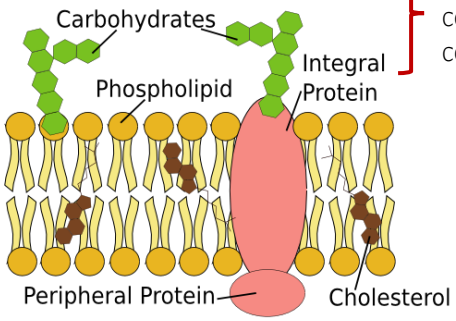


Image credit: Medical Sciences 2nd ed.

The cell membrane is a lipid bilayer containing cholesterol for stability and a wide variety of proteins (e.g. channels, receptors). The cell membrane is in contact with the cytoplasm, intercellular space and extracellular matrix (see below and later: this messy structure outside the cell may form a basement membrane for specialised functions, such as in the kidney).



Glycocalyx: molecules for cell-cell recognition, communication and adhesion

Image credit: study.com

The "heads" of the phospholipid molecules are hydrophilic, so are attracted to the water of the cytoplasm and extracellular fluid and hence form the boundaries of the cell membrane. The hydro-carbon "tails" are hydrophobic and are directed inwards towards each other. Molecules with both of these properties are known as amphipathic.

Extracellular matrix (ECM): a network of molecules (secreted by cells) that provides structural support to surrounding cells, as well as adhesion and communication. To help you visualise this, plasma is the ECM of blood.

Functions of the cell membrane:

- Physical protective barrier between living components and the outside environment
- Regulates membrane transport (molecule uptake or secretion) and hence controls internal environment
- Use of ion gradients across membrane in excitation, communication and transport
- Holds receptors for cell-cell recognition and detecting/responding to stimuli
- Structural support: has anchoring sites for cytoskeleton and ECM
- Endo-membrane ("inside") system forms compartments and stable binding / catalysis sites for enzyme processes
- Dynamic movement involving vesicles

Organelles can be membranous (creating cell compartments for separate processes) or non-membranous.

Membranous	Structure	Function
Nucleus	Nucleoplasm, nucleoli and chromatin in nuclear membrane (which has pores)	Control centre of cell, stores genetic material that dictates protein synthesis
Mitochondria	Double membrane forming cristae on inside surface for binding enzymes	ATP synthesis (chemical form of energy)
Rough endoplasmic reticulum	Extension of nuclear membrane; has ribosomes attached	Scaffold for synthesis of secretory or membrane proteins. Vesicles containing proteins bud off to Golgi
Smooth endoplasmic reticulum	Membrane system of sacs/tubules (called sarcoplasmic R in muscle)	Synthesis of lipids, steroids and sex hormones. Detoxifies drugs
Golgi apparatus	Stacks of membrane sacs; always regenerating forming/maturing faces	Protein modification and packaging into vesicles for secretion, containing in lysosomes or adding to cell membrane
Lysosomes	Membrane sac containing acid hydrolase enzymes and H+ pumps	Intracellular/extracellular digestion, autophagy, autolysis. Destroy pathogens
Peroxisomes	Membrane sacs containing oxidase enzymes	Detoxification, H_2O_2 breakdown
Non-membranous	Structure	Function
Ribosomes	Ribosomal RNA + protein; on RER or free in cytoplasm	Site of protein synthesis; moves along mRNA carrying amino acids
Cilia	Motile processes on cell surface, composed of microtubules	Unidirectional movement of substances over cell surface (e.g. mucus in trachea)
Flagella	Larger cilia	Movement of cell (e.g. sperm tail)
Centrosome	Two perpendicular centrioles, each composed of microtubules	Chromosome movement in cell division, base of cilia/flagella, organisation of microtubules in cytoskeleton

The **cytoskeleton** is another non-membranous organelle. It is a 3-D protein scaffold throughout the cell, giving mechanical strength and carrying out a wide array of functions via its different constituents:

Component	Actin microfilaments	Tubulin microtubules	Intermediate filaments
Functions	Cell movement	Structural support	Membrane support
	Cell shape changes	Intra/extracellular transport	Localisation of organelles
	Microvilli structure	Spindle in cell division	Oppose mechanical force

Kinesin motors are structures that carry vesicles/organelles along microtubules/nerves using ATP hydrolysis.

Interactions

Viruses can use receptor-mediated endocytosis to circumvent the immune system

Endocytosis: -**phagocytosis**: the cell membrane invaginates to engulf large molecules in a phagosome

-**receptor-mediated (specific) endocytosis**: clathrin forms a coat which deforms the cell membrane to produce a vesicle in which molecules are taken into the cell e.g. insulin, LDL

-**non-specific endocytosis**: a liposome containing large molecules fuses with lipid layer and contents are released into cell

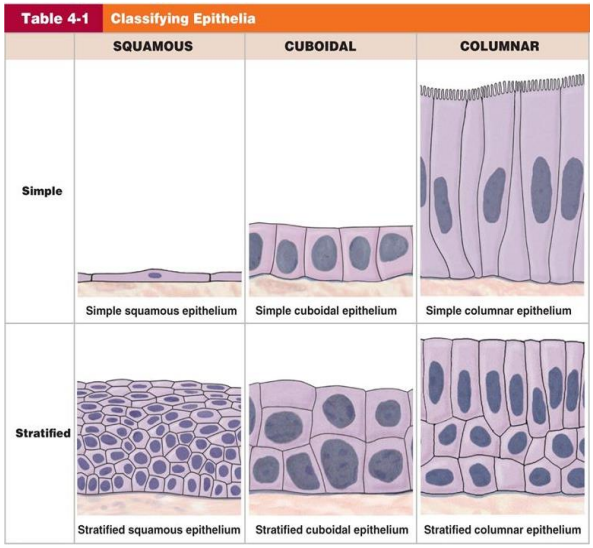
Exocytosis: Constitutive secretion = newly synthesised cell membrane components (released from Golgi in lysosomes) fuse with CM randomly. Regulated secretion = hormone stimulates the above process.

Tissues

Tissues are groups of the same cell type carrying out a particular function. Numerous types of tissue may be present in an organ. The four major tissue types are **epithelial, connective, muscle** and **nervous** (see Topic 5).

Epithelia (e.g. skin, capillaries, gut, alveoli)

Image credit: Pearson Education Inc.



Epithelia form the boundaries between environments and must be crossed by substances entering or leaving the internal environment. They develop from all three embryological layers and their functions include exchange, transport, protection and secretion.

Epithelia are classified by the number of layers (simple or stratified) and the morphology (shape) of cells. The type of stratified epithelia can be identified by the shape of the top layer of cells. Epithelia are also highly cellular (so regenerate rapidly due to friction) with little ECM, and avascular but innervated so substances must diffuse to and from blood.

Cells are polarised: apical surfaces have microvilli to maximise surface area and bases have a basement membrane for mechanical support.

Type	Examples
Simple squamous	Heart lining, blood vessels, lymphatics, alveoli, kidney glomerulus
Simple cuboidal	Ovary, pigmented layer of retina, kidney tubules
Simple columnar	GI tract lining, gall bladder, glandular ducts, fallopian tubes
Stratified squamous	Skin (keratinised), mouth/oesophagus (non-keratinised), urinary tract (transitional)
Stratified cuboidal	Ducts of sweat glands, oesophageal glands - RARE
Stratified columnar	Urethra lining, large gland ducts

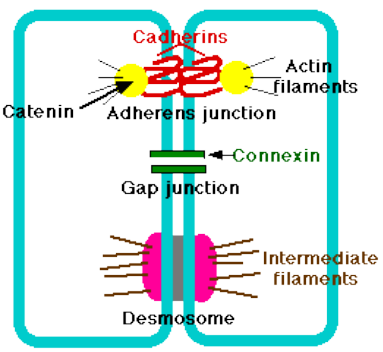
Note that the type of epithelium follows its function: **simple squamous epithelium** allows filtration, diffusion, osmosis and secretion but little protection due to its thin nature. All **stratified epithelia** afford protection due to their regenerative capacity and multiple layers. All **cuboidal and columnar epithelia** permit secretion – bigger cells have more space to synthesise - and absorption (except stratified columnar; it does not carry out absorption).

Glands are epithelia that secrete and store products such as hormones. Goblet cells are unicellular glands. Endocrine glands are ductless so hormones are released directly into extracellular fluid or blood to travel to target organs. Exocrine glands secrete products through a duct onto epithelium. There are three types:

- Merocrine: secretory product released from glandular cell in exocytosis
- Apocrine: vesicle containing products is secreted from glandular cell
- Holocrine: glandular cell dies and becomes secretory product

Cell junctions

Image credit: biology-pages.info



Epithelial cells function together as a tissue, so are tightly associated via intercellular junctions.

Junction type	Structure	Function
Tight	Occludins (interconnecting membrane protein links)	Interlocking protein links fuse membranes to give small intercellular space that limits permeability between cells
Gap	Connexons (protein tubes)	Protein channels through plasma two membranes or intercellular space allow transport between cells
Desmosome	Cadherins (cell adhesion molecules)	Cadherins bind to the basal lamina of cells at adhering junctions, giving stability to the tissue.

Connective tissues

This is core understanding for the Locomotor module so it's worth getting to grips with now!

Connective tissue is the most abundant tissue in the body and develops from the embryonic mesenchyme. Functions include support, load-bearing, protection, binding and transport. It is not inert, but responds to its environment. Its poor repair potential underpins many clinical conditions such as osteoporosis and arthritis.

- Connective tissue proper: fat, ligaments
- Fluid: blood, lymph
- Supporting: bone, cartilage

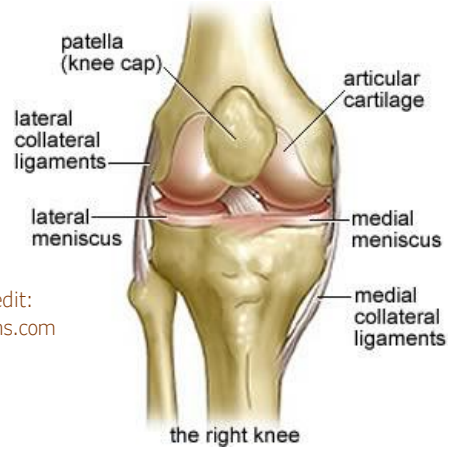


Image credit: aclsolutions.com

Structure of connective tissues

Connective tissue is largely mechanical so has an extensive extracellular matrix for structural and biochemical support and relatively few cells. Throughout the ECM network, connective tissues have varying compositions of interstitial fluid, nerves, cells and capillaries. Load-bearing, dense tissues such as cartilage have low vascularity, while loose tissues such as adipose have high vascularity.

Structure of extracellular matrix

The ECM consists of a framework of extracellular fibres (collagen, elastic fibres and reticular fibres) with ground substance (proteoglycan molecules) filling in the gaps between the framework and any other structures present (cells, nerves, blood vessels and fluid). The connective tissue is therefore a complex mesh with a range of properties depending on the proportion of different components:

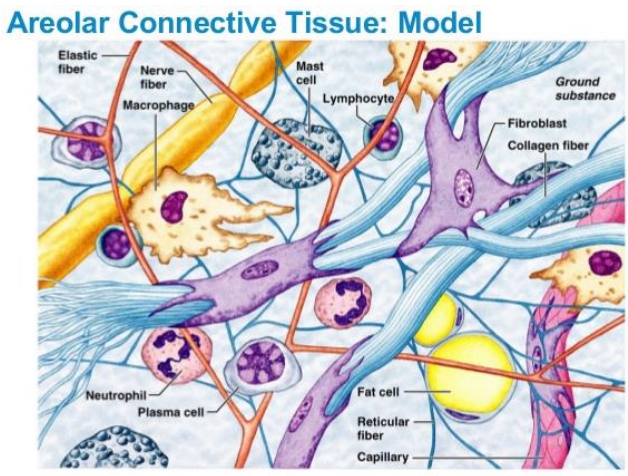


Image credit: Pearson Education

- Collagens give tensile strength to the network
- Elastic fibres give elasticity
- Reticular fibres are thinner and branching

- Proteoglycans are highly hydrated molecules allowing compressive strength, as water can be pushed out of the tissue under weight

Integrins are proteins that signal between the ECM and metabolic systems, allowing it to react to its environment e.g. wound healing. Cartilage thickening with exercise is an example of an ECM adaptive response to mechanical stress.

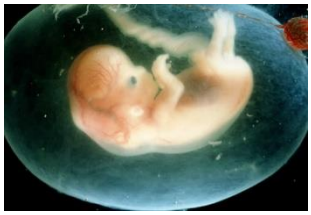
Note: anatomical terminology is also tested in Body Basics

Topic 2: Embryology

LEARNING OUTCOMES

- human reproduction, including formation of germ cells and conception
- embryogenesis (cleavage, gastrulation, neurulation and somatogenesis to organogenesis)
- growth control
- sex determination
- concept of stem cells

Image credit: medicinenet.com



Sexual reproduction (as opposed to asexual cloning)

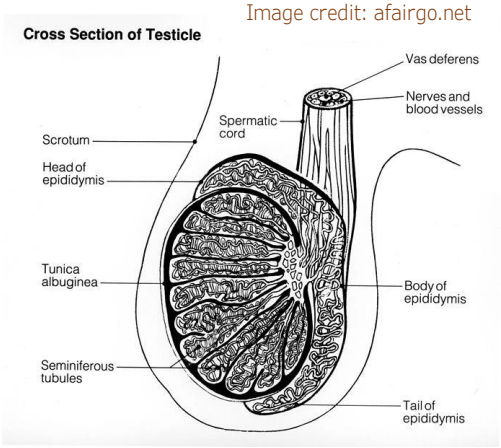
Advantages: each individual has a different genetic makeup and therefore resistance to pathogens and survival in various environments, allowing the species to continue in spite of environmental or biological pressures.
Disadvantages: a partner is needed, which can be problematic if the population density is low, and genes do not pass on undiluted so a "perfect set" could not be transmitted to the next generation.

- The male produces many small gametes that provide no nurture
- The female produces few large gametes with many resources (yolk, placenta and milk) to compensate

Germ line cells are set aside early in embryonic development to only produce gametes (the rest of the body being somatic cells, which cannot). Germ line cells have no environmental influences, only DNA as information.

Male reproductive anatomy and spermatogenesis

Image credit: afairgo.net



Seminiferous tubules are surrounded by connective muscle stroma. The layers of the tubule, moving inwards to the lumen, are:

- Myoid ("muscle-like") cells
- Spermatagonia – germ line stem cells on basal side undergoing mitosis ("2n") and differentiating to become
- 1° spermatocytes undergoing meiosis I to become
- 2° spermatocytes undergoing meiosis II to become
- Spermatids – haploid cells ("n")
- Maturing sperm, released into the lumen

These are the stages of spermatogenesis. Mitotic proliferation begins at puberty. The purpose of meiosis (remember: it has **two** divisions) is to shuffle the DNA (forming chiasmata) and produce haploid cells.

Sertoli cells release the mature spermatozoa. There are continuous tight junctions between these cells to prevent an immune reaction during puberty against newly-produced sperm cells, which could be detected as "foreign". Damage to these junctions results in infertility as sperm would be destroyed, as can mumps (uncommonly).

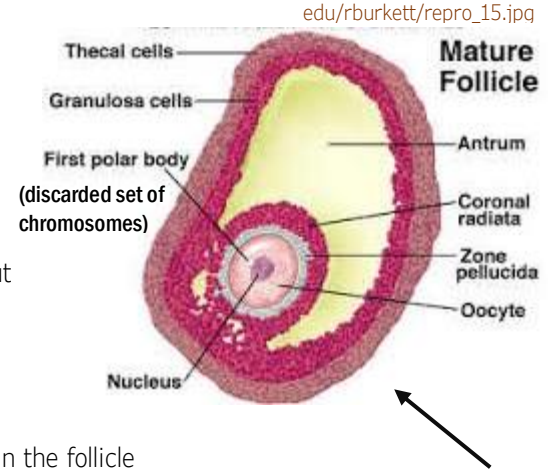
10^8 sperm/ml semen is average concentration. <2 x10^7 sperm/ml semen is classed as infertility - this is a common exam question.

The mature sperm flow passively into the epididymis, which secretes molecules into the seminal fluid that activate the sperm and allow them to swim.

Female reproductive anatomy and oogenesis

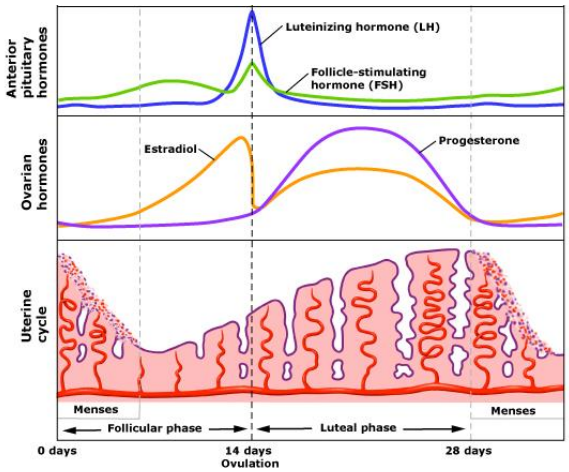
Adult women have no germ line stem cells that are constantly undergoing mitosis: this occurs in the foetal female, and female babies are born with their oogenesis paused in prophase I. At this stage, the incomplete gametes are known as primary oocytes, surrounded by granulosa cells.

Image credit: http://faculty.southwest.tn.edu/rburkett/repro_15.jpg



The continuation of oogenesis:

- The pituitary gland starts producing follicle stimulating hormone (FSH) at puberty
- This causes around 50 follicles in the ovary to resume development each month, but only one usually makes the full development to undergo ovulation
- Primary oocytes in follicles enlarge and synthesise RNA but still do not proceed any further through meiosis
- They produce a glycoprotein zona pellucida and the granulosa layer proliferates, surrounded by a theca layer Granulosa cells communicate via gap junctions
- Granulosa cells secrete follicular fluid to form an antrum in the follicle
- Luteinising hormone (LH) from the pituitary binds to thecal cells and causes maturation into a Graafian (pre-ovulation) follicle by completing meiosis I and arresting again in meiosis II as a secondary oocyte
    - If LH is not received at the right time, the cell undergoes atresia and dies
- The oocyte detaches from the granulosa and ruptures the follicle, moving out of the ovary and into the fallopian tube
- The remains of the ruptured follicle are known as the corpus luteum and produce progesterone and oestrogen to develop the uterine lining. If pregnancy does not occur, it soon dies.
- Meiosis II is **only** completed if fertilisation occurs.

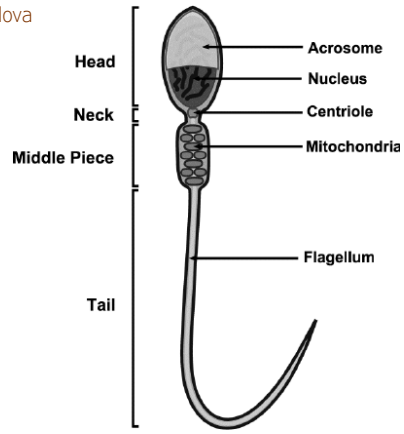


You will note from the parallel stages of gametogenesis in males and females (although not compared in great detail here) that each cell produced by the mitotic proliferation of stem cells yields **four** viable gametes in males but only **one** in females.

Image credit: assignmenthelp.net

Capacitation

Image credit: Nova Publishers



Fertilisation can **only** occur naturally in the female reproductive tract because proteases in the cervical fluid remove the glycoprotein coat acquired in the epididymis, which renders the cell membrane more permeable to calcium ions and therefore enables tail lashing and destabilises the membrane of the acrosome (see below).

The cervical mucus modifies during the menstrual cycle to allow sperm past the cervix. They can bind to the epithelium at the start of the oviduct until ovulation, allowing them to live in the body for days.

Fertilisation

Acrosome reaction: when a sperm meets the zona pellucida of the egg, the membranes of the acrosome and the egg fuse, so the contents of the acrosome (a remnant of the Golgi apparatus) can digest the ZP. It burrows towards the oocyte and fuses with its membrane, causing repeated waves of calcium entry. This:

- releases cortical granules which make the ZP impenetrable to sperm so it is only fertilised once
- recommences meiosis II in the oocyte, producing a second polar body

Both sets of chromosomes de-condense into male and female pronuclei which fuse to form a zygote.

Assisted fertilisation

This may be required due to advancing maternal age, blocked vas efferentia/deferentia, impotence, low male fertility or blocked/absent oviducts which can arise for a variety of reasons.

The first stage involves promoting oogenesis, which involves either exogenous FSH or blocking oestrogen detection to stimulate endogenous FSH production. The eggs and sperm to be used are then harvested and the latter are artificially capacitated. They are mixed and observed (genetically tested) and then implanted.

Intra-cytoplasmic sperm injection: sperm are removed from the testis and injected directly into an oocyte if they are unable to succeed in the above process. This raises ethical questions as to whether sperm that are this incapable should be used to produce a child.

Embryonic development

The embryo now has to use its genes to grow one million times bigger, create different cell types from the existing one and establish the axes and anatomy of the body.

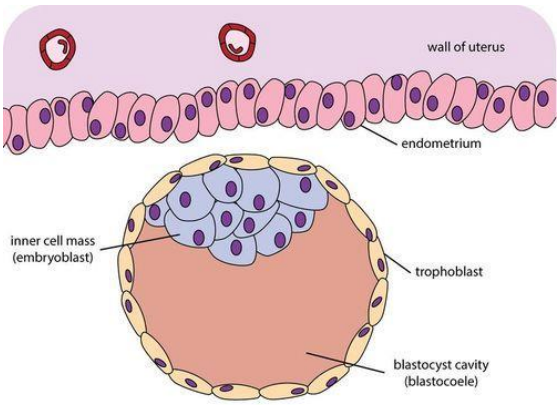
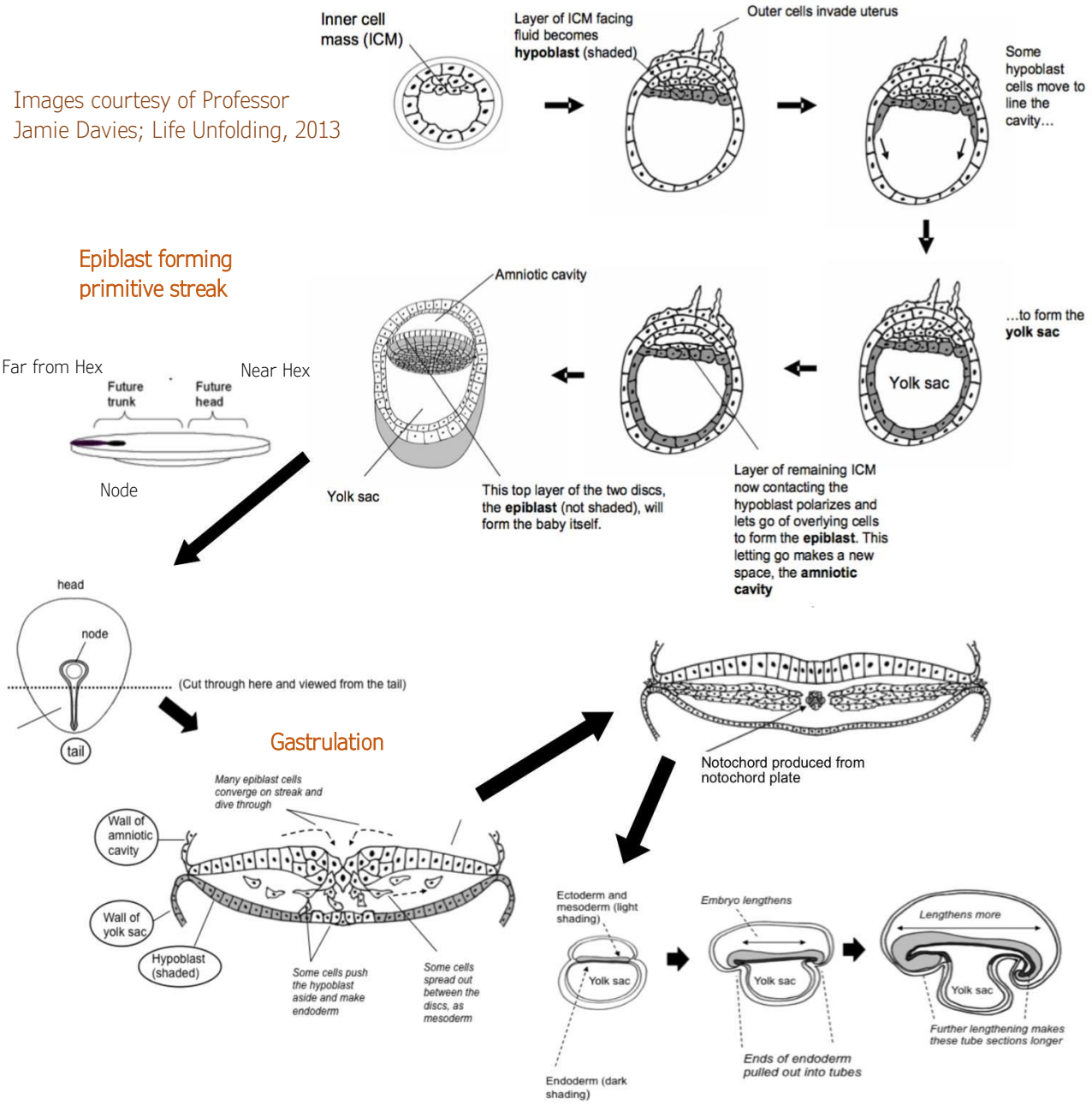


Image credit: freelearningchannel.com

- To produce different cell types, the single cell first undergoes cleavage (mitosis where the clump of cells stays the same overall size). At the 4-cell stage, this blastocyst starts to synthesise its own mRNA and enzymatically destroys maternal mRNA.
- When lots of these identical cells are available, the only difference between them for the embryo to work with is basic geometry – cells on the inside of the clump are in contact with others all around, while the outside ones have a free surface.
- Fluid is then pumped into the blastocyst to arrange an inner cell mass (which will become the body) and an outer trophoblast layer (which will form the placenta) surrounding the ICM and the cavity.
- The blastocyst hatches out of the zona pellucida and travels down the fallopian tube to the uterus.
- The trophoblast then invades the uterine epithelium (endometrium) in implantation (see diagram).

- The ICM is initially all one cell type so it uses geometry as before to make internal differences. Essentially, cells touching different surfaces specialise in a different way. (This differentiation is a 3-D process and difficult to visualise.)
- The layer of ICM in contact with the fluid is the hypoblast and some of these cells migrate down the edges of the cavity to line it, forming the yolk sac.
- The layer of remaining ICM touching this hypoblast detaches from the rest of the ICM and drops down to form the amniotic cavity. This dropped layer is called the epiblast and later becomes the baby itself. The embryo is now like a hollow ball with two stacked discs inside. It is radial, so has to create a third dimension.
- Again, the embryo makes differences out of nothing to produce a body axis. Cells in the centre of the hypoblast produce Hex protein and these move out towards the edge of the disc as a clump.
- The clumped cells secrete proteins that oppose the production of a primitive streak in the epiblast layer above. Therefore, epiblast cells furthest from the clump will pile up to give a primitive streak (spine precursor) and moves towards the centre to form a node, while cells above the clump will form the head.

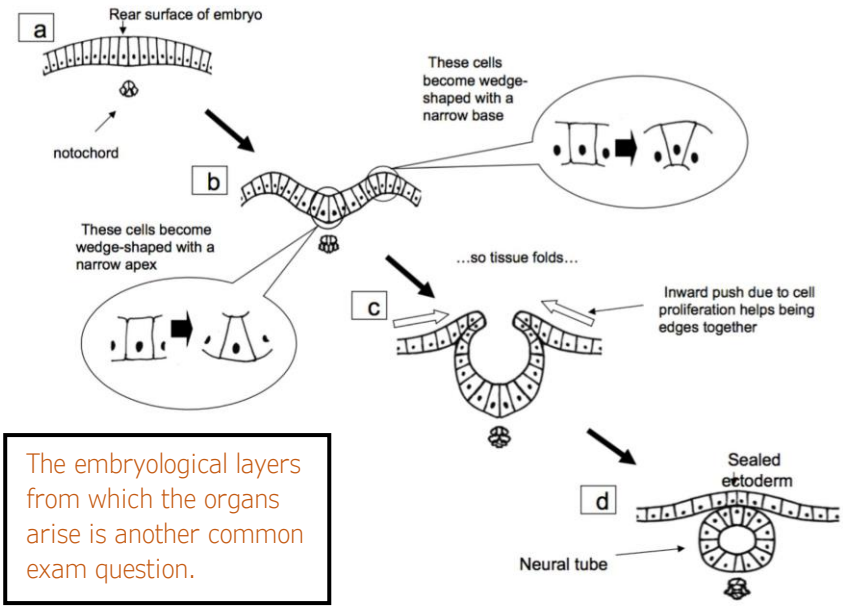
Images courtesy of Professor Jamie Davies; Life Unfolding, 2013



- Gastrulation is the process of making a gut (central tube). Epiblast cells fall down from the streak and squeeze into the midline of the hypoblast to make an endoderm ("inner skin"), and others spread out between the discs as a mesoderm (layer between ectoderm and endoderm).
- An aggregation of cells in the middle of the lower endoderm rises up as a notochord plate and then break off to form a notochord (later obliterated by vertebrae).
- The embryo lengthens and so this endoderm becomes a long tube, with the yolk sac connected by a branch. The lungs, liver and pancreas also later branch from the gut.

The gut is an open tube and the CNS is a closed tube.

- Neurulation is the production of a central nervous system (another central tube). The dorsal ectoderm folds inwards (see diagram below) to give off a neural tube (above the notochord) and seal up again. The sealing of the two separate structures is possible because they have two different cell adhesion molecules.
- If this closure fails, spina bifida or anencephaly occur. Very rarely, a twin embryo may be caught up in this closure and engulfed, creating an abnormality known as fetus-in-fetu.
- Later, the neural crest (ectodermal cells in the gap above the neural tube) migrates towards organs and these processes become the peripheral nervous system, adrenal gland and melanocytes in skin.



The embryological layers from which the organs arise is another common exam question.

- Next, the mesoderm around the neural tube divides itself into somites (segments on either side of the neural tube) which are the precursors to vertebrae and skeletal muscle.

- Differentiation is the production of more internal differences from the existing ones. Signals (such as SHH from the notochord) spread to neighbouring tissues to switch on other genes in them and hence cause them to specialise. These differences ripple out through the entire body to produce different cell types.

- Tissue migration is another aspect of development. The neural crest is an example, one product of which is the face. These cells come from the back of the head, and where they meet is the facial centre line. Another example is the secondary palate, which divides the nose and mouth. If this does not seal, cleft palate occurs.

Twinning

Monozygotic ("identical") twins can arise at three different stages in embryonic development:

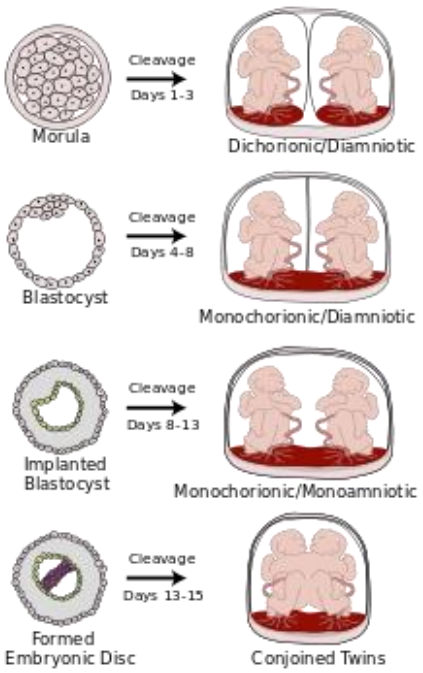


Image credit: wikipedia.org

- After the first division of cleavage the two blastocyst cells produced lose contact with each other and so two embryos implant separately in the womb, each with their own placenta.
- The newly-created ICM splits into two separate clumps within the trophectoderm and so the twins share a placenta but have separate amnions. This is the most common cause of monozygotic twins. It carries a risk of foetal transfusion syndrome, where one twin takes more of the nutritional supply than the other.
- Two primitive streaks form in the epiblast which produces twins in the same amniotic cavity (and likely conjoined). Even less commonly, the primitive streak may split and the embryo has two heads. This is rarely compatible with post-natal life.

Growth control

Organs must be proportional in size to function with the others depending on them. Body size is in part controlled growth hormone (GH) secreted by the pituitary gland; therefore, pituitary tumours making excess GH may cause gigantism. Some tissues take the GH signal and relay it on to other cells via insulin growth hormones. In Laron syndrome, cells have no receptors for growth hormone so the body is smaller but with Vitruvian proportions.

GH affects liver and muscle growth directly but other tissues only indirectly through these. Rabbit experiments have demonstrated a number of principles regarding growth: limbs "know" what size they are meant to be, fast growth can occur at any time and the two paired limbs grow independently. Bones know when to stop growing as they lose the ability to respond to GH with increasing number of divisions. The feedback loop of growth signals allows the correction of mistakes and knowing when to cease growth.

Limb growth occurs very quickly and needs a rapidly growing vascular system to support its oxygen demands. Therefore, anything that inhibits blood vessel growth will impair limb growth and permit non-Vitruvian conditions. **Thalidomide** was a drug prescribed for morning sickness and depression in pregnancy, which was found to kill developing blood vessels. Therefore, foetuses only grew short limbs in a condition known as phocomelia.

Achondroplasia involves a mutation stopping proper development of chondrocytes (cartilage cells). Cartilage is the precursor to bone, so when it is not produced, individuals have an average-sized trunk and short limbs.

Non-Vitruvian conditions can also be genetic, such as pycnodysostosis. Cathepsin K is a protease that releases GH from its storage receptors so it can exert its effects, but in this disease it is mutated and cannot work. Only limb growth is affected, which shows that not all parts of the body keep up with each other; but the amounts of different tissue are proportional to each other and the size of the limb (hence we know that this is dictated by bone).

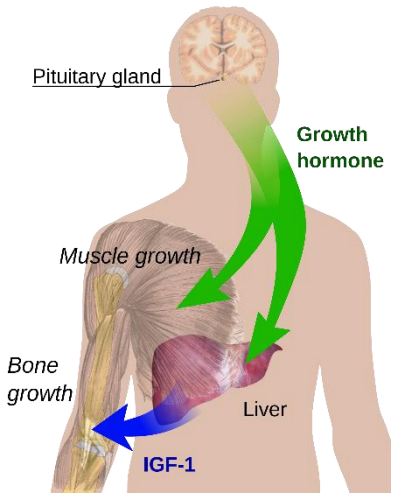


Image credit: jjideabioorganic.com

Skin growth is driven by mechanical forces and not genetics, which is why the amount of skin is always proportional to the body. Where skin is stretched, mitosis occurs to reduce the tension. On the other hand, "mechanically isolated" organs (such as the spleen, but not the thymus) will grow the correct **total volume** of tissue in the body due to a feedback loop.

Sex determination

Embryos all begin development in the same way but must start to express a gender when gonad growth commences. Germ line cells are removed from the epiblast to the yolk sac (outside the body) and so are not included in gastrulation. The germ cells migrate from the yolk sac, though the gut and then mesentery, to the gonadal ridge. The mesentery is a sheet of tissue that anatomically connects the gut (later the abdominal organs) to the cavity wall.

> Germ line cells = cells which divide to produce gametes
>
> Somatic cells = all other cells that form the body

The SRY gene on the Y chromosome determines gender. Embryos are female by default, so they will only become male if the somatic cells of the gonad express SRY. If SRY is engineered to shut down in an XY organism, a female is produced. Switched-on SRY make these gonadal somatic cells become testes (instead of ovaries) that secrete androgenic hormones. This is the sole signal to the rest of the body's somatic cells to become "male". These hormones are testosterone and anti-Mullerian hormone (AMH), the latter of which destroys the early oviducts and uterus. The XX/XY status of the non-gonadal somatic cells themselves has **no** effect on their behaviour; these cells are influenced only by these androgenic hormones.

Androgen insensitivity syndrome: if the androgen receptor in an XY embryo does not work, the exterior "default" female features will be present but with male gonads, as the rest of the body cannot detect the male hormones.

> Testosterone is produced by Leydig cells and forces the Wolffian ducts survive, which go on to become the vas deferens.

Testosterone is a weak activator of androgen receptors and has to be converted by 5**α**-reductase to a more effective ligand. Therefore, XY children with a deficient 5**α**-reductase enzyme (due to mutation) have female bodies before puberty. At puberty, when the level of testosterone rises to a high enough level to stimulate androgen receptors sufficiently even without the enzyme, they become young men. These are known as guevedoces, meaning "eggs at twelve" (slang for testes).

- Other intersex phenotypes exist and therefore ethical questions regarding neonatal or paediatric surgery so they can conform to one gender should be considered, including consent.

Topic 3 - Genetics

Image credit: scienceclarified.com

LEARNING OUTCOMES

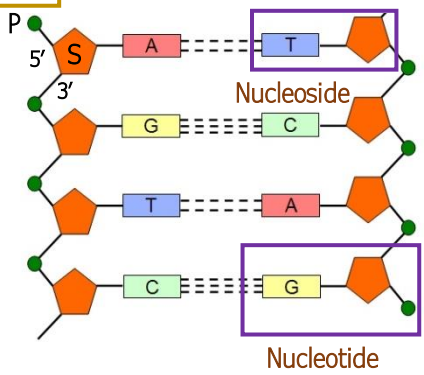
- chromosome structure and function; how DNA is compacted in chromosomes
- structure and function of DNA
- DNA replication, including error-correction
- gene expression array profiling
- types of RNA, and mechanisms of RNA synthesis
- transcript structure including introns, exons, splicing, polyadenylation, and mechanisms of RNA processing
- mechanisms of protein synthesis, including the genetic code and ribosome action
- mitosis, meiosis and chromosomal crossing over, ploidy
- genotype and phenotype correlation
- ill health due to environmental and genetic factors
- epigenetic modification of chromatin

Genetic information must be stored, retrieved and interpreted by cells in order to make and maintain an organism. Reproductive cells must be able to pass this on to the next generation.

DNA

Phosphodiester bonds link the nucleotides to give a polymer

Image credit: thinglink.com



Information dictating protein synthesis and therefore the functioning of an entire organism is encoded as a series of bases on deoxyribonucleic acid. This translates to produce a sequence of amino acids assembling the primary structure of proteins (including enzymes, which hence control other biochemical reactions).

The molecule takes the form of a double helix – a twisted "ladder" where pairs of hydrogen-bonded complementary bases form the rungs between anti-parallel backbones of phosphate and pentose sugar (deoxyribose).

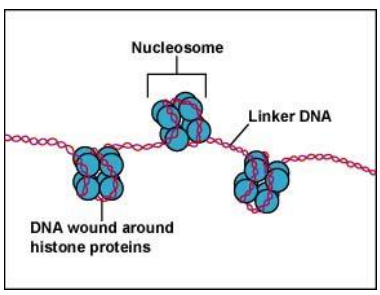
The long double helix shape of DNA explains two of its fundamental processes: how the plan for an organism can be carried in chemical form, and how it is copied when a cell divides.

- As a very long molecule, a huge number of sequences combining the four bases is possible, and therefore this precise sequence **is** the code carrying the genetic information
- As a double helix, complementary base pairing provides a copying mechanism

The four bases (adenine, cytosine, guanine and thymine) are nitrogenous rings. A always bonds to T with two hydrogen bonds and C always bonds to G with three hydrogen bonds. C and T are small (contain one ring) and known as pyrimidines (suffix –idine as a nucleoside), while A and G consist of two rings and are called purines (suffix –osine as a nucleoside). Therefore, a pyrimidine always bonds to a purine to give a "rung" **three** rings long.

Chromosomes

Image credit: schoolworkhelper.net



DNA molecules are wrapped around proteins to form nucleosomes, which are in turn wound in a helix into fibres, and these are then folded into loops which compose the chromosome. This allows a massive amount of information to be compacted into a tiny space.

The DNA molecule is associated with histone proteins and other proteins to compose chromatin, the substance of which chromosomes are made. This

nucleosome (subunit of chromatin) allows the packaged DNA to still be accessible for use. Each nucleosome is a length of negatively-charged DNA wrapped around a positive histone core (a protein octamer with tails). They are highly conserved in evolution, showing little difference across species.

The loops are unravelled to allow gene expression: ATP-dependent chromatin remodelling complexes slide the DNA around the nucleosome to expose the code. Gene expression can be increased or decreased by chemically modifying the histone tails (by methylation, acetylation or phosphotylation) to alter the accessibility of the chromatin.

The centromere region is a repetitive DNA sequence of millions of base pairs that uses kinetochore protein complexes to bind sister chromatids together and to attach the spindle to the chromatin for mitotic separation.

The telomere is a structure on the end of a chromosome which is a chain of many base repeats, resulting in a T-loop that folds back on itself. These prevent fusion with other chromosomes and protect the ends from natural exonuclease enzyme action in the cell. No telomere indicates an abnormality.

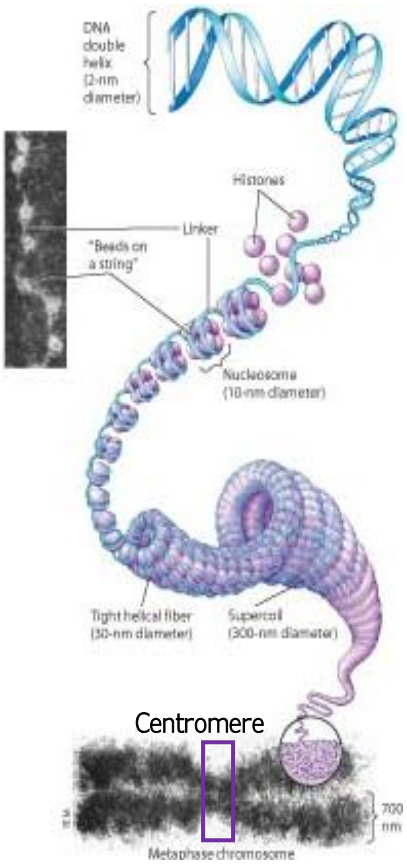


Image credit: Pearson Education Inc.

Sister chromatid: one of two identical chromatids produced by the replication of a single chromosome. One chromatid moves into each daughter cell in mitosis.

Homologous chromosome: one of the two chromosomes forming a pair. The two have the same genes but possibly different alleles of them. One homologous chromosome in each of the 23 pairs comes from one parent.

The karyotype refers to the full set of 46 chromosomes (23 pairs of homologous chromosomes) of which 22 pairs are autosomes and one pair are sex chromosomes (XX in females, XY in males). These images are often taken in metaphase when DNA is condensed into chromosomes. For that reason, each pair of chromosomes may be composed of four chromatids (two sets of sisters bound at centromeres) as the DNA has just been replicated.

Duplication and segregation – the cell cycle

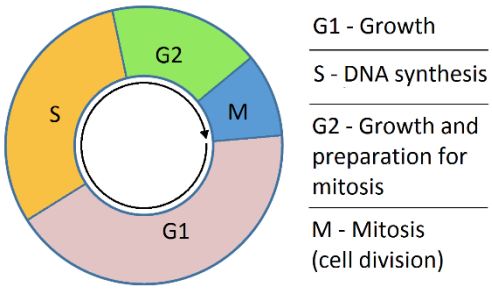


Image credit: Wikipedia.org

- Chromatin is not condensed into chromosomes during interphase (G1, S and G2)
- DNA replication occurs in S-phase so there is twice as much genetic material during G2 and M until cytokinesis
  - After S phase cohesin rings hold sister chromatids together
  - These rings are removed during anaphase so the chromosome duplicates segregate to produce two diploid daughter cells
- Organelles are formed in G1 and spindle proteins in G2

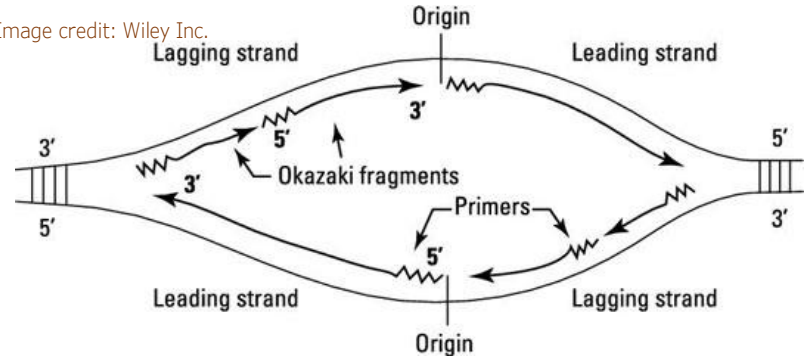
DNA replication

For cells to divide and produce new ones, the DNA of the parent cell must be replicated so there is enough genetic information for two daughter cells. Each strand of DNA acts as a template for the synthesis of a complementary strand, due to base pairing rules.

Semi-conservative replication: one original strand and one newly synthesised strand in each daughter cell

- DNA synthesis is initiated at replication origins; a short stretch of A=T bases is a good place to start as there are only two hydrogen bonds to break
- Initiator proteins recognise the origins and open the helix, allowing DNA helicase to separate the two strands
- Single-stranded binding proteins keep the helix unwound
- Two forks form at each origin so replication progresses in both directions
- DNA polymerase adds free nucleotides to 3′ ends in the form of deoxyribonucleotide triphosphate – the high-energy phosphate bond is broken to release energy

Image credit: Wiley Inc.



NOTE: DNA polymerase can only work in the 5′ to 3′ direction, but at each fork from the origin there is a leading and a lagging template. Replication on a leading strand proceeds as expected, but on a lagging strand Okazaki fragments (lengths of DNA) are synthesised discontinuously and joined into a strand by DNA ligase.

DNA polymerase can only continue adding nucleotides to an existing strand, not initiate a brand new one. Primase (RNA polymerase) makes an RNA primer at the start of the new strand that can be extended by DNA polymerase. The primer is later removed by nucleases. The lagging strand continually needs primers to produce fragments.

DNA repair

Mutations (changes in the sequence of bases) in the codon can alter the amino acid it codes for and hence the protein produced if it is not repaired. DNA must be checked as it is replicated, because if a wrong base is added this error is propagated by base pairing. Checking is carried out by DNA polymerase during replication.

If there is a base pairing mismatch, DNA mismatch repair proteins remove the damaged section of the new strand and the gap is repaired by DNA polymerase and ligase. Mutations in the genes for mismatch repair predispose to cancer (uncontrolled cell growth). DNA can be damaged by chemicals, viruses, or radiation (exposure to UV light can produce thymine dimers). Information from the correct strand is used to fix the damaged one in several ways.

Deleterious (fatal) mutations are eliminated by natural selection while necessary ones are conserved in evolution – therefore, sequences found in the entire species pinpoint necessary genes. This applies to non-coding regulatory DNA as well as protein-coding. Sections of DNA that code for proteins are known as exons, while the rest (regulatory DNA between genes) are called introns. Introns with no function can mutate with no effect.

DNA to RNA - transcription

RNA is another nucleic acid that assists in protein synthesis from DNA. It is encoded by DNA.

DNA → transcription → RNA → translation → protein

Nucleic acid	DNA	RNA
Length	Long	Short
Structure	Double-stranded	Single-stranded
Shape	Double helix	Complex, varying
Sugar	Deoxyribose	Ribose
Bases	A, C, G, T	A, C, G, U (uracil)
Stability	Stable	Unstable

There are three forms of RNA:

- Messenger – carries the code out of the nucleus to the ribosomes to be used
- Ribosomal – makes up ribosomes
- Transfer – carries amino acids to the ribosome and mRNA in translation

Transcription: mRNA polymerase II makes an mRNA copy of the coding/sense strand (of the length of DNA to be used) via complementary base pairing to the template/antisense strand. This requires free nucleoside triphosphates (as in DNA replication) but not primers. Many RNA polymerases can transcribe a gene at once and while errors are more common they are not passed on to any offspring.

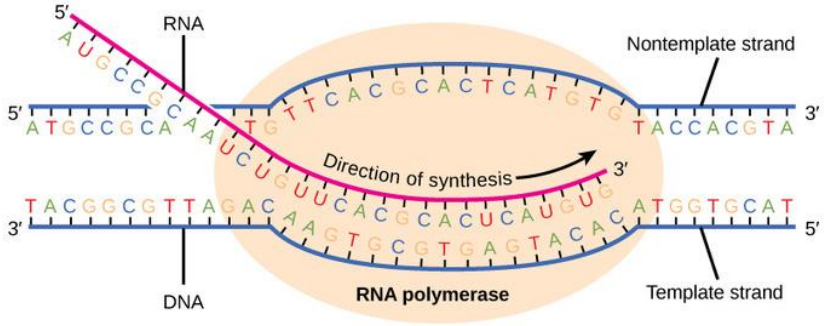


Image credit: Boundless Biology

A gene is a length of DNA coding for one discrete characteristic. All cells have all genes present in the karyotype but each type of cell expresses a different complement of genes. The expression pattern may be tissue-specific, developmentally-regulated, a combination of the two or always on.

Control of gene expression

- Promoter: a DNA sequence immediately before the gene to be expressed that dictates the transcription rate
- Enhancer: a sequence increasing transcription of a gene that can be far away from the one it influences
- Transcription factor: a protein that binds to specific DNA sequences within promoters or enhancers to turn expression of a gene on or off i.e. dictating whether RNA polymerase binds. They are specific to a tissue type.

Splicing removes introns from the transcribed strand to give functional RNA (exons only). A single gene can be spliced differently to produce a different product. Polyadenylation is the adding of a string of adenylate residues (a "polyA tail") to the 3′ end of an mRNA molecule for stability. Untranslated regions on the 5′ (start) and 3′ (finish) ends of the mRNA molecule may control translation and affect mRNA stability respectively.

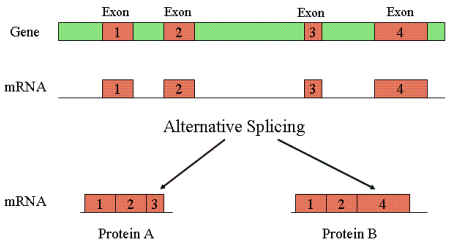


Image credit: NCBI

RNA to protein - translation

- A base triplet (sequence of three bases) or codon codes for one amino acid (or "start" or "stop")
- The code is redundant or degenerate: there are multiple codons for the same amino acid
- Translation must start at the right base or the codons will be read completely differently downstream

tRNA is an adaptor molecule that carries amino acids to the correct place on the mRNA template, allowing the mRNA code to be read and produce a sequence of amino acids. tRNA molecules are specific to different amino acids; a high-energy bond forms between the two.

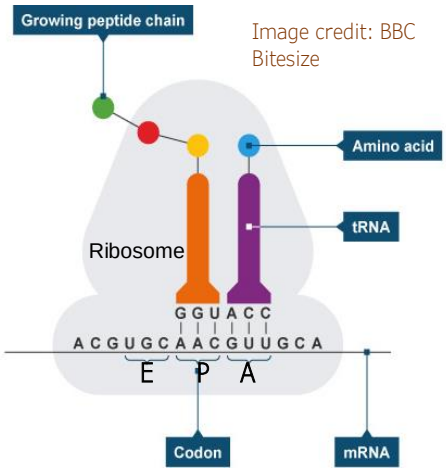


Image credit: BBC Bitesize

Ribosomes are made of rRNA and are large, complex organelles with large and small subunits. They move along the mRNA transcript (5′ to 3′) catalysing peptide bond formation between amino acids and holding the structures in place for translation.

- The ribosome holds the mRNA template which indicates the amino acid sequence of the protein to be produced.
- The free amino acid binds to the top 3′ end of the tRNA and moves to the vacant A site of the ribsosome
- The tRNA anticodon triplet base-pairs to the codon on mRNA in the A site
- The ribosome provides enzymes to form a peptide bond between the P and A amino acids
- The large subunit translocates so the last two amino acids are in the E and P sites
- The small subnit translocates so the A site is vacant and the tRNA in the E site is realeased

Translation stops at stop codons: these bind release factors which cause the ribsosomal subunits to dissociate

Genetic inheritance

Meiosis produces gametes (haploid sex cells) and gives rise to variation by producing four genetically different daughter cells from one. Some distinguishing features:

- Two divisons producing four gametes
- Duplicated homologous chromosomes pair up as bivalents in single file in metaphase I
- Homologous chromosomes segregate in MI
- Sister chromatids segregate in MII

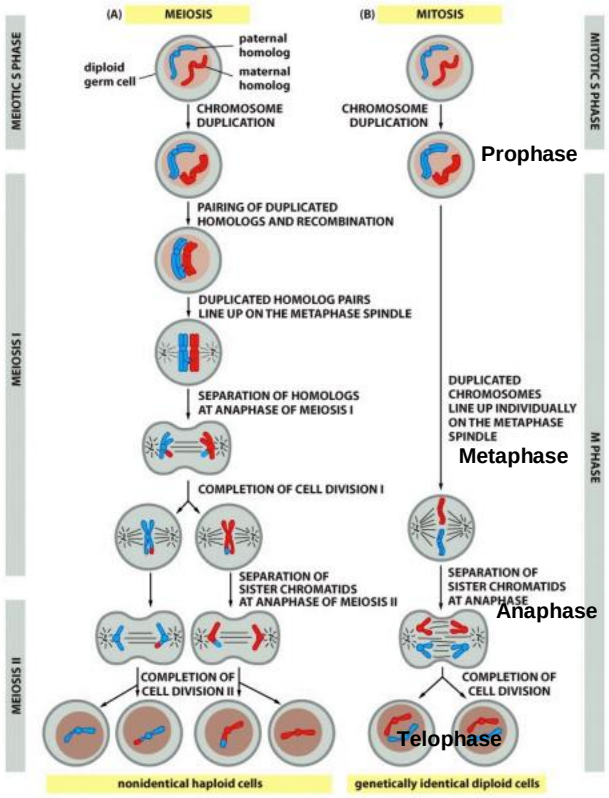


Image credit: lecture

Variation arises due to:

- Homologous recombination (swapping of genes) between non-sister chromatids in prophase I, forming chiasmata (NB: genes that are close togther are likely to be inherited together)
- Independent assortment of maternal and paternal chromosomes lining up in metaphase I (IA of genes occurs within pairs of chromosomes in homologous recombination)
- Fertilisation is random and combines genetic information from two different individuals

Genes come in different forms or **alleles**, coding for different versions of the same characteristic e.g. blue eyes or brown eyes. An organism will have two alleles for each trait; one maternal and one paternal. If the alleles are the same they are homozygous for that trait; if not, they are heterozygous. Some alleles are dominant over others for the same characteristic i.e. mask the effect of the recessive one and thus show in the phenotype (appearance).

Law of segregation: the two alleles present in the genotype segregate in gamete formation and combine at random in fertilisation

Law of independent assortment: alleles for different traits segregate independently so both maternal and paternal are present in a gamete

Mutations

Genetic variation occurs within an individual as we have a nuclear genome from each parent. Mutations can lead to a disease phenotype by altering the amino acids in a protein produced (see DNA repair) or the amount of product yielded. They can be caused by damage or errors in segregation, replication, repair etc. For example, xeroderma pigmentosum is a disease in which the repair mechanism for thymine dimers (caused by UV rays) is defective, leading to skin cancer (see Topic 9).

Single base substitutions in a codon can be **synonymous** (codes for the same amino acid) or **non-synonymous**:

- Missense replaces one amino acid with another
- Nonsense replaces an amino acid with a stop codon
- Splice site mutations remove or create splicing signals
- Mutations in regulatory seqeunces alter when, where and how much a gene is active

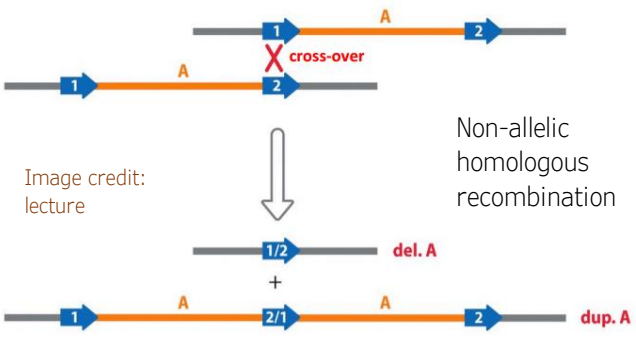


Image credit: lecture

Non-allelic homologous recombination

Deletions of sections of DNA result in a partial protein, while **duplications** disrupt protein function or produce more product. If chromatids align

incorrectly, non-allelic segments cross over in homolgous recombination so the segment is deleted in one chromosome and duplicated in the other.

Frameshift mutations: the deletion or insertion of one or two base pairs in a coding region shifts the codon triplet sequence so completely different amino acids are translated.

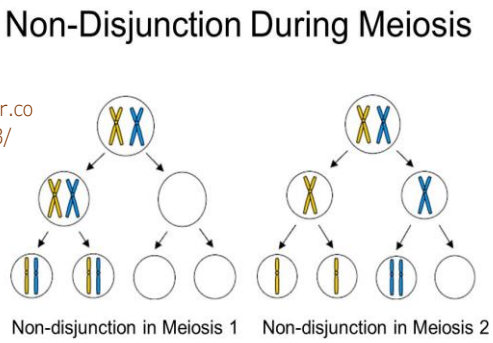


Image credit: http://slideplayer.com/slide/6262073/

Chromosomal abnormailites – ploidy

These are defined as the loss or gain of complete chromosomes.

- Polyploidy is an additional extra genome; a triploid embryo is an egg that has been double fertilised (3n) and hence could not survive to term
- Aneupolidy is absent or extra copies of **one** chromosome in a diploid
  - Trisomy is three copies of a particular chromosome (such as trisomy 21, known as Down syndrome)
  - Monosomy is the lack of one chromosome in a pair (such as monosomy X, known as Turner's syndrome)
  - These are caused by nondisjunction in anaphase I or II, when segregation fails and both duplicated chromosomes or sister chromatids move to the same daughter cell

Sex-linkage

Some diseases are not autosomal but carried on the sex chromosomes. Most are X-linked as the X chromosome is much bigger than the Y; sons who inherit a recessive pathogenic gene do not have another healthy X to mask its effects, unlike daughters, so will express the disease phenotype. Girls would have to possess two disease alleles.

Modifying effects of allelic interactions

- A disorder carried by a recessive allele will only be expressed if both alleles at the locus are pathogenic
- A disorder carried by a dominant allele only requires one copy of the pathogenic gene
- Dominant conditions that cause a gain of function mutation (adding a new harmful ability) cannot be fixed by gene therapy as the poisoning effect would still be present even if a normal allele was introduced

Modifying effects of genetic background

β-thalassemia is a blood disorder where the gene for β-globin is deficient. Normally the two globin proteins in heamoglobin are produced in equal amounts, so excess α-globin protein leads to red blood cell death. Patients with the same alleles can have siginificantly different outcomes due to the variety of possible mutations causing it.

Modifying effects of environment

Phenylketonuria (PKU) is a disease where the PAH liver enzyme is mutated. PAH normally metabolises toxic phenylalanine, but enzyme deficieny means it builds up in the body. This can be rapidly diagnosed using the heel prick test in newborns and the condition is simply treated by putting the child on a low-phenylalanine diet.

- This is an excellent example of the **interaction** between genetics (PAH mutation) and the environment (diet) in producing a disaese phenotype.

Some genetic diseases can be variegate: each family has its own mutation apparently producing the same disease phenotype, so genetic screening in the general population is very difficult.

Topic 4 - Cells, components and communication

LEARNING OUTCOMES

- structures of amino acids and proteins, including prosthetic groups
- enzymes; activity and regulation
- metabolic pathways and their control
- signalling between cells, including hormones, second messengers and signalling cascades
- cell membranes: structure, function, membrane proteins, transport mechanisms across membranes

ATP = adenosine triphosphate, the chemical form in which energy is stored and used

Protein structure and amino acids

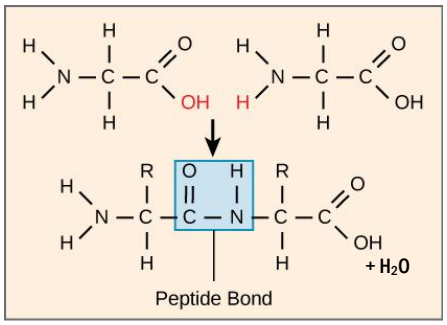


Image credit: boundless.com

Proteins are a group of molecules capable of carrying out a huge range of functions; enzymes, hormones, haemoglobin and immunoglobulins (antibodies) all fall into this category. All proteins are polymers of amino acids, which are molecules consisting of an alpha-carbon with a carboxyl group (-COOH), an amino group ($-NH_2$), a hydrogen atom (-H) and a unique side group (denoted –R) bonded to it. These side chains dictate the properties of the amino acid and hence the protein.

Peptide bonds between the carboxyl group of one acid and the amino group of the next form the polymer chain. The first amino acid of the polypeptide has a free amino group and the last has a free carboxyl (acid) group, which can simply be remembered as "amino~~~acid". At body pH 7.4, the carboxyl group is dissociated (negatively charged: $-COO^-$) and the amino group is protonated (positively charged: $-NH_3^+$) – not shown on the diagram.

The classifications of the named amino acids can come up as an exam question

Categories of amino acids

Non-polar side chain: e.g. alanine, glycine. Repelled by water (which is polar) so involved in hydrophobic interactions that determine the shape of the protein, e.g. grouping themselves together in the interior of soluble proteins in aqueous environments (cytoplasm) OR on the exterior of those in lipid environments (membranes)

- Proline has a secondary amino group, as the side chain bonds to it to form a rigid ring structure that disrupts alpha helices and allows the formation of fibres in collagen

Polar but uncharged side chain: These have no actual electronic charge but an unequal distribution of charges between atoms. Serine, threonine and tyrosine have hydroxyl (-OH) groups in their side chains which can form hydrogen bonds, while asparagine and glutamine can also do so through their amide ($-CONH_2$) groups (via both the amine and carbonyl components). Cysteine's side chain contains a sulfhydryl group (-SH) which is integral to many enzyme active sites. It can be oxidised to form a covalent cross link (-S-S-) between two cysteines within a protein, known as a disulphide bridge. The cysteine dimer is known as cystine.

Acidic side chains: the –COOH in side groups of aspartic acid and glutamic acid ionise at pH 7.4 to become $-COO^-$.

Basic side chains: the $-NH_2$ in side groups of lysine and arginine ionise at pH 7.4 to become $-NH_3^+$. They accept protons in the same way the primary amino group of the amino acid does. Histidine is weakly basic and as a free amino acid it tends not to change at pH 7.4. When it forms part of a protein its ionic environment may influence it to be neutral or positive, which is important for the function of haemoglobin.

Protein structure

The sequence of amino acids gives all the information needed to form the entire 3-D structure of the protein due to the properties and actions of side chains.

Primary: amino acid sequence

Secondary: α-helices and β-pleats

Tertiary: 3-D folding interactions

Quaternary: multiple polypeptides

The **primary structure** of a protein is the sequence of amino acids in the polypeptide bound by peptide bonds, dictated by the base sequences of DNA (see Topic 2). Therefore, mutations in DNA change the folding and function of proteins which manifest as the symptoms of a genetic disease. These mutated proteins can also accumulate in large inclusions that cause cell death in neurodegenerative diseases such as Alzheimer's and Parkinson's diseases.

Naming dipeptides: "amino~~~acid"

The first acid (with a free amino group) is given the suffix "yl" and is followed by the same of the second acid (with a free carboxyl group).

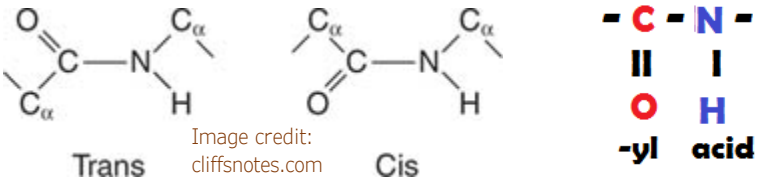


Image credit: cliffsnotes.com

Hydrolysing peptide bonds requires specific enzymes because they are covalent. These bonds are partial double bonds as they are charged, short, rigid and planar so no free rotation is possible between them.
However, the single bonds between the residues of the bond and the alpha carbons are free to rotate and can give rise to **cis** or **trans** configurations. The trans arrangement is more common due to steric hindrance of bulky side groups on alpha carbons.

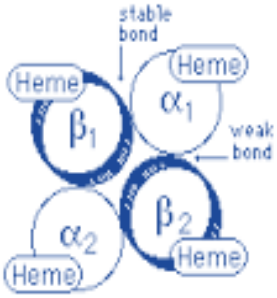
The most common **secondary structures** are the α-helix and the β-pleat. The α-helix is a right-handed coil structure held in place by hydrogen bonds, with the side groups facing outwards. The bonds run parallel to the spiral and are between carboxyl and amid groups on every fourth amino acid in the chain. Proline, charged amino acids and tryptophan disrupt helices through rings, ionic bonds or repulsion and bulky side chains respectively.

The **tertiary structure** is the final folding of the polypeptide via trial and error, assisted by chaperone proteins. This folding gives domains (functional areas) of the protein, sometimes by combining different secondary structures (motifs). Interactions between the side chains give the final shape stability: disulphide bridges, hydrophobic interactions, ionic bonds and hydrogen bonds as already discussed.

The **quaternary structure** is the organisation of multiple polypeptides to form a protein. Non-covalent interactions hold these multimeric proteins together. Polypeptides can function independently or co-operatively, such as in haemoglobin where oxygen binding to one subunit increases the affinity of the others (co-operative loading).

Globular proteins

Image credit: med-ed.virginia.edu



Haemoglobin carries oxygen in red blood cells because O_2 is non-polar and hence has limited solubility in blood plasma. It can also transport H^+ and CO_2 to the lungs. Its quaternary structure is two dimers composed of α polypeptide and a β polypeptide each, giving a total of four globin protein chains (tetramer). The two polypeptides in each dimer are held together by hydrophobic interactions and the dimers themselves linked by ionic and hydrogen bonds. Each polypeptide has a haem prosthetic group containing iron bound to it via a histidine residue, which reversibly binds oxygen. It is therefore an example of a haem protein and can bind four oxygen molecules.

Myoglobin is another haem protein which acts as an oxygen store, so it is present in skeletal and cardiac muscle. It is a single polypeptide containing many α-helices which are partly terminated by proline. Again, histidine binds to the haem group. As there is only one globin with a prosthetic group, each molecule can only hold one O_2.

Fibrous proteins

Collagen and elastin are structural fibres of the extracellular matrix (see Topic 1). They are found in connective tissues, skin, blood vessels and the eye.

Collagen is a long, rigid molecule of three polypeptides wound together by hydrogen bonds, and comes in different forms depending on its constituent polypeptide. Type I collagen has two α1 chains and one α2 while Type III is composed of three α1 chains. Glycine is present every three amino acids in the primary structure. There are three categories of collagen: fibril-forming, network-forming and fibril-associating.

Elastin is present in the lungs, arteries and ligaments; connective tissues that must stretch. In the extracellular space, tropo-elastin is deposited on a fibrillin glycoprotein scaffold to produce the protein.

Enzymes

Enzymes are proteins that catalyse all the reactions in the body, increasing the rate of reaction without being used up themselves. Active sites are the region to which substrates bind specifically to form an enzyme-substrate complex and undergo the reaction. Some enzymes need other non-protein molecules to become active. The active enzyme with this non-protein component is known as a holoenzyme, and without it is known as an apoenzyme. If the component is a metal ion it is called a co-factor, but if it is an organic molecule it is called a co-enzyme. Coenzymes may be permanently bonded to the enzyme and called a prosthetic group (such as the haem group in haemoglobin), or transiently associated with it and known as a co-substrate.

Cells are compartmentalised (mitochondria, lysosomes, nucleus) to separate substrates/products from competing reactions and also to prevent digestion of the entire cell by acid hydrolases intended for damaged structures.

Chemical reactions have their substrates, intermediates and products at different energy levels. The free activation energy is the difference in energy between the substrate and the high-energy intermediate, and is a barrier to be overcome in a reaction. Enzymes work by providing an alternative reaction pathway with a lower activation energy than the un-catalysed pathway, so more substrates have the energy required to become a product.

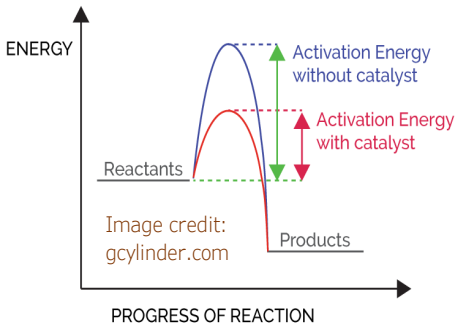
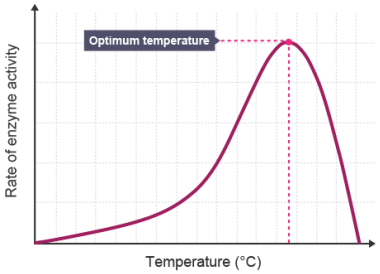


Image credit: gcylinder.com

The **rate of reaction** is defined by the amount of substrate converted into product per unit of time, and can be influenced by several factors:

- **Substrate concentration**: increased concentration of substrate increases the rate of reaction (RoR) until all the active sites available are saturated and further augmented concentration has no effect
- **Temperature**: increased temperature provides substrate molecules with more energy to overcome the energy barrier up to a certain point, after which RoR decreases due to denaturation of the enzyme as a result of excess heat energy breaking bonds within the protein. In humans the optimum temperature is 35-40°C.
- **pH**: some substrates or active sites may require a certain concentration of H^+ and hence ionisation in order to catalyse a reaction. Extremes of pH also affect ionic bonding within enzymes and denature them. Their optimum pH will vary depending on the body region, such as stomach enzymes requiring acidic conditions.



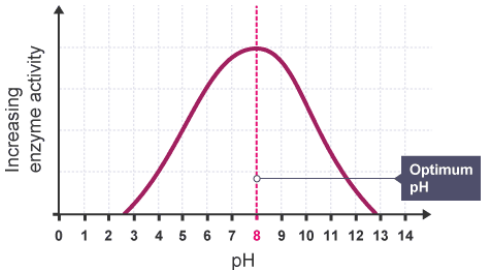


Image credit: BBC Bitesize

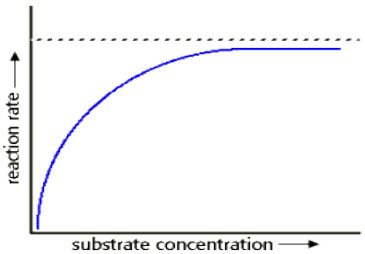


Image credit: biology.arizona.edu

Regulation of enzyme activity

Organisms have to control enzyme activity for proper functioning. Metabolic activity is mostly influenced by substrate concentration, but also via three other processes:

- **Allosteric regulation**: positive or negative effector molecules bind non-covalently to a non-active site, therefore producing non-competitive inhibition or activation. This causes a conformational change in the protein of the enzyme and hence alters the enzyme's affinity for its substrate or its catalytic activity.
  - Negative feedback of a metabolic pathway is an excellent example of this. The end product of a series of reactions will act as a negative allosteric effector on the enzyme that catalyses the regulated rate-limiting step of the series when it reaches a sufficiently high concentration, hence reducing production.
- **Covalent modification**: enzymes add or remove phosphate molecules to certain residues in the enzymes, again causing a conformational change that may activate or inhibit enzyme activity. Kinases catalyse phosphorylation of the enzyme using ATP as a source of phosphate, while phosphatases carry out the reverse.
- **Adjusting enzyme synthesis and degradation**: increasing or decreasing enzyme production or destruction will change the number of active sites available and hence the overall enzyme activity. This type of regulation involves protein synthesis and is therefore much slower than the other two methods, taking hours or days.

Cell signalling

Cells must be able to communicate in order to co-ordinate processes in an organism. Target cells must be able to interpret signals and respond by changing their behaviour. They detect signalling molecules (produced by the signalling cell) using receptor proteins that cause downstream reactions to cause the change. **Signal transduction** is the conversion of an extracellular signal to an intracellular effect. There are several types of signal:

- Endocrine: hormones secreted into the bloodstream by an endocrine cell, to signal to target cells far away e.g. insulin
- Paracrine: signal molecules diffuse across the extracellular fluid to nearby cells (see also exocrine, Topic 1)
- Autocrine: signal molecules affect the cell that produces them
- Neuronal communication: electrical signals move to synapses where they are converted to chemical signals (neurotransmitters)

When blood glucose is high, insulin increases the synthesis of enzymes controlling glucose metabolism. This is an example of enzyme regulation.

Cells may not respond to every signal being sent in the environment; it will only do so if it carries a specific receptor with high affinity for the signal (often on the plasma membrane). The sequence of transduction is:

Ligand (1º messenger) → Receptor → Intracellular 2º messenger → Effector protein causing change in cell

Binds to	Causes conformational change to activate enzyme producing	Starts downstream cascade of reactions activating

Small, hydrophobic extracellular signalling molecules (ligands) can diffuse across the lipid plasma membrane and bind to cytoplasmic receptors (e.g. steroids, which modulate gene expression). Large, hydrophilic ones cannot and instead bind to transmembrane receptor proteins on the cell surface to cause a signalling cascade (process above).

Secondary messengers

Some of the intracellular signalling molecules in these cascades act as molecular switches, which receive a signal causing them to become active and as a result activate other molecules downstream. There are two types of protein switches. The first type is switched on by phosphorylation (carried out by kinases, see covalent modification) and off by de-phosphorylation by phosphatases. The other type, GTP-binding proteins, are active when they have GTP bound and inactive when they have GDP bound. These proteins have intrinsic GTPase activity, so when they are activated by GTP binding they quickly hydrolyse GTP to GDP and switch themselves off.

Receptors: G-protein coupled receptors

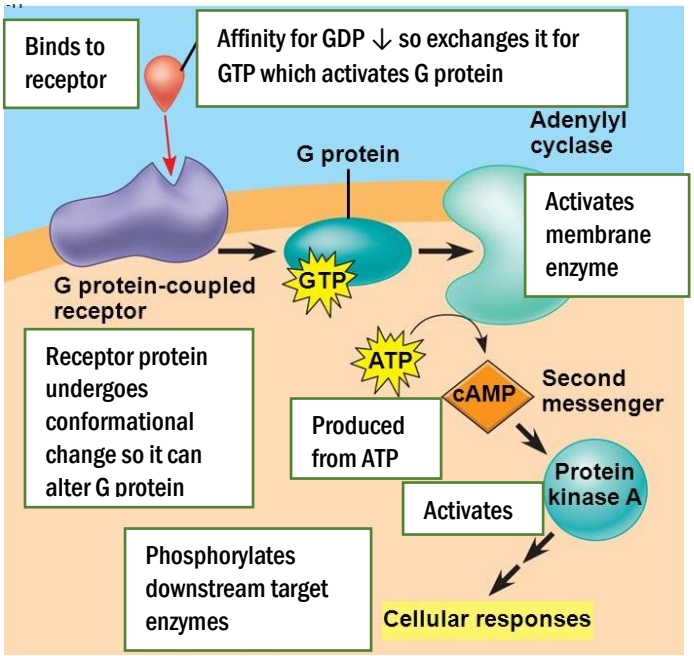
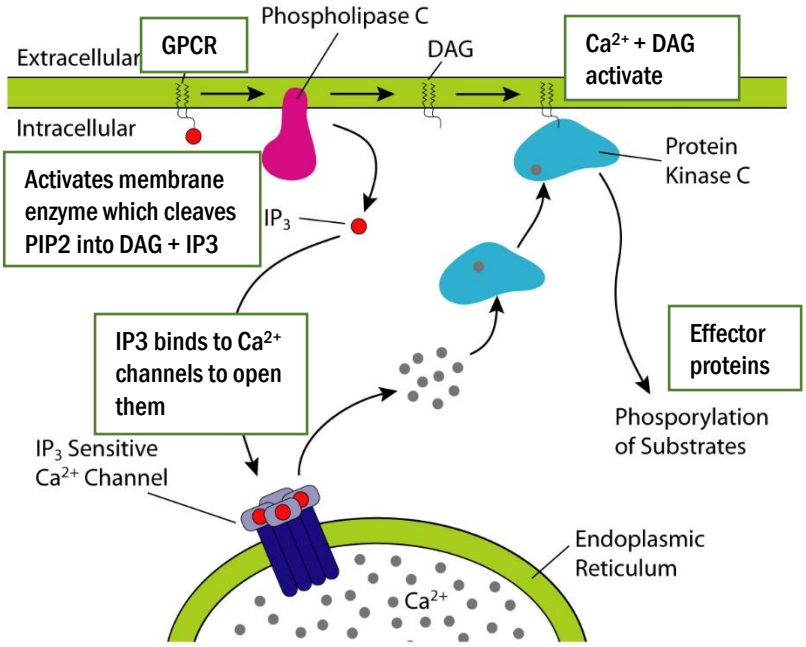


Image credit: Pearson Education Inc.

GPCRs commonly activate two membrane-associated enzymes: **adenylyl cyclase** and **phospholipase C**, which activate different downstream effector pathways.

The G protein is composed of three subunits – α, β and γ. When the α subunit binds GTP and becomes activated it dissociates from the other two to activate them, allowing them to go on and activate other molecules.

The α subunit is a GTP-binding protein (see above) so switches itself off after a period of time and the G protein returns to its inactivated state.

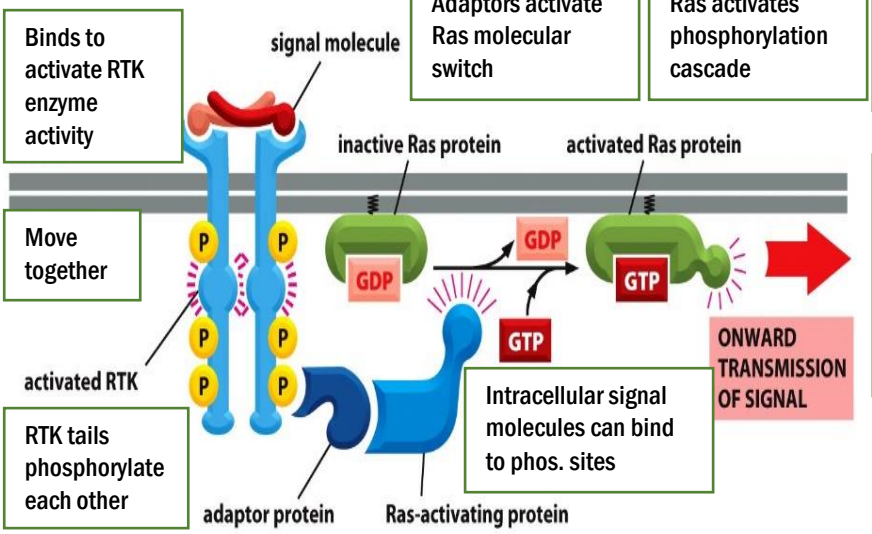


Calcium is ordinarily concentrated in the ER and ECF, giving a steep electrochemical gradient for it.

Increased cytoplasmic calcium is detected by Ca response proteins such as calmodulin. When they bind together it causes a conformational change which allows the protein to wrap around target proteins to activate them, such as CaM-kinase, which phosphorylates others.

Image credit: Pearson Education Inc.

Receptors: Enzyme-coupled receptors



Receptor Tyrosine Kinase (RTK) is a kinase enzyme, with tails in the cytoplasm that act as receptors for the 1° messenger.

Ras is a GTP-binding protein (see above) and is the α subunit of G proteins. This diagram shows the cascade of signals where various molecules activate each other in turn.

Image credit: Essential Cell Biology 3rd Ed.

One of the phosphorylation cascades activated by switching on Ras includes MAP kinases, which stands for mitogen-activated protein kinases. Mitogens are 1° messengers that promote cell proliferation, and this cascade eventually leads to signals that cause gene transcription in the nucleus.

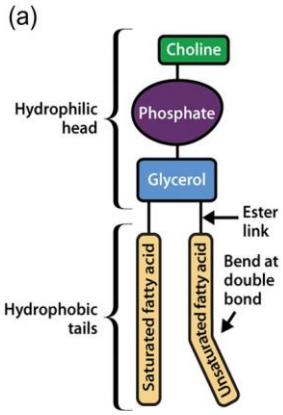
Ras → MAPKKK → MAPKK → MAPK → downstream signalling molecules

The sequence of phosphorylation activation via MAP kinases

If the Ras molecular switch is inactivated and unable to respond to ligand bonding, cells cannot proliferate. On the other hand, if the intrinsic GTPase activity of the Ras protein is faulty, it will always have GTP bound and hence be constantly activated, causing continued cell proliferation. Cancer is uncontrolled cell growth due to DNA damage: mutations inactivating the GTPase activity of Ras, so it cannot switch itself off, may be found in cancer cells. (see Topic 9)

Cell membranes

The function and structure of biological membranes was explored in Topic 1.



Phosphatidylcholine, the most common phospholipid. Image credit: biochemistry.org

The composition of these selective barriers varies according to their function and location within the cell. The lipid bilayer is asymmetric and fluid, meaning there is lateral movement, flexion and rotation of lipid molecules (but movement between the two monolayers is rare). This fluidity depends on the nature of the hydrocarbon tails of the phospholipid molecules. Hydrocarbon chains containing double bonds (known as unsaturated) have kinks, which prevent close, regular packing of the phospholipids and thus give rise to more fluid membranes. Saturated tails give a more viscous bilayer. Glycolipids are always in the external monolayer (see glycocalyx, Topic 1) while lipids on the interior tend to contain phosphate. This is asymmetry.

Cholesterol is a small, rigid steroid molecule that also has an impact on membrane fluidity by filling the spaces between hydrocarbon tails where the kinks have left gaps. It makes the bilayer less fluid and less permeable. The significance of this comes into play in cell signalling, dictating whether or not signalling proteins can move through the bilayer. Greater fluidity also permits membrane fusion and transport in this way.

Proteins within the membrane are functional, not structural, encompassing enzymes, transporters receptors, and anchors. Membrane proteins can be transmembrane/ integral (spanning the whole bilayer) or associated with one monolayer (peripheral).

Transport

ATP-binding cassette: an ATP-driven pump that moves substrates such as **drugs** out of a cell. ABC inhibitors could prevent **multi-drug resistance**.

The cell membrane controls the exchange of molecules between the interior of the cell and the external environment. The ion composition is very different on either side of the membrane, and this must be controlled for cellular processes. Na^+, Cl^- and Ca^{2+} are concentrated in the extracellular fluid while K^+ is enriched inside cells.

This produces both a concentration gradient and a voltage gradient across the cell membrane for each ion. These two forces combined are known as the electrochemical gradient. When both components are acting in the same direction across the membrane, the EC gradient is very steep; but in the opposite case, the EC gradient is smaller.

The overall difference in voltage across the membrane is known as the membrane potential, where the inside of the cell is negatively charged relative to the outside (see Topic 5) so a cell will strongly pull positive ions (Na^+) in.

Transport method	Molecules	Process
Simple diffusion (down concentration gradient)	Small, non-polar; CO_2, O_2	The hydrophobic bilayer prevents large or water-soluble molecules simply passing through, but some very small, uncharged, polar ones can do so due to their size (e.g. water, ethanol).
Channel proteins – facilitated diffusion (down conc. grad.)	Small, charged (ions)	Transmembrane proteins have a hydrophilic inner lining due to polar amino acid side chains. These form channels allowing selective passage through the membrane for specific ions. They may be opened by signals such as ligand binding or voltage.
Transporters – facilitated diffusion (down conc. grad.)	Various (including large, polar)	Transmembrane proteins have an active site to which specific molecules bind. As a result, the bonds within the protein alter and this conformational change transfers the molecule to the other side of the cell membrane.
ATP-driven pump - active transport (against EC grad.)	Na^+/K^+ /ATPase , Ca^{2+}/ATPase	Na^+ binds to the pump so it hydrolyses ATP to ADP (giving energy). The pump's phosphorylation causes a conformational change in it, moving 3 Na^+ out of the cell and binding 2 K^+. This causes de-phosphorylation and restores the pump to its original conformation, moving K^+ into the cell.
Coupled transporter - active transport (against EC grad.)	Glucose/Na^+, Ca^{2+}/Na^+	A pump couples the transport of a solute down its EC gradient with the movement of another against it. In the glucose/Na^+ symport, Na^+ moving down its steep EC gradient drags glucose in the same direction, against its EC gradient. This glucose then moves on via a uniport, and the Na^{2+} through **Na^+/K^+/ATPase**. In the Ca^{2+}/Na^+ antiport, the movement of Ca^{2+} out of the cell against its EC gradient is powered by the movement of Na^+ into the cell following its EC gradient (in the opposite direction).

Topic 5: Neuroscience

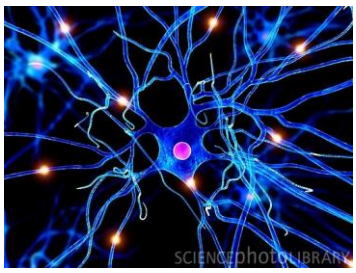


Image credit: Science Photo

LEARNING OUTCOMES:

- nervous system structure and function, from cells to systems
- ionic composition of intracellular and extracellular fluids
- electrical properties of cell membranes, resting gradients, excitability
- action potentials and voltage-gated ion channels; determinants of conduction velocity
- sensory inputs, transmission and spatial and temporal integration of information, synapses
- neurotransmitters: synapses as drug targets
- skeletal muscle cells and neuromuscular junctions; motor units and contraction
- cardiac and smooth muscle cells: reflexes

Nerves and muscles are made up of discrete, charged, excitable cells that use currents (the movement of ions in a biological solution) to detect stimuli, conduct signals and carry out responses.

Law of dynamic polarisation: there is a preferred cell-to-cell direction in which currents move

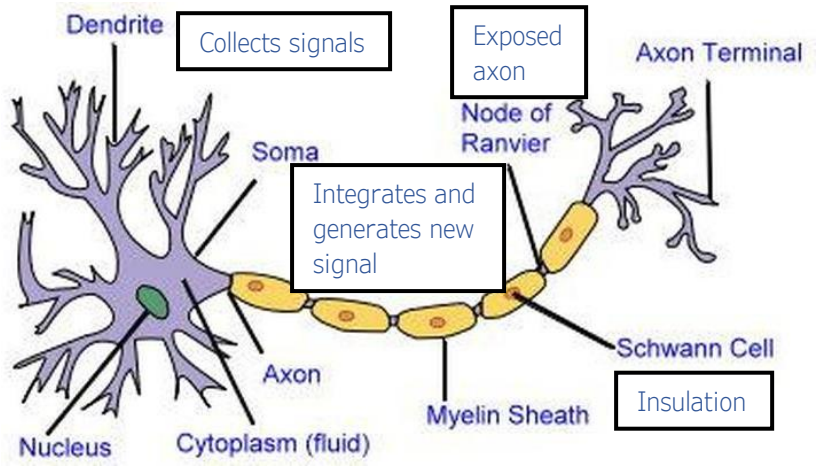


Image credit: en.wikibooks.org

General motor neurone

Sensory neurones (afferent, dorsal)

↓ (input)

CNS

↓ (output)

Motor neurones: muscles or glands (efferent, ventral)

Specialised glial cells

Astrocytes: fill spaces between neurons in brain and are most numerous cell in organ. Mop up chemical messengers that accidentally diffuse into intercellular space to stop signal going to wrong cells.

Schwann: oligodendroglia, make up myelin – wrap repeatedly around axon to insulate it, speeding up transmission

Electrical excitability

The Nernst Equation finds the potential for only one ion. You will not have to learn it for the exam as the computer calculator is extremely simple.

- The movement of ions across a membrane can only occur through pumps (active) or channels (passive) (see Topic 4).
- K^+ and negatively-charged proteins are concentrated in the cytoplasm while Na^+, Cl^- and Ca^{2+} are amassed in the ECF, giving the interior of cells a net negative charge relative to the outside environment.
- The Na^+/K^+/ATPase pump is particularly important in maintaining this distribution for biochemical purposes.
- At rest, leak potassium channels are open (these are not voltage-dependent) and respond to pH, oxygen potential and mechanical stretch
- At rest, sodium channels are closed

Setting the resting potential

When not excited, K^+ moves out of the cell down its conc. gradient

Proteins are too large to diffuse so the cell has a negative charge

This charge causes K^+ to flow back into the cell, down its EC grad.

It then reaches equilibrium (no **net** movement) at -65mV.

Action potentials

Action potentials are short pulses of electricity fired when a neurone is stimulated. They are propagated along its length, carrying information.

- The information is encoded in the frequency and patterns of the APs
- The firing rate (not amplitude) shows the intensity of the stimulus
- A sequence of APs shows a long stimulus
- APs give fast, long-range, precise communication (compared to hormonal/chemical messages)
- The current produced has to reach a threshold to generate an AP ("all or nothing").
- A charged cell is one poised to be depolarised (which is what produces action potentials)

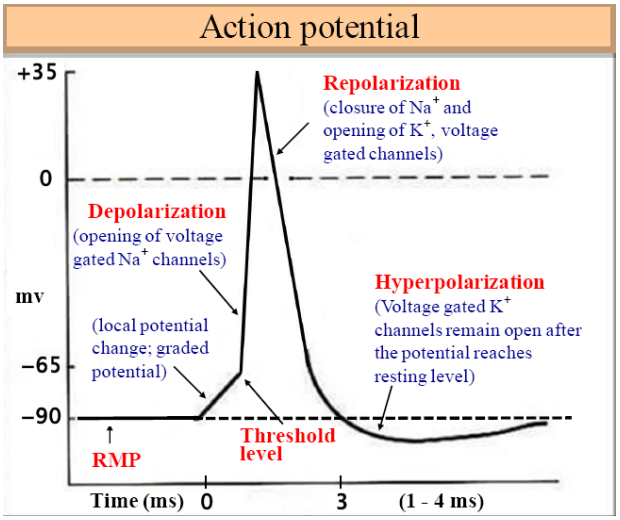


Image credit: howmed.net

After-hyperpolarisation is when the K^+ tries to reach its own equilibrium potential of -80mV after repolarisation, so the membrane potential undershoots before the K^+ VGICs can close.

Depolarisation: a change of polarity in the membrane potential (voltage) of a cell

Voltage-gated ion channels

Voltage-gated ion channels are sensitive to changes in membrane potential.

- Na^+ VGICs are closed at rest to maintain the resting membrane potential
- A stimulus depolarising the membrane to -40mV distorts the protein to open it specifically to Na^+ ions
- This rapidly causes much faster depolarisation for 1ms
- The channel inactivates by covering the pore and then closes when the protein returns to its resting configuration after repolarisation of the membrane. The physical pore cover is removed.

K^+ VGICs are much slower to open after depolarisation, as entry of K^+ resets the membrane potential to resting. During the refractory period after an AP the cell cannot generate another AP, so that depolarisations are discrete.

Action potential conduction along neurones

In unmyelinated neurones, depolarisation at one point of the membrane (production of an action potential) sets up local circuits, so depolarisation continues as a wave down the length of the neurone – propagation.

In myelinated neurones, APs are propagated in salutatory conduction. Between myelin (insulating sheaths of Schwann cells), there are gaps called Nodes of Ranvier where there is a high density of ion channels. APs are initiated in an axon hillock and jump from one node to the next in a much faster method than unmyelinated.

Myelin, increased axon diameter and higher temperatures increase conduction velocity.

Sensory input

- Mechanoreceptors: unmyelinated fibres in the skin sensitive to stretch/bend/pressure
- Mechanosensitive ion channels: gates opened by stretching of membrane

Dermatome = the area of skin with innervation supplied by one nerve (do not need to know these)

Touch receptors	Stimulus detected
Free nerve endings	Pain, temperature, crude touch
Meissner's corpuscles	Touch, dynamic pressure
Pacinian corpuscles	Deep pressure, vibration
Merkel's discs	Touch, static pressure
Ruffini's corpuscles	Skin stretching
Muscle spindles	Muscle length
Golgi tendon organs	Muscle tension
Joint receptors	Joint position

Neurotransmitters

Neurones exchange information via sending chemical messengers across synapses (gaps between neurones). Synapses allow fine-tuning and integration of signals.

A neurotransmitter is an endogenous chemical messenger that conveys neuronal information from a pre-synaptic terminal to its post-synaptic target. Many different molecules act in this way.

- Enzymes for the synthesis of NTs are produced in the neurone cell body and move down the axon on microtubules
- They produce the NT from precursors in the pre-synaptic terminal, which is then stored in vesicles
- These bind to the cell membrane at the synapse (docking)
- When an action potential reaches the terminal, the membrane is depolarised
- This causes voltage-gated calcium channels to open
- Ca^{2+} makes the NT vesicles fuse with the membrane (calcium sensing)
- The NT is released into the synaptic cleft (exocytosis)
- It binds to receptors on the post-synaptic neurone (below)
- Enzymes break down the NT and its constituents are taken up into the pre-synaptic terminal for re-use
- A new empty vesicle is pinched off the membrane into the pre-synaptic terminal (endocytosis)
- This is filled with NT (loading) for the next AP

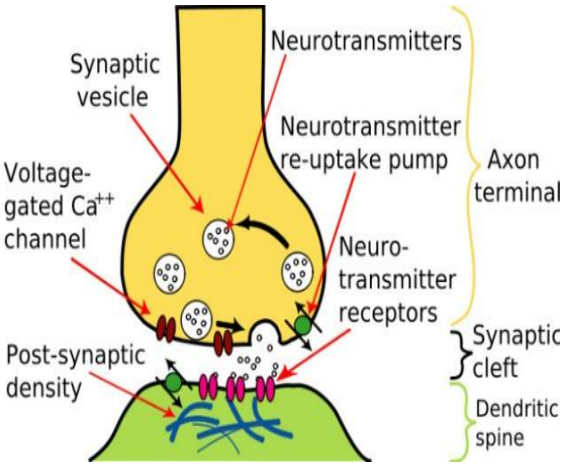


Image credit: brainimmune.com

Endogenous = originating from within an organism

Receptors

Ionotropic receptors are **fast** ligand-gated ion channels that open when the NT binds

Metabotropic receptors are **slow** because they activate a second messenger system (via GPCRs, see Topic 4)

The NT released at a synapse governs whether it is excitatory (acetylcholine, noradrenaline and glutamate increase the excitability of the post-synaptic neurone) or inhibitory (GABA and glycine do the reverse).

At an excitatory synapse, if sufficient NT binds to Na^+ ionotropic receptors the membrane will depolarise to produce an excitatory post-synaptic potential (EPSP). IPSPs occur at inhibitory synapses because Cl^- channels are opened instead, further polarising the membrane (making it more negative instead of reversing its voltage, so it is harder to generate an action potential) e.g. a GABA-gated Cl^- channel (ionotropic receptor).

These allow EPSPs to add up and, if the threshold potential is met, generate an action potential.

Integration of signals

Spatial summation is when a neurone combines multiple EPSPs from different synapse connections

Temporal summation is when a neurone combines multiple consecutive EPSPs from the same synapse

Synapses as drug targets

- **Agonists** are molecules that produce the same effect at receptors as endogenous NTs
- **Antagonists** are molecules that block the effect of NTs
- Nicotinic receptors are ionotropic receptors that respond to acetylcholine (ACh). They are named for another of their agonists, nicotine, and are antagonised by curare.
- Muscarinic receptors are metabotropic ACh receptors that are agonised by muscarine and antagonised by atropine.
- GABA inhibitory receptors are agonised by ethanol and other depressants which reduce stimulation.

This will come up again in Pharmacology (Topic 10).

Muscle innervation

Also important in Pharmacology (Topic 10).

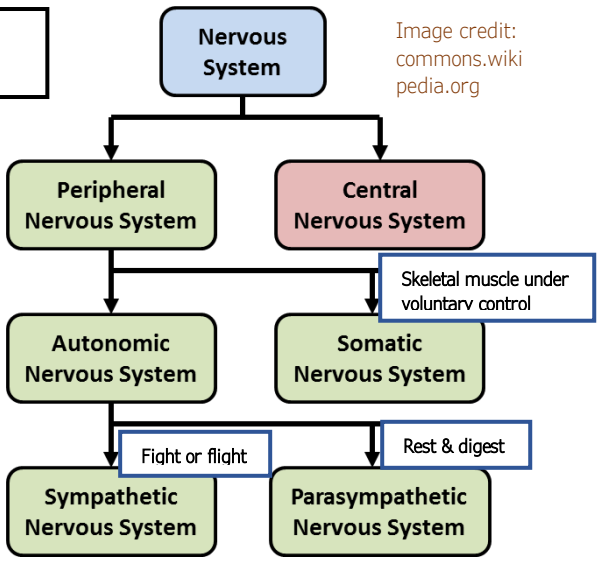


Image credit: commons.wikipedia.org

Somatic nervous system

The SNS signals to **skeletal muscle** to contract and is under conscious control, so it reflects **behaviour**. Just one nerve fibre connects the CNS to the skeletal muscle it innervates and is known as a somatic motor fibre.

In the horn of the spinal cord, axial muscle neurones are medial to those of distal muscles. Flexor muscle neurones are dorsal to extensor muscle neurones.

The motor end plate is the pre-synaptic terminal for one skeletal muscle fibre. Many motor end plates split off from one nerve so that it can innervate the whole muscle and co-ordinate its contraction.

A motor unit is an alpha motor neurone from the spine and the fibres of the muscle that it innervates (causes to contract) which then splits into many synapses (motor end plates).

The motor neurone pool is the set of alpha motor neurones that innervate one muscle, so damage to a single motor unit will not prevent normal muscle activity.

Muscle fibre = one muscle cell

Autonomic nervous system

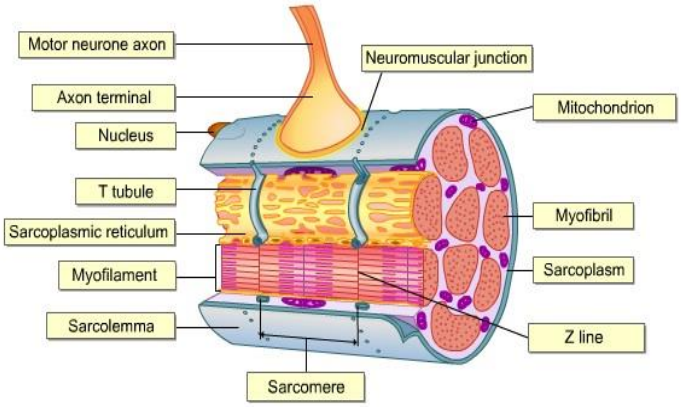
The ANS controls involuntary reactions and innervates **smooth muscle**, **cardiac muscle** and **gland cells**. The cell bodies of these neurones are clustered in ganglia, a chain of which runs down beside the spinal cord. It has two components which are always acting in different proportions depending on the situation: the sympathetic and parasympathetic nervous systems.

Sympathetic nervous system: stress (such as being chased) causes stimulation of sympathetic neurones that initiates a huge variety of responses necessary to deal with the situation: the heart rate increases to pump more blood to skeletal muscles, the airways dilate to get more oxygen and digestion is inhibited so that energy is not used unnecessarily. This is known collectively as the "fight or flight" response, evolutionarily preparing the body to fight or run away from a predator. Pre-ganglionic neurones release ACh and post-ganglionic release noradrenaline (NA), so the receptors within the ANS are known as adrenoceptors. Adrenaline (Adr) stimulates the body using this pathway and as such is used to treat cardiac arrest.

Parasympathetic nervous system: has the opposite effect on the body to the sympathetic NS and dominates during rest, so reduces the heart rate and promotes digestion and excretion. All neurones within this system release ACh and neurones travel long distances to target organs, since most originate in the cranial (neck) spinal cord.

Muscle contraction

Image credit: biomhsblogspot.com



The contraction of muscles leads to the shortening of muscle fibres and a variety of actions. Smooth muscle is present in blood vessel walls and the digestive tract lining, so this action impacts on bloods pressure and gut motility as well as body movement, heart contractility and a plethora of other bodily functions.

The synapse between the neurone and the muscle fibre is known as a neuromuscular junction. ACh crosses this synapse and stimulates receptors to initiate contraction, then is degraded by acetylcholinesterase enzymes so that the products, acetic acid and choline, diffuse back to the pre-synaptic neurone to be re-synthesised into ACh.

Muscle contraction at a molecular level depends on the movement of myofilaments past each other in the cell cytoplasm to shorten the fibre. Thick myosin filaments have heads which can bind to sites on thin filaments of actin.

- The EPSP generated in the post-synaptic membrane (sarcolemma) travels through the T-tubules (transverse tubules) into the fibre
- This causes the Ca^{2+} channels of the sarcoplasmic reticulum to open
- Ca^{2+} diffuses into the sarcoplasm and binds to troponin C so that the myosin heads are free to attach to binding sites on the actin filament
- This binding causes a conformational change in the heads, which pivot and slide the actin filament along the myosin filament to shorten the sarcomere
- ATP is hydrolysed so the head detaches and swings back to its original position, ready to repeat the process for as long as APs and Ca^{2+} are present

Sarcolemma = excitable cell membrane covering a muscle fibre (one cell) that has infoldings at intervals known as T-tubules

Sarcoplasmic reticulum = membrane sac storing calcium

Sarcoplasm = cytoplasm of muscle cell

Sarcomere = contractile unit between two Z lines

Reflexes

A reflex is an involuntary movement in response to a stimulus. Instead of being processed by the brain and producing a conscious response, they travel through a reflex arc as far as the spinal cord and back.

- Myotatic: in antagonistic pairs of muscles, they are innervated so that one is inhibited when the other is excited to allow movement, e.g. in the knee jerk reflex so that extensors contract and flexors relax
- Crossed extensor: one leg extends when the other flexes, so gives stability when one leg is moved from pain
- Vestibulo-ocular: when the head is rotated, extraocular muscles (around the eyes) are inhibited on one side and excited on the other to fix the position of eyes (and hence line of sight)

Clinical conditions

Disorders of electrolyte balance (ions in solution) - change membrane resting potential and excitability

Condition	Impact on ECF	Causes	Symptoms
Hyponatremia	↓ Na^+ ∴ ↓ excitability as less Na to move in for depolarisation	Diuretics, cirrhosis, CHF, renal disease (salt reabsorption)	Cramps, weakness, fatigue, confusion, reduced consciousness/coma *brain oedema*
Hypernatremia	↑ Na^+ ∴ ↑ excitability (reverse of above)	Water loss: renal failure, fever, vomiting, diarrhoea	Tremor, seizures, hyper-reflexia, thirst, lethargy, convulsions *brain shrinkage*
Hypokalaemia (most common)	↓ K^+ ∴ ↓ excitability as K will diffuse out of cell so harder to depol.	Diuretics, cirrhosis, renal disease, malnutrition or malabsorption in GI tract	Mild: weakness, fatigue, constipation, *arrhythmias*. Severe: paralysis of muscles, including in vital systems
Hyperkalaemia	↑ K^+ ∴ ↓ excitability as less polarised	Drug interactions on kidney function	Impaired vital organ systems, *cardiac arrest*, ventricular fib arrhythmia

- Disorders of ion channel activity and excitability: channelopathies are diseases which affect ion channels (e.g. mutations) and thus depolarisation/repolarisation of cells. Na^+ channel blockers are used as local anaesthetics, preventing pain signals being sent. Peripheral nerve blocks inhibit one nerve, epidurals are injected at the spinal level and Bier's blocks are infused into a whole limb.
- Disorders of action potential conduction: demyelinating diseases damage the Schwann cell insulation of neurones so ↓ AP conduction e.g. multiple sclerosis, Charcot-Marie-Tooth disease, Guillain-Barré syndrome (autoimmune).
- Disorders of peripheral nerves: diabetic and autonomic neuropathies damage nerves themselves, while neuritis is inflammation of cranial nerves leading to loss of function: Bell's palsy and trigeminal neuralgia.
- Disorders of synaptic transmission: in myasthenia gravis, the immune system attacks ACh receptors so signals cannot be sent at the neuromuscular junction, leading to weakness and fatigue.
  - Uses of drugs at synapses are explored in more detail in the Pharmacology topic
- Disorders of reflex excitability: hypo-reflexia may be caused by lower lesions on or loss of motor neurones, thyroid deficiency or denervation of muscles. Hyper-reflexia can result from upper motor neurone lesions, spinal injury, brain haemorrhages or drug abuse. Spinal shock (paralysis) follows spinal cord trans-section.

Topic 6: Microbiology

LEARNING OUTCOMES:

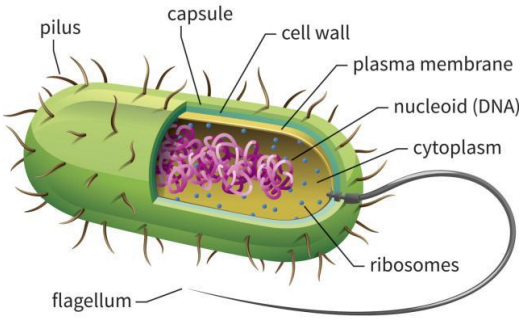


Image credit: about.com

- properties of different classes of infectious agents
- bacterial structure (Gram-positive and Gram-negative)
- bacterial growth, replication and gene transfer
- virulence and determinants of virulence
- mechanisms of pathogenesis (molecular, cellular)
- normal flora, and how normal balance of the body flora can alter and lead to infection
- host-pathogen interactions
- virus structure, genetic material, and replication
- mechanisms of viral disease

Infection is the invasion of a host organism by a pathogen. These agents often multiply and their actions can have numerous effects on host tissue, as can the immune response itself towards the microorganism. The mechanism of this host response is explored in much greater detail in Topics 7 and 8, so this section will focus on the five main groups of infectious agents explored in this module: bacteria, viruses, fungi, prions and parasites.

General pathogenesis

- Pathogenicity is the ability of an agent to damage the host, while virulence is the relative ability of this in comparison to others. This is produced by virulence factors e.g. toxins, adhesins, capsules, anti-phagocytics.
- Disease occurs when pathogens are not controlled by host defences (because of wounds in the skin barrier, antibiotics destroying natural competitive flora or foreign objects). The response is influenced by environmental and host factors such as age, genetics, immunocompetence and other conditions.
- Primary pathogens can instigate disease in healthy subjects, while opportunistic ones (especially fungi) do so in immunosuppressed organisms when host defences are reduced.
- The host response often involves inflammation and innate and adaptive immunity (see Topics 7). These can be damaging themselves, in addition to the direct pathogen damage, if the response is excessive.
- The brain, muscle, bone and bladder are the few areas without commensals (natural bacterial flora).

Bacteria

- Bacteria are single-celled prokaryotes (organisms with no membrane-bound organelles). They multiply by either binary fission (splitting) or spore formation (spores are resistant to heat and sterilisation, e.g. C. diff).
- DNA is carried free in the cytoplasm and additional genes are carried on plasmid loops. Horizontal gene transfer can confer genetic information for other abilities between bacterial cells, by either transformation (release of free DNA), transduction (DNA carried by a virus that can invade bacterial cells) or conjugation (DNA moves through a pilus that physically connects the cells).
- Bacteria have cell walls containing peptidoglycan, chains of which are linked by peptide bridges (which are an antibiotic target). This aspect of cell wall structure can help to classify cells using a Gram stain. The PG layer is thick in some bacteria and it stains blue-black (may look purple), so they are known as Gram-positive bacteria. In the remaining species, the PG layer is much thinner and has a lipopolysaccharide layer surrounding it, so they stain pink and are called Gram-negative. This blocks the PG cell wall and so makes them resistant to penicillin and vancomycin. LPS is an endotoxin that triggers harmful inflammation.
- Bacterial cells can be round (**cocci**), rod-shaped (**bacilli**), curved or spiral-shaped.

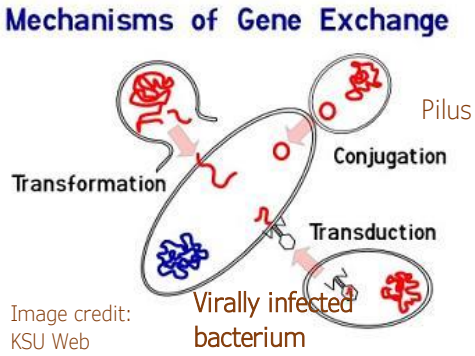


Image credit: KSU Web

The **tree diagram** at the end of this chapter categorises all of the bacteria on the syllabus.

Gram-positive - cocci

Sensitive to penicillin/vancomycin

Episome: plasmid that can replicate independently

Staphylococcus

- **S. aureus** is the main coagulase-positive species in this genus (test for the enzyme to identify it), and it stains golden on a blood agar plate. It produces pus-forming soft tissue infections that are carried in the bloodstream, and its importance lies in the methicillin-resistant strain (MRSA), a major healthcare-acquired infection. This is via its abundance of virulence factors (ways in which it survives):
    - Protein A on the cell wall helps evade phagocytosis by immune cells
    - Coagulase enzyme produces a fibrin coat/capsule that protects against phagocytosis
    - Hyalurorindase enzyme breaks down host tissue
    - Haemolysins (exotoxin) that produce pores in red blood cells
    - Toxins causing toxic shock, boils and pneumonia
- **S. epidermidis, S. capitis and S. saprophyticus** are coagulase-negative and are only pathogenic on a foreign body such as a prosthetic.

Streptococcus

-itis = inflammation

α-haemolytic (incomplete haemolysis on a blood agar plate, showing a green colony):

- **S. pneumoniae** causes pneumonia, meningitis and bronchitis in COPD patients
- **S. oralis and S. salivarius** are viridans natural mucosal flora, which can cause endocarditis if they infect valves

β-haemolytic (complete haemolysis on a blood agar plate, so clear when held up to light), classed by wall toxin:

- Group A: **S. pyogenes** can cause tonsillitis, cellulitis, soft tissue necrosis and puerperal sepsis, and some of the toxins it produces lead to scarlet fever and toxic shock syndrome. Other virulence factors include phagocyte-resistant M protein in the cell wall and various destructive enzymes.
- Group B: **S. agalactiae** is part of the normal vaginal/rectal flora but can cause neonatal meningitis, bacteraemia and pneumonia plus fever in labour.

Bacteraemia = bacteria in blood

Enterococcus

These invade the gut to elicit urinary tract infections (UTI) and intra-abdominal sepsis. Poor prognosis if in blood.

Gram positive – bacillus

Bacillus

- **B. anthracis** from soil has multiple toxins/virulences and causes anthrax by inhalation or cutaneous entry.

Listeria

- **L. monocytogenes** from soft cheese in pregnancy causes intrauterine or neonatal septicaemia or meningitis.

Corynebacterium

- **C. diphtheriae** is the most pathogenic of the normal skin commensals and is transmitted via respiratory droplets. Inflammation of the throat pseudomembrane can cause suffocation and bull neck. Exotoxins inhibit protein synthesis in the host, leading to cell death in the heart and peripheral nerves.

Gram positive - anaerobes

Sensitive to metronidazole

These bacteria are soil organisms and commensals that become opportunistic when in damaged tissue or abnormal locations. They have a putrid odour and are sensitive to metronidazole.

Clostridium

- **C. tetani** invades wounds. Tetanus produces an exotoxin that stops the release of the inhibitor GABA, hence neurones are constantly excited. Voluntary = rigid paralysis, autonomic = hyper-sympathetic state.
- **C. botulinum** via food. Exotoxin stops ACh release so leads to flaccid paralysis, leading to use in botox.
- **C. difficile** alcohol-resistant spores cause CD colitis (diarrhoea), which is often a result of antibiotic use.
- **C. perfringens** contains damaging enzymes (a-toxin = lysis) causing soft tissue damage and food poisoning.

Gram negative – cocci

Resistant to penicillin/vancomycin

Neisseria gonorrhoeae, **Neisseria meningitidis** and **Moraxella cattharalis** (causes COPD exacerbations) are obvious.

Gram negative – bacilli

Fastidious – grow slowly and require enriched agar

- **Haemophilus influenzae**: uncapsulated causes pneumonia, capsulated causes meningitis
- **Legionella** species are carried by amoebae in water and causes severe pneumonia
- **Helicobacter pylori** produces buffers stomach acid to survive and causes gastritis, which produces ulceration

Non-fastidious sugar fermenters (enterobacteriaceae commensals)

- **Escherichia coli** has filaments that make it stick in GI and urinary tracts. Also produces haemolysins and anti-phagocytic capsule. Causes UTI, diarrhoea or bacteraemia.
- **Klebsiella** causes UTI, bacteraemia, pneumonia and liver abscesses.
- **Shigella** causes gastroenteritis and colitis in humans only.
- **Salmonella typhi** causes typhoid and traveller's diarrhoea, while non-typhoid species cause gastroenteritis.

Non-fastidious non-fermenters

- **Pseudomonas aeruginosa** has a huge range of virulence factors and AB resistance mechanisms. It complicates cystic fibrosis and leads to ventilator pneumonia plus bacteraemia in the immunocompromised.
- **Burholderia cepacia** has a very poor prognosis in cystic fibrosis patients.
- **Vibrio cholerae** is carried in water and produces rice water stools. Toxin increases cyclic AMP and so reduces ion concentration in GI cells, so water is not absorbed by osmosis and leads to diarrhoea and dehydration.
- **Campylobacter** from contaminated animals causes most bacterial gastroenteritis – self-limiting diarrhoea.

Gram negative - anaerobes

Sensitive to metronidazole

- **Bacteroides** are gut/mouth commensals but cause intra-abdominal/skin/soft tissue infections below the waist
- **Fusobacteria** cause head/neck infections (brain abscesses) and mixed intra-abdominal infections

Small virus-like bacteria

- **Mycoplasma pneumoniae** causes penicillin-resistant pneumonia, so treat with clarithromycin
- **Chlamydia trachomatis** is an STI exhibiting genital inflammation ("chlamydia", treat with macrolides)
- **C. pneumonia** and **c. psittaci** both cause pneumonia that is treated with macrolides
- **Rickettsia** species are transmitted by parasites and present as spotted fever or typhus (rash, fever, headache)
- **Mycobacterium tuberculosis** causes a pneumonia with latency and requires intense combination treatment

Most microorganism names hint at the disease they cause, so apply this knowledge in the exam.

Viruses

Nucleic acid + protein coat = nucleocapsid

Viruses consist of single-stranded or double-stranded DNA or RNA inside a protein coat (capsid, made up of capsomere subunits). They may also contain enzymes (if not present in the host) or a cell membrane-derived coat from the host. They are not considered alive until they have invaded a host cell and use its functions to replicate. Families of viruses exhibit similar nucleic acid features, symmetry and structures so they are easy to categorise.

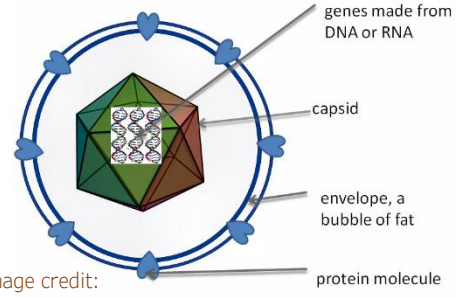


Image credit: Wikipedia.org

Replication can occur anywhere except the blood: sense nucleic acid strands (1 mRNA) can go straight to ribosomes for protein synthesis, while antisense cannot. Various modes of propagation as well as chemical markers aid virus identification. Host transmission routes include oral, skin, droplet, sexual, trans-placental (vertical) or directly through trauma, injection or bites. Modes other than trans-placental (between generations) are known as horizontal.

Replication: Virus adsorbs to host cells via their receptor binding proteins linking to existing host receptors. The membranes fuse or the virus enters the cell via receptor-mediated endocytosis. The capsid is shed to uncoat the nucleic acid, and this replicates to synthesise more RNA and viral proteins. These are then assembled into new nucleocapsids and released; this is either by egress (cytolysis, e.g. herpes destroys neurones in this way) or budding (glycoproteins inserted into existing cell membrane which buds off to release a viral envelope).

Actions in the host

Acute lytic infection has a shorter duration and host cells burst to release the replicated viruses. Persistent infection with shedding, such as with **Hepatitis B**, is chronic and replication is slow. Latent viruses such as **herpes** may remain dormant in the host and re-activate later (such as during a cold). **HIV** is a provirus that integrates its nucleic acid into the host genome in a persistent slow infection. Transformation mutates the DNA for use in host cell growth systems, precipitating uncontrolled and increased growth, crowding, new antigens and loss of contact – the cardinal signs of cancer. For example, **Epstein-Barr virus** is associated with lymphomas (see Topic 9) while **HPV** is an agent in cervical carcinoma. Unsurprisingly, **Hep B** and **Hep C** are associated with liver cancer.

Examples:

- **Poliovirus** is composed of single-stranded RNA and a 4-capsid icosahedral coat with no envelope. It infects the gut (lymphoid tissue cytoplasm) and spreads through the blood to motor neurones. 99% of infections are asymptomatic, but when nerve cells are destroyed, meningitis and the paralysis associated with polio occur.
- In contrast, **herpes simplex virus** carries double-stranded DNA in an enveloped icosahedron. It replicates in the nucleus and uses kinesin motors to ascend neuron axons. They remain latent in these ganglion areas and later reactivate, so move back down the same axons to epithelia and produce cold sores on the skin.
- **HIV** is a retrovirus; reverse transcriptase produces DNA from its RNA and integrase inserts it into the host genome. This is irreversible, making HIV impossible to cure, and leads to AIDS by destroying leukocytes at the end of latency (8-10 yrs). Anti-retrovirals prolong life.

Antivirals can target any stage of the replication cycle and often are used in combination to fight an infection. The best-known is acyclovir, which inhibits viral DNA polymerase. The drug is activated (phosphorylated) by viral kinases and so only exerts its actions on infected cells.

Fungi

Fungi are multicellular, multinucleate eukaryotes that reproduce by budding. They have a thick carbohydrate wall of chitin, glucan and mannan. They either grow as yeasts (unicellular), filaments ("branches" with hyphae) or dimorphs (yeast in patient but filamentous in lab – severe disease in healthy people). They can also be classified by the type of infection: superficial, cutaneous, subcutaneous (tissue under skin), systemic or opportunistic. The latter is exemplified by candida, aspergillus, c. neoformans and p. jirovecci: they are only pathogenic in the immunocompromised, such as patients with congenital immune defects, AIDS or receiving transplant drugs.

Examples

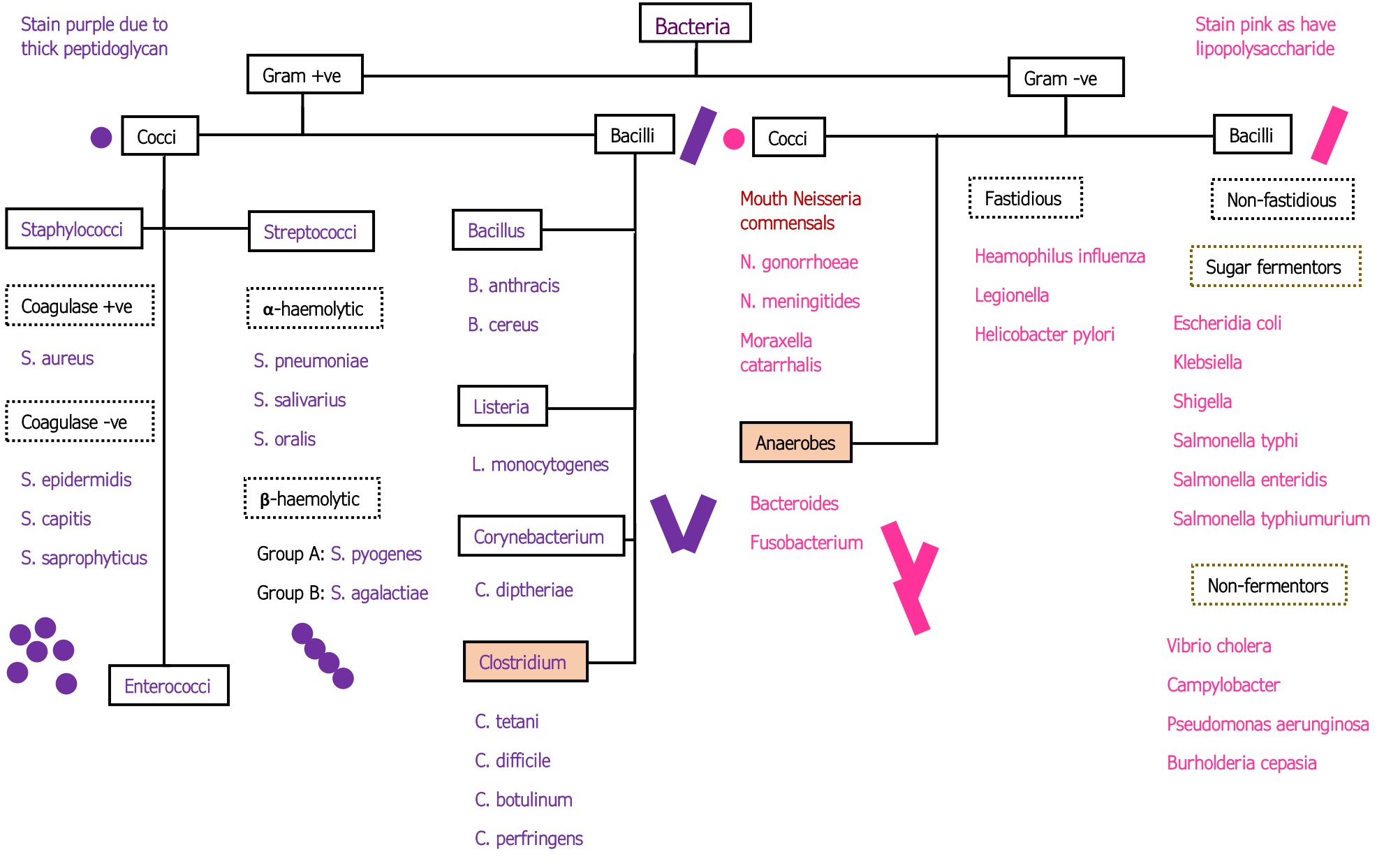
- **Candida**: causes athlete's foot (cutaneous), mucosal inflammation (thrush) and candidemia (systemic in blood). Candidemia has a high mortality and a risk of infecting the heart, eyes, liver and spleen.
- **Aspergillus**: invasive, opportunistic and often fatal; hypersensitivity = allergic bronchopulmonary aspergillosis.
- **Cryptococcus neoformans**: indicates AIDS and causes fungaemia and meningitis.
- **Pneumocystis jirovecci**: causes pneumonia and consequently hypoxia in immunocompromised patients.

Parasites

Parasites live on other organisms and benefit from this relationship at the host's expense. **Protozoa** are single-celled and cause malaria, toxoplasma and leishmania. **Helminths** are worms (which may cause schistosomiasis) and **arthropods** can carry Lyme disease or yellow fever, for example.

Prions

Prions are host proteins that are abnormally folded and so disrupt cell function. For example, they form fibrils in the brain or cause refolding of other proteins. They are transmitted by contaminated items or food and are resistant to treatment. Prions causing Creutzfelt-Jakob disease (transient dementia) can cross the species barrier.


Stain purple due to thick peptidoglycan
Bacteria
Gram +ve
Gram -ve
Stain pink as have lipopolysaccharide
Cocci
Bacilli
Cocci
Bacilli
Staphylococci
Streptococci
Bacillus
Mouth Neisseria commensals
Fastidious
Non-fastidious
Coagulase +ve
α-haemolytic
B. anthracis
N. gonorrhoeae
Heamophilus influenza
Sugar fermentors
S. aureus
S. pneumoniae
B. cereus
N. meningitides
Legionella
Escheridia coli
Coagulase -ve
S. salivarius
Listeria
Moraxella catarrhalis
Helicobacter pylori
Klebsiella
S. epidermidis
S. oralis
L. monocytogenes
Anaerobes
Shigella
β-haemolytic
Salmonella typhi
S. capitis
Bacteroides
Salmonella enteridis
Group A: S. pyogenes
Corynebacterium
Fusobacterium
S. saprophyticus
Salmonella typhiumurium
Group B: S. agalactiae
C. diptheriae
Non-fermentors
Clostridium
Vibrio cholera
Enterococci
Campylobacter
C. tetani
Pseudomonas aerunginosa
C. difficile
Burholderia cepasia
C. botulinum
C. perfringens

Topic 7: Inflammation

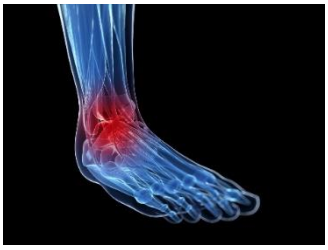


Image credit: verywell.com

LEARNING OUTCOMES

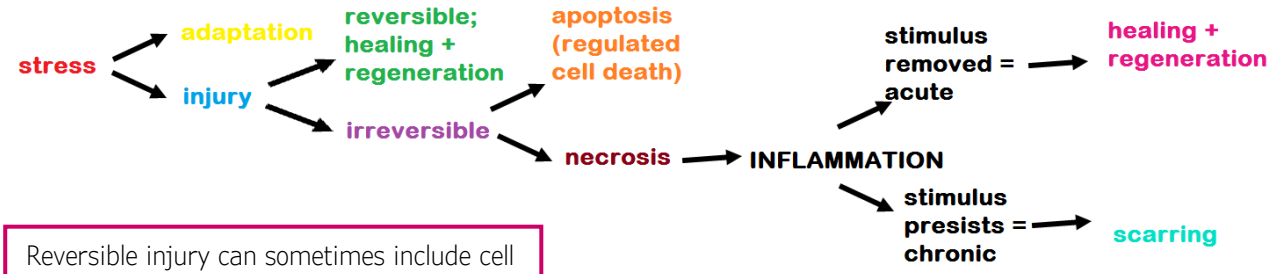
- mechanisms of cell injury: infection, hypoxia; necrosis and apoptosis
- acute inflammation: development and causes
- chronic inflammation: development, causes and consequences. Granulomas
- healing and repair especially in skin, bone, brain and heart

Inflammation is the response of living tissue to cell damage or infection so is **essential** in medicine. The physiological purposes of this vascular and cellular response are **minimisation of the injury, defence against infection** (there is a great overlap with immunology; see Topic 8), **tissue repair** and **return to normal function**. The five cardinal signs of inflammation are heat, redness, swelling, pain/tenderness and loss of function. The symptoms of inflammation are integral to this healing process but are often uncomfortable, and as such a balance must be found between allowing the body to repair and suppressing the process. When the inflammatory process goes beyond normal repair, pathologies occur, which damage tissue. Inflammation can be either acute or chronic.

Ischaemia = loss of blood supply to area; often leads to hypoxia

Cell injury

Stress describes a variety of environmental difficulties encountered by a cell. If a cell cannot adapt to it, it becomes injured, which is what causes inflammation. Stresses include chemicals/drugs, hypoxia (lack of oxygen), infection, physical agents, immune reactions, nutritional issues and genetic mutations. Mild, transient injury is reversible and can the cell can recover and return to homeostasis. Severe, progressive injury leads to cell death.



Reversible injury can sometimes include cell swelling and fat accumulation

Apoptosis = programmed cell death	Necrosis = unregulated cell death due to injury
• Energy-dependent • Only affects necessary cells • Membrane blebs (bubbles form) • Chromatin condenses • Blebs bud off to break cell up into apoptotic bodies • Fragments digested by phagocyte • **Intrinsic (mitochondrial) pathway**: removal of growth factors/hormones leads to cascade through mitochondrion, eventually producing caspases (protease enzymes) that disintegrate the DNA and cytoskeleton to cause blebbing • **Extrinsic (death-receptor mediated) pathway**: Fas death ligand binds to surface receptor to initiate protein cascade that produces caspases (above) Apoptosis is used in physiological development and homeostasis; if cells ordinarily divide, old ones may need to be destroyed	• Passive process • Indiscriminate with bystander damage • Membrane blebs but then breaks down • Cell bursts (lysis) and spills contents → **inflam** • Activated by toxins released/macrophages Types of necrosis: • **Coagulative**: microscopically, nuclei stain darker and "jelly" appearance of tissue as proteins denature, e.g. ischaemia in heart attack • **Liquefactive**: protein digested in lipid-rich tissue so tissue structure lost; pus, e.g. stroke in brain • **Caseous**: "cheese" appearance of granulomatous inflammation (see later) e.g. tuberculosis • **Gangrenous** (dry): coagulative necrosis in extremity due to slow vascular blockage e.g. toe gangrene in diabetes. Wet gangrene = infection • **Fat:** chalky deposits from degraded fat tissue e.g. acute pancreatitis

Inflammation is not a simple linear flow scheme but a complex interplay of simultaneous processes.

Acute inflammation

- → This irreversible cell injury (**necrosis**) releases signals known as DAMPs (damage-associated molecular patterns), which are molecules that trigger the inflammatory process. These include DNA, RNA and histones – things that will only be outside a cell if something is wrong!
- → PAMPs (pathogen-AMPs) on the surface of microbes trigger inflammation when infection occurs. These can be foreign cell wall components (such as lipopolysaccharide, LPS) or nucleic acids.
- → These are detected by pattern recognition receptors (PRRs) in/on immune cells which then cause the release of inflammatory mediators – histamine, prostaglandins, leukotrienes, bradykinin, cytokines (ILs, TNFs and IFNs) and chemokines. (Intial vasoconstriction occurs in the area, which appears to have little function.)
  - In trauma, mast cells in blood vessel walls degranulate to release the mediator histamine, which leads to early local vasodilatation (NB: blocking this is the action of the anti-inflammatory drug antihistamine).
  - This directs more blood carrying inflammatory components to the area, and causes **redness**.
  - Blood vessel endothelia contract and gaps open (active leak) to deliver exudate to the damaged tissue, containing white blood cells (leukocytes), opsonins, complements and fluid – this causes **swelling**.
  - Macromolecules (large) may also be transported across cells in vesicles and released in transcytosis.
  - It is more efficient to keep this defence system in the circulation and only activate it where required, due to the high energy demands of the inflammatory process.
  - Bradykinin maintains vasodilatation via endothelial nitric oxide, and also activates **pain** receptors.
- → Chemokines are a group of cytokines that recruit circulating immune cells, e.g. they promote selectins that bind neutrophils (potent phagocytes and the first leukocytes to arrive) to the venule wall, which then roll and move into tissue in transmigration down the IL-8 gradient. They engulf bacteria, dead cells and debris.
  - Capillaries are small and would get blocked by leukocyte binding, but this is not a problem in the liver or lungs as they have huge networks. This is initiated by pyrogens (bacterial molecules producing fever).
  - These also cause chemotaxis – the movement of cells along a fibrin scaffold.
  - Neutrophils are short-lived due to their short telomeres (Topic 3) – their many lysosomes are dangerous
  - Phagocytes are dynamic, varying by the info they collect about targets and responding differently
  - Phagocytosis can be challenged by slippery capsules, foreign bodies, slime or intracellular pathogens
- → Cytokines (messenger hormones) activate resident, endothelial or circulating immune cells to maintain vasodilatation and cause lysis in infected cells, so that the pathogen is exposed to the immune system.
- → Opsonins mark out cells for phagocytosis. Phagocytosis may be oxygen-dependent or oxygen-independent.
- → Macrophages (monocytes in tissue rather than blood) arrive later to phagocytose and produce cytokines.
- → The complement cascade is a series of reactions either marking bacteria for phagocytosis (opsonisation) or forming membrane attack complexes (MACs, see Topic 8). The clotting cascade may also be initiated.
- → Nerves release substance D
- → Temperature is raised to increase the rate of action of immune cells. At peripheral cutaneous sites, **heat** will be perceived as they are normally cold relative to core body temperature.
- → The last resort stimulated by PRRs is neutrophil extracellular traps. If a parasite/fungus/bacterium cannot be phagocytosed, an enzyme moves into the neutrophil nucleus, releasing its contents, which act as a net to prevent spread. Resistance: Strep. have fibrin-degrading enzymes and S. aureus hides within fibrin layers.
- → The inflammatory mediators will also bind to their complementary receptors all over the body, producing **systemic effects**. These include malaise and ↑ temperature, heart rate, respiratory rate and leukocyte count.
  - IL-1 and TNF-α (and possibly IL-6) change the hypothalamic set point by producing prostaglandins so body temp rises, leading to **shivering/fever** (aspirin blocks this, see Topic 10)
  - Acute phase proteins produced by the liver have various functions and can be measured in blood tests to indicate the level of inflammation e.g. CRP
  - IL-8 moves to the bone marrow and stimulates the production of more neutrophils

The lymphatic system is a network of fine vessels draining excess fluid from tissue. Exudate can flow through this into nodes, where lymphocytes process foreign molecules and instigate an immune response (explored in Topic 8).

The evolutionary function of pain is to signal that something is wrong, prompting us to change our behaviour; to move the affected part from the stimulus and/or rest it to allow healing.

Resident macrophages are mononuclear phagocytes that stay in one body tissue e.g. alveoli. In the liver, they are known as Kupffer cells and they search the blood for signals of injury or infection, prompting a response if necessary. They can also trap bacteria for neutrophils. Resident monocytes carry this out in

Other possible features of inflammation

Effects of fluid accumulation (oedema): third degree burns – there is no skin to hold the exudate so fluid is lost. Brain – there is no give in the skull but the brain is slack so fluid compresses it. Meningitis – leads to septicaemia or inflamed meninges (protective membranes) so brain is compressed.

Types of exudates:

- Pus: neutrophils, enzymes, bacteria and fibrin; often shows on micrograph. Yellow-green peroxidase gives it its colour
- Fibrinous: sticky fibrin strands, few cells
- Serous: mostly fluid, few features (e.g. burn blister)
- Haemorrhagic: vascular destruction, hence leak of blood

Types of lesions:

- Sinus: cavity lined with granulation
- Abscess: pus in newly-formed cavity, surrounded by granulation
- Fistula: abnormal tract linking epithelia that should not be linked
- Erosion: loss of some epithelial layer but basement membrane left to guide healing
- Ulcer: loss of epithelial and basement membrane layers (e.g. exposing muscle to inflammation); may fissure
- Granuloma: organised cluster of large macrophages with ruffled membranes, which form in response to a stimulus that cannot be removed. Low-turnover versions form around foreign bodies and have long-lived macrophages; high-turnover ones are immune-related and contain T-cells and short-lived macrophages.

Consolidation: light patches on micrograph due to exudate congestion in a tissue

Outcomes of acute inflammation:

- **Resolution**: if the trigger subsides, pro-resolving agents (immunoresolvants clearing mediators, cells, debris and pathogens) or anti-inflammatory influences terminate inflammation and the tissue is restored to its healthy condition. After resolution, changes to gene expression may change tissue response to injury.
- **Organisation**: tissues are replaced by granulation tissue (pre-scar tissue) which is more "sticky" and may be problematic. Scarring at a focus is protective, but if widespread is destructive to tissue e.g. in cirrhosis.
- **Chronic inflammation**: see below
- **Death**

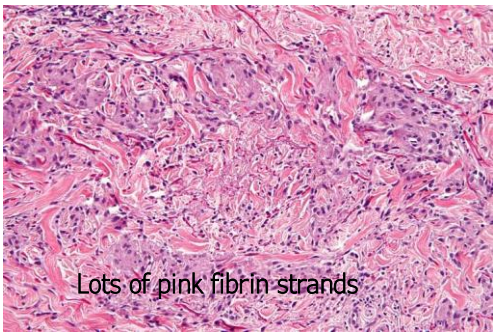
Granulation tissue: red, shiny, new connective tissue consisting of fibroblasts, new blood vessels and new ECM. It surrounds dead tissue, pus or irritants to protect body tissue and deliver nutrients.

Chronic inflammation

Definition: LONG-TERM INJURY + INFLAMMATION + REPAIR (often caused by a persistent immune response)

Macrophages and granulation tissue are characteristic features. Macrophages come in many different states in chronic inflammation, highlighting their wide range of functions in protecting the body: killing and clearing (phagocytosis), production of cytokines, acute phase reactions and antigen presentation (see Topic 8).

Image credit: answers.com



Lots of pink fibrin strands

Tuberculosis is a form of chronic inflammation as the stimulus cannot be removed. The mycobacterium has a protective coat which prohibits phagocytosis, so granulomas form around them.

Amyloidosis is the aggregation of mutated (misfolded) proteins, causing atrophy (wasting) in chronic inflammation or dementia.

Cystitis (bladder inflammation) can be caused by urinary catheters, which are both foreign bodies and reservoirs of microbes. UTIs can lead to urinary incontinence and confusion.

The hazard gradient is where high-risk responses in the blood can be damaging to tissues and may lead to septic shock. Sepsis or "blood poisoning" is the inflammatory response to infection, a cytokine storm that in the worst cases leads to septic shock (low blood pressure and inflammatory tissue damage). Immunity and coagulation is suppressed, and patients have susceptibility to 2^{o} infection. A vicious circle of necrosis and inflammation ensues.

Tissue healing

Not all tissues have the ability to **regenerate**, where new cells are produced to replace old ones. Some only **repair**, where fibrous scars patch up the damaged tissue but it does not return to full functioning.

- Labile cells: always proliferating so regenerate – epithelia such as skin, GI tract
- Stable cells: do not normally proliferate but can do so after injury – liver, kidney
- Permanent: cannot regenerate so must scar – neurones, heart muscle

In regeneration, cell numbers are regulated to balance loss and growth. If growth exceeds loss (e.g. uncontrolled cell division or interrupted apoptosis), neoplasia occurs (see Topic 9).

Regeneration can be stimulated by soluble growth factors which bind to receptors to initiate a downstream cascade of reactions (see Topic 4), cumulating in the release of transcription factors that bind to DNA and alter gene transcription. These may promote proliferation, prevent apoptosis or stimulate angiogenesis (growth of new blood vessels). Another signal for regeneration is physical cell-cell and cell-matrix interactions via the ECM and cell junctions, mediated by integrin proteins (see Topic 1) that again produce a cascade of signals.

Scarring (fibrosis)

Bleeding → Clotting cascade → Acute inflammation (neutrophils) becomes chronic inflammation (macrophages and granulation) → Fibroblasts migrate to lay down new ECM → Angiogenesis and collagen → Scar maturation

Angiogenesis is prompted by growth factors (signal hormones such as VEGF). New vessels branch off existing ones and mature.

Fibrotic tissue is weaker and less elastic than healthy tissue.

Fibroblasts are connective tissue stem cells. Growth factors cause them to migrate and proliferate. They secrete ECM components, collagen and elastin.

Tissue remodelling: the extra-cellular matrix of granulation tissue must be degraded to remodel tissue in the healing process. Matrix metalloproteinases (MMPs) are enzymes that each break down one component of this ECM. They mediate long-term scar maturation and degradation, which is why lesions can look better over time. The action of MMPs is regulated by TIMPs, which inhibit their action.

Pathology of symptoms

Toxins from infection and the inflammatory response itself may destroy tissue and require repair.

Pus often shows on a micrograph as fibrin and many nuclei of neutrophils. The consolidation of exudate (fluid and defensive cells) in airways blocks them, and patients may have breathing difficulties leading to hypoxia.

Destruction of blood vessel walls can allow blood to leak into airways, can may be coughed up with sputum. Bleeding from any abnormal area must ALWAYS be investigated by a doctor.

The inflammatory response releases cytokines, leading to fever, shivers and tachycardia (raised heart rate).

Inflammatory bowel disease

Ulcerative colitis and Crohn's disease are chronic inflammatory diseases of the GI tract, with symptoms such as diarrhoea, cramps and weight loss. They may be autoimmune diseases where the immune system attacks its own mucosa or natural gut flora. Treatment is via corticosteroids or immunosuppressant drugs.

Q4.4	Ulcerative colitis	Crohn's disease	Comment (Q4.5)
Macroscopic Features	Colon	Whole gut	
Fibrous thickening	No	Yes	Fibrosis (repair)
Fissuring ulceration	No	Yes	Acute inflammation
Mucosal pseudopolyps	Yes (typically)	Rarely	Regeneration
Microscopic Features			
Crypt abscesses	Yes (typical)	Rarely	Acute inflammation
Granulomata	No	Yes	Chronic inflammation
Granulation tissue	Yes	Yes	Tissue organisation and repair

Topic 8: Immunology

LEARNING OUTCOMES

- the immune system: cell types and their function, development and intracellular communication
- the innate immune system: barriers, humoral factors, complement, NK cells, phagocytosis
- the acquired immune response: T cells, B cells, antigens, epitopes
- clonal selection theory, the MHC
- lymphoid organs: structure, location and function
- antibodies: classes, isotypes, structures, functions
- antibody class switching and affinity maturation

CD = cluster of differentiation; surface makers on body cells which do not always have a function

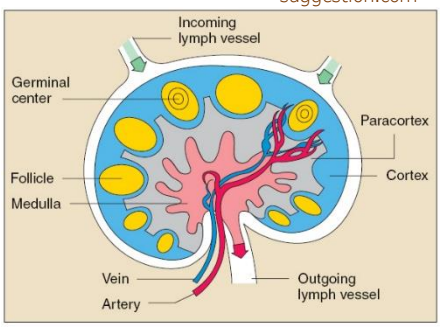
The immune system is a set of mechanisms that aim to protect the body from infection. It is alerted by foreign molecular patterns (PAMPs and DAMPs), altered cell processes and sometimes by its own "self" tissues when recognition of these tissues as "self" is defective.

Infection → Innate response → Adaptive response → Neutralisation of pathogen

Leukocytes (white blood cells) are derived from bone marrow stem cells and many have several roles in different elements of immunity.

Cytokines = immune messenger hormones (IFNs, ILs, TGFs and chemokines, which are chemoattractants). Type I interferons (α and β) are anti-viral; Type II (γ) is from T-cells

Image credit: keywords-suggestion.com



Lymphoid organs

Hydrostatic pressure in the capillaries forces plasma to filter out of the blood and into the tissues as interstitial fluid. Some of this later moves back in via osmosis due to oncotic pressure, but the excess fluid is returned to the bloodstream via lymphatic vessels (in which it is known as lymph). The lymphatics drain into lymph nodes at junctions, where the adaptive immune system scans the fluid for pathogens and initiates a response if necessary. The lymphatic system drains into the blood via the thoracic duct, the only part of the system usually visible.

- Primary lymphoid organs (bone marrow) produce lymphocytes. T cells mature in the **thymus** until puberty
- Secondary lymphoid organs (nodes, spleen, tonsils, Peyer's patches in gut) are where immune responses start

Antigens = molecules on pathogens capable of provoking an immune response

The innate immune system

The innate immune system responds to infection or damage to yield a non-specific, rapid response common to all organisms. There is a significant overlap with the knowledge in Topic 7, Inflammation.

→ Infection or necrosis releases PAMP and DAMP signals and also activates basophils, mast cells and resident macrophages (non-specific phagocytes **fully** activated by IFNγ from other macrophages, NKs or T-helper cells)
  - Basophils (in blood) and mast cells (in tissue) are leukocytes with granules which release histamine and heparin when activated, causing vasodilatation. These cells act in allergies or parasite infections, along with eosinophils

→ These signals bind to PRRs (toll-like receptors, C-type lectins or nod-like receptors) on phagocytes, stimulating chemotaxis of neutrophils and releasing inflammatory mediators (cytokines) - IFNs, ILs, TNFs and kinins

→ Neutrophils phagocytose debris/pathogens and IL-8 stimulates their production in bone marrow. Pseudopodia extend around the pathogen and meet to engulf it in a phagosome. This fuses with a lysosome to give a phagolysosome, which actively pumps in H^+ to activate acid proteases such as cathepsins

→ Dendritic cells (in the periphery) are poor killers, but come into play in adaptive immunity (see later)

→ Natural killer cells (NKs) are lymphocytes which provoke apoptosis in cells missing MHC I markers (see later)

→ PAMPs and DAMPs activate the complement cascade, causing extravasation, opsonisation and MACs

→ Use of NETs on parasites/fungi as a last resort (explained in Topic 7)

The complement cascade

The complement cascade is a sequence of soluble blood proteins/enzymes that are cleaved downstream. There are three pathways by which it can be stimulated: the classical, lectin and alternative pathways, all of which converge at the stage producing the enzyme C3 convertase, which cleaves the protein C3 into C3a and C3b.

- **C3a** (an anaphylatoxin) increases the inflammatory response, stimulating mast cell degranulation
- **C3b** acts as an opsonin on cells and coats immune complexes to promote their removal by the spleen. It also leads to the production of C5b
- **C5b**, along with other cascade products, produces a membrane attack complex (MAC). This forms a small pore in the membrane of a pathogen so water rushes in and ions out, leading to lysis which kills the cell

Development of adaptive immune cells

The **acquired** immune response is **specific** to the pathogen and has two components: humoral (B cells) and cell-mediated (T cells), taking place in fluid and tissues respectively.

B and T lymphocytes (named because they mature in the **b**one marrow and **t**hymus respectively) are found mostly in 2° lymphoid organs (especially nodes) but also circulate to search the body for antigens. B cells recognise soluble antigens in fluid and produce antibodies to clump the pathogen, to prevent binding to host receptors which would allow entry to host cells. T cells must have the antigen presented to them. Both signal to other areas of immunity.

Lymphocyte receptors – detecting antigens

- B cell receptors (BCRs) have the same structure as the antibody produced (i.e. complementary to antigen)
- T cell receptors (TCRs) are linear, so they must have antigens presented to them by other cells (see later)
- Receptors have constant (common to all) and variable (specific to antigen) regions on them
- An array of methods is used to promote **variation** in lymphocytes and thus allow a greater range of protection
  - The variable regions of receptors are constructed using VDJ gene segments. V, D and J parts of the receptor genes are used and joined in numerous different combinations (somatic recombination), producing a huge number of different receptors from a limited amount of DNA (combinatorial diversity)
  - This is also achieved by using different combinations of heavy and light chains in receptors
  - Junctional diversity results from random base insertions or deletions, but this may code for null regions
  - Secondary diversity arises from random mutations (somatic hypermutation) in the variable chain genome of cloned B-cells, produced after activation. This alters the affinity of the receptor for its AG
  - Only B cells with a higher affinity for the AG bind and receive survival signals; the rest die by neglect (clonal selection). This progressively makes the 2° response more effective, known as affinity maturation
- Tolerance destroys T cells in the pool that would respond to antigens on self cells **(see MHC explanation below)**
  - **Central**: T cell selection in the thymus. In positive selection, T cells are exposed to cortical cells with MHC molecules, and any which bind weakly to them undergo apoptosis. This ensures that all T cells can recognise MHCs. In negative selection, medullary dendritic and epithelial cells present self antigens to the T cells and those which bind strongly to them are forced to undergo apoptosis
  - **Peripheral**: regulatory T cells suppress other immune cells (e.g. reactions to self MHC II) to prevent excessive responses. Not all self antigens are present in the thymus so this regulation is important. Anergy is when co-stimulation does not occur, so the cell dies by neglect (see below)

SCID = a lack of B and T cells, caused by failure in recombination

The adaptive immune response

→ In cell-mediated immunity, T-cell receptors must have antigens chopped up into epitopes and presented to them on a major histocompatibility complex (MHC) by antigen-presenting cells (APCs) in order to activate
  - **Class I MHC**: present on all nucleated cells, except nerves. Present endogenous antigens and mutations to **CD8** (T-killer) lymphocytes
  - **Class II MHC**: present on specialised APCs only, of which dendritic cells are the most important type, but this function is also performed by macrophages and B cells. Present exogenous antigens to **CD4** (T-helper) lymphocytes. Remember using "**ex**tra **help for** you **too**" (exogenous, helper, CD4, class II)

- **NK cells** (innate) kill cells with downregulated MHC I molecules (e.g. cancerous or virally infected cells), recognising them as harmful. Inhibitory receptors on NKs bind MHC I, while activating receptors bind stress signals from harmful cells. Activated NKs prevent viral proliferation by downregulating protein tissue factor and destroying mRNA, and producing TI IFNs to message an anti-viral state to nearby cells.

→ Specialised APCs (especially dendritic cells in the periphery) take up the antigen, present it on an Class II MHC and upregulate cytokine production
→ They drain into the lymphatics and present to CD4 (helper) cells in the lymph nodes (call this signal 1)
→ The CD4 cells then send a co-stimulatory signal back to the APCs (signal 2); a way of saying that they also recognise the foreign antigen and giving a "go-ahead" for the rest of the immune response. This prevents autoimmunity, as any T-cells recognising self should have been destroyed in the thymus
→ This **full** activation of T helpers permits them to divide rapidly, mature into different types of helper – which act as commander cells for the whole immune response – and survive
→ Different CD4s release different cytokines to direct B cells, CD8s and APCs (e.g. **fully** activating macrophages)
→ APCs (remember: almost any cell, not just specialised immune cells in this part of immunity) present antigens to T-killer cells (CD8) using an MHC I in order to activate them so that they proliferate
→ CD8s then identify infected cells by their MHC Is and induce apoptosis in them using the Fas death ligand or perforin and granzyme, both of which stimulate the caspase cascade (see Topic 7 for apoptosis)
→ Memory T cells are also produced, and circulate to mount a faster 2° response if re-infection occurs
→ In humoral immunity, BCRs complementary to a specific antigen detect it and present it on a class II MHC
→ Co-stimulation from activated T-helpers activates the B cells, which divide to produce many clones
→ These either differentiate into plasma cells, which produce antibodies (also called immunoglobulins), or memory cells, which remain in the bloodstream long after infection so that the 2° response is faster if the pathogen re-enters. Antibodies also activate complements.
→ Certain pathogens provoke T cell-independent immunity, where antibodies can be generated directly after antigen detection. The response will be faster but less effective.

Antibodies (immunoglobulins)

Antibodies are Y-shaped soluble proteins which are complementary to the specific antigens to which they bind. They can be membrane-bound as receptors. Their roles are to kill, neutralise or opsonise (mark out for death) pathogens. There are 5 classes of antibodies, remembered by MADE-G: IgA, IgD, IgE, IgG and IgM.

Isotype	Structure	Role
IgG	Monomer	Opsonise, neutralise, activate complements and ADCC. Can cross placenta.
IgE	Monomer	Activate mast cells, basophils and eosinophils in allergy/parasite infection
IgD	Monomer	Uncertain
IgA	Dimer	Neutralise and flush mucosal pathogens
IgM	Pentamer	Trap pathogens and activate complements; first to be produced

- **Opsonisation**: antibody/complement attaches to pathogen and binds to Fc receptor on phagocyte, boosting phagocytosis by "marking" it out for engulfing
- **Neutralisation**: blocking binding of toxin to receptor on tissue cells (e.g. as in tetanus, diphtheria, cholera, flu)
- **ADCC** (antibody-dependent cell-mediated cytotoxicity): antibodies bind to surface antigens presented by an infected cell (opsonisation without phagocytosis). NKs and neutrophils therefore release cytotoxic granules (degranulate) containing perforin, granzyme and TNFα, which induce apoptosis in the cell
- **Eosinophils**: leukocytes that bind to IgE on the surface of multicellular parasites, which causes them to degranulate and release compounds which are toxic to both parasite and host as well as inflammatory mediators
- **Complements**: see above

Class switching occurs in response to cytokines in order to produce immunoglobulins with different roles. IgM is produced first, and then B cells are made to produce different isotypes through enzymatic removal of sections of Ig DNA. It is a permanent change because the constant region produced (and hence cell interactions in different parts body) is changed, but the antigen affinity stays the same because the variable region is retained.

Immunopathology

Organ transplantation can provoke an immune response due to foreign antigens, foreign MHCs or foreign antigens on self MHC, as the donor's immune system is unique. Identical twins are the safest donor option, known as syngeneic, due to their identical genes. Rejection can be hyper-acute (Type II), acute (Type II and III) or chronic (Type IV), where deposits in blood vessels leads to ischaemia of the transplant. Immunosuppression prevents this reaction.

Hypersensitivity is an excessive immune reaction giving harmful side effects. There are four types:

- Type I: rapid immune response (due to pre-existing IgE in the host) to molecules that would not normally incite an immune response. **Process**: Dendritic cells present antigen → CD4 sensitised → B cells produce antibodies → IgE cross-links between antigen and mast cells → degranulation → inflammatory response. **Examples**: hay fever, asthma, anaphylaxis (allergic reaction, producing systemic effects such as oedema, hypotension and bronchoconstriction). **Treatment**: adrenaline, beta-agonists, corticosteroids (see Topic 10)
- Type II: components of host cells are attacked by autoantibodies, such as in an incorrect blood transfusion. **Process**: IgG or IgM binds to self structures → phagocytosis by macrophages → lysis by complements. **Examples**: haemolytic anaemia in ABO blood group contact, Rhesus blood group reaction.
- Type III: reaction to immune complexes (antigen + antibody). **Process**: persistent infection forms complexes → deposited in tissue → acute and chronic inflammation→ tissue destroyed. **Example**: serum sickness.
- Type IV: delayed, T cell-mediated response to infection. **Process**: in tuberculosis, macrophages and lymphocytes are activated → cannot clear infection → granuloma forms. **Examples**: TB, contact dermatitis

Autoimmune diseases are caused by a failure in self-tolerance, multigenic disorders or the environment, leading to immune system attacks on the body. They follow the same classifications as hypersensitivity reactions:

- Type I: infection by a common antigen causes upregulation of APC co-stimulators and so a greater immune response. Infectious agents can instigate an immune response against host self cells by modification, inflammation or molecular mimicry.
- Type II: in myasthenia gravis, an autoantibody against the ACh receptor leads to their destruction, causing fatigue, muscle weakness and drooping eyelids. In Graves' disease, there is an autoantibody for thyroid-stimulating hormone receptors and so the hormone thyroxin is always produced, leading to hyperthyroidism. Symptoms include nervousness, tremor, bulging eyes and weight loss, as thyroxin is the body's "accelerator".
- Type III: systemic lupus erythematosus is a condition in which antibodies against nucleic acids and phospholipids are present. This may produce the distinctive "butterfly rash", pericarditis and arthritis.
- Type IV: diabetes mellitus Type I involves T cell reactions to pancreatic β cells, which produce insulin. Some demyelinating diseases (see Topic 5), such as multiple sclerosis, attack the nervous insulation. Ulcerative colitis and Crohn's disease are chronic inflammatory bowel diseases mediated in this way.

Treatments typically include immunosuppressants, such as corticosteroids or cytokine blockades.

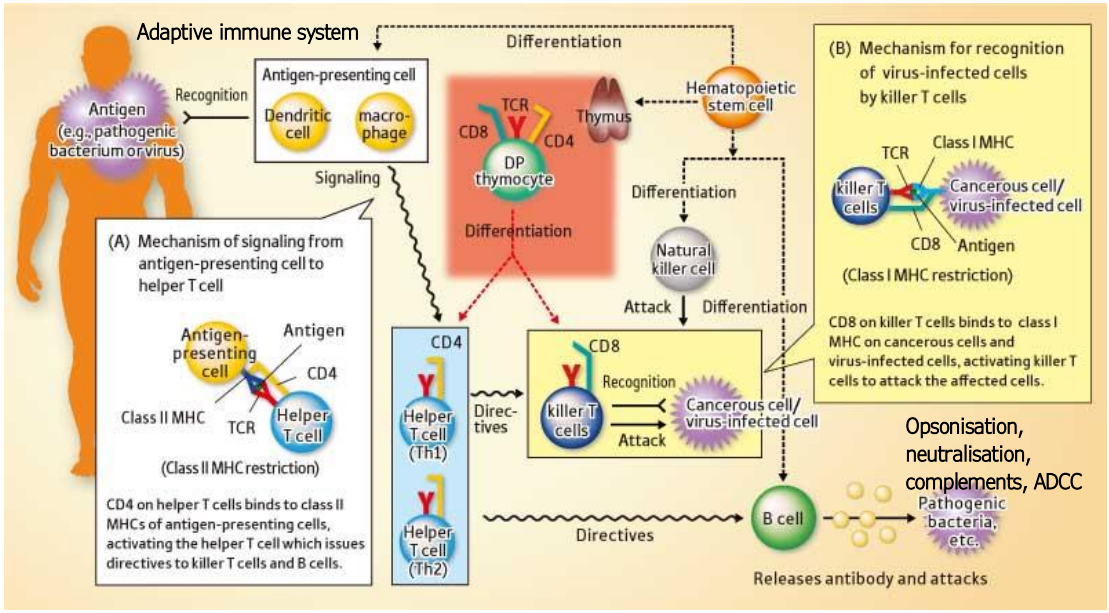


Image credit: medicalxpress.com

This diagram doesn't include every aspect of the immune response, but is a good illustration of most cell interactions. Making your own version would be a helpful revision method.

Topic 9 – Neoplasia

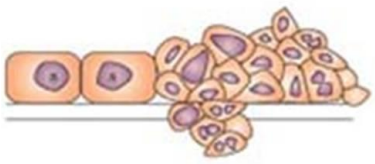


Image credit: Wikispaces

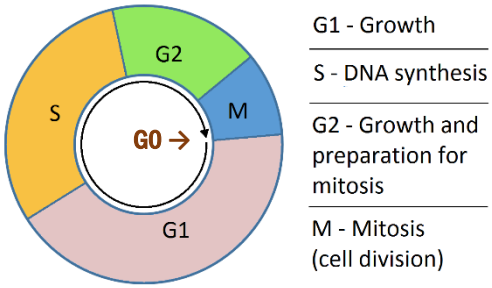
LEARNING OUTCOMES

- principles of neoplasia: normal cell cycle control and causes of its failure
- how tumours cause morbidity and mortality
- classification and description of tumours
- stepwise progression in oncogenesis; oncogenes and tumour suppressor genes
- causes and prevention of cancer

In health, cells progress normally through the cell cycle and respond to signals accordingly. Uncontrolled cell growth that persists in the absence of stimuli is known as **neoplasia**, which manifests clinically as cancer.

The cell cycle

Image credit: Wikipedia.org



Healthy cells pass through the cell cycle, carrying out different stages of duplication. Cyclin-dependent kinases (CDKs, which activate downstream proteins by phosphorylation; see Topic 4) signal to control the movement through these stages. The three stages other than mitosis are collectively known as interphase.

In a stage of interphase known as G0, cells are dormant (effectively resting outside of the cell cycle, in quiescence) and will only move on to differentiation, proliferation or death if they detect signals from other cells. These may be ligands (such as growth factors), ECM physical interactions or cell-cell adhesion changes, and stimulate the intracellular cascade that culminates in the action of transcription factors on DNA for protein synthesis. Ordinarily, when hyperplasia (cell proliferation) is demanded it is tightly controlled. When it is uncontrolled and irreversible it is known as neoplasia, which is due to damage to DNA controlling the cell cycle and the subsequent downstream effects.

Different types of body cells act differently in relation to the cell cycle. Permanent cells such as neurones and cardiac myocytes cannot duplicate, while conditionally-renewing cells such as hepatocytes will proliferate and differentiate to repair injury. Labile tissue such as epithelia and bone marrow have stem cells, which are constantly dividing in health – one daughter cell remains as the stem cell and one differentiates with respect to its function.

Definitions

- Hypertrophy: increase in cell size and hence organ size e.g. uterus in pregnancy
- Hyperplasia: increase in cell number and hence organ size e.g. lactating breasts, prostate with age. Organ architecture is maintained and proliferation stops once the stimulus is removed
- Metaplasia: one differentiated cell type becomes another due to persistent injury, but reverses once the injury is removed e.g. bronchus due to tar, cervix due to pH. Named after the cell type it becomes
- Dysplasia: irreversible cytological changes in neoplasia (see below) – **a descriptive appearance, NOT a stage**
- Neoplasia: abnormal tissue mass of uncontrolled growth that does not resolve upon removal of stimulus
- Invasion: neoplasm (mass = tumour) infiltrates surrounding tissue and/or organs
- Metastasis: neoplasm spreads to other parts of the body via blood, lymph or body spaces and proliferates
- Benign: neoplasm proliferates but does not invade or metastasise; can produce pressure, obstruction or excess hormones but are not normally life-threatening. Often have an intact capsule and cells more normal

Tumour classification

Only malignancies are known as "cancer"

-oma = tumour

Tumour classification refers to the type of cell and absence or presence of malignancy. **Benign**: surface epithelia = papilloma, glandular epithelia = adenoma, fat = lipoma, fibrocytes = fibroma, cartilage = chondroma, smooth muscle = leiomyoma, skeletal muscle = rhabdomyoma, bone = osteoma, germ cells/gonads = teratoma.
Malignant: surface epithelia = squamous cell carcinoma, glandular = adenocarcinoma, all connective = -sarcoma instead of -oma, teratocarcinoma = germ cells/gonads, melanoma = skin, lymphoma = lymph nodes, glioma = astrocytes, leukaemia = white blood cells (NB: leukaemias uniquely exhibit distinctive mutations + no aneuploidy).

Morbidity and mortality

The effects of tumours depend on their location. For example, tumours of the rectum may obstruct the tract, ulcerate and bleed, leading to blood in faeces. In the lung, obstruction causes a cough and a hoarse voice; many symptoms are general, but adrenal gland metastasis is unique to this cancer. In the brain or meninges, tumours put pressure on brain tissue (as the skull cannot expand) and so cells die. In the prostate, urethra blockage and bladder hypertrophy are common. Destruction of vital tissue, haemorrhage or opportunistic infection cause death.

The route of spread will also depend on physical location as well as the type of tissue – for example, colon cancer often spreads to the liver via the hepatic portal venous blood or coelom, while breast cancer may spread to the bone as they have similar adhesive molecules. This latter mechanism is known as the "seed and soil" hypothesis: particular cancer cells require the right tissue/receptors if they are to spread, so clinical course may be predicated.

Cancer is graded by the degree of tissue differentiation (1-3); poorly differentiated cells hardly look like tissue and have the worst prognosis. TNM staging also measures advancement and guides treatment, scoring by tumour size, lymph node involvement (as this is where immune cells drain to) and extent of distant metastases.

Carcinoma in situ = neoplasm just before the stage of invasion

Oncogenesis

Damage to DNA is at the root of the varying multi-stage pathways involved in the development of cancer. The **dartboard diagram** at the end of this chapter indicates the interacting cardinal behaviours of neoplastic cells, their cellular (microscopically visible) effects and the macroscopic symptoms produced. Here, they will be explained in more detail. The process of escaping normal genetic control involves re-programming metabolism, evading the immune system and promoting pro-tumour inflammation.

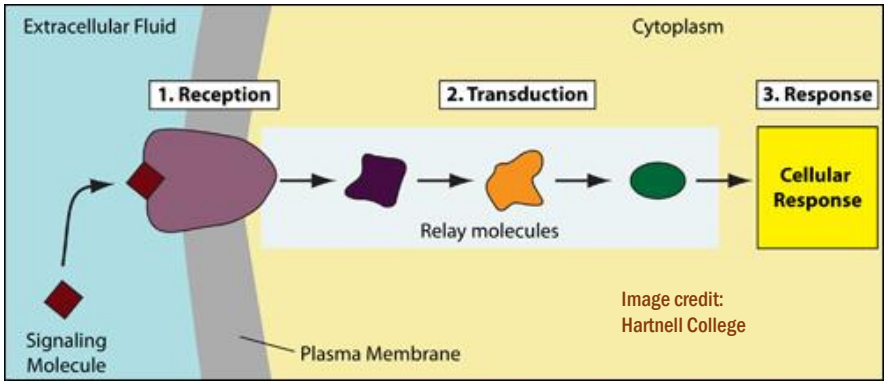
Several somatic mutations are required to develop a malignant cancer: initiation makes a normal cell cancerous and promotion drives proliferation. A further mutation (progression) provokes metastasis. The stroma is a supporting vascular connective tissue around carcinomas, and varies in composition according to the molecular signals from the tumour cells. This angiogenesis (blood vessel growth) is necessary for tumour growth.

Two kinds of gene are altered in neoplasia: oncogenes and tumour suppressor genes (TSGs), as well as DNA repair proteins. These mutations influence cell proliferation, apoptosis, signalling, DNA repair and cell adhesion.

Oncogenes

Oncogenes are normal genes which, if mutated, precipitate cancer. These are often regulatory genes and only **one** allele needs to be altered in order to activate it as an oncogene, which can occur by several mechanisms.

- Retroviruses may insert oncogenes into the host cell genome or introduce viral promotors, so transcription factors express host genes inappropriately e.g. using c-myc (see Topic 3 for gene promotion).
- Ras genes can become an oncogene through a point mutation; the altered amino acid in the protein causes the Ras molecular switch (see Topic 4 for its role in signal transduction) to become constitutively active.
- The epidermal growth factor receptor (EGFR) can become overactive by either amplification producing multiple copies of the gene or truncation of the gene causing the receptor produced to become constitutively active. The cell will act like it is constantly receiving growth factor signals and so proliferate excessively.
- Growth factors such as sis can also be altered. **Any stage of growth signalling cascades can be modified.**



Cellular response = gene transcription influencing the processes above, so the cell proliferates uncontrollably

"p" before a gene abbreviation simply denotes the protein produced from that gene

TSGs

Tumour suppressor genes are normal regulatory genes that are protective against neoplastic behaviours e.g. by restraining cell proliferation. **Both** alleles must be inactivated by a mutation to produce cancer.

- The retinoblastoma (Rb) TSG codes for the pRb protein, which must be phosphorylated (inactivated) in the cell cycle by CDK in order for the cell to pass from G1 to S phase – it acts as a "brake" on the cell cycle. Rb blocks progression through the cell cycle by binding to the transcription factor E2F, which would otherwise be free to bind to DNA and express S phase entry genes.
- p53 is the "guardian of the genome" – the cellular concentration of this protein increases when DNA is damaged and it acts as a transcription factor for the protein p21. p21 is a CDK inhibitor, binding to it so that it can't phosphorylate Rb, and hence arrests the cell cycle so the DNA can be repaired. If repair is impossible, apoptosis occurs. p53 is an exception in that only **one** damaged allele can cause cancer – a cell with damaged DNA is free to proliferate dangerously.
- APC is a regulatory protein in the Wnt ligand pathway (signalling pathway in tissue organisation and cell-cell signalling). APC regulates β-catenin, which acts as a transcription factor for growth promoting genes. Therefore, mutated APC will not bind β-catenin and so growth becomes uncontrolled (especially in colon).

DNA repair mutations

Mismatch repair proteins correct mismatched base pairs after DNA replication. When these are defective, mutated proteins are free to be produced from DNA. Mutated nucleotide excision proteins are seen in xeroderma pigmentosum, where damaged DNA results in skin cancer. Altered strand break repair proteins (such as BRCA) also allow mutations to propagate, and so patients are more susceptible to cancers. Lastly, aneuploidy is common in solid tumours as a result of p53 deregulation.

Telomerase

Cells only undergo a limited number of divisions before dying by apoptosis. Viral oncogenes can give cells the ability to escape this and become immortal by activating telomerase (e.g. by HPV in cervical cancer). Telomere end structures (see Topic 3) are gradually lost over time as the ends of DNA cannot be completely synthesised from Okazaki fragments, and complete loss of them promotes apoptosis. Telomerase enzymes make new repeat sequences on the telomere (normally only seen in stem and germline cells) and hence the cell becomes immortal.

Hallmarks of cancer

- **Self-sufficiency in growth signals** is where the cancerous cell produces its own signals for proliferation (ligands/ECM component interactions/cell-cell adhesion molecules) instead of receiving them from other cells - it gains the ability of autocrine stimulation. The mutated Ras protein is truncated, so the GPCR loses its intrinsic ATPase activity and acts like it is receiving growth signals all the time. In addition, tyrosine kinase receptors for these may be over-expressed so that the cell is hyper-responsive to growth factors (Topic 4).
- **Insensitivity to growth inhibition signals** includes genetically inactivated pRb, TGFβ receptor downregulation/ mutation and c-myc overexpression. C-myc is a transcription factor stimulating proliferation and regulating apoptosis. TGFβ normally suppresses c-myc and pRb phosphorylation, so damage will promote proliferation.
    - **Transcription factor:** a protein that binds to a gene to activate or suppress its protein synthesis
- **Evasion of apoptosis** is a result of decoy death ligands or downregulated/mutated death receptors. Ordinarily, changes in ECM signals provoke apoptosis, but cancerous cells have lost this ability. Mutated p53 also cannot cause apoptosis in response to damaged DNA. Lastly, the anti-apoptotic BCL2 mitogen is overexpressed.
- **Immortality** is a result of the first three processes plus upregulated, inappropriate telomerase activity.
- **Angiogenesis** is necessary - as the tumour enlarges and spreads, it requires a greater blood supply for growth. Tumour cells and infiltrated macrophages secrete angiogenic factors (e.g. VEGF) which switch on the process. ECM breakdown also releases sequestered angiogenic growth factors, such as FGF. In addition, the Ras oncogene upregulates these factors. The vessels produced are wide and inefficient at exchange, so drugs have difficulty reaching tumour cells to be effective and necrosis develops at the centre of the tumour.
- **Invasion and metastasis** require the loss of cell-cell adhesion, ECM proteolysis and cell movement. Cadherins and integrins are mutated for this purpose and also to affect cell signalling stimulating proliferation and survival. Tumour cells widen spaces by oedema to allow movement. Tumour cells secrete matrix metalloproteinases (MMP enzymes) which carry out ECM breakdown, and MMP inhibitors are downregulated.

High-grade intra-epithelial neoplasms (carcinoma in situ; stage before invasion through the basement membrane) and other advanced lesions exhibit the cytological features of dysplasia, which are seen on pathology slides. In addition to these signs, the cells appear more crowded and sometimes with mucus. Microscopically, you can observe pleomorphism (larger cell nuclei, producing a greater nucleus:cytoplasm ratio), hyperchromasia (darker staining nuclei), variation in cell size/shape, reduced differentiation, loss of cell polarity and abnormal, increased mitoses. These reflect the altered genetic material within the nucleus and the subsequent excessive proliferation.

Causes of cancer

Altered DNA produces the effects seen in the behaviour of neoplastic cells and gives them advantages over other cells. Exposure to carcinogens seems to follow a dose-response pattern and require a threshold-level dose for neoplasia to occur. This can be caused by:

PET scans show tumours up brightly as they use up a lot of glucose for growth

- Viral transduction transforming DNA using an oncogene e.g. Ras switch
- Deletion of a tumour suppressor gene that would inhibit proliferation or promote apoptosis (e.g. p53 or pRb), which may in turn activate an oncogene
- Defects in a DNA repair gene (e.g. those coding for mismatch repair proteins)
- Electrophiles activated from chemical carcinogens (these are specific to the tissue, stage and species)
- Tar metaplasia from smoking
- Radiation e.g. UV rays may precipitate DNA strand breaks or thymine dimers, which disrupt base pairing

Risk factors

- Increasing age – accumulated mutations and decreasing telomerase
- Smoking – metaplasia due to carcinogens in tar
- Genetic predisposition
- Environment, occupation and diet – including carcinogen exposure (most important factor)
- Radiation exposure
- Infectious agents – viruses carrying out transduction
- Parasites – induce chronic irritation, which is a promoting agent for tumours

Philadelphia chromosome: as mentioned above, leukaemias are often caused by specific, known base mutations. One such defect is the translocation of the ends of chromosomes 9 and 22 onto the other, producing what is known as a Philadelphia chromosome. This fusion creates an oncogene that is responsible for chronic myeloid leukaemia - so when additional carcinogenic mutations occur (Ras, p53), the disease appears.

Treatment includes surgery to remove tumours, chemotherapy (if metastasised), bone marrow transplants, radiotherapy, angiostatic agents, pain relief, palliative care and immunotherapy. The latter stimulates the immune system to recognise the cancer cells as foreign and attack them. Radiation and chemotherapy aim to provoke apoptosis in cancer cells, but are also toxic to healthy cells and so exhibit many side effects. The cancer cells can also evolve, such as developing channels to extrude drugs (see Topic 4 for ABCs) or other survival mutations. For this reason, therapy targeting a combination of disease aspects and tailored to the individual patient is essential.

Pathology tips:

The easiest way to tell the difference between a slide of a benign cancer and a malignant one is the pattern of growth: a benign tumour will be clearly **circumscribed** and not extend into the tissue but into a lumen, for example (**exophytic** growth), while a malignant tumour will exhibit a messy, crab-like spread into the affected tissue (**endophytic** growth).

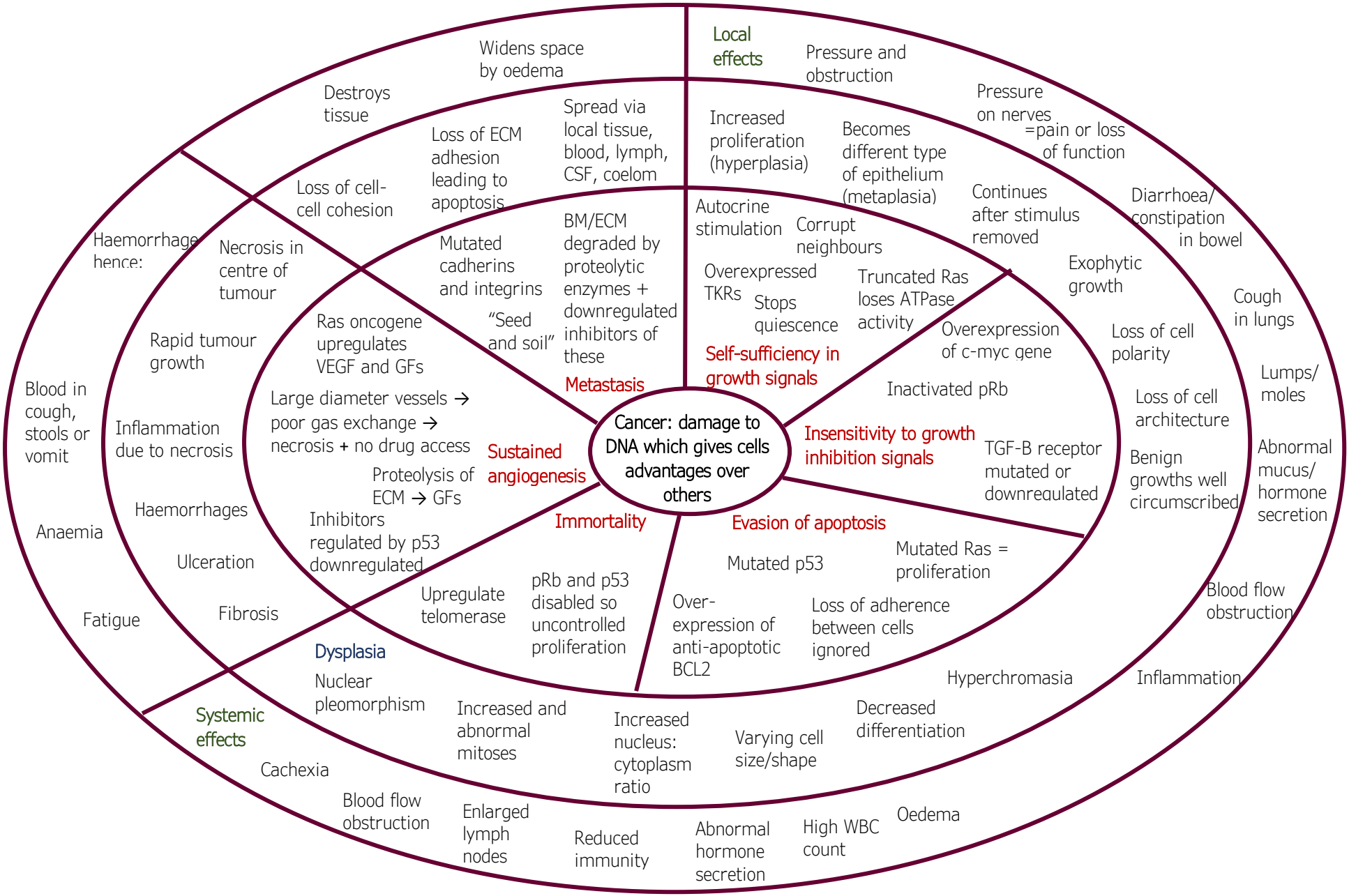
Polyps are abnormal outgrowths connected to the affected tissue by a stalk.

In slides with chronic inflammation (Topics 7 and 8 as well), smooth muscle proliferation and bright pink fibrin deposits in scarring can also be seen.

Slides of the majority of tumour cells will demonstrate many of the signs of **dysplasia**, so if you are familiar with them you will have a good idea of what to look for.

It is possible to see areas of benign and malignant neoplasia on the same sample!

The tubules of lymph nodes may be stretched and show dysplastic signs.


Cancer: damage to DNA which gives cells advantages over others
Local effects
Widens space by oedema
Destroys tissue
Pressure and obstruction
Pressure on nerves =pain or loss of function
Metastasis
Spread via local tissue, blood, lymph, CSF, coelom
Loss of ECM adhesion leading to apoptosis
Loss of cell-cell cohesion
Haemorrhage hence:
Necrosis in centre of tumour
Blood in cough, stools or vomit
Anaemia
Fatigue
Self-sufficiency in growth signals
Increased proliferation (hyperplasia)
Becomes different type of epithelium (metaplasia)
Autocrine stimulation
Corrupt neighbours
Continues after stimulus removed
Diarrhoea/ constipation in bowel
Exophytic growth
Cough in lungs
Lumps/ moles
Abnormal mucus/ hormone secretion
Blood flow obstruction
Inflammation
Mutated cadherins and integrins
BM/ECM degraded by proteolytic enzymes + downregulated inhibitors of these
"Seed and soil"
Rapid tumour growth
Inflammation due to necrosis
Haemorrhages
Ulceration
Fibrosis
Sustained angiogenesis
Overexpressed TKRs
Stops quiescence
Truncated Ras loses ATPase activity
Overexpression of c-myc gene
Inactivated pRb
Loss of cell polarity
Loss of cell architecture
Benign growths well circumscribed
Insensitivity to growth inhibition signals
TGF-B receptor mutated or downregulated
Ras oncogene upregulates VEGF and GFs
Large diameter vessels → poor gas exchange → necrosis + no drug access
Proteolysis of ECM → GFs
Inhibitors regulated by p53 downregulated
Immortality
Upregulate telomerase
pRb and p53 disabled so uncontrolled proliferation
Evasion of apoptosis
Mutated p53
Mutated Ras = proliferation
Over-expression of anti-apoptotic BCL2
Loss of adherence between cells ignored
Dysplasia
Nuclear pleomorphism
Increased and abnormal mitoses
Increased nucleus: cytoplasm ratio
Varying cell size/shape
Decreased differentiation
Hyperchromasia
Inflammation
Systemic effects
Cachexia
Blood flow obstruction
Enlarged lymph nodes
Reduced immunity
Abnormal hormone secretion
High WBC count
Oedema

Topic 10 – Pharmacology

Image credit: shutterstock.com

LEARNING OUTCOMES

- basic pharmacological terminology
- pharmacodynamics and mechanisms of drug action
- dose-response relationships; interaction between antagonists and agonists
- selectivity of drugs; beneficial and adverse effects (and how they can be plotted on a D-R curve)
- the therapeutic index
- pharmacokinetics and drug metabolism; alternative routes of administration
- inter-patient variation in response to drugs
- uses of different dosing regimes
- drug allergy
- adverse reactions
- use of drugs to modulate the sympathetic and parasympathetic nervous systems
- discovery, development, approval and regulation of drugs
- marketing of drugs (potential abuses of this process)
- complementary and alternative medicines

100,000 prescriptions are written every year in Scotland

A **drug** is a substance which has pharmacological effects on the body by either initiating events or blocking the actions of other substances. When used in medicine, they attempt to restore or preserve the normal functioning of systems by regulating dysfunctional pathways.

Examples of drug targets

- Ligand-gated ion channel
- GPCR: target protein is enzyme or channel (Topic 4)
- Kinase-linked receptor
- DNA-linked receptor: ligand affects gene transcription
- Enzymes (active site/cofactor)
- Transporters/pumps

Pharmacodynamics

Pharmacodynamics is what the **drug does to the body**. This includes the effects of the drug (both positive and negative), the mechanisms by which they act, and the relationship between concentration and effect.

As seen in Topic 4, changes in cellular function can be mediated by the binding of ligands (endogenous molecule or drug) to target sites (often but not limited to receptors), which may initiate a cascade of reactions. Drugs act by binding to these specific sites on cells, with protein components (receptors, enzymes, channels and transporters) being the most common target. Mechanisms of these are explored below. Complexes may be reversible or irreversible. Side effects occur when the drug binds to and exerts its effect on a molecule which is not a target.

Modes of action

Not all targets are receptors, but these terms are often used interchangeably

Agonist: drug binds to complementary target protein to induce conformational change in intra-protein bonding, which signals for biological response. Receptors are often named for their principal agonists.

Antagonist: binds to complementary receptor but causes no change. May be competitive or non-competitive.

Partial agonist: does not produce a full effect and hence reduces the overall impact of fully saturated receptors.

Inverse agonist: produces the opposite effect to the agonist by switching off receptors that are constitutively active (by default active without agonist binding). These are very unusual.

Dose-response curve

Linear (hyperbolic in shape) or log dose-response graphs can be used to show measured clinical values (the body's biological response to the drug). A log graph (left) will be sigmoidal in shape and shows the effective dose range as the straight region.

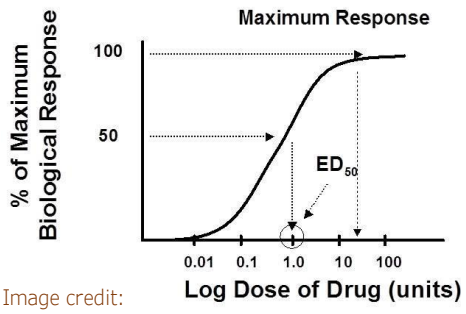


Image credit: studyblue.com

E_{max} is the maximum biological response. ED_{50} is the dose at which 50% of the maximal response is produced.

Competitive drug antagonists will move the D-R curve to the right, as a higher concentration of agonist would be needed to achieve the desired effect. Increased antagonist concentration moves it further to the right.

Non-competitive drug antagonists move the curve to the right, and also mean that the E_{max} is reduced - increased drug concentration will not offset the effects of this mechanism, as it does not compete with ligand binding.

Partial agonists move the curve slightly to the right, but again E_{max} is not reached as the efficacy is reduced.

The affinity is the strength of the complex between the drug and receptor, dictating how quickly it dissociates. Observing a drug with **low** affinity for the receptor (e.g. ACh), any changes in drug concentration will quickly be reflected in the level of binding/effects. Therefore, fine control can be carried out by changing the concentration. In drugs with **high** affinity (e.g. growth hormone), many molecules will be well-bound at a low drug concentration so effects will be prolonged even when the concentration falls.

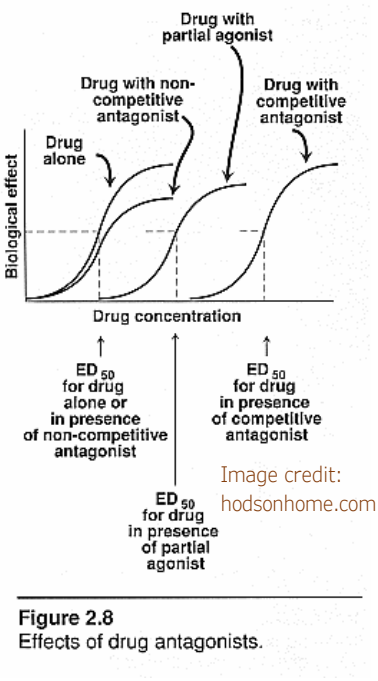


Image credit: hodsonhome.com

Figure 2.8
Effects of drug antagonists.

The efficacy of a drug is the extent of the response when all receptors are full (E_{max}). A full agonist will have the greatest efficacy at a receptor whereas a partial agonist will have a lower efficacy. It can also refer to drugs with the same effect but using different mechanisms.

The potency of a drug is the amount required for a given level of response and is influenced by the affinity. A high potency is not a reason to pick one drug over another – the dosage of a less potent drug can simply be increased.

Efficacy and potency vary according to individual variation amongst patients, such as receptor number or structure. As such, the dose-response curve for a drug (recommending dose ranges) is an average. Very importantly, how the body handles a drug (pharmacokinetics) and hence its concentration in the relevant tissue varies drastically amongst patients.

The therapeutic index of a drug is the ratio of the concentration at which the drug is harmful to the concentration at which it is beneficial. Drugs with a lower therapeutic index are harder to prescribe a dosage for. Blood tests must be carried out to monitor the dose.

Drug and receptor selectivity

Receptors are named for their agonists, and their sub-types describe their selectivity (relative responsiveness) for ligands i.e. the concentration required to produce a biological effect, with 1 requiring less than 2. While effects will be greatest at one receptor subtype, a particular drug can still have great effects at a less selective subtype in sufficient concentrations. Selectivity is achieved at the lowest effective dose, so adverse effects do not occur.

Desensitisation – reduced response to a drug over time

Tachyphylaxis = **fast** desensitisation to a drug, such as through depletion of chemical mediators of the pathway

Tolerance = **slow** desensitisation to a drug after prolonged exposure, via a number of mechanisms. Downregulation is where receptor numbers are reduced (receptors are endocytosed into the cell and destroyed) to prevent over-stimulation of the cell by the ligand. Receptor structure changes are conformational changes that affect the target's action after binding, such as phosphorylation of a GPCR or closure of an ion channel due to overexposure. The body counteracts the **effects** of the drug in physiological adaptation – dampening biochemical reflexes are activated to oppose the biological response and restore homeostasis.

Pharmacokinetic changes can also desensitise the body to a drug; it may be actively removed from cells or metabolised more when the body increases enzyme production after extensive exposure.

In the case of withdrawal, the dampening reflexes against the drug are left with no drug to act upon, so withdrawal effects will occur. Either the intrinsic ability mimicked by the drug has been lost through lack of use, or the counteracting reflexes are still present without the drug.

Pharmacokinetics

Pharmacokinetics is **what the body does to a drug**. It includes the rate and extent of absorption and distribution, rate and methods of elimination and plasma concentration of drug over time. The drug is absorbed at the site here it is given, Mnemonic: ADME

Absorption

A drug can be given by a variety of routes, all of which then flow into the circulation. They have various features:

- **Oral**: patient cannot be vomiting; drug must be swallowed, survive gastric acid, not bind dangerously to food, be absorbed by mucosa, survive FPM and EHC (see below). The method is simple and can be self-administered for **long-term non-acute** illnesses. Buccal, sublingual and rectal are other enteral routes (pass through the gut).
- **IV**: 100% of dose absorbed into the bloodstream, reaching a high plasma concentration quickly; no FPM but risk of infection and systemic side effects
- **Intramuscular**: simple method but can be painful and have unpredictable absorption
- **Subcutaneous**: can be self-administered but must be absorbed from fat (e.g. insulin)
- **Inhaled**: co-ordination required to get to lungs (asthma drugs)
- **Topical**: adverse effects limited to area of application on skin

Nitrates are given under the tongue as they do not survive the gut

Parenteral routes

Distribution

Distribution of the drug through the body depends on the level of protein binding in plasma (mostly to albumin, which causes slower distribution) and water/lipid solubility. Prescribers must check whether it will be carried to desirable or undesirable areas. Movement is mostly via passive diffusion and some active transport until equilibrium is reached. The speed of distribution depends on the solubility, binding and size of the drug molecule.

The "concentration" of the drug is the **free** (non-bound) drug present in the fluid compartment of the body – these are blood plasma, interstitial fluid (between cells) and intracellular fluid. The **volume of distribution** is defined as the total volume of body fluid compartments that the dose has distributed into immediately following IV injection. This value is calculated using the initial plasma concentration of the drug given and the amount of drug administered. A smaller V_d value indicates that there is a lot of drug still present in the plasma and possibly interstitial fluid.

$V_d = D / C_o$

Where D = mg drug given and C_o = mg/L initial plasma conc.

Metabolism

Metabolism of a drug (largely in the liver) reduces its bioactivity and increases its water solubility for excretion. Phase I is oxidation by cytochromes, which can be affected by other drugs (such as warfarin) and makes most drugs inactive. Phase II is conjugation, where molecules are added on to make the drug water-soluble. Drug metabolism is affected by a variety of both drug and patient factors, and when it is decreased (such as in cirrhosis), toxic effects or accumulation may occur.

Interaction: the presence of one drug affects the metabolism of another. This is by either inducing metabolism via stimulating metabolic liver enzyme production (alcohol, rifampicin) or by inhibiting these enzymes through competition (ciprofloxacin). This is important for drugs such as warfarin, oral contraceptives or anti-epileptics.

First pass metabolism: early metabolism of the drug by enzymes in gut wall or liver, which reduces later plasma concentration and possibly body response. This then must pass through the portal venous system to get to the systemic circulation. This can be circumvented by the use of capsules or alternative routes of administration.

Excretion

- **Renal**: drug/metabolite is eliminated through urine if it has a low molecular weight and is water soluble enough not to be reabsorbed into the body fat from kidney tubule. Some are actively secreted into tubules.
- **Biliary**: drug/metabolite enters bile after liver metabolism, is carried through gut and excreted in faeces if it has larger molecular weight (including conjugated drugs).

Entero-hepatic circulation: sufficiently lipid-soluble conjugates or drugs excreted in bile may be reabsorbed through the gut wall and return to the liver instead of continuing through the GI tract, prolonging their effects in the body.

For example, this recycling of glucuronide conjugates requires bacteria capable of hydrolysing them to release the drug again. Broad-spectrum antibiotics can reduce this reabsorption and hence drug availability.

Bioavailability

Bioavailability is the % of the dose administered that enters the systemic circulation unchanged, calculated as the area under the plasma concentration-time graph for the admin route over that for IV admin. For IV admin the bioavailability is 100%, as the drug enters the circulation directly with no destruction, metabolism, binding etc.

Clearance

Clearance (due to metabolism and excretion) is the volume of plasma cleared of drug per time.

First-order kinetics: a constant **fraction** of drug is cleared per time, because its clearance (by enzymes or renal filtration) depends on its concentration (see half-life). This gives an exponential curve of plasma conc. over time.

Zero-order kinetics: a constant **amount** of drug is cleared per time, because clearance enzymes for them are limited so become fully saturated quickly. The drug will accumulate if more is given than its clearance can cope with. A drug can change from first-order to zero-order kinetics if it reaches a certain concentration.

Dosing

The **half-life** (length of time that it takes for the drug plasma concentration to halve) of the drug dictates the dose frequency – the drug concentration has to remain within the effective range for the treatment period. Repeated doses are given before the preceding ones have been cleared, so that the drug steadily accumulates and the elimination equals administration. If the drug has a long half-life, a loading dose may be required to quickly reach a steady state concentration. If it has a short one, it reaches a steady state quickly and can be controlled accurately, but oral administration may not be appropriate. Individual factors affecting drug handling (age, gender, weight, genetics, liver/kidney function, interactions and environment) are as important as the dose with regards to response, so monitoring may be necessary (but possibly difficult/dangerous).

Modified release formulations: tablet design that releases drugs with a short half-life over a sustained period of time so they can be given less frequently

Adverse drug reactions

Adverse drug reactions are undesirable responses to a drug that are common, avoidable and often soon after administration. Reactions are more likely in drugs with a low therapeutic index. Prescribers must be especially careful with the elderly due to their reduced elimination ability. They occur with all drugs so must be weighed up with the benefits, and may reduce patient adherence. A common reaction is GI bleeding due to NSAIDs.

- **Adverse reaction**: harmful effects of a drug requiring withdrawal or dose reduction
- **Toxic effects**: harm at a cellular level e.g. mutations, cancer
- **Drug toxicity**: when drug plasma concentration exceeds therapeutic range

Type A reactions are predictable from known pharmacology because they are often dose-dependent and mild. Type B reactions are rare, severe, not predictable and as a result often only discovered when they become widely available to a large population of great individual variability. Types C-G are less important. Caution is required when prescribing during pregnancy, due to a whole host of extra possible reactions (e.g. steroids are teratogenic).

Drug allergies: hypersensitivity (see Topic 8 for mechanisms Type I-IV) can also apply to drugs, and can be very severe so a thorough history is imperative. Penicillin is a well-known example of a Type I reaction.

Complementary and alternative medicine: it is important to take any non-medical therapies into account, as they may form part of the presenting complaint or interact with drugs prescribed. CM is used alongside conventional medicine while AM is used in its place. This may be due to a fear of conventional medicine, the more holistic approach used and a desire to exercise more control over treatment. The most important example is St John's Wort, which is a herbal remedy for depression – herbal remedies may exert pharmacological effects (many drugs are plant-derived). It may cause serotonin syndrome when taken with other depression medication, leading to over-excitability, and reduces the effects of warfarin and other important drugs by inducing their metabolism.

Pharmacogenetics

Genetic background is one source of variation leading to unique responses to drugs among patients (see above). DNA code variations for transporters, enzymes and receptors dictate pharmacokinetics and pharmacodynamics. Pharmacogenetics refers to variations in single genes, while pharmacogenomics relates to multiple genes. This has sparked great interest in the area of personalised medicine, where drugs could be tailored to individual patients. Further understanding could also prevent the loss of drugs that are only effective for a small patient population.

Trials and regulation

Trials are required to assess dosage, uses and beneficial/adverse effects of new and existing drugs. There are many types of trial and four phases of testing. Confounding factors (e.g. differences in lifestyle and disease) must be explored because people are fundamentally different, and unnecessary risks must be avoided. Bias can come from a variety of sources and every step should be taken to minimise it, such as stratification and blinding. There must also a be control group against whom the outcome is compared and an end-point (or a surrogate biomarker for this). Ethical considerations include consent, inclusion of all results, correct interpretation and critical appraisal. New drug therapies have great potential but are limited by the speed of trials and cost. Medicines management (by NICE/SMC/a DTC) describes deciding which drugs to pay for based on cost-effectiveness, safety and efficacy.

Drugs at the neuromuscular junction

Motor nerves generally synapse in the spinal cord with either alpha motor neurones (voluntary) or interneurons (reflexes) that signal to the muscles they control. The neuromuscular junction is the synapse between the end of a motor neurone and the sarcolemma of the muscle it innervates (see Topic 5 for the process of excitation-contraction coupling). This is a common drug target as responses can be modulated easily.

Drug classes

ACh release blockers: e.g. botulinum toxin. The toxin acts as a protease enzyme on proteins that assist the docking of vesicles containing the neurotransmitter to the pre-synaptic membrane, stopping contraction. The prevention of ACh release can cause muscle paralysis and death, but can also be used cosmetically or to relax muscle spasm.

Nicotinic ACh receptor blockers: non-depolarising blockers competitively antagonise ACh (cholinergic) receptors to prevent depolarisation in the post-synaptic membrane (sarcolemma). They are based on the natural compound curare and are reversed by anticholinesterase. Can be used as an anaesthetic. Depolarising blockers (Sux is the only one used) agonise the receptor and continue to bind, to keep the ion channel shut. Sux is a dimer of ACh and is broken down slowly by cholinesterase, so has a number of side effects e.g. hypotension and hypothermia.

Anticholinesterases: prevent the enzymatic breakdown of ACh and so prolong its presence and effects in the NMJ. They are used to diagnose/treat myasthenia gravis (edrophonium, Topic 5), reverse anaesthesia and increase cholinergic activity. Irreversible anticholinesterases are dangerous and can lead to paralysis and cholinergic crisis.

Muscle relaxants: relieve spasticity by acting in the CNS, PNS or both to block signals for contraction (MS, c. palsy). Dantrolene prevents calcium release from the SR to block contraction, but lack of muscle use can worsen disability.

Drugs and the autonomic nervous system

As seen in Topic 5, the autonomic nervous system is the division of the peripheral nervous system that is **not** under conscious control. Its two components, the sympathetic and parasympathetic nervous systems, have opposing effects and the body is constantly varying the balance between the two according to the environment.

Sympathetic nervous system – fight or flight

- The sympathetic nervous system provokes actions in smooth muscle and glands in response to **stress or threat**. These responses are what would be expected if one was being chased by a predator, for example.

- Afferent autonomic nerves are sensors and efferent nerves are effectors (sending information to organs). The ganglionic chain (clusters of cell bodies synapsing with other ANS neurones) runs down beside the spinal cord.
- Pre-ganglionic neurones release ACh into synaptic clefts while post-ganglionic ones release noradrenaline (except in the pathway to sweat glands, where both release ACh, and it is uncertain why this is the case).
- There are four different kinds of sympathetic receptors which are present in various parts of the body – α_1, α_2, β_1 and β_2. They are known as adrenergic receptors because their ligands are the catecholamine neurotransmitters adrenaline and noradrenaline/NA (also known as epinephrine and norepinephrine). They are synthesised in the adrenal medulla and released in response to stress or threat signals from sensory nerve fibres.
- The receptors are named **α** and **β** because A and NA have different effects on blood vessel smooth muscle at them (don't worry about the difference between the two molecules, just know the overall effects). At **α_1** receptors in the **gut and skin blood vessels**, ligand binding causes vasoconstriction – blood flow is being prioritised for the muscles in case of escape. At **β_2** receptors in **skeletal muscle blood vessels**, ligand binding causes vasodilatation to allow greater blood flow. The distribution of receptors translates into the effects seen.
- α_2 receptors mediate NA feedback inhibition, while **β_1** increase heart contractility (stroke vol.) and heart rate.

Actions of the sympathetic nervous system:

- Vasodilatation in skeletal muscle
- Vasoconstriction in smooth muscle
- Venoconstriction so ↑ venous return
- Bronchodilation (SM relaxation, β_2)
- Activation of renin-angiotensin system
- ↓ secretions (except ↑ sweat)
- Pupil dilation
- Constricts sphincters & relaxes bladder
- Platelet aggregation

Drugs

You can easily figure out what drugs do and what their side effects may be by knowing what happens at each receptor

α agonists: e.g. NA, A and phenylephrine. Indicated for cardio shock and nasal congestion (by vasoconstriction)

α antagonists: "-zosin". Indicated for hypertension (by vasodilation), prostatic hypertrophy (contracts prostate).

β agonists: adrenaline (non-selective) indicated for anaphylaxis and cardiac arrest. Dobutamine for β_1 (heart), indicated for heart failure. Salbutamol for β_2 (bronchi), indicated for asthma and via IV for premature labour.

β antagonists: "-olol". Indicated for hypertension, heart failure, angina (**β_1**) and tremor (β_2). Beta-blockers should not be given to asthmatics, as bronchospasm is a side effect and will exacerbate their condition.

Parasympathetic nervous system – rest and digest

- The parasympathetic nervous system essentially exerts the **opposite effects** to the sympathetic nervous system and dominates at rest.
- Cholinergic receptors mediate effects and so the primary NT is ACh. The two subtypes of receptor are nicotinic (ligand-gated ion channels) and muscarinic (GPCRs), named for their specific ligands.
- The ganglia (relay centres/synapses, described above) are located close to the effector organ, in contrast the sympathetic NS.
- Muscarinic receptors M_1, M_3 and M_5 **activate** the phospholipase C pathway, while M_2 and M_4 **inhibit** the adenylate cyclase pathway. M_2 also opens K^+ channels and closes Ca^{2+} channels to reduce heart activity.

Actions of the parasympathetic nervous system:

- Bronchoconstriction
- Bradycardia
- Hypotension
- Salivation
- Lacrimation
- Sweating
- Pupil contraction
- Peristalsis

Cholinomimetic drugs imitate the effects of NTs at cholinergic receptors, and so will have **parasympathetic** effects:

- Direct acting agonists: acetylcholine, bethanechol, pilocarpine and muscarine (latter three muscarinic)
- Indirect acting agonists: -stigmines are reversible anticholinesterases; organophosphates are irreversible
- **Uses**: glaucoma (promotes drainage), intestinal/bladder atony (peristalsis) and Alzheimer's disease (↑ACh)

Anti-muscarinic drugs are often competitive antagonists of M receptors, and as such will have **sympathetic** effects:

- Ipratropium: used in asthma and COPD to promote bronchodilation
- Atropine: used in myocardial infarction (to stimulate heart), pre-anaesthetic (to prevent respiratory secretions), ophthalmological tests (to dilate pupil) and to counteract cholinomimetic poisoning
- Benztropine: used in Parkinson's to restore cholinergic/dopaminergic balance