IASM Notes

Semester II

(Adapted from M20 lecture notes)

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Content

L50 Non-communicable Diseases

A. Non-communicable Diseases

- Broad definition: essentially defined as NOT communicable/maternal/perinatal/nutritional diseases and intentional/unintentional injuries (WHO)
- Examples: cancer, cardiovascular disease, metabolic disorder, psychiatric disorder, atopic disorder, reproductive outcomes,…
- May also involve infectious agents in **aetiology** (source of disease):
  - Eg. HPV in cervical cancer
  - Eg. *Helicobacter pylori* in peptic ulcer and stomach cancer
  - Eg. Hepatitis B virus in liver cancer
- Present a major public health burden:
  - NCDs expected to account for 73% of deaths and 60% of disease burden by 2020
  - ↑ NCDs affects poor and disadvantaged population disproportionately → widening health gap
- Cause of mortality: chiefly NCDs in high-income countries, communicable disease in middle/low-income countries
- NCD rates rising in many developing countries due to changing demographic → double burden of infectious and non-infectious diseases
- Also note changing tobacco use patterns may affect NCD prevalence

B. Cancer

- **Cancer**: group of diseases in which abnormal cells divide without control
- Accounted for ~8M (14%) deaths globally (2008)
- Generally classified according to **primary anatomic site** where they originate
- Contributed to by a variety of factors including environmental (eg diet, smoking)

1. Lung Cancer

- Extremely rare cause of death 100y ago → most common cancer cause of death in men (in most high income countries)
- Heavily associated with **tobacco smoking** (gender, time lapse, countries) and **air pollution** (urban/rural difference)

2. Breast Cancer

- **'Disease of affluence'**: ↑ incidence for ↑ income
- Complex aetiology, major risk factors include:
  - Hormonal factor (oral contraceptives, hormone replacement therapy (HRT))
  - Alcohol (↑ oestrogen)
  - BMI (∵ main source of oestrogen after menopause is fat tissues → ↑ fat ↑ oestrogen level)
  - *In utero* and childhood growth
  - ↓ by childbearing and breastfeeding
- Note 'peaks' seen in epidemiology graphs may be due to screening

3. Stomach Cancer

- Nearly 1M new cases in 2012, 5th common in world
- Falling epidemiologic trends since discovery of *Helicobacter pylori* as cause of peptic ulcer (1975, stomach cancer was most common neoplasm at the time)
- Epidemiologic distribution:
  - Men 2x women
  - >70% in developing countries, 1/2 in E Asia
- Risk factors: smoking, obesity, nitrosamines

C. Cardiovascular Disease

- Diseases of heart and blood vessels that include:
  - Hypertension
  - Coronary heart disease (CHD)
  - Cerebrovascular disease (CVD)
  - Peripheral vascular disease
  - Rheumatic and congenital heart diseases
  - Cardiomyopathies
- Two major sources of mortality: CHD and CVD with different epidemiological trends → different aetiology (not yet understood)
- Risk factors: hypercholesterolemia, high BP, tobacco smoking
- Note CVD may be related to adverse socio-economic circumstances and deprivation in early life

*__Rheumatic heart disease__ arises from strep A infection of heart

D. Psychiatric Disorders

- Often ignored problem but constitute a major burden of morbidity (36% of total YLD)
- High treatment costs → often denied insurance
- Also note high prevalence of **comorbidity** (prevalence of psychiatric disorder in patients with physical illness)

E. Musculoskeletal Disorders

- Major contributor of morbidity and YLD (34%)
- Also extremely high prevalence among population → health burden (eg hip fractures)

F. Major Risk Factors of NCDs

- Many risk factors identified but many unconfirmed or only responsible for a small fraction of disease burden
- Still a number of risk factors with aetiological significance firmly established:
  - **Blood pressure**: important risk factor for cardiovascular disease
  - **Smoking**: associated with a variety of disease (eg. lung cancer) → account for millions of deaths around the world, figure still rising
  - **Obesity**: risk factor for cardiovascular disease, DM II, musculoskeletal problems; rising trend esp in high-income countries worrying

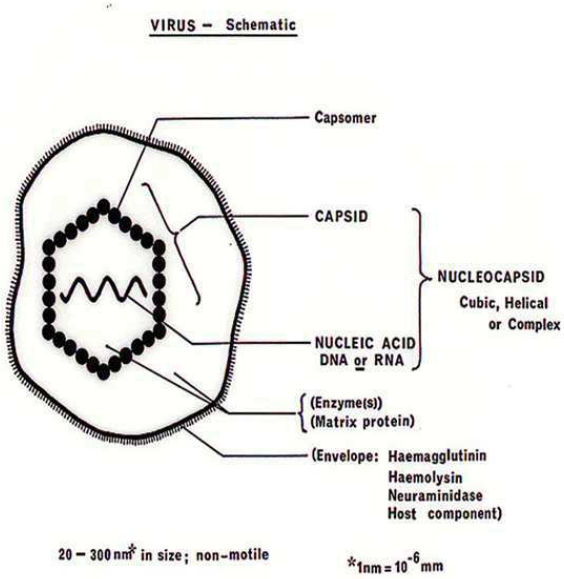
G. Measurement of Disease Burden

- **Years of life lost (YLL)**: measurement of mortality compared to life expectancy
  - Can account for age difference (difference between death of an infant and an elderly)
- **Disability adjusted life years (DALY)**: measurement of morbidity, YLL + YLD (years lived with disability)
- Note psychiatric and musculoskeletal disorders rise significantly in 'league tables' for DALY when compared to YLL and death tables

L51 What are Viruses and Prions?

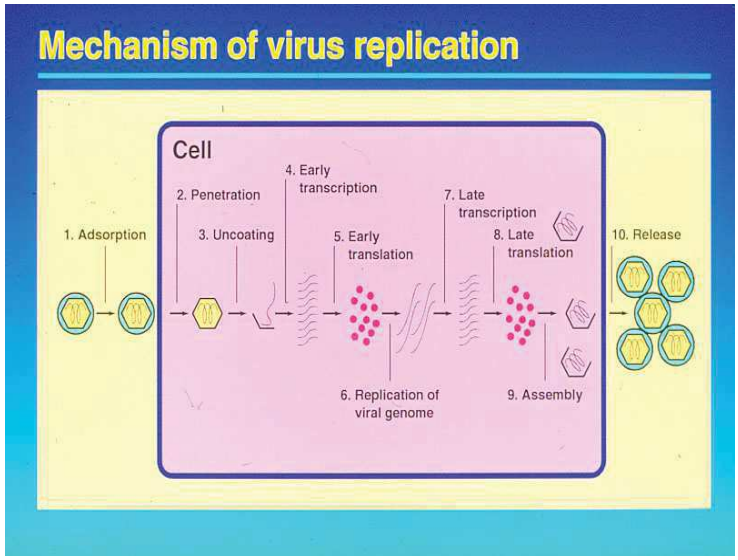
A. Virus

- Small obligate intracellular parasites that may cause contractible illnesses
- Size in the scale of 100 nm, can only be seen via EM
- Structure: a set of viral genetic materials protected by a protein covering (**capsid**)
  - Capsid made up of subunits called **capsomers**
  - Genetic material is composed of DNA or RNA
  - Some have an additional **envelope** around capsid
    - → Enveloped viruses more easily destroyed by heat etc due to more delicate structure



- Viral shape: icosahedral, helical, complex
- Small genome: no. of genes may not exceed 100 → obligate intracellular parasites to hijack host cell machinery for own use
- **Defective viruses**: a virus that does not encode all the structural proteins required for viral replication
  - Needs a **helper virus** to provide key genes for virus replication cycle
  - Eg. Hep D can only replicate in hep B infected cells (∵ it uses hep B antigen but does not carry gene to encode it)

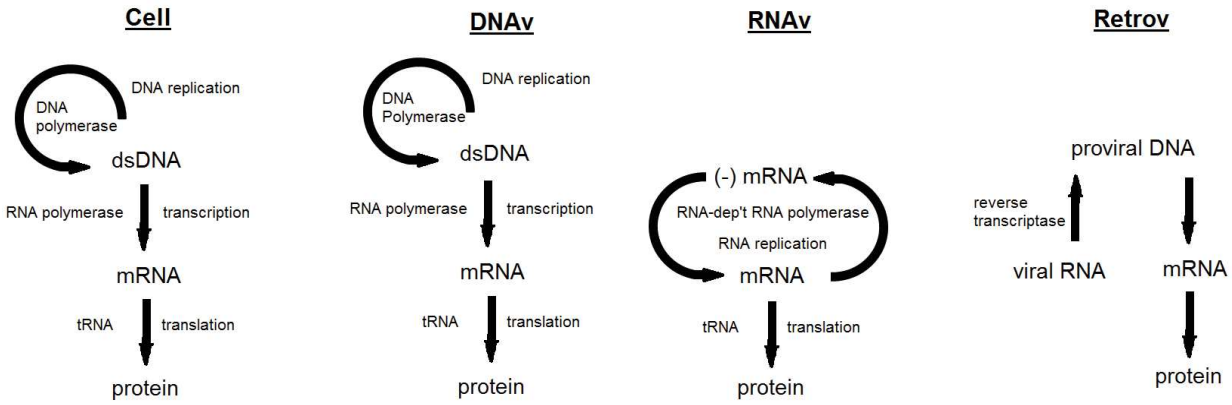
1. Virus replication cycle



1) **Attachment (adsorption)**: specific process involving viral component and cell membrane components (eg. HIV only infects T cells ∵ recognizes CD4 molecule on T cells);
2) **Penetration (endocytosis)**;
3) **Uncoating**: mostly by host digestive enzymes in phagosomes;
4) **Viral protein synthesis**: virus hijacks host protein synthesis machinery to synthesize viral protein;
5) **Viral nucleic acid replication**;
6) **Virus assembly**;
7) **Viral release**: by exocytosis.

2. Viral Genetics

- Three types of virus in terms of genetic materials: **DNA virus**, **RNA virus**, **retrovirus**



Note that **RNA-dependent RNA polymerase** is found in RNAv only and **proviral DNA** is in fact integrated into the host genome

B. Viral Disease

1. Viral Disease Progression

- **Incubation period**: time interval between entry of virus into bod and commencement of clinical symptoms
- **Clinical disease**: period in which the patient exhibits disease symptoms
- **Convalescence**: period in which patient recovers from the disease
- **Viral shedding**: period in which viruses are expelled from host cells following successful reproduction, may start at the end of incubation period (eg. influenza) or throughout clinical disease period

2. Viral Pathogenesis

- **Pathogenesis**: a series of events and processes that combine to produce disease
- **Localized infections**: virus does not enter blood (**viraemia**)
  - Multiplies at epithelial surface at or near site of entry into body
  - Disease symptoms may be local or systemic (due to immune response not viral infection) (eg respiratory infection, viral diarrhea)
- **Systemic infections**: virus spread by bloodstream or other means to distant sites
  - May have **tissue tropism**: localization in target organs
  - May cause organ damage $\rightarrow$ major disease
- **Viraemia**: presence of virus in blood
  - **Acute viraemia**: in early phase of many disseminated viral infections
  - **Chronic viraemia**: in chronic infections such as hep B and HIV
- **Cell/organ tropism**: virus may be attracted to certain organs (eg. HIV has a $CD4^+$ T cell tropism)

a. Mechanism of Viral Pathogenesis

- **Cytolysis** (cytopathic effect):
  - Uncontrolled viral replication
  - Switching off of host cell function (due to hijacking of host cell machinery)
  - Induced apoptosis
- **Immunopathological**:
  - Pathogen may induce strong immune response
  - Some immune response, esp unsuccessful ones leading to prolonged activation, may cause tissue damage
  - Eg. hepatitis B: immune response kills virus infected cells → damage of liver tissue
- **Oncogenesis**:
  - Some viruses may induce tumors (due to integration of viral genome in host genes)
  - Eg. hepatitis B → hepatocellular carcinoma
  - Eg. HPV → cervical cancer

C. Viral Diagnosis

- Two main ways: detect virus or detect host immune response

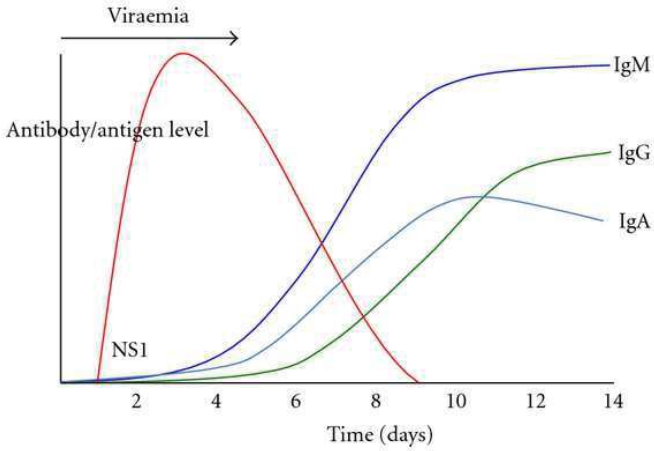
1. Detection of Virus

Method	Timespan	Details
EM	Hours	- Identification by morphology seen in EM - Require >10^6 virus particles per mL → normal sample usually not enough (exception: fecal sample of diarrhea, vesical fluid for HSV)
Culture	Days – weeks	- Virus cultured to provide more sample for identification - Eg. **embryonated egg inoculation**: use of different sites in embryonated egg for viral culture - Eg. **live animal culture** - Eg. **cell culture**: use of normal cells → look for virus **cytopathic effect** (structural changes in host cells caused by viral invasion)
Ag detection	Hours	- Viral antigen can be detected by immunofluorescence
Nucleic Acid detection	Hours – days	- Use of PCR to amplify target DNA - Detection using northern blot EIA
Viral inclusion bodies	Days	- Histology of some viral inclusion bodies (in infected cells) can be used to identify virus - Eg. Negri bodies in rabies

*Virus sample transported in **virus transport medium**: with water (for moisture), buffer with pH indicator (for pH maintenance), protein (for maintenance of viral stability), antibiotics (for prevention of bacterial overgrowth)

2. Detection of Antibody Response

- Single Ab test +ve can only mean infection at some time during lifetime
- **Antibody titre** measured for two times 10-14 days apart
- ≧ **4 fold increase** → rising Ab titre → **seroconversion**
- **IgM +ve**: recent infection
- **IgG +ve**: infection in lifetime



D. Prions

- **Prions**: infectious protein without nucleic acid
- Responsible for Mad Cow disease or Creutzfeldt-Jakob disease (CJHD)

L52 Medically Important Viruses

A. Picornaviruses

- **Picornavirus**: a family of small RNA viruses belonging to viral group IV ((+) ssRNA)
- Important types:
  - **Rhinovirus**: cause URTI (eg. common cold)
  - **Enterovirus**: causes systemic infection (eg. poliovirus, enterovirus 71)
  - **Hepatovirus**: one cause of viral hepatitis (eg. hepatitis A virus)
- No lipid envelope but very tough capsid: can survive in external environment, gastric acidity and faecal enzymes → can be transmitted by **faeco-oral route**

*Viral group denotation:

(+): viral RNA as mRNA i.e. directly translated

(-): viral RNA complement to mRNA, i.e. need RNA polymerase before translation

ss: single-stranded; ds: double-stranded

**Another common cause of common cold is coronavirus

1. Enteroviruses

- Stages of disease
  - Entry by ingestion, tough coat confers resistance to gastric acidity
  - Multiplication in intestinal lymphoid tissue (asymptomatic infection)
  - Entry into bloodstream → viraemia (flu-like illness)
  - Spreading to other organs → replication lead to cell damage → organ dysfunction → disease
    - → Anterior horn cells of spinal cord → paralysis (poliomyelitis, enterovirus 71)
    - → Meninges → meningitis
    - → Brain → encephalitis
    - → Cardiac muscles → myocarditis (Coxsackie B)
    - → Skeletal muscles → **myalgia** (muscle pain) (Coxsackie B)
    - → Skin/mucosa → skin rash, mucosal vesicles (eg mouth)
- Stopping of infection at different stages will correspond to different severity of disease
- Notable feature: **asymptomatic infection**
  - 95/100 poliovirus carriers are asymptomatic
  - Asymptomatic carriers can still infect others
  - Phenomenon known as '**iceberg effect**'
- Lifelong immunity after infection
- Stability of virus allow survival in environment for a prolonged period → efficient transmission
- Eg. poliovirus – **polio** (characterized by paralysis of lower limb)
- Eg. enterovirus 71 – **hand, foot and mouth disease** (can lead to neurological complications when infecting anterior horn cells or brain stem)

a. Polio Vaccines

- Polio has three types of antigens (1, 2, 3) → need for a multivalent vaccine
- **Oral live attenuated vaccine**: lost neurotropism traits
  - Note that only one subtype will establish due to **viral interference**
  - Need to take 3 doses for immunity to all subtypes
  - Advantage: natural (lead to formation of intestinal antibodies → mucosal immunity) and convenient (oral introduction)
  - Disadvantage: may develop into disease in 1/750000 cases
- **Killed vaccine**: three injected doses (primary, secondary and booster)

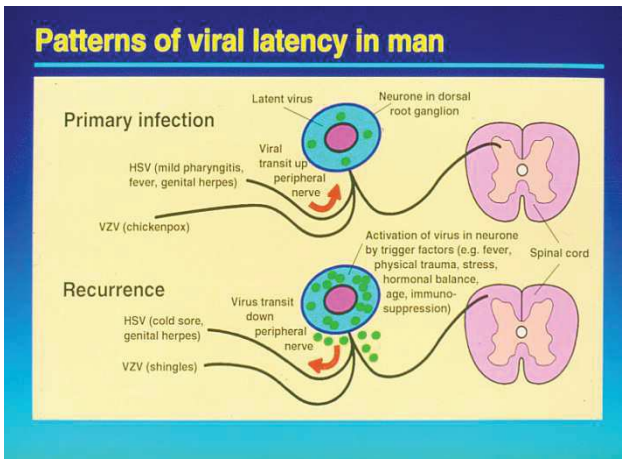
*Due to risk, oral vaccine gradually switched to killed vaccine as polio is slowly being eradicated

B. Herpesviridae

- **Herpesviridae**: a type of enveloped DNA virus belonging to virus group I (dsDNA)
- Examples: HSV-1, *Varicella-zoster virus*, *Epstein-Barr virus*
- Notable feature: **persistent (life-long) infection**
  - **Virus latency** in body
  - Integration of viral genome into host genome
  - No viral protein expressed on cell surface → virus can persist in cell despite active host immune response
  - Symptomatic or asymptomatic reactivation → transmission

1. Herpes Simplex Virus (HSV)

- **Type 1**: above waist (eg mouth (oral lesion); finger (herpetic whitlow))
- **Type 2**: below waist (eg genital herpes (STD))
- Primary infection often asymptomatic
  - Symptoms may range from mild oral lesions to severe stomatitis
- Latent infection in sensory / autonomic ganglia
- Reactivated by trigger factors → recurrence at <u>primary site</u>



2. Varicella-zoster Virus

- Primary infection lead to **chicken pox** (all over body)
- Latent infection in multiple levels of spinal cord
- Reactivation at one level of spinal cord (i.e. one ganglion) → **shingles** (pustules on area innervated by one ganglion)
  - Shingle patients can further infect others, causing **chicken pox**

*Skin lesions:

Macule: mere change in colour, no elevation

Papule: mere elevation, no fluid

Vesicle: elevation with clear fluid

Pustule: with cloudy fluid (i.e. pus)

Ulcer: complete loss of epidermis or even dermis/subcutis (possibly caused by bursting of pustules)

C. Influenza Virus

- Influenza virus: a group of RNA viruses belonging to the group **orthomyxoviridae** of virus group V ((-) ssRNA)
- Three types (distinguished by core protein):
  - Type A: major type of influenza that may cause serious illnesses
  - Type B: almost exclusively infect humans, low mutation counts confers immunity
  - Type C: only cause minimal disease
- 8 pieces of segmented genomes
- Especially note **haemagglutinin (H1-16)** and **neuraminidase (N1-9**) subtypes of type A influenza viruses
  - **Haemagglutinin**: binding to cell receptor (sialic acid) in host cells and facilitate entry of viral genome into cytoplasm
  - **Neuraminidase**: cleaves terminal sialic acid to promote virus release (after viral replication) → virus can spread through mucin of respiratory tract
- Antivirals:
  - Amantadine / rimantadine: block virus uncoating
  - Oseltamivir (Tamiflu) / Zanamivir (Relenza): neuraminidase inhibitors → virus release blocked
- Transmitted via aerosol: droplets and droplet nuclei
- Pathology:
  - Destroys epidermal cells of trachea → only basement membrane left
  - Systemic symptoms caused by cytokines (of host defense system)
- Host immunity conferred by Ab to haemagglutinin and neuraminidase (neutralises virus infectivity) and by cell-mediated immunity (helps recovery)
- Nomenclature: type / (host) / place of isolation / lab number / year of isolation [HN subtype]
  - Eg. A / Chicken / New York / 14009 / 93 [H5N2]
  - Eg. A / Hong Kong / 156 / 97 [H5N1]
- Notable feature:
  - **Antigenic drift**: mutation leading to minor antigenic change (due to lack of proofreading ability by RNA polymerase)
  - **Antigenic shift**: complete change of genetic fragments due to genetic reassortment between different viruses (only in influenza A)
- Prevention: annually-renewed vaccine and good hygiene (↓ transmission)

L53 Outbreak

A. Outbreak

- **Outbreak (epidemic)**: occurrence in a community or region, of cases of an illness, specific health-related behavior or other health-related events clearly in excess of normal expectancy
- Differences between **endemic**, **epidemic** and **pandemic**:
  - **Endemic**: sustained infection in a specific group without need of input
  - **Epidemic**: sudden, localized outbreak
  - **Pandemic**: widespread epidemic (in multiple regions and groups of population)
- Note **zoonosis** (animal diseases that can be naturally transmitted to humans) as a possible source of outbreaks
- **Epidemiology**: study of disease distribution (who / how many / where) and determinants (when / how / why)

1. Identification of Pathogen

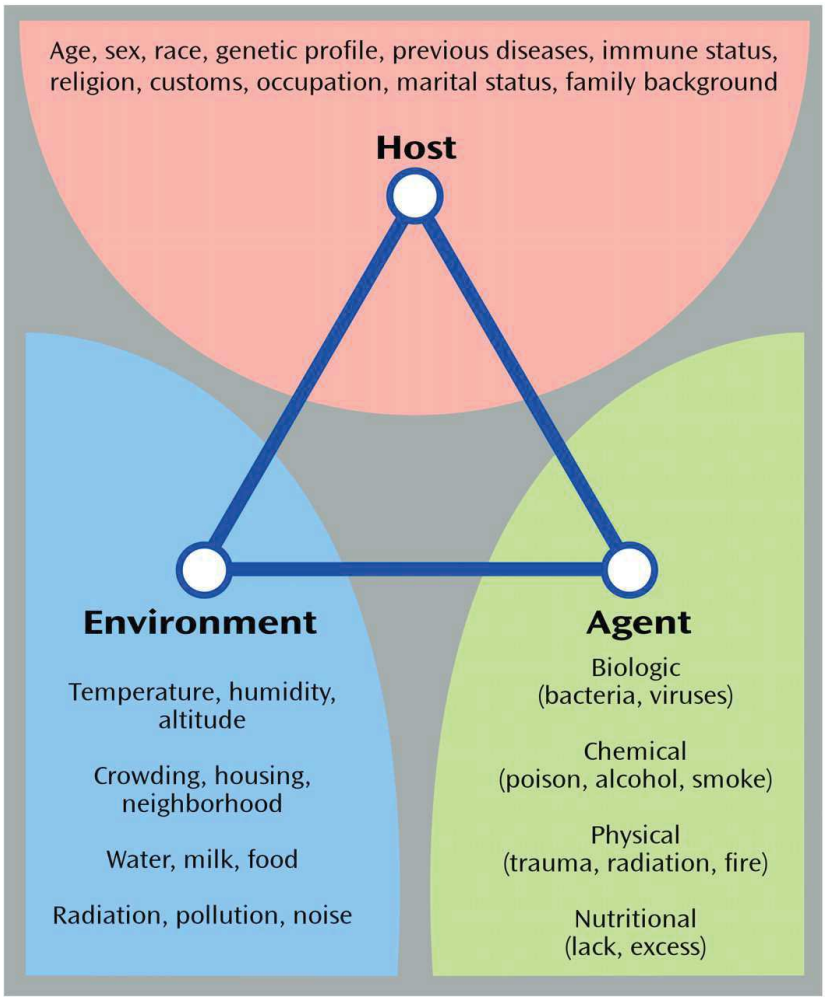
- **Koch's postulates**:
  - Organism is demonstrable in cases of the disease but not in healthy individuals
  - Isolated in pure culture
  - Inoculation of a pure culture of organism should reproduce disease in a susceptible animal
  - The organism can be re-isolated from experimentally infected animal

- **Fredricks & Relman criteria**:
  - Reason:
    → Not all pathogens can be cultured
    → Asymptomatic carriers exists
    → Not all diseases have experimental animal models
  - A nucleic acid sequence belonging to a putative pathogen should be present in most cases of an infectious disease and preferentially in gross anatomic sites known to be diseased, and not in those organs that lack pathology;
  - None or fewer copies of pathogen-associated nucleic acid sequences should occur in hosts or tissues without disease;
  - Sequence copy number of pathogen should decrease with resolution of disease and correlates with severity of disease or pathology;
  - Organism inferred from sequence should be consistent with the known biological characteristics of that group of organisms;
  - Pathogen sequences should be found in diseased tissue;
  - These evidences for microbial causation should be reproducible

2. Study of Epidemiological Distribution and Determinants

- Line-list of cases is studied in outbreaks
- Contact, travel and occupation histories of cases are obtained
- Source of outbreak can be traced in this way

a. Epidemiological Triangle



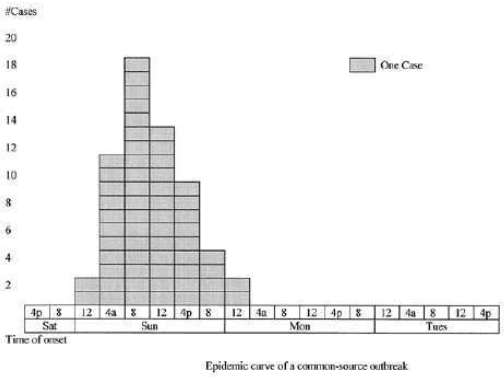
3. Surveillance in Prevention of Possible Outbreaks

- **Surveillance**: ongoing, systematic collection, analysis and interpretation of data
- Methods of surveillance:
  - **Passive** (routine data) by notifications, lab specimens, registries
  - **Active** by special studies and serologic surveys (↓ iceberg effect)

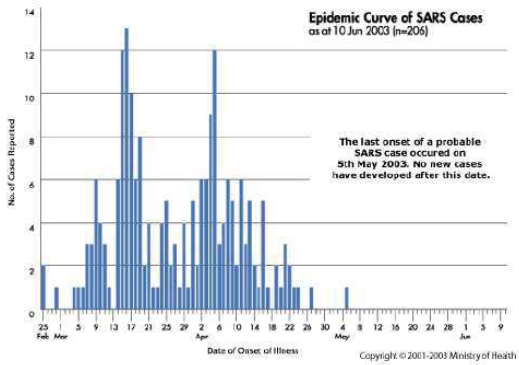
4. Interpretation of Epidemic Data

a. Epidemic Curves

- **Epidemic curve**: number of cases plotted against time
- Note the difference in epidemic curves of **point-source epidemic** (bell shape curve) and **propagated epidemic**



Epidemic curve of a common-source outbreak



Point-source epidemic

Propagated (person-to-person spread) epidemic

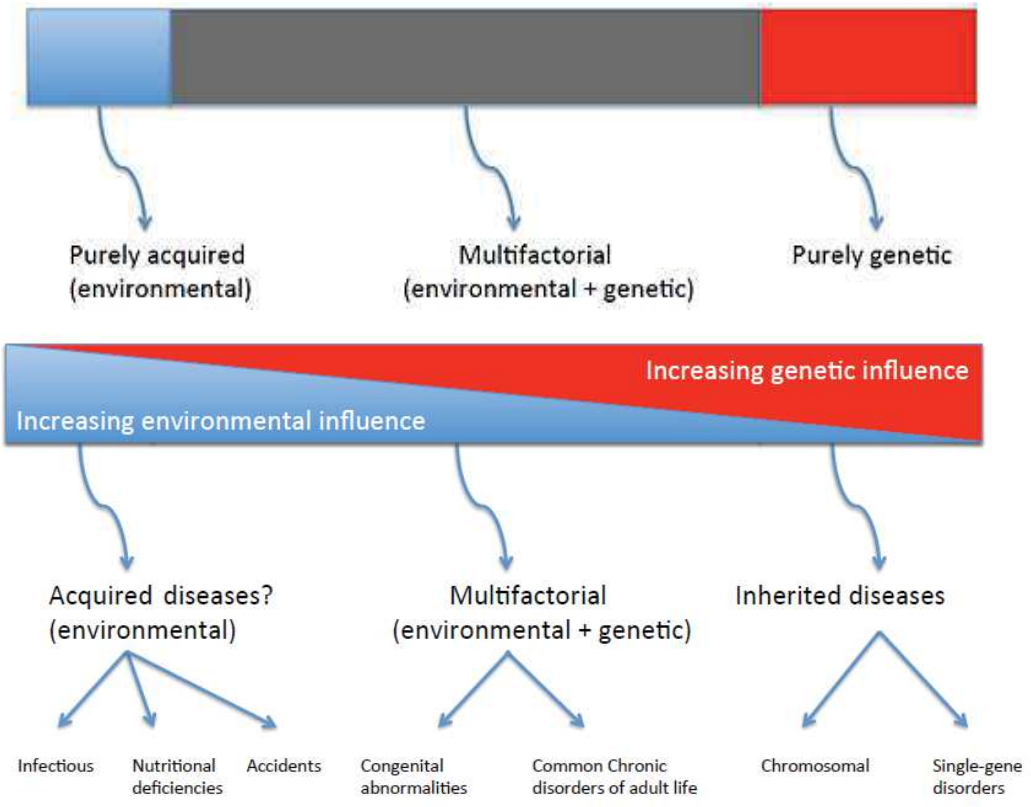
b. Reproductive Number

- **Basic reproductive number (R_0)**: the number of cases one case generates on average over the course of its infectious period in a **non-immune** population
- **Reproductive number (R_t)**: number of secondary cases generated by one case later in epidemic when part of population is non-susceptible (immune)

L54 Does Genetics Matter?

A. Evolution of Genetics in Medicine

- Genetics development is advancing rapidly (note implication of human genome project)
- Application in medicine also changes from **medical genetics** (clinical medical care of inherited disorders) to **community genetics** (prevention of monogenic diseases) to **public health genomics** (clinical management, prevention and risk prediction for common diseases)
- Understanding of aetiology also changed: there are no 'purely acquired' or 'purely genetic' diseases
  - Genetic factor interplays with environmental factor to give rise to diseases
  - Example: genetic predisposition affects survival rates of some diseases eg. SARS
  - Example: lack of HIV-attaching surface proteins confers resistance



- Spectrum of disease:
  - **Monogenic disease**: high penetrance, high PPV, amenable to genetic service
  - **Polygenic disease**: low penetrance, poor PPV, rarely amenable to genetic service

B. Monogenic Diseases

- **Monogenic disease**: disease caused as a result of a **single gene defect**
- Inherited in a clear and predictable simple Mendelian pattern
- Usually of **high penetrance** and **full expressivity:**
  - Strong association between genotype and phenotype
  - High **positive predictive value** of genotype for disease phenotype
  - **Positive predictive value (PPV)** = $\frac{true+ve\ cases}{all+ve\ cases}$
- Accurate risk estimation possible for counseling

1. Phenylketonuria (PKU)

- **Phenylketonuria**: inability to metabolize phenylalanine due to lack of phenylalanine hydroxylase deficiency → accumulation of phenylpyruvic acid
- An autosomal recessive genetic disorder
- Progressive mental retardation, brain damage and seizures
- Enormous benefit in early intervention ∵ adverse effects can be prevented by avoiding phenyl-rich food

2. G6PD Deficiency

- **G6PD deficiency**: defective production of **glucose-6-phosphate dehydrogenase**
  - G6PD located at entry of **pentose phosphate pathway** for formation of NADPH
  - NADPH helps regenerate GSH to eliminate free radical (only source of GSH in RBCs)
  - Oxidative stress → all GSH consumed → ROS accumulation
  - Enzymes and proteins damaged by ROS → electrolyte imbalance → phagocytosis of damaged RBC → non-immune **hemolytic anaemia**
- X-linked recessive disease
- Common in tropical regions ∵ confers protection against *Plasmodium falciparum* (i.e. malaria parasite)
- Symptom: non-immune hemolytic anaemia when exposed to oxidative stress
  - Examples of causative substances: broad beans, antimalarial drugs (primaquine and chloroquine), sulfonamides (sulfamethoxazole, thiazide), aspirin

3. Huntington's Chorea

- **Huntington's chorea**: progressive neurodegenerative genetic disorder affecting muscle coordination and cognitive functions
- Autosomal dominant disease
- Typical onset between 35 and 44
- Variable severity (depending on repeat counts) but predictable pattern of symptoms progression
- No effective intervention, surveillance or treatment
- Controversy regarding presymptomatic genetic testing due to adulthood onset
  - Optimal timing for testing
  - Right of parents to test children
  - Confidentiality and disclosure of test results

Classification of the trinucleotide repeat, and resulting disease status, depends on the number of CAG repeats[15]

Repeat count	Classification	Disease status	Risk to offspring
<26	Normal	Will not be affected	None
27–35	Intermediate	Will not be affected	Elevated but <<50%
36–39	Reduced Penetrance	May or may not be affected	50%
40+	Full Penetrance	Will be affected	50%

C. Polygenic Disease

- **Polygenic disease**: a disease that is the cumulative effect of a large number of genes at different loci
  - Each inherited according to Mendel's rule
  - Complex overall disease inheritance each with a small effect on phenotype
- Tendency to 'run in families' albeit with low heritability compared to monogenic disorders (eg. ~2-5% of close relatives of DM also suffer from DM)
- Higher influence by environmental factors
- Examples: heart disease, diabetes, asthma, diabetes, epilepsy, hypertension, **manic depression** (bipolar disorder), schizophrenia, …

D. Use of Genetic Tests in Medical Context

1. Diagnostic Testing

- Available for definitive genetic diseases (eg. Down Syndrome)
- Employed for making a diagnosis (by karyotyping or other genetic tests) upon clinical suspicion of a certain genetic disease

2. Newborn Screening

- Public health programs designed to screen infants shortly after birth for a list of conditions that are treatable but not yet clinically evident in newborn period
- Performed on all newborns soon after delivery
- Aim: identify genetic disorders that can be treated early in life
- Criteria for screening:
  - Simple and sensitive test
  - Effective treatment/management is available
  - Serious outcomes if left undetected → benefit in early intervention
  - Relatively common in population
- Examples: G6PD deficiency, congenital hypothyroidism, phenylketonuria (not carried out in HK)

3. Prenatal Diagnosis

- Aims to test for diseases or conditions in a fetus or embryo before it is born (eg. Down syndrome)
- Carried out in antenatal follow-up in an obstetric setting
- Offered to couples with an increased risk factors (eg. family history, known carrier status)
- Affects pregnancy decisions

a. Prenatal Diagnosis for Down's Dyndrome

- Risk score-based screening test
  - Blood test results (α-fetoprotein, β-hCG, inhibin-A, estriol, h-hCG)
  - Ultrasonography (**nuchal translucency**: accumulation of fluid at back of the neck)
  - Maternal age + gestational age of fetus
- Maternal plasma for cell-free fetal DNA testing:
  - Identifiable by differences in methylation pattern of specific DNA sequences
  - Quantified using massive parallel sequencing technology
- Invasive confirmatory tests:
  - Chorionic villus sampling
  - Amniocentesis

4. Carrier Testing

- **Carrier testing**: evaluation of carrier status in family members of patients suffering from an inheritable disease (by testing for single mutant genes for recessive diseases)
- No health implications but on reproductive decision
- Eg. cystic fibrosis mutation test for a woman whose brother has the condition
- Eg. thalassemia gene carrier tests

5. Presymptomatic Testing

- **Presymptomatic testing**: testing in healthy individuals for likelihood of having certain diseases with a genetic basis **prior to onset of symptoms**
- Implied **certainty** of developing condition at some point in the future
- Eg. disease that begins later in childhood / adolescence:
  - **Charcot-Marie-Tooth disease** (hereditary motor and sensory neuropathy)
  - **Familial amyloid polyneuropathy** (a type of **amyloidosis**)
- Eg. disease that begins in adulthood:
  - **Huntington's Disease (HD)**

6. DNA Sequencing

- Since completion of Human Genome Project, sequencing technology availability, affordability, accessibility, accuracy all improved
- Great potential for application in multiple sectors of medicine:
  - **Personalized medicine**: guiding individualized treatment
  - **Pharmacogenomics**: guiding dosage adjustment
  - **Nutrigenomics**: guiding personalized nutritional advices or therapy
  - **Toxicogenomics**: individualized response to toxins → better estimation of risk of toxins to patients or public
  - **Disease susceptibility prediction**: guiding personalized risk assessment ± lifestyle advices and preventive approaches
  - Better understanding of **disease mechanisms** and **categorization**
  - New **diagnostic potential** (↑ accuracy and ↓ side effects)
- Problems and challenges:
  - Huge business potential
  - Potential benefit/harm of awareness of a genetic risk
  - Difficulties (esp for laymen) to interpret genetic associations
  - Utility of genetic prediction in different settings (equity issues)
  - Complexity of social policy issues
  - Logistical challenges in preparing for new changes
  - Practical problems in genetic data handling
  - **Ethical, social and legal implications (ELSI)**

a. Application of Genomic Medicine

i. Guiding Individualized Treatment in Breast Cancer

- ***HER2* (human epidermal growth factor receptor 2)**
  - Proto-oncogene at long arm of human chromosome 17
  - Code for a Y-kinase: a cell membrane surface-bound receptor normally involved in signal transduction pathways for cell growth and differentiation
- Amplification of *HER2*/neu gene in 15-20% of breast cancer
  - Prognostic implication (higher aggressiveness and disease recurrence)
  - Predictive in response to **trastuzumab (Hercceptin)**
    - → Monoclonal Ab binding to HER2 receptors
    - → Only effective in breast cancer where HER2/neu receptor is overexpressed
    - → Halts cell proliferation

*CA breast = carcinoma of breast = breast cancer

ii. Guiding Dosage Determination for Warfarin

- **Warfarin**: an anticoagulant for prevention of **thrombosis** and **embolism**
- Narrow therapeutic window: overdose → bleeding risk
- Blood level affected by many common medications and some fluid
- Frequent blood testing (prothrombin/international normalized ratio for blood-clotting tendency) for dosage monitoring and adjustment
- Genetic test of *VKORC1* and *CYP2C9* → assessment on patient's rate of warfarin metabolism
  - Guide initial dose of warfarin
  - Achieve optimal dose more quickly
  - ↓ risk of bleeding

*Note 2014 meta-analysis shows no benefit in reducing excess or little anticoagulation

iii. Guiding Treatment Option Selection to Avoid Potential Complications

- **6MP (mercaptopurine)**: immunosuppressive drug for treating many different types of cancer (leukemia, pediatric non-Hodgkin's lymphoma, polycythemia vera (cancer with excess RBC) and inflammatory bowel diseases (eg. Crohn's disease and ulcerative colitis)
- 6MP metabolized by **thiopurine methyltransferase (TPMT)**
  - 90% have high (wild-type, WT) activity
  - 10% with intermediate activity
  - 0.3% with low activity → toxicity from 6-MP → need for dose reduction
- Testing of TPMT genotype → predict severe hematological toxicity

iv. Guiding Risk Prediction for Familial Breast Cancer

- **BRCA1/BRCA2** carrier ↑ risk of developing breast and/or ovarian cancer at an early age (before menopause)
  - Lifetime risk of CA breast: 60% (carrier) and 12% (non-carrier)
  - Lifetime risk of CA ovary: 15-40% (carrier) and 1.4% (non-carrier)
- Account for only 3-10% of breast cancer cases overall (patients with multiple close family members with CA breast or ovary)
- Individuals with above genes: advise screening and **prophylactic** (preventive) **surgery**

b. Disease Susceptibility Testing

- **Disease susceptibility testing**: testing for presence of genetic sequence variants that have been implicated to be associated with an increased risk for a disease
- Current main application:
  - Hereditary breast/ovarian cancer syndromes (mutated *BRCA1* and *BRCA2*)
  - Familial adenomatous polyposis coli (*APC* gene)
  - Hereditary nonpolyposis colorectal cancers (*MSH2*, *MLH1*, *MSH6*, …)
- Tests for cardiovascular diseases, DM and Alzheimer's under development
- Early notification of risk can cause a lot of problems due to delayed and probabilistic outcome (esp unnecessary anxiety and misleading assurance)

	Traditional genetic testing	New genomic risk profiling
Disease type	Rare	Common
Inheritance	Mendelian	Complex
Number of genes involved	One (or few)	Multiple
Type of variants involved	Coding	Mostly non-coding
Prevalence of each variant	Low	High
Penetrance	High	Low
Assay type	Targeted sequencing	Genome-wide arrays
Test result	Highly predictive	Probabilistic
Individual impact	High	Low
Family impact	High	Low
Potential population impact	Low	High

i. Direct to Consumer (DTC) Testing

- Over-the-counter or at-home genetic testing for known genes or more questionable susceptibilities
- Advantages:
  - Promote awareness of genetic diseases
  - Enables greater consumer awareness of and access to tests
  - Allow consumers to take a more proactive role in their healthcare
  - Help consumers improve their health and make beneficial treatment and lifestyle decisions
  - Provides a privacy advantage over testing through a healthcare provider
- Problems:
  - Suitability of test indication
    - → Clinical validity not been sufficiently proven
    - → Inconsistencies and lack of consensus on which genes are genotyped for a particular disease
    - → **ACCE framework** aimed at eliminating tests without scientific basis
  - Healthcare provider may not provide sufficient guidance
    - → Problems on interpretation and follow-up of results
    - → Important decision-making about treatment or prevention based on inaccurate, incomplete or misunderstood information
    - → Also note only 29% American physicians deem themselves adequately trained to provide **genetic counseling**
  - Potential on invasion of genetic privacy
    - → Possible abuse of genetic information by testing companies
  - Genetic discrimination
    - → May be unfair for individuals having certain type of attributes
    - → Consequences may be detrimental to society
    - → **Genetic information nondiscrimination act (GINA)** in US to protect against genetic discrimination esp in insurance and employment
- Control of genetic testing: France, Germany, Portugal and Switzerland banned DTC genetic testing (can only be carried out by a doctor) and US will soon follow

L56 Microbes and Disease

A. Microbiology

- **Microbiology**: study of organisms which are usually small in size, simple in structure
- Microbes can be classified as:

Microbes	Size	Tools	Remarks
Worms (parasites)	1 mm – 10 m	Hand lens	
Protozoa Fungi	3 μm – 10 μm	LM	
Bacteria	0.5 μm – 5 μm	LM	without mitochondria (and other organelles) and thus has to rely on an enzyme cascade producing energy
Viruses	0.02 μm – 0.1 μm	EM	only consists of genetic material enclosed by a protein coat (and possibly also an envelope
Prions	< 1 nm	Molecular techniques	infectious proteins

- **Clinical microbiology**: study of effects of microbes on human as a pathogen or/and commensal
  - Concerns with **diagnosis**, **management** and **control** of infectious disease
- **Infectious diseases**: communicable disease caused by microbes (**contagion**) → can cause **outbreaks** and associated **terror** and **social stigmatization**
- Disease is caused by overwhelming of defense mechanism by **virulence factor** of pathogens leading to disruption in normal physiology and thus a clinically symptomatic disease
- Battlefields against infectious disease:
  - **Community/hospital setting**: epidemiologist fighting population outbreaks by source control
  - **Clinical setting**: doctors fighting diseases (in individual patients) by prescription and infection control
  - **Laboratory setting**: microbiologists fighting microbes (in specimen) by identification
- Cellular locations of invading pathogens:
  - **Cytosolic pathogens** in any cells
  - **Intravesicular pathogens** in macrophages
  - **Extracellular pathogens** with toxins taken up by B cells

- Typical steps taken by a microbiologist when coming across with a new pathogen:

1) Find the microbe;
2) Develop Dx test;
3) Define disease syndrome;
4) Study disease progression;
5) Study pathogenesis (how virus produces host damage).

1. Koch's Postulates

- Used to ascertain the role of a certain species of microbes in causing a particular disease

1) Found in abundance in all patients but not in healthy organisms;
2) Must be isolated and grown in pure culture;
3) Cause the disease when introduced to a healthy organism;
4) Must be re-isolated from the inoculated host and identified as identical to the original sample.

- Later revised as more knowledge about microbiology is discovered

B. Pathogenesis of Human Diseases

- Diseases ∵ **virulence** mechanism of microbe overcome **defence** mechanism of the host → disturbance in normal **physiology** → symptoms
- If physiological disturbance is minimal → **asymptomatic** / **subclinical** infection; chronic → **carrier** state
- Damage to host mediated by:

1) Direct **cytolysis**: taking over of protein synthesis machinery for intracellular replication (→ induce apoptosis) or secretion of toxins or enzymes
2) **Molecular mimicry (immunopathological damage)**: stimulation of immune system against cross-reactive host antigen (causing aberrant immune response against itself)
3) **Oncogenesis**: result of integration of viral genome into host chromosome (Hep B virus causing liver cancer or *Epstein Barr virus* causing naspharyngeal cancer) or a result of chronic inflammation (*helicobacter pylori* causing stomach cancer)

C. Diagnosis of Infectious Diseases

1. Using Microbial Factors

- **Visualisation** of characteristic **morphology** of microbe in clinical specimen by **Gram stain** and LM for bacteria and EM for viruses
  - **Gram stain**: Gram + or – depend on structure of cell wall → important criteria in classifying bacteria
- **Culture of microbe**: type of nutritional supplement/temperature highly variable for different bacteria and fungi → used to identify
  - Cell culture system used for virus
  - **Streaking**: swab stroke on agar plate → dilute specimen → single bacteria colonies isolated → prevent complex biochemical interaction between different colonies
- **Detection of specific microbial components**:
  - **Proteins**: enzyme immunoassay (EIA using antigen (Ag) – antibody (Ab) reaction
    - → Recombinant antigen/antibody manufactured by recombinant DNA technology
    - → Ab/Ag arranged in assay to combine with sample Ag/Ab (from specimen or gel electrophoresis)
    - → Anti-Ig enzyme added and breaks down substrate to form fluorescent product
  - **Lipids**: gas liquid chromatography or high-performance liquid chromatography (HPLC) (esp volatile fatty acid metabolites by anaerobes)
  - **Polysaccharides**: EIA
  - **Specific DNA/RNA sequences**: PCR (for DNA), RT-PCR (for RNA) and/or probe hybridization)
    - → Use of primers to amplify DNA/RNA fragments for detection in northern/southern blot
    - → Much faster than culture → preferred in developing rapid tests for diseases

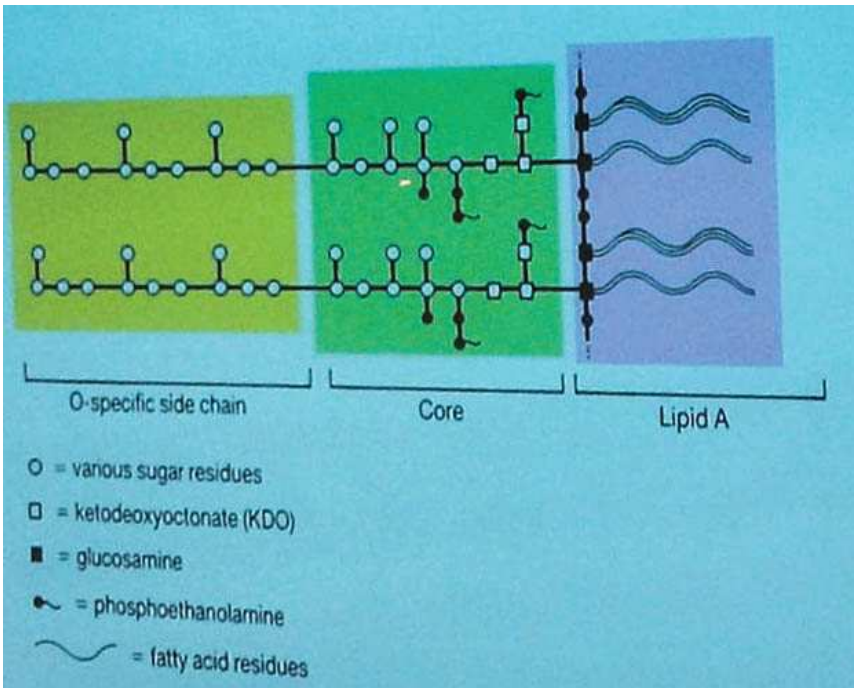
2. Using Host Factors

- **Antibody response** of host towards microbial components (using crude or recombinant antigen) (esp during convalescent phase)
- **Cellular immune response** of host (eg. **Mantoux test**, **lymphocyte proliferation** or **cytotoxic lymphocyte response** towards specific antigens)
  - Rarely used

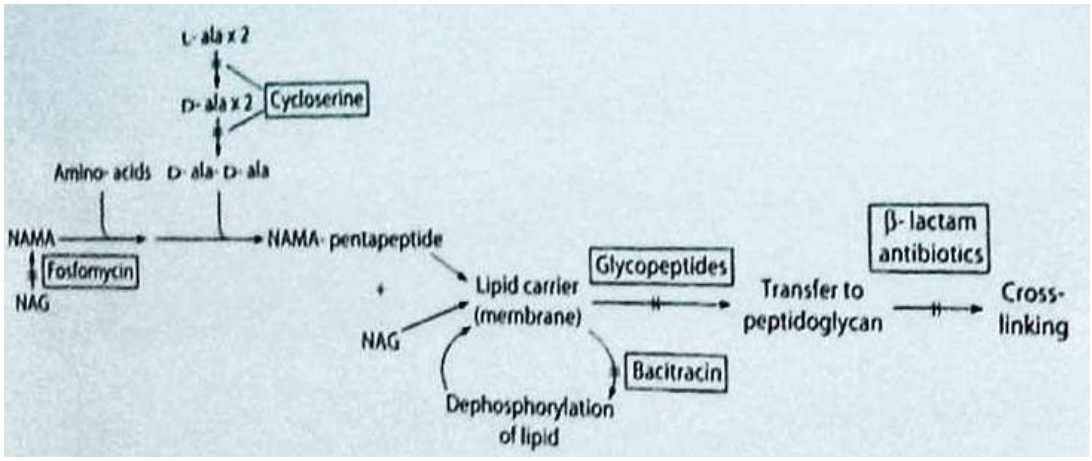
*__Mantoux test__: intradermal tuberculin injection test for tuberculosis

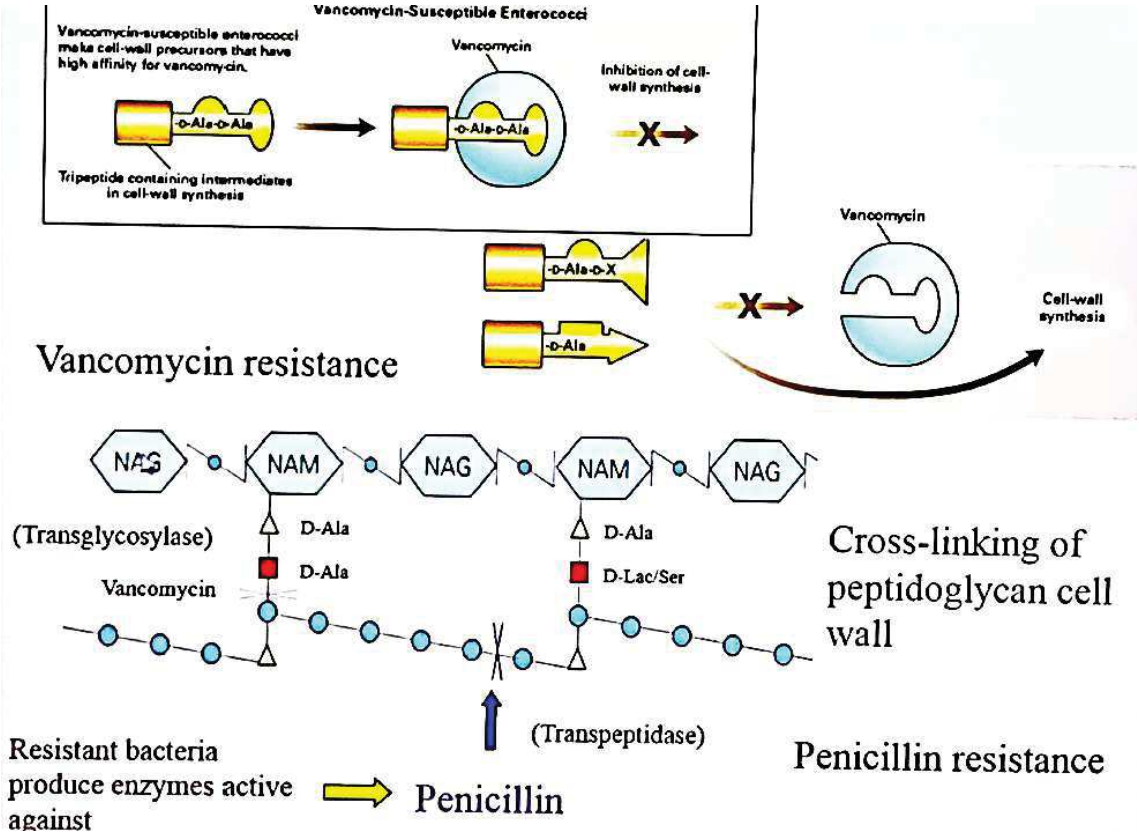
D. Bacterial Structures

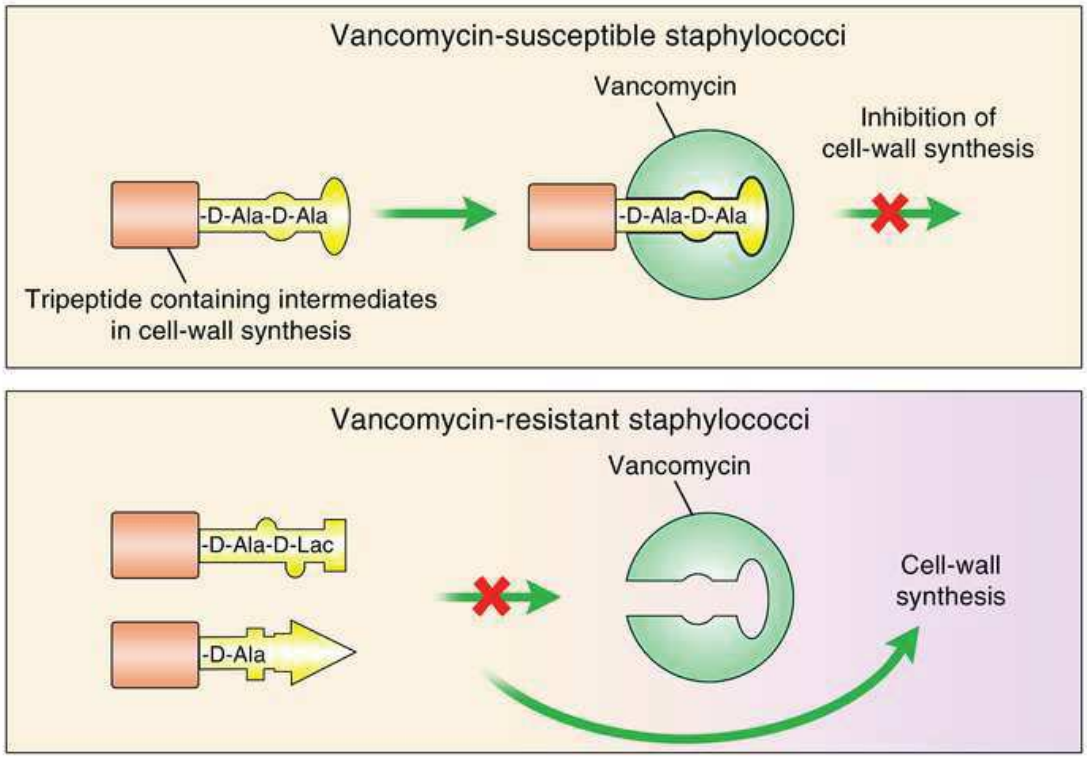
- **Bacteria** are prokaryotes with no true nucleus
- Usually has a single chromosome of 1-6Mbp in size attached to **mesosome** on cytoplasmic membrane
- Cytoplasm contains many ribosomes made up of smaller 30S and 50S subunits (different from eukaryotic ribosomes)
  - Can be an antibiotic target: gentamicin, tetracycline, erythromycin etc. have high affinity with bacterial ribosome → bacterial protein synthesis stopped
- Cytoplasm enclosed by cytoplasmic membrane containing phospholipids (also cholesterol in **mycoplasma**) and proteins
- Energy production effected by enzyme system for oxidative phosphorylation, ion transport and electron transfer (attached to cytoplasmic membranes)
- **Gram stain** used to classify bacteria:
  - **Gram positive** (deep purplish blue) has **thick peptidoglycan** layer as cell wall (trapping stain colour complex i.e. crystal violet and iodine)
  - **Gram negative** (light pink) has a very **thin peptidoglycan** layer and an additional **outer membrane** (lipid in nature) of **lipopolysaccharide (LPS)**
    → Outer membrane also ↓ permeability to antibiotics (esp hydrophilic (eg vancomycin) antibiotics
- LPS also called **endotoxin** ∵ potent stimulus of host pro-inflammatory cytokine production → most important bacteria factor causing **sepsis** and **shock** in hospitalized patients
  - Structure: **lipid A** + core oligosaccharide + O antigen
  - **Lipid A** accounts for most physiological propertoes of endotoxin
    → **Septic shock**: hypotension, **oliguria** (low urine output), hypoxia, acidosis, microvascular abnormalities, disseminated intravascular coagulation, multiple organ failure (in 20-40% of Gram –ve bacteraemia cases → 20-50% mortality with Gram –ve bacteremia)
    → Biological effects: activation of cytokine cascade



- Rigid peptidoglycan layer is a polymer of repeating subunits of 1-4 linked **N-acetyl glucosamine** and **N-acetyl muramic acid** cross-linked by pentapeptide bridges
  - Enzyme cross-linking these peptides can be an antibiotic target eg. penicillin, vancomycin




Vancomycin-Susceptible Enterococci
Vancomycin-susceptible enterococci make cell-wall precursors that have high affinity for vancomycin.
Vancomycin
Inhibition of cell-wall synthesis
-D-Ala-D-Ala
Tripeptide containing intermediates in cell-wall synthesis
-D-Ala-D-X
-D-Ala
Vancomycin
Cell-wall synthesis
Vancomycin resistance
NAG
NAM
NAG
NAM
NAG
(Transglycosylase)
D-Ala
D-Ala
D-Ala
D-Lac/Ser
Vancomycin
Cross-linking of peptidoglycan cell wall
(Transpeptidase)
Resistant bacteria produce enzymes active against
Penicillin
Penicillin resistance


Vancomycin-susceptible staphylococci
Vancomycin
Inhibition of cell-wall synthesis
-D-Ala-D-Ala
-D-Ala-D-Ala
Tripeptide containing intermediates in cell-wall synthesis
Vancomycin-resistant staphylococci
Vancomycin
-D-Ala-D-Lac
-D-Ala
Cell-wall synthesis

- Other specialized structures:
  - **Capsule**: additional covering of polysaccharides or polypeptide → possible virulence factor conferring antiphagocytic (eg hyaluronic acid content) or adhesive properties
  - **Pili** and **fimbriae**: hair-like appendages → used in conjugation (sex pili)and adhesion to mucosa
- Under very adverse environmental conditions, may form highly resistant spores protected by a thick cortex of peptidoglycan and protein coat

***Mycoplasma**: a genus of bacteria that lacks cell wall

****Sepsis**: acute full-body inflammation

*****Some types of bacteria are NOT normally stained by routine stains:

- Gram positive:
  - Acid-fast bacilli: eg. *Mycobacterium*
- Gram negative:
  - Obligatory intracellular pathogens: eg. *Chlamydia*, *Rickettsmia*, *Orlentia*, *Coxiella*
  - Spirochaetes: eg. *Treponema*, *Leptospira*, *Borrelia*
- Cell-wall deficient bacteria: eg. *Mycoplasma*, *Ureaplasma*

E. Bacterial Growth

- Bacteria multiply by **binary fission**
- Bacterial growth exhibit 3 phases:
  - **Lag phase**: newly introduced bacteria slowly adapting to the utilization of nutrient in the medium, no change in cell number
  - **Log phase**: number of bacteria increases exponentially
  - **Stationary phase**: amounts of nutrients becomes exhausted and bacterial growth gradually slows down, no change in cell number
- **Generation time**: time required for number of bacteria to double (~30 min for *E. coli*)

F. Bacterial Nutrition

- **Strict aerobes**: exclusively use oxygen as final electron acceptor
  - Eg. *Pseudomonas aeruginosa* commonly found in hospital environment
- **Strict anaerobes**: use other inorganic substances (eg NO_2, SO_4^{2-}) as final electron acceptor
  - O radicals toxic to such bacteria ∵ lack (or deficient in) required detox enzymes to deal with such radicals
  - Produce **foul-smelling volatile fatty acid** as by-products of metabolism
  - Eg. *Bacteroides fragilis* and *Clostridium perfringens* found in human intestines
- **Facultative anaerobes**: can utilize both oxygen and other organic substances (eg. pyruvate) as final electron acceptor
  - Most medically important bacteria belong to this category
  - Eg. *Staphlococcus epidermis* and *Staphylococcus aureus* on skin, *Streptococcus viridans* and *Streptococcus pyogenes* found in throat, *Escherichia coli* and *Enterococcus faecalis* found in intestines
- **Microaerophilic bacteria**: prefer to grow under **low oxygen tension** and in the **presence of CO_2**
  - Eg. *Helicobacter pylori* (causing gastritis) and *Campylobacter jejuni* causing diarrhea

*Bacteria can exist both within and outside human cells: intracellular → protected against defense mechanism; extracellular → greater opportunities for growth, reproduction and dissemination but have to face harsher conditions incl. host immune system

G. Microbial Genetics

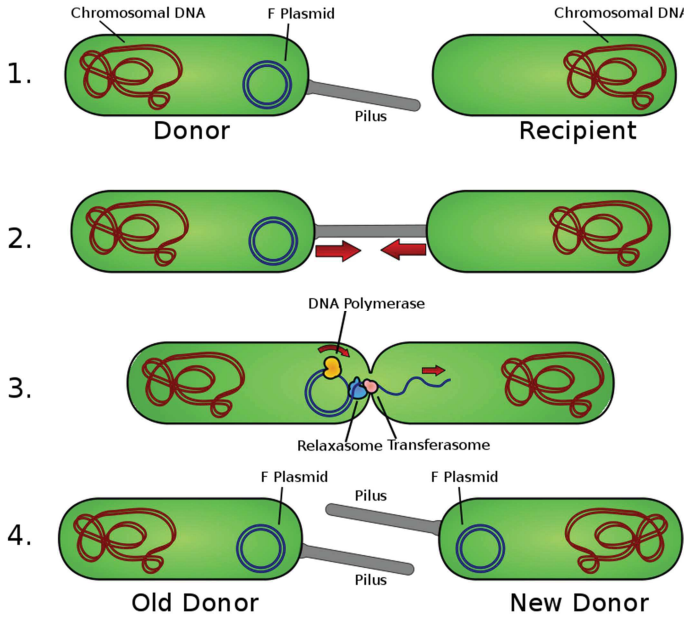
1. Ways of Modifying Bacteria's Genome

a. Transformation

- **Transformation**: direct uptake of external genetic materials from the surrounding
- Origin: dead smooth *Streptococcus pneumoniae* (Gram +ve cocci that cause pneumonia and meningitis) can transform rough *S. pneumonia* to smooth virulent species
- Foundation of molecular genetics

b. Conjugation

- **Conjugation**: genetic exchange between bacteria by close contact
- Mostly done via exchange of **plasmids** in sex pilli
- **Plasmid**: exchanging self-replicating extra-chromosomal double-stranded circular DNA
  - F-plasmid (prototype) carries a tra and trb locus consisting of ~40 genes
  - Tra locus contains **pilin gene** and **regulatory genes** → form pili on cell surface



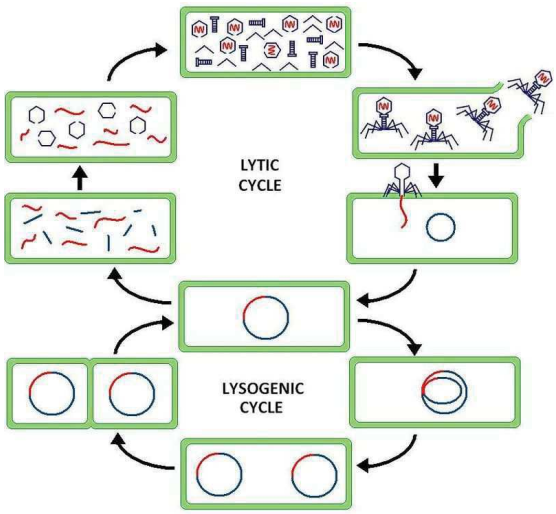
  - Also contains genes encoding proteins that attach themselves onto surface of F- bacteria (recipient) → initiate conjugation
  - Several proteins encoded by tra/trb locus can open a channel between bacteria and traD enzyme (at base of pillus) → initiate membrane fusion → allow passage of plasmid from F+ to F-
  - Note that only one strand of DNA in plasmid is transferred → then replicated in both bacteria → result is 2 F+ cells
- Importance: dissemination of bacterial resistance genes among bacterial population

c. Transduction

- **Bacteriophages (phages)** (virus that infect bacteria) may mediate genetic material transfer among bacteria
- Many toxigenic bacteria (eg. toxigenic *Corynebacterium diphtheriae*) are latently infected with a phage
- Toxin gene contained in phage genome → integrated into bacterial chromosome
- Usually only repressed form is expressed (lysogeny) → stable association of phage with bacterial host
- **Phage conversion** occurs (due to a variety of reasons) when the toxin gene is allowed to express → elaboration of toxin

i. Virus Reproduction Cycle

- **Lytic cycle**:
  - Viral genome integrated into host genome
  - Protein manufacturing machinery seized to produce more virus
  - Host cell cannot survive → apoptosis → release of more virus
- **Lysogenic cycle**:
  - Viral genome integrated into host genome
  - Host genome containing viral genome replicate and produce new offspring with viral genetic materials
  - **Lysogenic conversion**: toxin gene activated in certain events (eg UV irradiation) → switch to lytic cycle



d. Point Mutations

- Point mutation → change in pathogen characteristics incl. antigenicity, virulence, transmissibility and antimicrobial susceptibility
- Eg. *Mycobacterium tuberculosis*:
  - Brought about by gene rearrangement or point mutation
  - Random occurrence
  - May lead to failure in drug treatment (eg. antibiotic resistance)

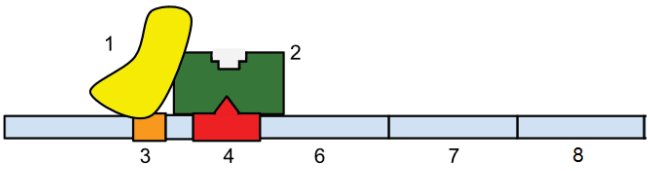
2. Gene Regulation

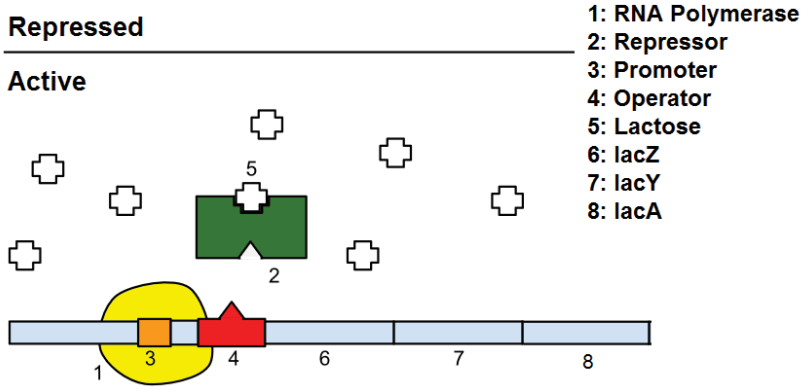
a. Catabolite Induction in Nutrient Utilization

- **Catabolite induction**: enzyme concerned with utilization of certain specific nutrients are only made when corresponding nutrient is present
- Example: *lac* operon:
  - 3 components:
    - → 3 functional genes for lactose utilization in linear array
    - → **Promoter** and **operator** upstream of array for control of expression
    - → **Allosteric repressor protein** binding to operator and lactose
  - Absence of lactose: repressor bind to operator → functional genes cannot be transcribed
  - Presence of lactose: lactose bind to repressor → change in conformation of repressor → repressor detached from operator → functional genes transcribed
- Importance: allow bacteria to respond to availability of nutrients in an economic manner

b. Antigenic Switching

- Importance: allow bacteria and parasites to escape surveillance of host immune system
- Example: *Salmonella* (a bacteria associated with diarrhea)
  - *Salmonella* expresses 2 related flagella proteins with different antigens
  - Genes under control of same promoter
  - Phase transition: transcription switched from one to other gene → antigenically distinct population of bacteria





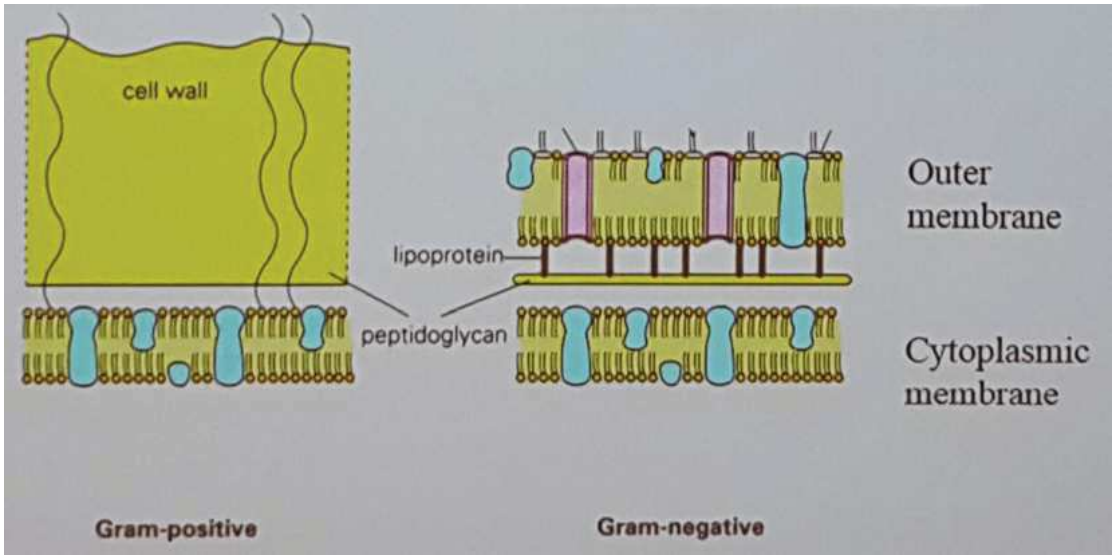
H. Bacteria Classification

- Asexual reproduction → cannot be classified based on possibility of interbreeding
- Classification largely based on **morphology**, **atmospheric requirement** and **cell wall structures**
- Other classification criteria:
  - Gram staining reaction (indicate cell wall structure)
  - Biochemical reaction profiles
  - Antigenicity
  - Ribosomal protein fingerprint (by mass spectrometry)
  - DNA sequence of 16S rRNA
- Terminology:
  - **Staphylo-**: cluster of grapes
  - **Strepto-**: chains
  - **Entero-**: guts
  - **Haemo-**: blood
  - **Helico-**: spiral
  - **Cocci**: spherical
  - **Bacilli**: rod
  - **Vibrio**: curved

Medically important bacteria	Distinguishing features
Treponema Borrelia Leptospira	Flexible, thin wall and in spirals
Mycoplasma	'No cell wall'
Mycobacteria Norcardia	Rigid wall containing long chain mycolic acid (acid fast stain positive)
Rickettsia Chlamydia Coxiella	Rigid walled, unicellular which is : (a) obligatory intracellular
Streptococcus Staphylococcus Enterococcus	1. Gram-positive cocci*
Corynebacterium Listeria	2. Gram-positive bacilli+, aerobic, non-sporing
Bacillus	3. Gram-positive bacilli, aerobic, sporing
Clostridium	4. Gram-positive bacilli, anaerobic, sporing
Actinomyces	5. Gram-positive bacilli, anaerobic, non-sporing
Neisseria	6. Gram-negative cocci
Escherichia Salmonella Shigella Klebsiella Proteus Vibrio	7. Gram-negative, enteric bacilli, found in intestine, facultative anaerobes
Haemophilus Brucella Yersinia Bordetella Legionella	8. Gram-negative, non enteric bacilli, facultative anaerobes
Pseudomonas	9. Gram-negative bacilli, aerobe
Bacteroides Fusobacterium	10. Gram-negative bacilli, anaerobe
Campylobacter Helicobacter	11. Gram negative curved bacilli, microaerophilic

1. Gram Staining Reaction

- **Gram stain** used to classify bacteria into two main types:
  - **Gram positive** (deep purplish blue) has thick peptidoglycan layer as cell wall (trapping stain colour complex i.e. crystal violet and iodine)
  - **Gram negative** (light pink) has a very thin peptidoglycan layer and an additional outer membrane (lipid in nature) of lipopolysaccharide (LPS)
    → This LPS also called endotoxin ∵potent stimulus of host pro-inflammatory cytokine production → most important bacteria factor causing sepsis and shock in hospitalized patients

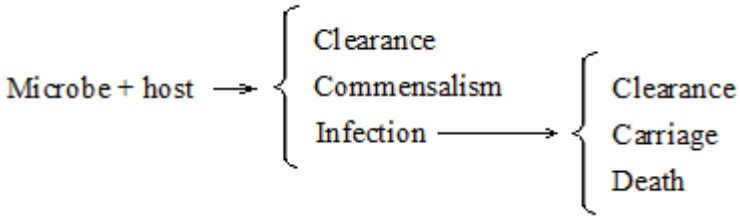


*Viruses classified in terms of presence of envelope outside protein coat

L57 Host-microbe Relationship and Microbial Pathogenesis

A. Host-microbe Relationship

- Interaction between host and microbe determines outcome of a host encountering a microbe:



- Hosts:
  - **Normal host**: normal non-specific and immune defenses
  - **Abnormal host**: abnormal non-specific and immune defenses
- Microbes:
  - **Pathogen**: virulent disease-producing microbe
  - **Non-pathogen/commensal**: non-virulent non-disease-producing microbe
- Probability of disease depends on:
  - Balance between **virulence of microbe** and **host defense ability**
  - Size and mode of inoculation
  - Medical treatment

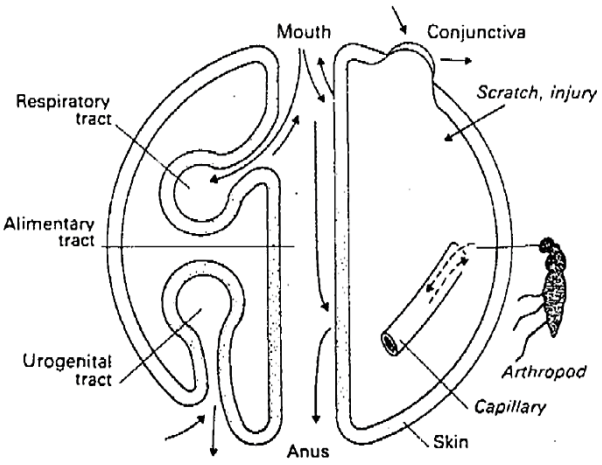
1. Host Defense Ability

- **Local defense**: mechanical barrier, natural flora
  - Defect: catheter, mucositis, burn, surgery
  - Consequences: invasion of normal flora (opportunistic infection)
- **Inflammatory response**: polymorphs, macrophages, acute phase proteins, interferons (IFN), lysozyme, complement
  - Defect:
    - → **Neutropenia**: abnormally low neutrophil counts
    - → **Chronic granulomatous disease (CGD)**: inability to form ROS (by **respiratory burst**) for phagocyte killing action (→ infection in multiple disease)
  - Consequence:
    - → **Pyogenic infection** (esp by catalase +ve bacteria):
      - pus cannot be formed without phagocytes → body susceptible to pyogenic (pus-forming) bacteria
      - lack of ROS synthesis means that body defense has to depend on H_2O_2 from normal metabolism → catalase +ve
    - → **Candida** and **aspergillus** infection
- **Acquired defense**: antibodies, T lymphocytes
  - Defects:
    - → **Congenital deficiencies** in humoral response
    - → **Multiple myeloma**: cancer of plasma cells
    - → **HIV infection**: cell-mediated immune response disabled
  - Consequence:
    - → **Encapsulated bacteria** (eg. pneumococci, meningococci, *H. influenzae* B.): phagocyte-resistant capsule meaning that they can only be eliminated by complement and Ab systems
    - → **Viral infection** (eg. HSV, CMV): intracellular→ invisible from HIR, must be targeted by CMIR
    - → **Intracellular pathogen** (eg. *Listeria*, *salmonella*, *Mycobaterium tuberculosis*): capable of dividing within phagocytes (will not digested)
    - → Some **fungus** (eg. *Cryptococcus*)

2. Microbe Pathogenesis

a. Microbe Invasion

- Source of microbe:
  - **Exogenous** (human, animal or environment)
  - **Endogenous** (normal flora)
- Mode of transmission:
  - Inhalation (droplet or aerosol)
  - Ingestion
  - Insect/arthropod bites
  - Contact (via mucosa, cuts or wounds)
  - Organ transplant
- Spread in body:
  - Local spreading through contiguous tissues
  - Distant spreading via bloodstream, lymphatic system or nerves



b. Pathogenesis

- Six steps of pathogenesis:

1) **Encounter**: superficial contact;
2) **Entry**: invasion;
3) **Spread**: local and beyond;
4) **Multiplication**: increase in number of microbes;
5) **Damage**: tissue destruction from agent, host response, or both;
6) **Outcome**: host and microbe

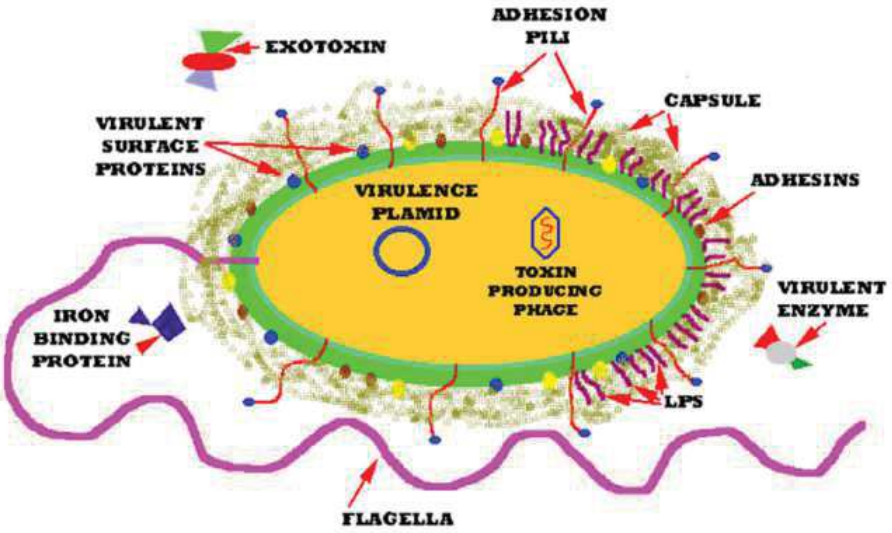
- **Virulence factors** helps facilitate the first five steps of pathogenesis

c. Microbe Virulence

- **Virulence**: degree or ability of a pathogenic organism to cause disease
- Characteristics of a <u>highly virulent microbe</u> (eg. *vibrio cholera*, *Bacillus anthrax*):
  - Low **infective dose** (threshold of pathogenesis)
  - High **attack rate**
  - Number and potency of **virulence factors**
  - High **morbidity** and **mortality**

i. Virulence Factor

- **Virulence factors**: microbial that allow organism to colonize, proliferate, invade or otherwise modify host tissue and organs



Encounter	fimbriae, adhesins, polysaccharides	
Entry	motility (flagella), enzymes	Phagocytosis resistance (eg capsule, **M protein**)
Spread		
Multiplication	acquisition of nutrients (eg. **siderophores**)	
Damage	toxins	

***siderophore**: high iron-affinity bacterial protein for acquisition of iron

****M protein**: a *streptococcus pyogenes* virulence factor resisting complement opsonization

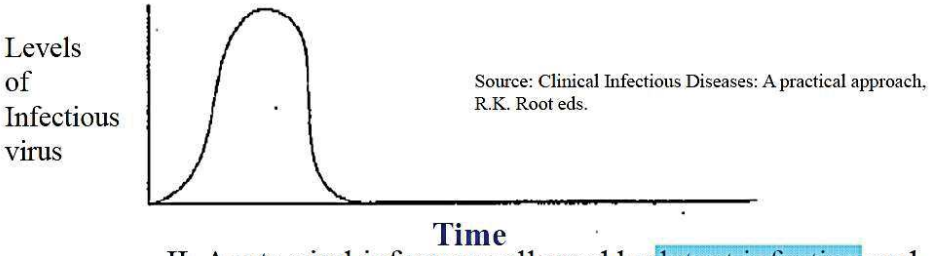
- **Exotoxin**: toxins secreted out of cell by pathogens
  - Found in both **Gram positive** and **negative** bacteria
- **Endotoxin**: integral components of cell wall or membrane, only released when pathogen is lysed
  - Especially note **lipopolysaccharide (LPS)**
  - Only found in **Gram negative** bacteria (in external membrane)

d. Examples of Pathogenesis

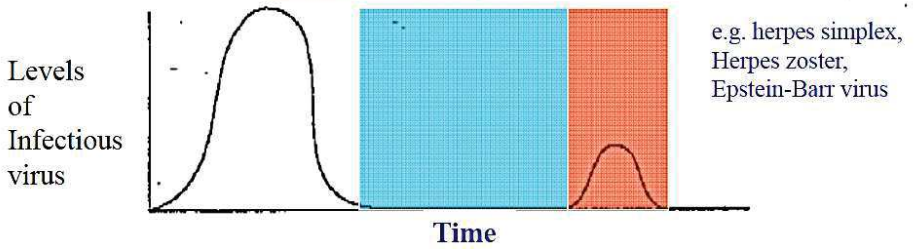
- **Cholera** caused by *vibrio cholera*
  - Toxin-mediated disease
  - Cholera toxin permanently cause **ribosylation** of Gsα subunit → adenylyl cyclase activated
  - Cellular cAMP level rises → H_2O, Na^+, K^+, Cl^- and HCO_3^- secretion → watery diarrhea
- **Diphtheria** caused by *Corynebacterium diphtheriae*
  - Toxin-mediated disease
  - Symptoms: mucosal inflammation, oropharyngeal oedema, myocarditis
  - Diphtheria toxin catalyzes binding of AD from NAD^+ to EF-2 → inactivation of EF-2 (essential for RNA translation)
- **Tetanus** caused by *Clostridium tetani*
  - Toxin-mediated disease
  - Tetanus toxin blocks release of inhibitory neurotransmitters glycine and GABA → muscular spasm and tetanus

3. Course of Disease

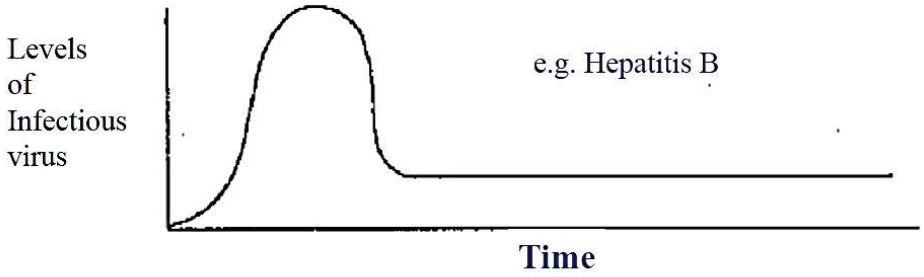
I. Acute viral infection followed by viral clearance



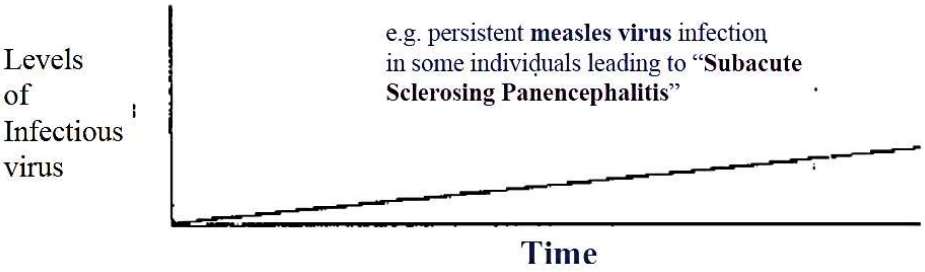
II. Acute viral infection followed by latent infection and periodic reactivation



III. Acute viral infection followed by chronic infection



IV. Slow chronic infection



*In reactivation following latent infection, the viral level would not be as high due to partial immunity

**Chronic infection does not mean no damage

- **Incubation period**: time elapsed between exposure to a pathogenic organism and when symptoms and signs are first apparent (characteristic to pathogen)
- **Latent infection**: phase during the course an infection during which the pathogens are dormant

*Not all pathogens have latent period

B. Normal Flora

- **90%** of cells making up human are **microbes**
- Commensal microorganisms (mostly bacteria) colonize skin, GI tract and lower GU tract
  - Internal organs are sterile
- Constitutes both a **protective host defense mechanism** and a **potential source of infection**
- May also interferes with culture results

1. Development of Normal Flora

- Development of normal flora:
  - Microbial colonization begins immediately after birth
  - Neonatal flora already remarkably similar to adult pattern within a few weeks
  - Composition of flora continues to evolve over time (due to health condition etc)
- Source: mother, other human contacts, contact with inanimate objects and food

2. Composition of Normal Flora

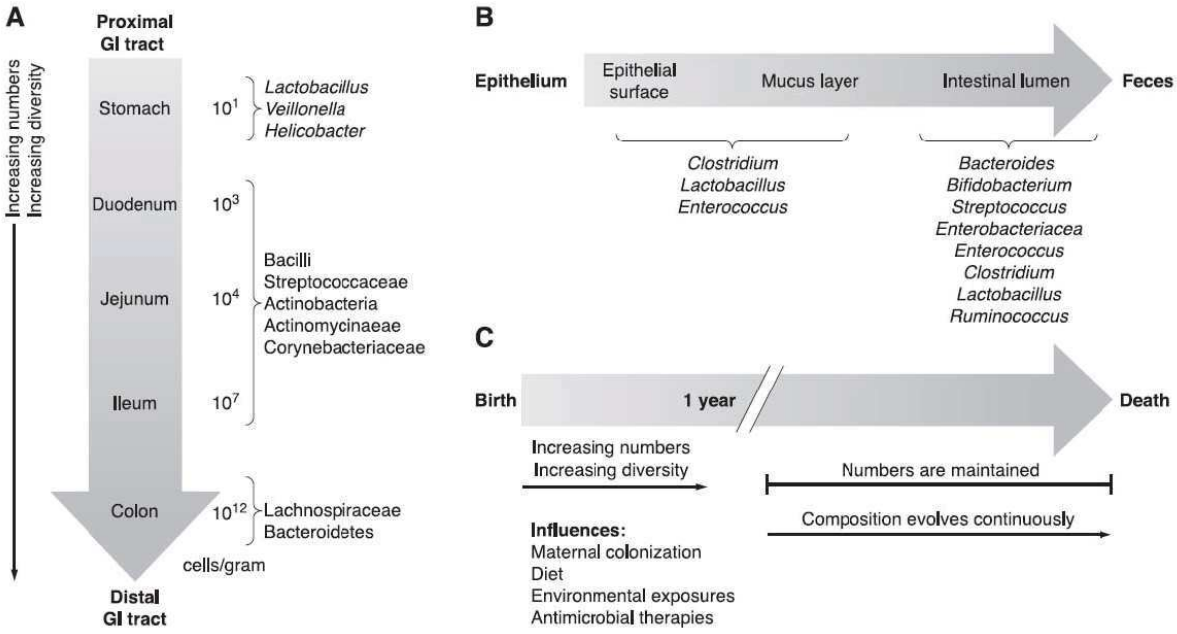


FIG. 2. Spatial and temporal aspects of intestinal microbiota composition. *A*: variations in microbial numbers and composition across the length of the gastrointestinal tract. *B*: longitudinal variations in microbial composition in the intestine. *C*: temporal aspects of microbiota establishment and maintenance and factors influencing microbial composition.

- Factors affecting flora composition:
  - Initial colonization **fortuitous** in nature: composition dependent on first suitable organism to arrive at a particular site
  - Patterns also dependent on host-bacterial factors

a. Skin Flora

- Reservoir and vehicle for transmission of many pathogens eg. MRSA
- Potential cause of disease: eg. overgrowth → *Propionibacterium acnes*
- Possible contaminants of blood culture → aseptic technique needed for blood withdrawal

b. Respiratory Tract Flora

- ***Streptococcus mutans***:
  - Found in large numbers (10^{10}/g) in dental plaque
  - Metabolize sucrose to form **glucan** → dental plaque
  - Metabolize sugars into acids → demineralization of enamel → tooth decay
- ***Streptococcus viridans***:
  - Possible transient bacteraemia due to dental cleansing → risk of infective endocarditis
  - **Antibiotic chemoprophylaxis** sometimes prescribed as precaution
- **Aspiration pneumonia**:
  - Inability in swallowing reflex → entry of foreign substance into lung → infection
  - Aetiology highly dependent on colonizing flora at the time of aspiration
    → Community acquired: usual oral flora esp. anaerobes
    → Hospital acquired: Gram negative bacilli

c. GI Tract Flora

- ***Clostridium difficile***:
  - Normally a harmless minor member of fecal flora
  - Resistant to multiple antibiotics ∴ antibiotic use → overgrowth → pseudomembranous colitis
- ***Escherichia coli***:
  - Normal inhabitant of GI tract
  - Commonest cause of urinary tract infection
  - Colon perforation → polymicrobial peritonitis

d. Urinary and Reproductive Tract Flora

- Anterior urethra: normal resident flora of *streptococcus*, *nesseria*, *bacteroides*, a few *enterobacteriaceae* and *Mycobacterium smegatis*
- Rest of urogenital tract: sterile

i. Vaginal Infections

- **Vaginal candidiasis**:
  - Yeast is part of normal flora
  - Overgrowth will cause vaginal candidiasis
  - Predisposing factors: antibiotics, pregnancy, increased warmth and moisture
- **Bacterial vaginosis**: Superficial infection of vagina
  - Characterized by overgrowth of some anaerobic flora (eg *Gardnerella vaginalis*, *Mobiluncus*)
  - Predisposing factor: sexual activity, use of IUD

3. Carrier State

- **Carrier state**: persistent carriage of a potential pathogen (eg. meningococcus, *Salmonella typhi*) in normal flora
- Possibilities:
  - Asymptomatic infection
  - Recent recovery from disease
- Significance: potential source of infection to contacts

L58 Innate Defense Mechanism

A. Body Defense Mechanism

- **Innate immunity**: non-antigen-specific and rapid immune response mechanism responsible for detection and destruction of most invading microbes within hours
  - Can help keep infection under control to allow time of activation of adaptive immune response
- **Adaptive immunity**: defense mechanism that are induced by exposure to foreign substances and will enhance after successive exposure to the particular molecule
- Generally, innate response are responsible for dealing with first 96 hours of infection
- Both systems complement each other well:
  - B lymphocyte secretes Ab for phagocyte opsonization
  - T lymphocyte secretes cytokines to activate phagocytes
  - Phagocytes help present antigens to T lymphocyte for activation of CMIR

B. Physical Barriers

- Skin, respiratory tract, GI tract and urogenital tract exposed to microbes
- **Mechanical**:
  - Keratinized layer of skin
  - Epithelial cells joined by tight junctions
  - Longitudinal flow of air or fluid across epithelium (esp note fx of cilia)
- **Chemical**:
  - FAs conferring waterproof ability to skin
  - Antimicrobial enzymes: lysozyme (saliva, sweat, tears) and pepsin (gut)
  - **Defensin**: antimicrobial peptide expressed by leukocytes and epithelial cels
  - **Dermicidin**: novel antibiotic peptide secreted by sweat gland
- **Microbiological**:
  - Normal flora (skin, gut, vagina): competition and production of substances toxic to other organisms

C. Phagocytosis

- **Phagocytosis**: ingestion and digestion of extracellular particulate material (incl. whole pathogenic microbe)
- Component: **macrophage** and **microphages** (neutrophils or polymorphonuclear leukocytes, PMNs (sometimes also include eosinophil))
  - **Macrophage** mature continuously from circulating monocytes
- Process:

1) **Chemotaxis**: chemical attraction of phagocytes to microorganisms
   - **Chemotactic** chemicals can be microbial products, components of WBCs, damaged tissue cells and complements;
2) **Adherence**: attachment of phagocyte's plasma membrane to surface of microbe or other foreign materials;
   - **Opsonization**: coating of microbe by **opsonins** (eg. Ab, complement proteins) that promotes adherence
3) **Ingestion**: plasma membrane of phagocyte extends projections forming **pseudopods** that engulf the microbe
   → microbe surrounded
   → pseudopods fuse
   → **phagosome** formed
4) **Digestion**: fusion of phagosome with **lysosome** with digestive enzymes and bactericidal substances
   → enzyme digest microbes in **phagolysosome**
   → **residual body** (phagolysosome with indigestible materials)
   → degraded product presented on cell surface (to initiate specific immune response)

D. Anti-microbial Proteins

1. Acute Phase Proteins

- **Acute phase proteins**: normal components of serum that increase markedly in concentration during first few days of infection
- Synthesized by liver cells upon microbe stimulation
- Eg. **C-reactive protein (CRP)**: binds to C-protein of pneumococci → binding and activation of complement promoted + opsonization

2. Complement

- **Complement**: very complex group of serum proteins that 'complements' antibody and phagocyte action
- Make up 5% of serum proteins in vertebrates
- Activated in a 'cascade' fashion by two ways:
  - **Alternative pathway**: activation predominantly by microbe (bacteria, yeast, fungi) surface components (less efficient)
  - **Classical pathway**: activation exclusively by Ag/Ab complex
- Consequences:
  - **Cytolysis**: C3b initiates a sequence of reactions involving C5, C6, C7, C8, C9
    → **membrane attack complex (MAC)** leading to formation of **transmembrane channels**
    → loss of ions
    → cytolysis
  - **Chemotaxis**: attraction of phagocytic cells to site of activation
  - **Opsonization**: organisms with complement by-products on surface are more easily phagocytosed
  - **Inflammation**: initiation of inflammatory response
    → ↑ blood flow + ↑ vessel permeability
- Deficiency: ↑ risk of infection and immune complex-disease (eg, SLE)

3. Interferons

- **Interferons** confer viral resistance at early stages of viral infection
- IFN-α (by virus infected leukocyte) and IFN-β (by virus infected fibroblasts and epithelial cells) are important in non-specific immune response
- IFN-γ is a component of specific immune response
- Important in reducing viral load prior to appearance of anti-viral Ab and/or T lymphocytes
- Application: therapeutic use for infection, cancer and auto-immune disorder

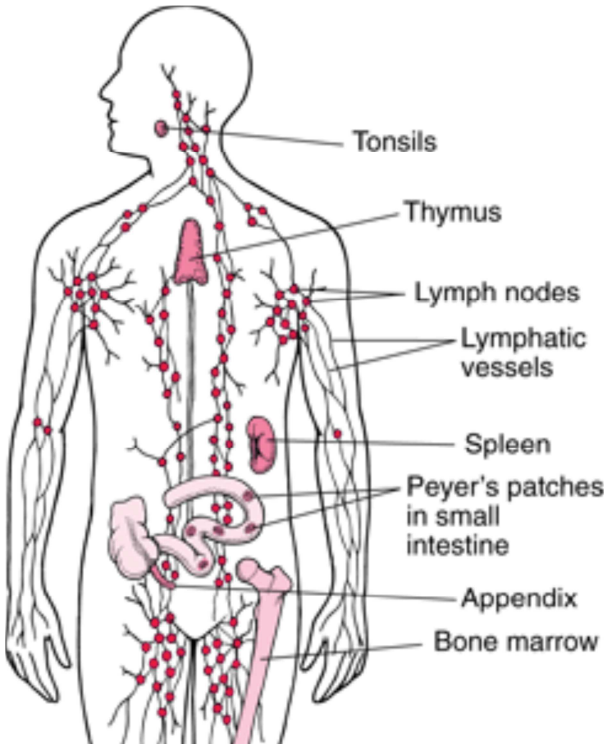
4. Natural Killer Cells

- **Natural killer (NK) cells**: non-phagocytic lymphocytes that is responsible for killing virus-infected tumor cells
- Make up 5-10% of recirculating lymphocyte population
- Lacks antigen-specific receptors
- Can recognize potential target cells by:
  - **NK cell receptors** to distinguish abnormal cells displaying reduced expression of MHC-I (→ cannot be recognized by T cells)
  - **Membrane receptor CD16** (for Fc region of IgG) and destroys cell via release of cytolytic granules (also known as **antibody-dependent cell-mediated cytotoxicity, ADCC**)
- Involved in early response to infection
- Activity stimulated by IFN-α, IFN-β and IL-2
- Produces a number of important cytokines (eg. IFN-γ) → critical role in immune regulation
- Field of major interest in immunology
  - Possibility of tumor-killing NK cells

L59 Specific Immunity: Antibodies and Lymphocytes

A. Immunology

- **Immunology**: study of all aspects of host defense against infection and of adverse consequences of immune response
- **Immune response**: cellular and molecular events that defend the host against pathogens/adverse events
- **Antigens (Ag)**: any molecules/cellular structures that elicit and immune response
- **Epitope**: antigenic determinant, a site on an antigen recognized by antigen recognition receptor of lymphocytes
- **Immunogenicity**: ability to induce either humoral and/or cell-mediated immune response
- **Immunological tolerance**: failure to respond to an antigen (eg. self-antigens or normal flora microbe antigens)



1. Lymphoid Organs

- **Primary lymphoid organs** (thymus and bone marrow) for development and maturation of lymphocytes from their precursors
- **Secondary lymphoid organs**:
  - Lymphocytes interact with antigens and other accessory cells
  - Differentiation of lymphocytes

B. Adaptive Immunity

- **Adaptive immunity**: part of immune system that is antigen-specific and tends to grow stronger with repeated exposure to the same pathogen
- Characteristics:
  - **Specificity**: specific for distinct antigens due to expression of **membrane receptors** that distinguishes differences between distinct antigens
  - **Diversity**: diverse lymphocyte repertoire in mammalian immune system → discriminate at least 10^8 distinct antigens
    → Result of numerous different **clones** of lymphocytes generated in an individual
  - **Memory**: exposure of immune system to a foreign antigen enhances its ability to respond to that antigen → more rapid and stronger secondary immune responses
  - **Discrimination of self from non-self**: ability by lymphocytes to recognize and respond to foreign antigens but not self-antigens (i.e. **tolerance**)

1. Humoral Response

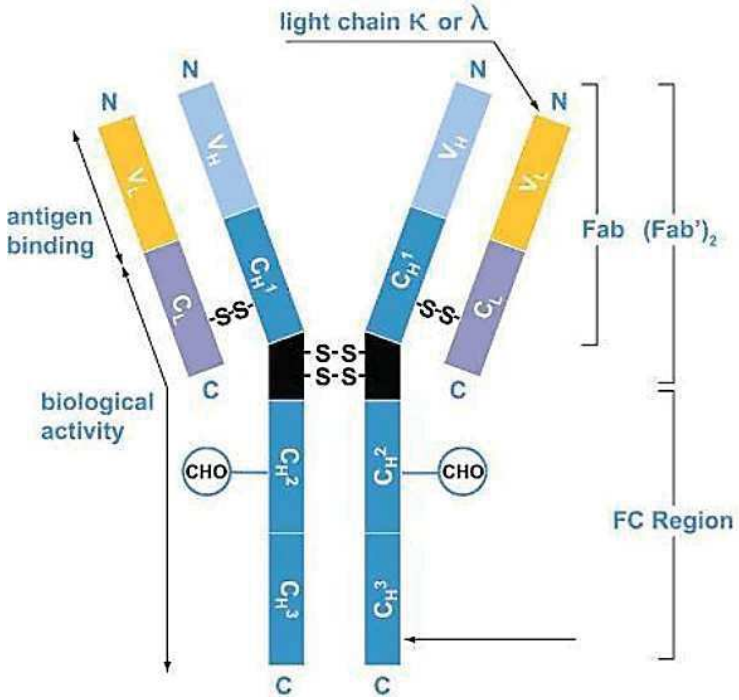
- **Humoral response**: part of acquired immune system that is mediated by macromolecules involving B cells and antibodies

a. B Lymphopoiesis

- **Haematopoietic stem cells (HSC)** in bone marrow differentiates to form naïve B cells with **IgM** and **IgD** as **B cell receptors (BCR)**
- Naïve B cells accumulate at spleen and lymph nodes
- Activation by pathogenic antigen triggers multiplication and differentiation into **plasma cells** (Ab-producing) and **memory cells**

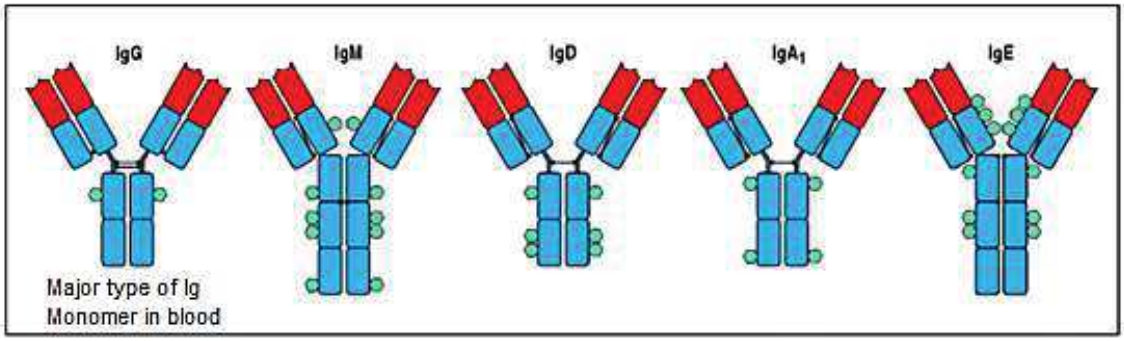
b. Antibodies

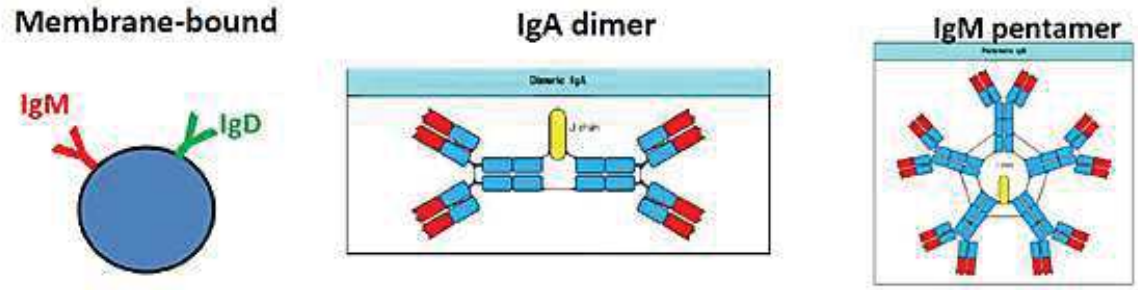
- **Antibodies** recognize the 3D conformational determinants of antigens:
  - Different Abs have different V domains that recognize different **epitopes** (antigenic determinants) → antigen-specificity
- Diverse repertoire: human B cells can generate >10^8 different antibody specificity



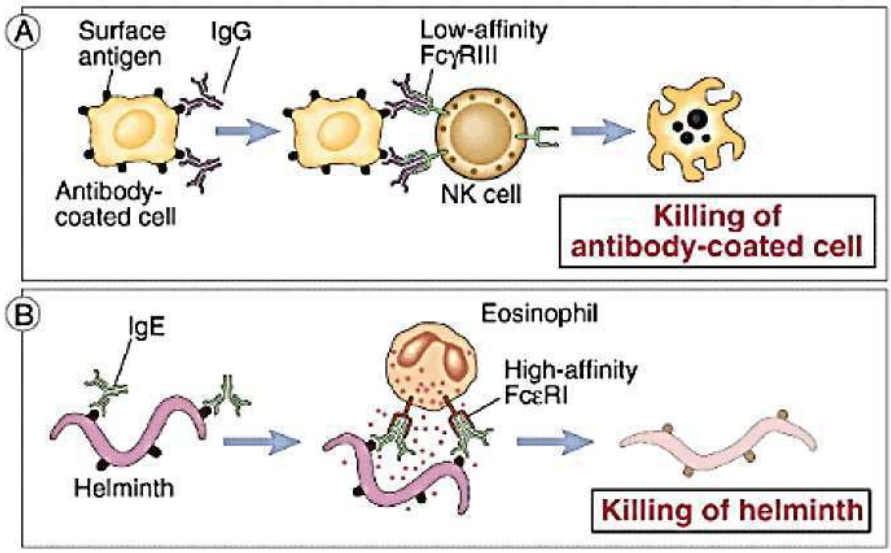
- An **immunoglobulin (antibody)** molecule composes of four polypeptides linked by disulphide bond:
  - Two identical **heavy chains** with 3 **constant** (C_H) domains and 1 **variable** (V_H) domain
  - 2 identical **light chains** with 1 **constant** (C_L) domain and 1 **variable** (V_L) domain
- **Antigen-binding site (Fab)** is formed by V_H and V_L domains
  - High variability in a.a. sequence in variable domains confers different antigen specificity
- **Constant region (Fc)** is formed by C_H and C_L domains (with limited variability)
  - Responsible for eliciting biological activity and functions of Ab
- Note **bivalence** of antibodies

- 5 isotypes of Abs based on differences in Fc region: **IgM**, **IgD**, **IgG**, **IgA**, **IgE**





- Antibody function:
  - **Opsonization**: neutralization, agglutination and precipitation of dissolved antigens enhances phagocytosis (via Fc receptors of macrophages)
  - **Activation of complement system**: complement system is activated via classical pathway → cell lysis by MAC
  - **Antibody-dependent cellular cytotoxicity (ADCC)**: IgG triggers killing of antibody-coated cells (via FcγRIII) by NK cells



Wenny's Immunology Webnotes

c. Process of Humoral Response

1) Each B cell has a single antigen specificity;
2) B cell binds with its own complement antigen via **B cell receptors** (i.e. IgM and IgD);
3) B cell internalizes antigen and present its epitope with MHC-II to Th-2 cells;
4) Th-2 cell interacts with B cells and secrete IL-4 and IL-6 to activate B cells;
5) B cells experiences **clonal expansion** and differentiates into **plasma cells** and **memory cells**;
6) **Plasma cells** synthesize and release large numbers of antibodies for neutralization of antigens.

2. Cell-mediated Immunity

- **Cell-mediated immunity**: part of immune system mediated by cells, specifically T cells, and does not involve antibodies

a. T Cell Lymphopoiesis

- **Haematopoietic stem cells (HSC)** in bone marrow differentiates to form T cells with **T cell receptors (TCR)**
- T cells mature at **thymus gland**
- T cells accumulate at spleen and lymph nodes
- Activation by pathogenic antigen (via T cell receptors) triggers multiplication and differentiation into **effector T cells** and **memory T cells**

b. T Cell Receptor

- Membrane-bound receptor composing of α and β chains
- Antigen-specific:

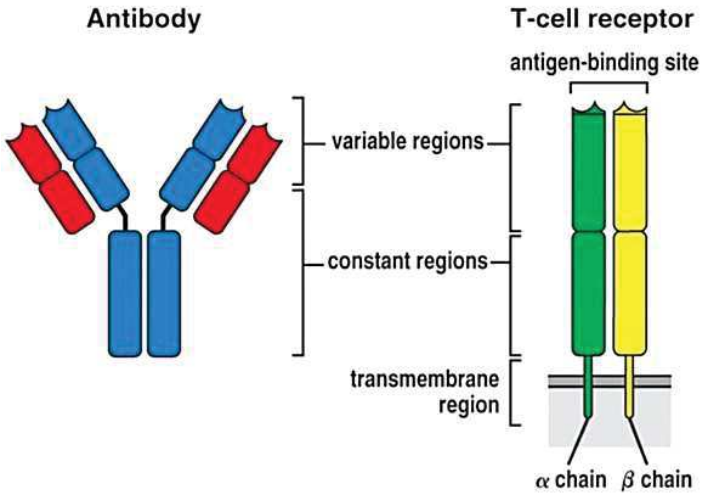


Figure 1-21 part 2 of 2 The Immune System, 2/e (© Garland Science 2005)

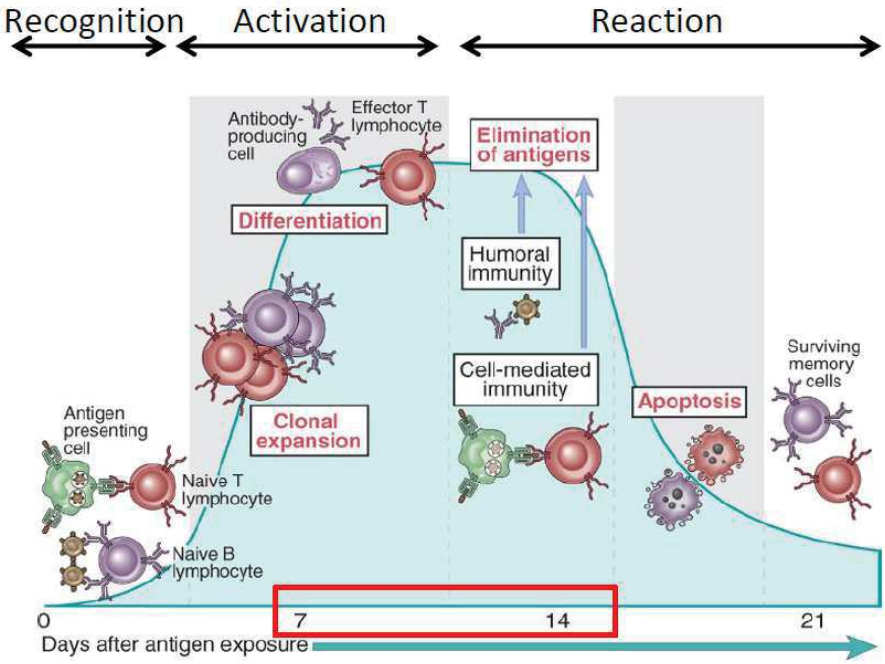
- Detects peptides presented by **major histocompatibility complex (MHC)** molecules on **antigen presenting cells (APCs)**
- Antigen binding aided by **co-receptors CD4** and **CD8**
- **CD8+ (cytotoxic) T cells** recognize **MHC-I**-presented peptides on **normal cells** → mediate **killing** of target cell through release of cytotoxic granules
- **CD4+ (helper) T cells** recognize **MHC-II**-presented peptides on **immune cells** to aid function of other immune cells (eg. B cells, NK cells):
  - **Th-1 cells** recognize MHC-II on phagocytes → proliferation and release of IFN-γ + IL-2 → activation of CD8+ T cells (to seek out the specific antigen) and phagocytes
  - **Th-2 cells** recognize MHC-II on naïve B cells → release IL-4 + IL-6 → activate naïve B cells to proliferate into plasma cells and memory cells
  - One of the most potent **cytokine** producers in body
    → Eg. IFN, tumor necrosis factor (TNF)-α, transforming growth factor (TGF)-β, lymphotoxin, interleukins (IL)-2/4/5/6/…
  - Development also modulated by cytokines
  - Can be further developed into different subtypes incl Th1, Th2, Th17 and Treg cells, specializing for producing specific types of cytokines

*Interaction between Th-2 and B cells determine type of Ig produced by B cells

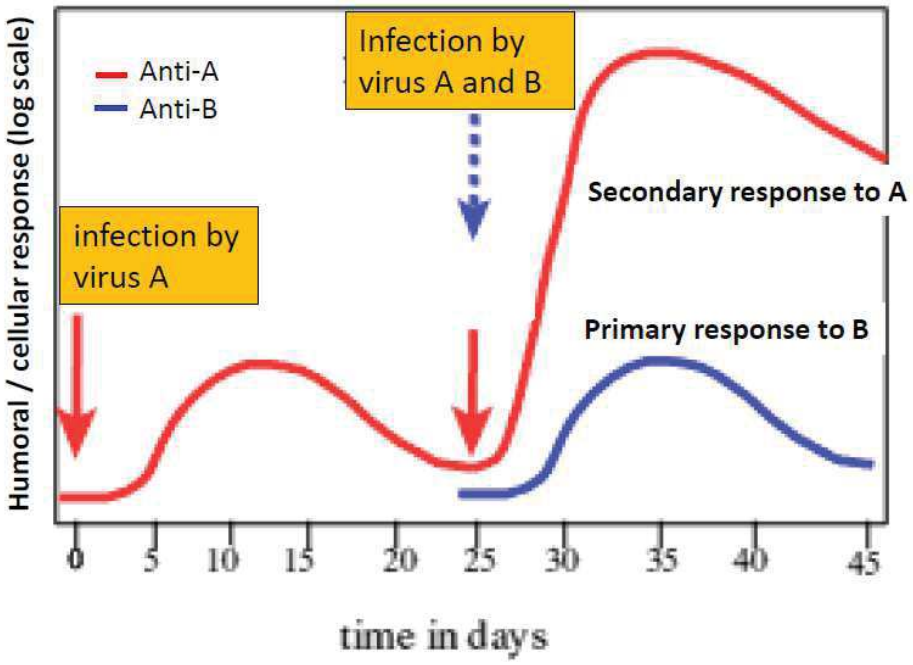
Note importance of **clonal selection in immune system: only very few lymphocytes for each antigen → diversity

But when infection strikes, clonal selection allows rapid expansion in population of that particular lymphocyte → create powerful specific immune response (and memory)

3. Phases of Specific Immune Response

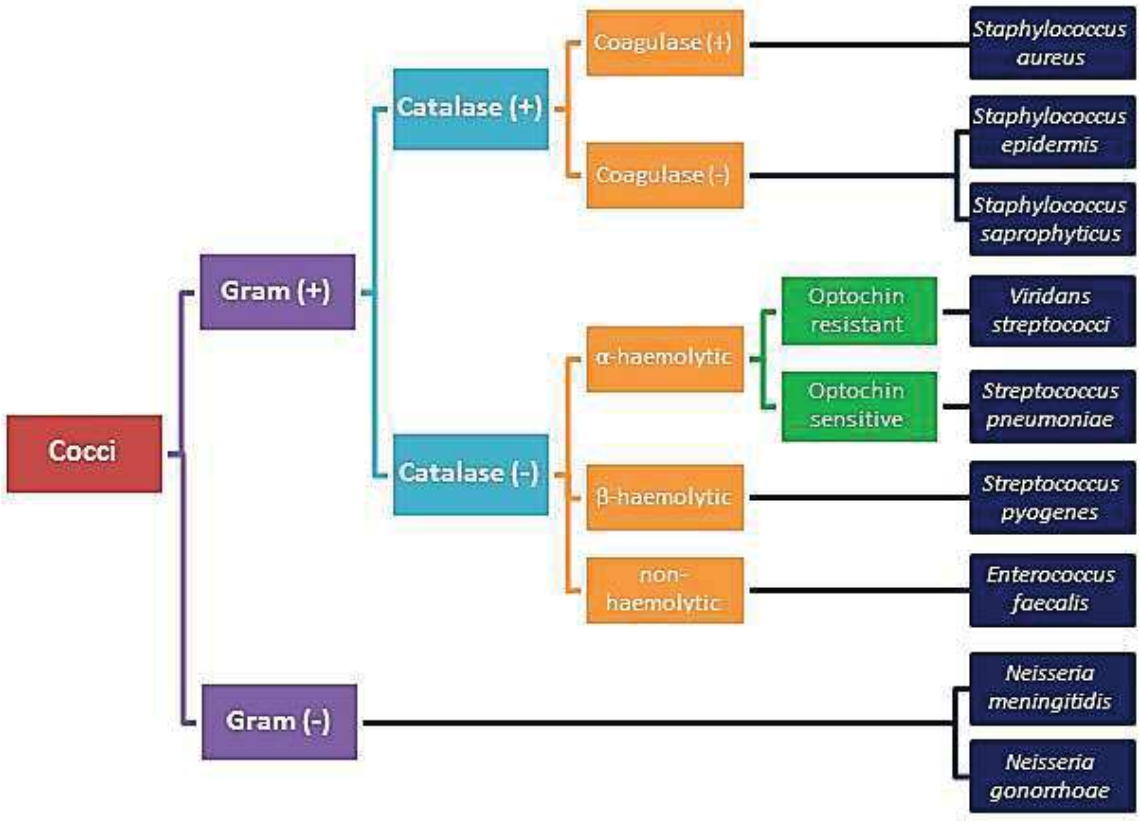


4. Immunological Memory



- Presence of memory B/T cells allow **secondary responses** to be:
  - Faster: shortened activation phase
  - Stronger: more efficient effector response (eg. ↑ Ab level and ↑ T cell proliferation)
- Provide long-lasting protection
- Serves as basis of vaccination: vaccines induce primary response and thus formation of memory B/T cells → protection from subsequent exposure of the same pathogen

L60 Medically Important Cocci



*Bacterial nomenclature:

- **Binomial nomenclature**: Genus (capitalized 1st letter) followed by species name
- Species/genus name either italicized or underlined in scientific usage
- Plural form can only be used in general English usage

A. Staphylococcus

- **Staphylococcus**: Gram-positive cocci in **clusters** (i.e. staphylo- = grape-like)
- 1 μm in diameter
- Features:
  - Facultative anaerobic
  - Salt tolerant (up to 7-10% NaCl)
  - Catalase +ve
    - → **Catalase test**: O_2 bubbles observed when H_2O_2 added to bacterial colony on agar
- Further classified using **coagulase test**:
  - Incubation with blood plasma on slide or tube
  - **Coagulase positive** (formation of clot): *Staphylococcus aureus*
  - **Coagulase negative** (absence of clot): *Staphylococcus epidermis*, *Staphylococcus saprophyticus*
- **Coagulase** responsible for converting **fibrinogen** to **fibrin**
  - Function: clotting plasma
  - A virulence factor: deposition of fibrin on surface hinders phagocytosis
  - Two types: **bound coagulase** and **extracellular coagulase**
- Examples:
  - ***S. aureus***
  - ***S. epidermis***: adherence to **prosthetic devices** (eg. implants, catheters) → **prosthetic infection**
  - ***S. saprophyticus***: normal vaginal flora, may cause **acute cystitis** in young women

1. Staphylococcus aureus

- Forms 'golden yellow' colonies on culture media (hence 'aureus')
- Forms **pink colonies** on **mannitol salt agar** (selective and differential medium for *S. aureus*)
- Virulence factors:
  - **Teichoic acid** for attachment (characteristic for gram +ve bacteria)
  - Evasion of host defense by:
    - → Coagulase for anti-phagocytic fibrin deposition
    - → **Protein A**: reacts with Fc of IgG → disables IgG from opsonization → anti-phagocytic
    - → **Catalase**: for breaking down H_2O_2 by immune cells
  - Invasion by hyaluronidase, lipase, DNase, protease
  - Toxins:
    - → **Epidermolytic toxins**: scalded skin syndrome (detachment of epidermis)
    - → **Toxin shock syndrome toxin**
    - → **Enterotoxin**: food poisoning
    - → **Panton-valentine leukocidin**: dermonecrosis
- Reservoir: humans (~30% of healthy people are nasal carrier)
- Transmission by direct contact
- Risk factors (~50% carrier): DM, haemodialysis, IV drug users
- Clinical diseases:
  - **Pyogenic infection**:
    - → Skin and soft tissue
    - → Wound and surgical site
    - → **Osteomyelitis**: infection and inflammation of bone and bone marrow
    - → **Arthritis**: infection and inflammation of joints
    - → Pneumonia
    - → Infective endocarditis
    - → Folliculitis
  - Toxin-mediated:
    - → **Scalded skin syndrome**: detachment of epidermis
    - → **Toxic shock syndrome**
    - → Food poisoning
- Antibiotic sensitivity:
  - Note almost all *S. aureus* are resistant to penicillin (via penicillinase) → methicillin and cloxacillin usage
  - **Methicillin-resistant *S. aureus*** (MRSA)
  - **Vancomycin-resistant *S. aureus*** (VRSA)

B. Neisseria

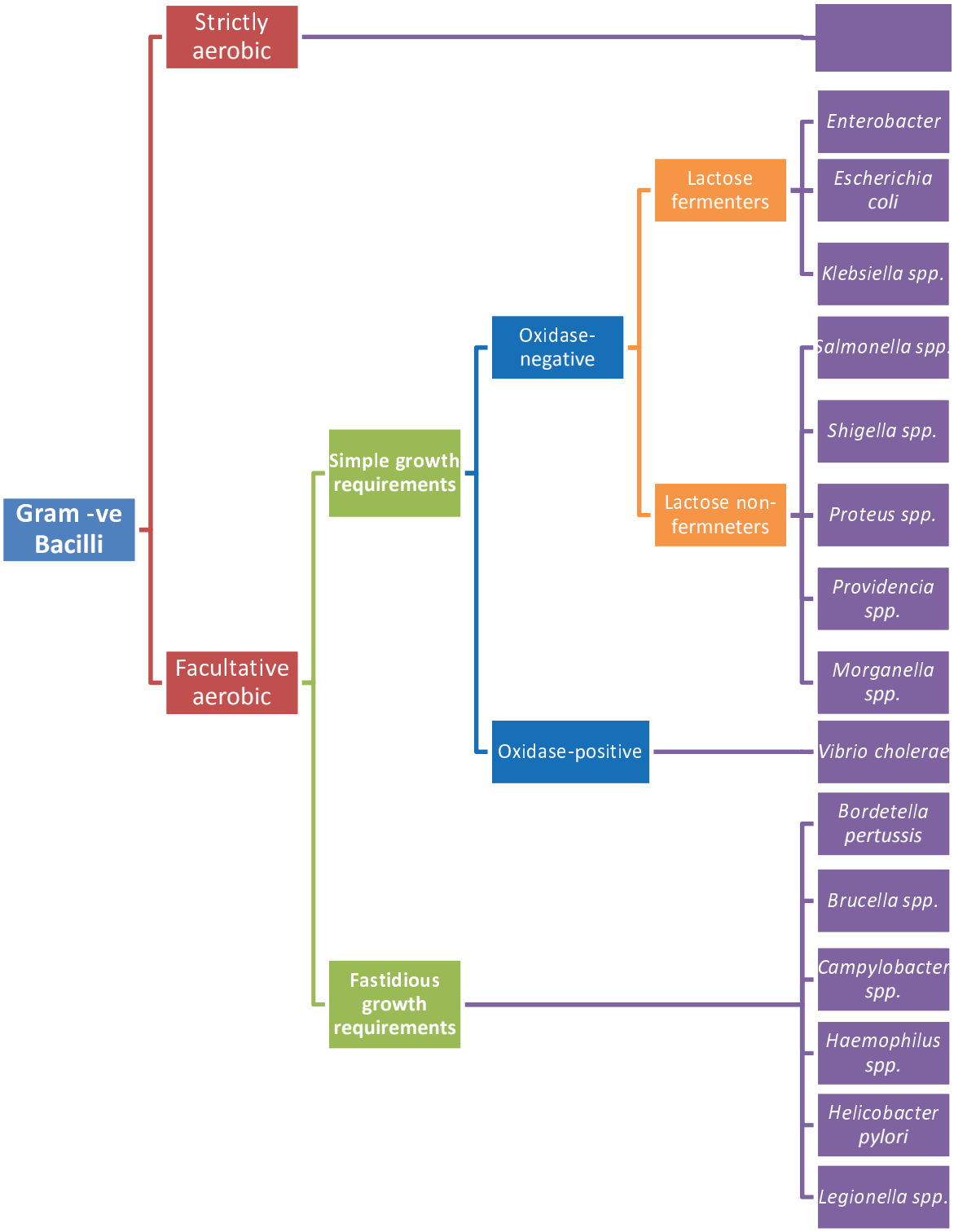
- Features:
  - Gram –ve **diplococcus** (occurring in pairs)
  - Strictly aerobic
  - **Cytochrome oxidase** (in e.t.c.) +ve
- Two important species:
  - *N. meningitidis* (meningococcus)
  - *N. gonorrhoeae* (gonococcus)
- Virulence factors:
  - Pili/fimbriae for attachment (characteristic for Gram –ve bacteria)
  - Anti-phagocytic capsule (for meningococcus)
  - Lipo-oligosaccharide as endotoxin

1. Neisseria gonorrhoae

- Clinical diseases:
  - **Urethritis** (in men): manifestation as pain during micturition and pus flowing out of urethra
  - **Endocervicitis** (in women): inflammation of inside of cervix
  - **Pelvic inflammatory disease**: inflammation of upper female genital tract
  - **Proctitis**: anal or rectal infection
  - **Pharyngitis**
  - **Gonoccoal ophthalmia neonatorum**: conjunctivitis of newborn contracted from maternal genital tract during delivery
  - **Disseminated gonoccoal infection**: non-localized gonorrhea (due to bacteremia)
  - **Arthritis**
- Note that antibiotic resistance of *N. gonorrhoeae* is rising
- Reservoir: human
- Transmission: sexual and mucosal contact
- Risk factors: prostitution, sexual promiscuity and unprotected sex
- Prevention: discouraging sexual promiscuity and usage of condom

L61 Medically Important Gram Negative Bacilli

A. Overview on Gram Negative Bacteria



- Method of classification of gram –ve bacteria:

1) Staining properties
2) Morphology: cocci, bacilli, coccobacilli, spirals
3) Dependence on oxygen: strictly aerobic, strictly anaerobic, facultatively anaerobic, microaerophilic
4) Physiological tests: eg. motility, optimal growth temp
5) Biochemical tests: eg. oxidase, sugar fermentation reactions
6) Phylogenetic studies
7) Profiling of cellular proteins by mass spectrometry

- Clinical significance:
  - Very broad spectrum of clinical disease esp urinary tract, GI tract, intra-abdominal infections and nosocomial infections
  - Relatively high mortality
  - Opportunistic infection in immunocompromised hosts
  - Increasing problem with antibiotic resistance

Some examples of medically important Gram negative bacteria.

	Oxygen requirement			
Cell shape	Strict aerobes	Facultative anaerobes	Microaerophilics	Strict anaerobes
Cocci	*Neisseria*			*Veillonella*
Bacilli	*Pseudomonas* *Acinetobacter* *Brucella*	*Enterobacteriaceae* *Vibrionaceae* *Haemophilus*		*Bacteroides* *Porphyromonas* *Prevotella* *Fusobacterium*
Curved or spiral	*Leptospira*		*Campylobacter* *Helicobacter* *Arcobacter*	

Non-cultivable (*in vitro*) spirochaetes: e.g. *Treponema pallidum*.

Differentiating characteristics of three important groups of Gram negative bacilli.

	Enterobacteriaceae	*Vibrionaceae* and related genera (*Aeromonas*, *Plesiomonas*)	*Pseudomonas aeruginosa*
Oxidase	–	+	+
Glucose fermentation	+	+	–

(*Plesiomonas* is grouped under *Enterobacteriaceae*, but biochemically it resembles members of *Vibrionaceae*.)

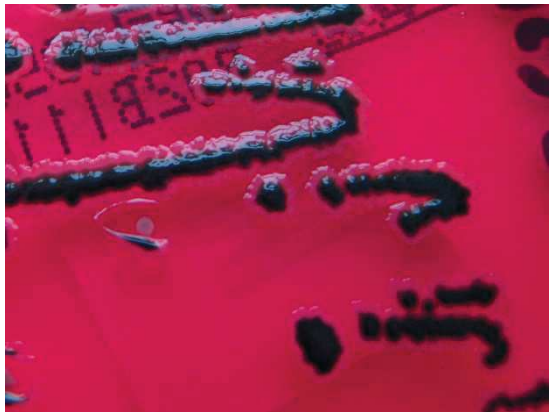
B. Enterobacteriaceae

- Also called **coliforms**
- Normal habitat: mainly **large bowels** of human and animals
- Examples: *Escherichia*, *Klebsiella*, *Proteus*, *Providencia*, *Morganella*, *Salmonella*, *Shigella*, *Enterobacter*, *Citrobacter* and *Serratia*
- Major surface antigens of *Enterobacteriacae*:
  - O antigen (somatic LPS antigen)
  - H antigen (flagellar antigen)
  - K antigen (capsular antigen)
- Diseases caused:
  - GI infections (gastroenteritis/dysentery): eg. *Salmonella*, *Shigella*, some *Escherichia coli* strains
  - Extra-intestinal infections: eg. urinary tract infection, bacteraemia, meningitis
- Note presence of carbapenem-resistant Enterobacteriaceae strains (eg. NDM-1, KPC)

1. Escherichia coli

- Commonest commensal *Enterobacteriaceae*
- One of the commonest Gram negative bacteria causing human infections
- Intestinal disease: e.g. **traveller's diarrhea** (enterotoxigenic *E. coli* leading to diarrhea), **haemorrhagic colitis** (*E. coli* O157:H7 and other serotypes carrying **Shiga toxin**)
- Extra-intestinal disease: e.g. urinary tract infection, meningitis in neonates, bacteraemia, intra-abdominal infections etc
- Lactose fermenter (pink and yellow colonies on MacConkey and XLD agar resp.)

2. Salmonella



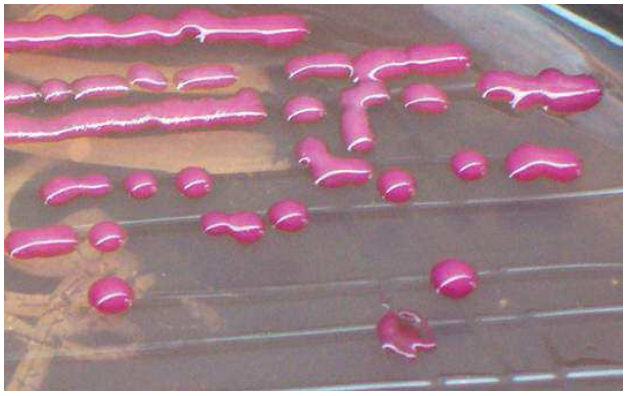
- *Salmonellae enterica*: >2500 **serotypes** (distinguished by different O and H antigen compositions)
- Widely distributed in nature as commensals or pathogens of animals
- Some serotypes (Typhi, Paratyphi) infect human only
- Generally classified as **typhoidal** and **nontyphoidal** salmonellae:
  - **Typhoidal**: human-only pathogen responsible for **typhoid fever** (characterized by systemic infection)
  - **Non-typhoidal**: only causes diarrhoea or disseminated/focal infections
- Important cause of foodborne (and sometimes waterborne) infections
- *Salmonella* infection is in general called **salmonellosis**: three types of clinical presentations:
  - Gastroenteritis: commonest
  - Typhoid and paratyphoid fever: systemic infection caused by *Salmonella* of Typhi and Paratyphi serotypes (only seen in human)
  - Disseminated or local infections: eg. osteomyelitis, meningitis, **abscess** formation
- Feature: black colonies (due to H_2S formation) on XLD agar

***abscess**: collection of pus within body tissue

3. Shigella

- May lead to **bacillary dysentery** (blood and mucus in stool), potentially fatal esp in children
- Examples: *Shigella dysenteriae*, *S. flexneri*, *S. boydii*, *S. sonnei*
- Transmission by contaminated food or water and **faecal-oral** human-to-human transmission
- Pathogenic mechanism mainly achieved due to **Shiga** toxin

4. Klebsiella



- *Klebsiella pneumonia*: commonest species causing human infections
- Observed as **mucoid** colonies on agar
- Diseases:
  - Community-acquired pneumonia
    - → Risk factors: elderly, alcoholics, other underlying diseases
    - → **Abscess** formation
  - Intra-abdominal infections: e.g. liver absecess , cholangitis
  - Urinary tract infection
  - Nosocomial infections

5. Urease-positive Enterobacteriaceae Genera

- Can secrete urea
- Examples: *Proteus*, *Providencia*, *Serratia*
- Can lead to urinary tract infection and other hospital-acquired infections

6. Citrobacter, Enterobacter, Serratia

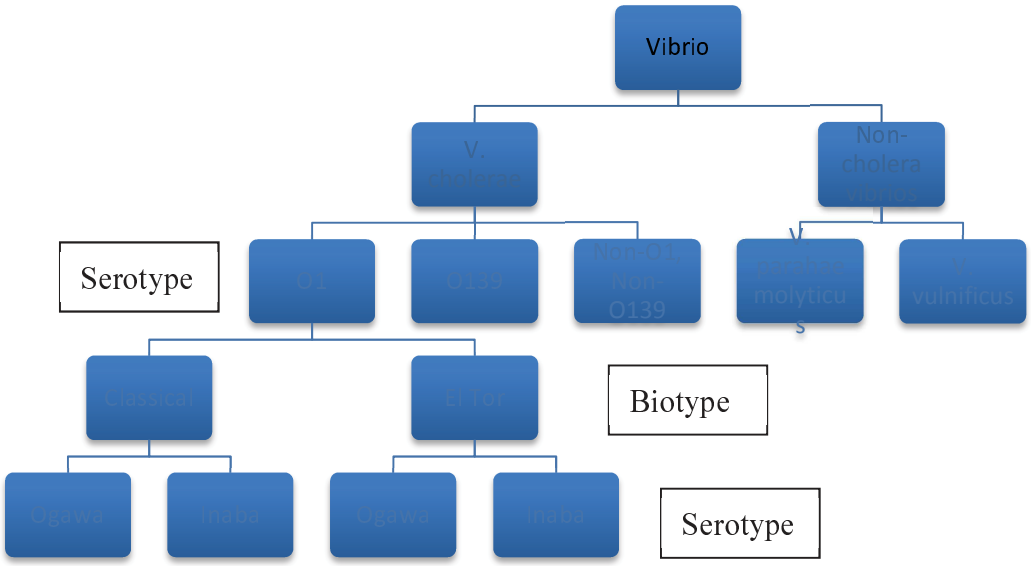
- Often associated with nosocomial infections
- Multi-antibiotic resistant

C. Vibrionaceae and Related Genera

- **Vibrio**: family of curved Gram –ve rods
  - Oxidase positive
  - Actively motile by polar flagellum
- Widely distributed in natural water bodies, seawater and fresh water
- Usually cause GI tract infections, occasionally severe systemic infections
- Examples:
  - *Vibrio cholera*: cause of **cholera**
  - *Vibrio parahaemolyticus*: a very common cause of bacterial gastroenteritis
  - *Vibrio vulnificus*: can cause **necrotizing fasciitis** (a severe form of soft tissue infection leading to tissue necrosis)

*Note that **necrotizing fasciitis** not limited to *V. vulnificus*, can also occur due to other bacterial infections

- Other related genera: *Aeromonas* and *Plesimonas* (under *Enterobacteriaceae*)
  - Similar to *Vibrio* species in many aspects
  - All oxidase positive curved bacilli
  - Natural environment of all is in aquatic
  - NOT classified in the family *Vibrionaceae*



1. Vibrio cholerae

- May lead to **cholera** or milder forms of gastroenteritis
  - **Cholera**: very profuse watery diarrhoea (rice water stool)
  - Can be rapidly fatal due to dehydration
- Serotypes (based on O antigen): O1, O139 (both can cause outbreaks), non-O1/non-O139
- Pathogenic mechanism via **cholera toxin**:
  - Raise intracellular cAMP at intestinal epithelium
  - Inhibits Na^+ and Cl^- absorption
  - Increase Cl^- and water secretion

D. Non-fermenters

- Gram negative bacilli that do not ferment glucose
- Most are saprophytes and widely found in soil, water and other moist environment (sometimes can also colonize the skin)
- Some are opportunistic pathogens
- Often very resistant to antibiotics
- Examples:
  - *Acinetobacter baumanii*
  - *Stenotrophomonas maltophilia* (intrinsic resistance to multiple antibiotics)
  - *Pseudomonas aeruginosa*

1. Pseudomonas Aeruginosa

- Feature: produces a green pigment (in nutrient agar) and characteristic fruity smell
- Often resistant to multiple antibiotics (incl. carbapenems) and even disinfectants
- Important cause of hospital-acquired infections

2. Acinetobacter

- Saprophyte in soil, water, sewage, occasionally as commensal on moist areas of human skin
- Example: *Acinetobacter baumanii* (multi-resistant incl. carbapenems)
- Survives well in hospital environment
- Causes nosocomial infections: e.g. pneumonia, catheter-related sepsis

3. Bordetella pertussis

- Cause of **pertussis** (whooping cough)

4. Legionella

- Environmental Gram negative bacilli
- Commonest human pathogen is *L. pneumophila*
- Cause of **Legionnaire's disease**

E. Haemophilus

- Short Gram –ve coccobacilli
- Haemo- (blood) + -phil (attracted to): requires blood or its components for growth
  - **X factor** (**haemin**: modified forms of heme)
  - **V factor** (NAD or NADP)
  - Provided by **chocolate agar** in culture
- Feature: pinpoint colonies on blood agar (indicating poor growth)
- Example: *Haemophilus influenza*
  - May lead to meningitis, pneumonia, sinusitis, otitis media and other invasive infections
  - Possess a polysaccharide capsule: a-f types and non-typeable
  - Type b causes most cases of invasive disease in children and infants
  - Vaccines against type b capsular polysaccharide is available

F. Campylobacter

- **Spiral-shaped** microaerophilic Gram –ve rods
- Widely distributed in nature (animals incl. livestocks)
- Human infections usually due to ingestion of contamination of animal products
- One of commonest bacterial causes of **acute infective diarrhoea**
- Can also cause systemic infections such as bacteraemia

G. Brucella

- Short Gram –ve coccobacilli
- Small, non-motile and non-sporing
- **Brucellosis**: disease caused by *Brucella* spp.
  - A zoonosis
  - Infection acquired through animal contact (incl. ingestion) in vasy majority of cases
- Main animal hosts: sheep and goat (*B. melitensis*), cattle (*B. abortus*) and pig (*B. suis*)

H. Helicobacter pylori

- **Spiral-shaped** microaerophilic Gram –ve rods
- Cause of duodenal/gastric ulcers, gastric cancer and gastric lymphoma

I. Anaerobic Gram-negative Bacilli

- Habitat:
  - Huge numbers in GI tract (oropharynx and large intestines)
  - Lower genital tract
  - Skin
- Diseases:
  - Mixed infections
  - Abscess formation
  - Intra-abdominal infections, genital tract infections, head and neck infections
- Examples: *Bacteroides* spp., *Porphyromonas* spp., *Prevotella* spp., *Fusobacterium* spp.

J. Spirochaetes

- Spiral-shaped organisms
- Usually not readily visualized by routine light microscopy
- Diverse ecology and clinical manifestations
- Examples:
  - *Leptospira interrogans*: cause of leptospirosis
  - *Borrelia recurrentis*: may lead to relapsing fever
  - *Borrelia burgderferi*: **Lyme disease** (systemic infection transmitted by ticks)
  - *Treponema pallidum*: **syphilis**

1. Treponema pallidum

- Not cultivable *in vitro*
- Cause of **syphilis** (STD)
- Organism can persist in body for decades if without treatment → chronic infection of CNS, CVS etc.
- Can also cause congenital infection of foetus and infant (if pregnant woman's syphilis left untreated)
- Much more prevalent in developed countries esp among homosexual male population

2. Leptospira interrogans

- Corkscrew-shaped bacteria with end hooks (other spirochaetes do not)
- **Leptospirosis**: zoonosis transmitted from vertebrate animals to humans
  - Occurs worldwide but is commonest in tropical and subtropical areas with high rainfall
  - Found mainly in situations when humans come into contact with urine of infected animals or a urine-polluted environment

L62 Fungi and Fungal Infection

A. Medically Important Fungi

- **Fungi**: a kingdom of eukaryotes that includes yeast and mould
- **Mycology**: study of fungi
- Cell wall:
  - **Glucan** (60%): polysaccharide, can serve as a target for antifungal drug
  - **Protein** (30%)
  - **Chitin** (10%): polymer of N-acetyl glucosamine (NAG)
- Cell membrane contain **ergosterol** (unique component that can serve as target for antifungal drug)

B. Classification of Medically Important Fungi

- **Yeasts**: unicellular fungi
  - 5 μm in diameter
  - Reproduce by **budding**: development of offspring from an outgrowth of parent cell
  - Examples:
    - → *Candida albicans*
    - → *Cryptococcus neoformans* (identified by **indian ink stain** (a negative stain)
- **Moulds**: multicellular fungi
  - Composed of **hyphae** (long branching filaments of 2-4μm wide)
  - Presence of **mycelium**: vegetative part of fungi consisting of mass of branching, thread-like hyphae
  - Asexual or sexual reproduction by **spores**
    - → 3μm in diameter for spores of *Aspergillus* (fungi infecting lungs)
    - → Morphology of spores can be used to identify *Microsporum*
  - Examples:
    - → *Aspergillus fumigatus*
    - → **Dermatophytes**: a group of three fungal genera (*Trichophyton*, *Epidermophyton*, *Microsporum*) that commonly causes skin disease in animal and humans
- **Dimorphic fungi**: yeast at 37∘C and mold at 25∘C
  - Examples:
    - → *Penicillium marneffei*

C. Epidemiology of Fungal Infections

- Geographical:
  - Global (*Candida*, dermotophyte, *Aspergillus*)
  - Local (*P. marneffei* (in HK) and other dimorphic fungi)
- Risk factors: immunocompromised patients
  - Neutropenia (*Aspergillus*)
  - HIV infection (*C. neoformans*, *P. marneffei*)

D. Infections Caused by Fungi

- Superficial infections:
  - **Candidiasis** by *Candida*
    - → **Oral thrush**
    - → **Vaginal thrush**
  - **Dermatophytosis** (ringworm) by dermatophytes (keratin-feeding fungal infections)
    - → **Tinea pedis** (feet, = athlete's foot)
    - → **Tinea capitum** (scalp)
    - → **Tinea unguium** (nails)
- Systemic infections:
  - Immunocompetent hosts: uncommon (except allergic reactions to spores)
  - Immunocompromised hosts:
    - → Systemic candidiasis
    - → *C. neoformas*
    - → *A. fumigatus*
    - → *P. marneffei*

E. Laboratory Diagnosis of Fungal Infections

- Detection of fungal components:
  - Direct visualization
    - → **KOH smear** for mould: dissolves skin cells but leaves fungi intact → hyphae morphology can be visualized under microscope
    - → **Gram smear** for *Candida*: yeast cells retain crystal violet dye → appears Gram +ve under microscope
    - → **Indian ink smear** for *C. neoformans*: negative stain for visualization of *C. neoformans* capsule
    - → Biopsy and histopathology
  - Culture by agar plates and broth culture
  - Antigen detection:
    - → β-D-glucan (fungal cell wall component)
    - → Capsular polysaccharide for *C. neoformans*
    - → **Galactomannan** (component of cell wall) detected by ELISA for *Asperigillus*
  - Nuclei acid detection for *Asperigillus*
- Detection of patients' response:
  - Antibody for *P. marneffei*
  - Biopsy and histopathology

F. Anti-fungal Agents

- Anti-fungal agents act on different sites of fungal life
- Action on cell membrane:
  - **Polyenes**
    - → Complex with **ergosterol** → disrupt fungal plasma membrane
    - → Examples: **nystatin**, **amphotericin B**
  - **Azoles**
    - → Inhibit **ergosterol** synthesis
    - → Examples: **fluconazole**, **itraconazole**, **voriconazole**, **posaconazole**
- Nucleoside analogues:
  - **5-flucytosine**: fluorinated uridine analogue → inhibit DNA synthesis
- Cell wall synthesis inhibitors:
  - **Echinocandins** (Caspofungin, micafungin, anidulafungin)
- Note that antifungal susceptibility testing is <u>rarely performed</u>
- Proper identification of fungus is more important since treatment is characteristic to type of fungus

G. Candida Albicans

- A type of yeast
- Most important species of *Candida*
  - Other species include *C. parapsilosis*, *C. tropicalis*, *C. krusei*
- Targeted mainly by intact skin and neutrophils/monocytes

1. Epidemiology

- Reservoir: normal commensals of human (skin, GIT, female genital tract) → endogenous infection
- Distribution: global
- Risk factors:
  - Recipient of broad-spectrum antibiotic treatment → selective pressure
  - Hospitalization (esp ICU)
  - IV lines
  - Immunocompromised patients (HIV, neutropenia, transplant, steroid treatment)

2. Clinical Disease

- Immunocompetent hosts: eg. vaginal thrush
- Immunocompromised hosts:
  - **Refractory** (recurring, hard to treat) **superficial infections**: eg. oral thrush, mucocutaneous candidiasis
  - **Systemic candidiasis**:
    - → **Candidaemia**: *Candida* found in blood, major cause of nosocomial bloodstream infection
    - → Disseminated infections: eye, skin, kidneys
    - → Hepatosplenic candidiasis
    - → Any organ

3. Diagnosis

- Diagnosis mainly done by detection of *C. albicans* or its components
  - Direct visualization by gram smear (appears Gram +ve in LM)
  - Culture:
    - → Agar plate culture of pus
    - → Broth culture of blood sample
  - Observation of **germ tube** (outgrowth by spores) formation in serum under LM
  - Biochemical tests
  - Antigen detection: β-D-glucan detection in serum

4. Treatment

- Superficial candidiasis:
  - Topic treatment (eg. nystatin)
  - Systemic treatment in refractory cases (eg. fluconazole)
- Systemic candidiasis:
  - Systemic treatment of fluconazole and amphotericin B

5. Prevention

- Avoid antibiotic overuse → prevent overgrowth of commensal *C. albicans*
- Fluconazole prophylaxis for transplant recipients
- Note that no vaccines are available against *C. albicans*

L63 Medically Important Parasites

A. Parasites and Parasitism

- **Parasitism**: any reciprocal association in which a species depends upon another for its existence
- **Parasite**: the species that derives all the benefit from the association
- **Host**: the harbouring species
  - **Definitive host**: harbours the adult or sexual stage of parasite
  - **Intermediate host**: harbours the intermediate or larval stages of parasite
- Classification of parasites by
  - Dependence on host: obligatory or facultative
  - Temporal relationship with host: temporary or permanent
  - Physical relationship with host: **ectoparasite** (**infection**) or **endoparasite** (**infestation**)
- Diagnosis by:
  - Clinical suspicion
  - Demonstration of parasites in appropriate clinical specimens by direct microscopic examination, staining or tissue sections
  - Serology: detection of antigens or antibodies in blood or other body fluids (not widely available)
  - Culture of parasites (only applicable in a small number of parasites)
  - Nucleic acid amplification (eg. PCR)
- Treatment:
  - Supportive and symptomatic
  - Specific anti-parasitic agents
- Prevention:
  - Elimination or reduction in number of parasites in reservoir
  - Elimination or reduction in number of vectors
  - Avoiding exposure to parasites and/or vectors
  - Chemoprophylaxis
  - Vaccines (no commercially available at the moment)
- Mainly classified into **protozoa**, **helminthes** (nematodes, trematodes and cestodes) and **arthropods**
- Importance of **parasitology** despite low incidence in Hong Kong:
  - Global significance of parasitic infections
  - International travel (leisure, work, missionary)
  - Increasing number of immunocompromised hosts
  - Increasing drug resistance in some parasites

1. Protozoa

- **Protozoa**: unicellular organisms (generally animal-like protists)
- Classification:
  - **Lumen-dwelling protozoa** (in gut or genital tract): eg. *Entamoeba histolytica*, *Giardia lamblia*, *Trichomonas vaginalis*, *Cryptosporidium* spp., *Cyclospora cayetanensis*, *Cystoisospora belli*
  - **Blood and tissue protozoa**: eg. *Plasmodium* spp., *Babesia* spp., *Toxoplasma gondii*, *Trypanosoma* spp., *Leishmania* spp.
- Major life form: **trophozoite** (active stage of protozoa parasite, non-reproductive)
- Transmission by:
  - Direct contact (by transferral of trophozoite)
  - **Cyst** transferral

2. Helminths

- Parasitic worms

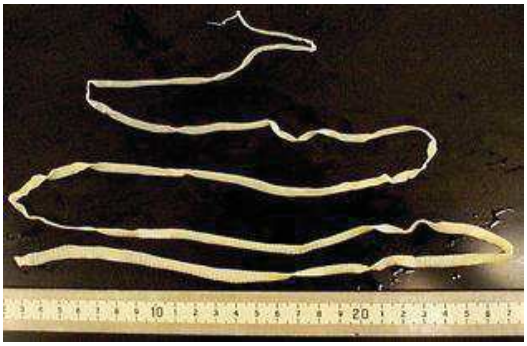
a. Nematodes

- **Nematodes**: cylindrical roundworms
- Types:
  - **Intestinal nematodes**: eg. *Ascaris lumbricoides*, *Enterobius vermicularis*, hookworms, *Trichuris trichiura*, *Strongyloides stercoralis*
  - **Blood and tissue nematodes**: eg. filariae (such as *Wuchereria bancrofti*, *Brugia malayi*), *Trichinella spiralis*, *Angiostrongylus cantonensis*

b. Trematodes (Flukes)

- **Trematodes**: flat, leaf-shaped worms
- Types:
  - **Liver flukes**: eg. *Clonorchis (Opisthorchis) sinensis* (commonest endoparasitic infection in HK, residing in bile duct, mostly asymptomatic but is carcinogen (cancer of bile duct)), *Fasciola hepatica*
  - **Intestinal flukes**: eg. *Fasciolopsis buski*
  - **Lung flukes**: eg. *Paragonimus westermani*
  - **Blood flukes**: *Schistosoma mansoni*, *S. japonicum*, *S. haematobium*

c. Cestodes



- **Cestodes**: tapeworms
- Consists of segments
- **Intestinal tapeworms**: eg. *Taenia solium*, *T. saginata*, *Diphyllobothrium latum*
- Tissue tapeworm infections can also be caused by larval stages of cestodes: eg. *Taenia solium* larvae (cysticercosis) and *Echinococcus granulosus* (hydatid disease)

3. Arthropods

- **Arthropods**: generally invertebrate animals with external skeleton
- Medical importance:
  - Directly causing injury, **envenoming** (impregnating with venom): eg. spiders, scorpions, ants
  - As **ectoparasites**: eg. lice, fleas, maggots, *Sarcoptes scabiei* (scabies mites)
  - Vectors for other infective agents: eg. mosquitoes, tsetse flies, lice, fleas, ticks, mites
- Classification:
  - **Insects** (3 pairs of legs): eg. mosquitoes, flies, bugs
  - **Arachnids** (4 pairs of legs): eg. spiders, scorpions, ticks, mites

B. Malaria

- **Malaria**: a disease caused by infection of *Plasmodium*

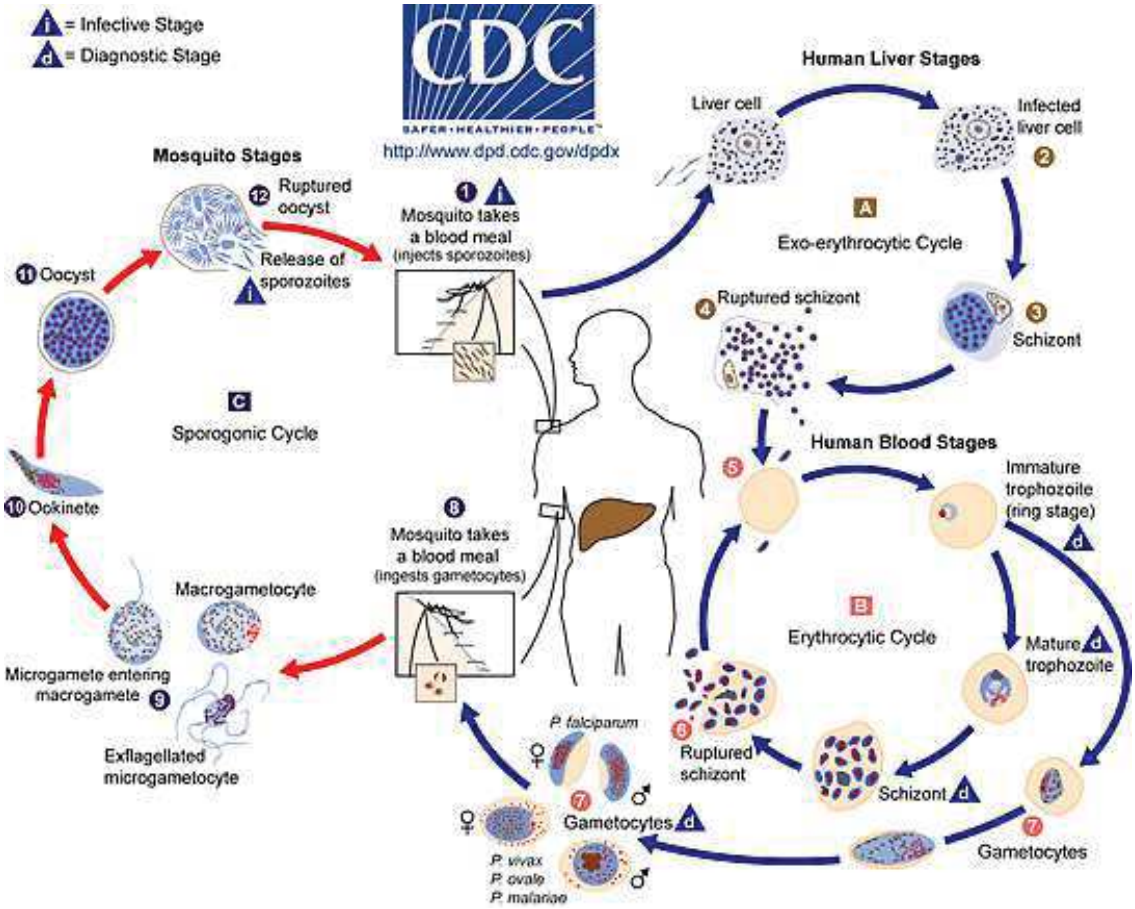
1. Epidemiology

- Five *Plasmodium* species can infect humans:
  - *P. vivax*: worldwide distribution
  - *P. falciparum*: generally in the tropics
  - *P. malariae*: usually sporadic
  - *P. ovale*: West Africa, some South Pacific islands, other parts of the world sporadically
  - *P. knowlesi*: in SE Asia, esp around Malaysia and Malaysian Borneo (primarily a simian (monkey) malaria that occasionally causes human infection)
- ~95% of all cases of malaria worldwide are due to *P. vivax* and *P. falciparum*

2. Transmission of Malaria

1) Vector for natural transmission: **female *Anopheles* mosquitoes**;
2) Blood transfusion and transplantation;
3) Contaminated needles or medical instruments;
4) Congenital (due to trans-placental transmission).

3. Life Cycle



1) **Sporozoites** introduced by mosquitoes into host circulation via biting action;
2) Sporozoites enter hepatocytes within half an hour;
3) Intracellular *Plasmodium* carries out **merogony** (a type of **schizogony**) to produce (and release) exo-erythrocytic **merozoites** in hepatocytes;
4) Other hepatocytes are infected while some (*P. vivax* and *P. ovale*) may undergo **secondary schizogony** and develop into **hypnozoites** (resting stage) and may persist for years;
5) **Merozoites** are released into bloodstream and invades circulating RBC;
6) Some *Plasmodium* carries out **merogony** to form (and release) merozoites to infect new RBCs;
7) Other *Plasmodium* undergoes **gametogony** to form **microgametocytes** (male) and **macrogametocytes** (female);
8) **Gametocytes** taken up by female *Anopheles* mosquitoes;
9) **Microgametocytes** fuses with **macrogametocytes** to form **ookinete** (zygote);
10) **Ookinete** undergoes **sporogony** to form an **oocyst** containing many **sporozoites** at outer stomach wall of the mosquito;
11) **Oocyst** ruptures to release **sporozoites** which can infect another new host.

***Schizogony**: intracellular *Plasmodium* undergoes repeated replication of its nucleus and organelles → formation of a multinuclear **schizont**

Cytokinesis of schizont can release numerous offspring

****Microgametocyte**: flagellated male gamete, of small size because multiple divisions of merozoites involved in its development

Macrogametocyte: female gamete, of large size because only differentiation of merozoites is involved in its development

*****Note that **sporozoites** are motile whereas **merozoites** are not

4. Pathogenesis of Malaria

- Increased RBC turnover due to:
  - Decreased **deformability** (ability to change shape without rupturing)
  - Parasite-induced cytolysis
  - Increased clearance in **splenomegaly** (increased spleen size due to infection)
- Cytoadherence in *P. falciparum* infection (adhesion of infected RBC to blood vessels due to parasite-induced expression of receptor-binding surface proteins) → microvascular obstruction
- RBC lysis leading to iron depletion, anaemia, **haemoglobinuria** (Hb in urine)
- Immune complex (Ab/Ag) deposition in kidneys → **nephrotic syndrome**
  - Mechanism: membranous nephropathy → ↑ protein contet in urine → excessive loss of protein from blood
  - Usually seen in chronic infections due to *P. malariae*
- Release of inflammatory cytokines during course of infection

5. Signs and Symptoms of Malaria

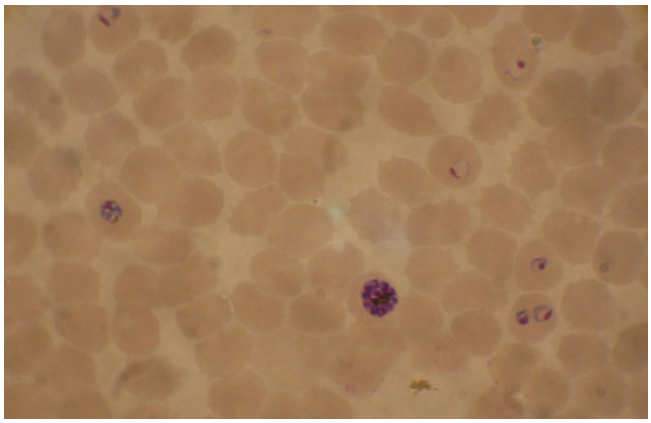
- **Paroxysms**: sudden recurrence/intensification of symptoms
  - Chills and rigor for 1-2 hours, followed by spiking fever in the next few hours
  - Marked sweating and **defervescence** (alleviation of fever) with a rapid drop in temperature
  - 48-hour cycle (in *P. vivax*, *P. ovale*, *P. falciparum*) or 72-hour cycle (in *P. malariae*)
- Severe malaria: cerebral malaria, severe anaemia, renal failure, shock, hypoglycaemi etc.
- Relapse: recurrence of symptoms after complete initial clearing of parasitaemia due to reinvasion of bloodstream by exo-erythrocytic stages (**hypnozoites**) for *P. vivax* and *P. ovale*
- **Recrudescence**: revival of symptoms after initial parasitaemia is reduced to a very low level (but not completely cleared)
- Most dangerous form of malaria is **falciparum malaria** (due to *P. falciparum*)

*In real life patients do not always present with the typical fever patterns

6. Diagnosis of Malaria

- Possibility of malaria must be considered in all cases of unexplained fever that starts after 7th day of stay in an endemic area
- Factors to consider:
  - **History**: travel history, fever pattern (not useful in most clinical cases), prophylaxis taken, blood or blood product transfusion etc.
  - **Specimens**: peripheral blood anticoagulated with EDTA
  - Thick and thin **blood films** stained with Giemsa, Wright or Field's stain

    → One set of negative blood smear does NOT rule out malaria



- Other diagnostic tests: **antigen detection** in peripheral blood, PCR

7. Treatment of Malaria

- Principles of therapy:
  - **Suppressive therapy** (used in chemoprophylaxis): destroys asexual erythrocytic stages to prevent development of clinical symptoms
  - **Clinical cure**: targets at elimination of asexual erythrocytic forms during acute attack
  - **Radical cure**: targets at elimination of gametocytes and hepatic resting stages (**hypnozoites** of *P. vivax* and *P. ovale*)
- Types of **antimalarial agents**:
  - **Blood schizonticides**: eg. chloroquine, mefloquine, atovaquone-proguanil, artemisinins
  - **Tissue schizonticides**: eg. primaquine

8. Prevention and Control of Malaria

- Vector control
- Avoidance of exposure
- Treatment of cases
- **Chemoprophylaxis**:
  - No antimalarial prophylactic regimens give complete protection → still need to implement other protective measures when travelling to an endemic area
  - Antimalarial must be started before leaving for an endemic area, taken with strict regularity during stay and continued for some time after returning
  - Different antimalarial agents may require different regimens or timing

L64 Principles of Disinfection, Sterilization and Infection Control

A. Nosocomial Transmission of Infection

- **Contact transmission**:
  - **Direct contact**: pathogen is spread directly from body surface to body surface (eg. *Sarcoptes sacbiei*, *Streptococcus pyogenes*, *Staphylococcus aureus*, respiratory viruses, rotavirus)
  - **Indirect contact**: pathogen spread via a contaminated intermediate object (eg. *Clostridium difficile*, *Stahpylococcus aureus*, vancomycin-resistant enterococci (VRE), respiratory viruses, rotavirus)
- **Droplet transmission**: transmission of pathogens by large-particle aerosols
  - Size: > 5μm in diameter and can go up to >100μm (depend on relative humidity, air velocity and temperature)
  - Sources: coughing, sneezing, talking or medical procedures (suctioning, bronchoscopy)
  - Travel distance typically 1-2 m from source (droplets <100μm may dry out before falling to ground) → short airborne time
  - Transmission by deposition on mucous membranes
  - Eg. most respiratory pathogens, *N. meningitidis*
- **Airborne transmission**: transmission of pathogens by droplet nuclei of evaporated aerosols
  - Size: < 5μm in diameter
  - Can remains airborne for prolonged period of time
  - Transmission by deposition in lower respiratory tract and lungs (deeper penetration due to smaller size)
  - Eg. *Mycobacterium tuberculosis*, measles virus, varicella-zoster virus, (influenza virus)

B. Infection Control on Nosocomial Infections

- **Nosocomial infections**: infection acquired by patients while they are in hospital, or by members of hospital staff
- Infection control done by disrupting transmission of infections: source → route of transmission → susceptible host
- Importance:
  - For patients:
    - → Prevent outbreaks and cross-infection
    - → Lowers morbidity and mortality
    - → Lowers cost in treatment and hospital stay
  - For staff:
    - → Provide a safe working environment
    - → Ensure safety and health
- Two-tier system: **standard precautions** and **transmission-based precautions**
  - Additional precautions for specific patient groups

1. Standard Precautions

- **Standard precautions**: precautions designed for the care of <u>all patients</u> in hospitals regardless of their diagnosis or presumed infections status
- Apply to:
  - Blood
  - All bodily fluids, secretions and excretions except sweat (regardless of whether or not they contain visible blood)
  - Non-intact skin
  - Mucous membranes
- Protocol:
  - Hand hygiene
  - Gloves (only when necessary)
  - Mask, eye protection, face shield, gown (when splashing / aerosol generation is likely)
  - Needles: never be recapped, bent, broken and disposed properly (in special containers and burnt in incinerators)

a. Hand Hygiene

- Handwashing
  - Social handwashing
  - Hygienic hand antisepsis
  - Surgical hand antisepsis
- Use of alcohol hand rub (some non-enveloped virus can be resistant to standard alcohol conc. used)

2. Transmission-based Precautions

- **Transmission-based precautions**: precautions designed for specific patients known to be infected or colonized with epidemiologically important pathogens
  - Neutropenic patients
  - Severe burns (>30% total body surface area (TBSA))
  - Organ transplant recipients (due to cytotoxic anti-rejection therapy)
  - Cystic fibrosis patients (highly damaged lungs with formation of biofilms → easy infection (∵ immune system cannot penetrate in biofilms))
- Used in addition to standard precautions
- Isolation procedures:
  - Hand hygiene
  - Gloving
  - Patient placement
  - Transport of infected patients
  - Masks, respiratory protection, eye protection, face shields
  - Gowns and protective apparel
  - Patient care equipment and articles
  - Linen and laundry
  - Dishes, glasses, cups, eating utensils
  - Routine and terminal cleaning

3. Other Aspects of Hospital Infection Control

- Staff health
  - Immunization
  - Post-exposure prophylaxis
  - Health counseling
- Waste disposal
  - Medical wastes
  - Sharps

C. Ways of Microbe Removal

- Classification:
  - **Physical**: heat, radiation, filtration
  - **Chemical**

1. Levels of Microbe Removal

	Procedure	Target microbial form	Object of application	Effect
Sterilization	Physical or chemical	All forms	Any	≥ 6 log CFU reduction of most resistant spores
Disinfection	Physical or chemical	Most forms (except spores or relatively resistant)	Inanimate object	3-5 log CFU reduction of microbe
Antisepsis	Germicide	Microbes	Living tissue	Inhibit or destroy microbes
Decontamination	Physical or chemical	Microbes	Any	Elimination of debris and proteins by cleaning; ≥1 log CFU reduction of microbes

*CFU = **colony-forming unit**: unit measuring the number of viable particles required to form one visible colony

**Delicacy of living tissues prevent usage of higher degrees of microbe removal

a. Spaulding Classification

- **Spaulding classification**: categorization of medical devices to determine the level of disinfection to be used
  - **Critical devices**: instruments that have contact with bloodstream or sterile body areas
    - → Eg. catheters, implants, needles
    - → Apply sterilization
  - **Semi-critical devices**: devices that come into contact with mucous membranes
    - → Eg. endoscopes, respiratory therapy equipments
    - → Apply sterilization or high-level disinfection
  - **Non-critical devices**: devices that come into contact with intact skin
    - → Eg. stethoscopes, sphygmomanometer cuffs, environmental surfaces
    - → Apply low-level disinfection or simple cleaning

2. Physical Removal

a. Heat

- Sterilization: heat is most effective method of sterilization
  - Dry heat by flaming or in hot air oven
  - Moist heat in **autoclave** (pressure steam sterilization)
    - → Pressure for penetration
    - → Steam help activate biochemical processes that kills the bacteria
    - → Protocol: 121∘C × 15 min or 134∘C × 3 min
- Disinfection: by moist heat in boiler and thermal washer disinfectors

b. Radiation

- Ionizing radiation
  - Commercial use
  - May damage some materials eg. some plastics
  - Forms:
    - → β rays from linear accelerator
    - → γ rays from ^{60}Co
    - → UV (254nm) (with poor penetrating power)

c. Filtration

- Membrane filters:
  - Different pore sizes: 1.2μm, 0.45μm, 0.22μm (standard for sterile filtration but virus can still pass through)
  - Use of sterilization of materials <u>sensitive to physical/chemical</u> methods (eg. pharmaceutical products) ∵ killing of bacteria may produce toxins
- High-efficiency particulate arrestance (HEPA) filters:
  - Removal of >99.9% of airborne particles (0.3μm)
  - Used in operating theatres, burn units, airliners
  - Expensive → rarely used

3. Chemical Removal

- Resistance to disinfectants (descending order):

1) Prions (contaminated products are simply discarded);
2) Spores (eg. *C. perfringens*) (due to thick spore wall);
3) Cryptosporidiidae (spore-like protozoa);
4) Mycobacteria;
5) Non-enveloped viruses (eg. coxsackie virus);
6) Fungi (eg. *Aspergillus* spp.);
7) Bacteria;
8) Enveloped viruses (eg. hepatitis B virus (HBV), HIV)

a. Aldehyde

- **Glutaraldehyde**:
  - Eg. Cidex
  - 2% glutaraldehyde activated alkaline solution
  - Bactericidal, fungicidal, virucidal, tuberculocidal, sporicidal
  - Non-corrosive but irritating
  - Protocol:
    - → 3 hours for sterilization
    - → 20 min for high-level disinfection
- **Formaldehyde**:
  - 37% for sterilization
  - 3-8% for disinfection

b. Halogens

- **Chlorine-releasing agents**:
  - Strong alkaline hypochlorite solutions (eg. Clorox)
  - Hypochlorite solutions (1-5% NaOCl)
  - Hypochlorite powders
  - Other chlorine-releasing compounds (eg. Presept)
  - Inactivation by proteins (due to binding action of chlorine to proteins)
- **Iodine**:
  - 1% iodine in 70% alcohol
  - Less readily inactivated by proteins
- **Iodophores**:
  - Complexes of iodine and solubilizers (increase I_2 solubility)
  - Same activity as iodine, but non-irritant and do not stain the skin
  - Mainly for disinfection of skin
  - Eg. Povidone-iodine

c. Chlorhexidine

- Highly active against Gram +ve bacteria
- Less active against Gram –ve bacteria
- Fungicidal, but limited activity against mycobacteria, spores and non-enveloped viruses
- A useful skin disinfectant (due to it being non-irritating and residual effect)
  - Eg. Hibitane, Hibiscrub (4% chlorhexidine gluconate), Savlon (cetrimide + chlorhexidine)
- Note **residual effect**: high residual antiseptic effect

d. Alcohols

- 70% ethanol or 60% isopropanol
- Bactericidal, fungicidal, tuberculocidal, virucidal
- Not sporicidal
- Poor penetrative powers
- Main use: rapid disinfection of clean surfaces and skin disinfection
- Danger: flammability

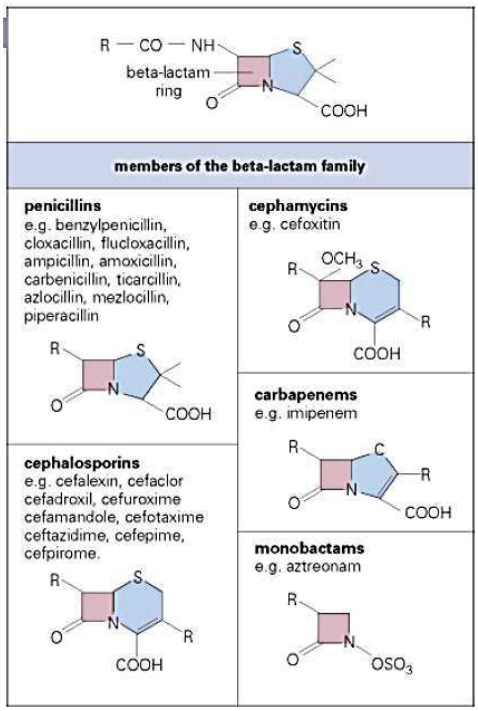
L65 Antimicrobials and Immunization

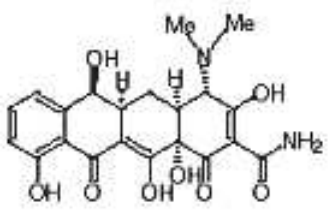
A. Antimicrobial Agents

- **Antimicrobial agent**: a substance that kills or inhibit growth of an infectious microorganism (incl. antibacterial, antifungal, anti-protozoal, anti-helminthic and antiviral agents)
- **Antibiotic**: naturally occurring antibacterial agent produced by a fungus or bacterium
- Classification can be by chemical structure, activity (bacteriostatic or bactericidal) or target site

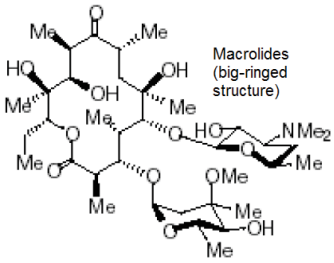
B. Antibacterial Agents

- Major groups by chemical structures:
  - **β-lactams**: penicillin, cephalosporins, cephamycins, carbapenems, monobactams
  - **Aminoglycosides**: amikacin, gentamcin, tobramycin
  - **Fluoroquinolones**: ciprofloxacin, levofloxacin
  - **Macrolides**: eg. erythromycin, clarithromycin
  - **Sulphonamides**: eg. sulfisoxazole
  - **Tetracyclines**: eg. doxycycline, minocycline
  - **Glycopeptides**: eg. vancomycin, teicoplanin

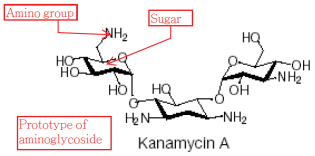




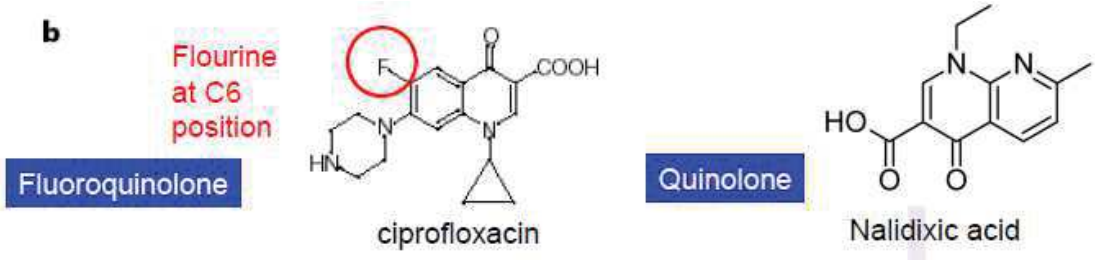
Tetracycline



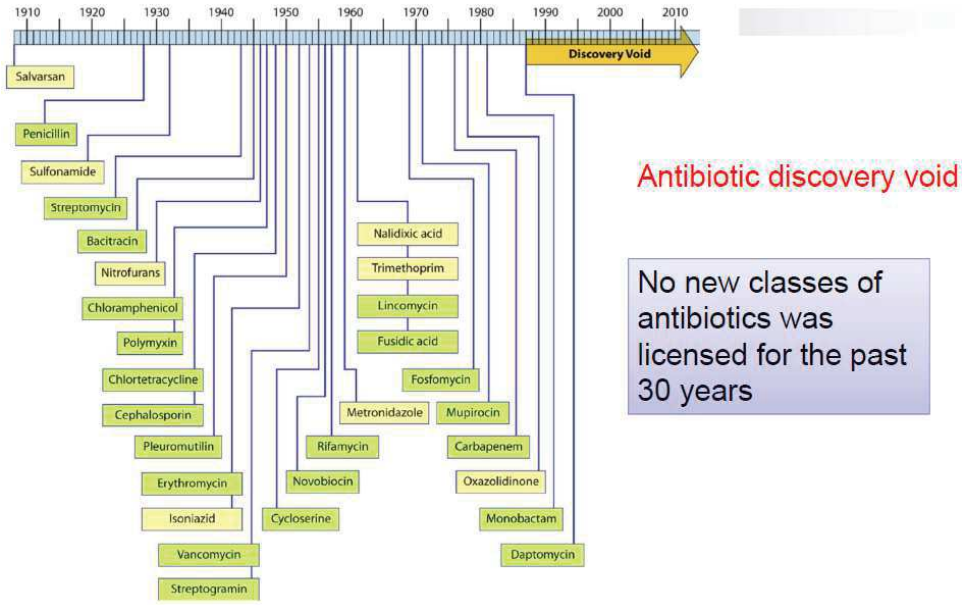
Erythromycin A



Kanamycin A



- No new classes of antibiotics was licensed for the past 30 years:



Antibiotic discovery void

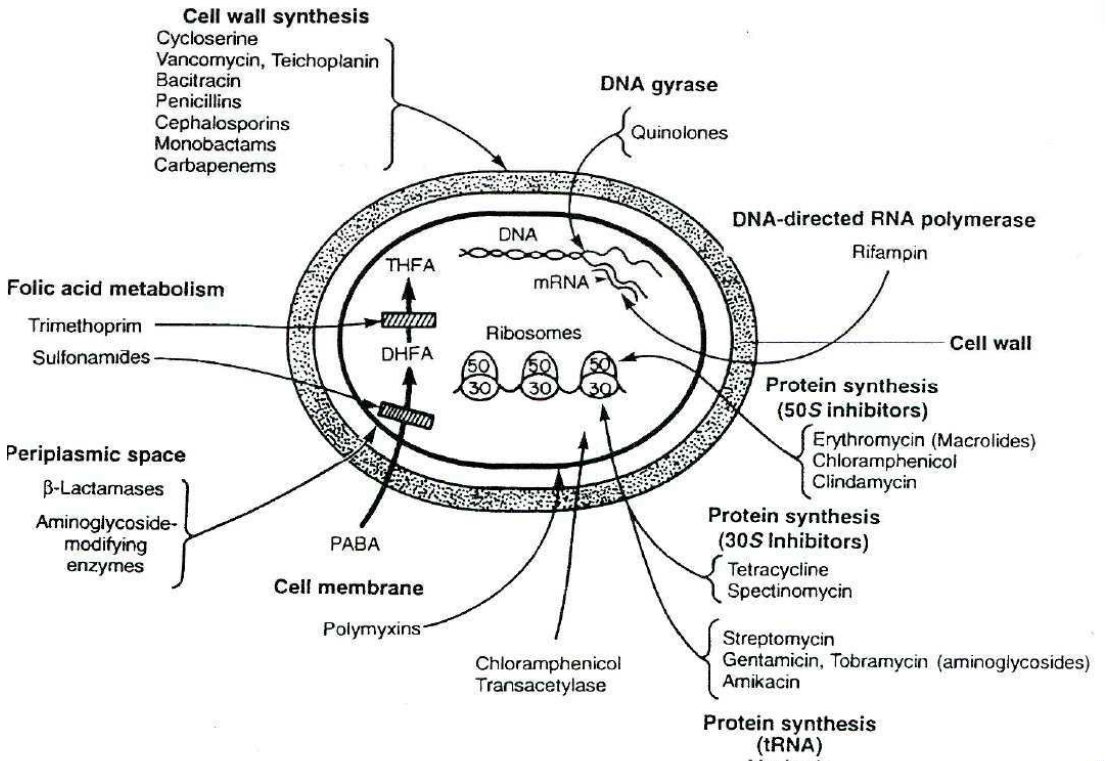
No new classes of antibiotics was licensed for the past 30 years

FIG. 1. Illustration of the "discovery void." Dates indicated are those of reported initial discovery or patent.

Silver LL. Challenges of antibacterial discovery. Clin Microbiol Rev 2011;24:71-109 5

1. General Principles of Antimicrobial Action

- Bacterial targets of antibacterials:
  - **Cell wall peptidoglycan** (absent in eukaryotic cells)
  - **Machinery of protein synthesis** (protein and RNA in prokaryotic cells differ from eukaryotic counterparts)
  - **Enzymes of bacterial nucleic acid metabolism**:
    - → **DNA gyrase**: critical in unwinding DNA
    - → **DNA dependent RNA polymerase**: responsible for transcription of bacterial DNA (differs from eukaryotic counterpart)
  - **Enzymes involved in folic acid synthesis** (pathway absent in humans)
- Antimicrobials targeting folic acid synthesis and **polymyxins** targeting protein synthesis (due to toxicity) are less commonly used



H.C. (1992). The crisis in antibiotic resistance. Science 257: 1064-1073.

a. Action of Penicillin

- **Penicillin** targets cross-linking (**transpeptidation**) of peptidoglycan to inhibit cell wall formation
  - Penicillin covalently binds to serine residue of **transpeptidase** → cannot catalyze peptidoglycan cross-linkage

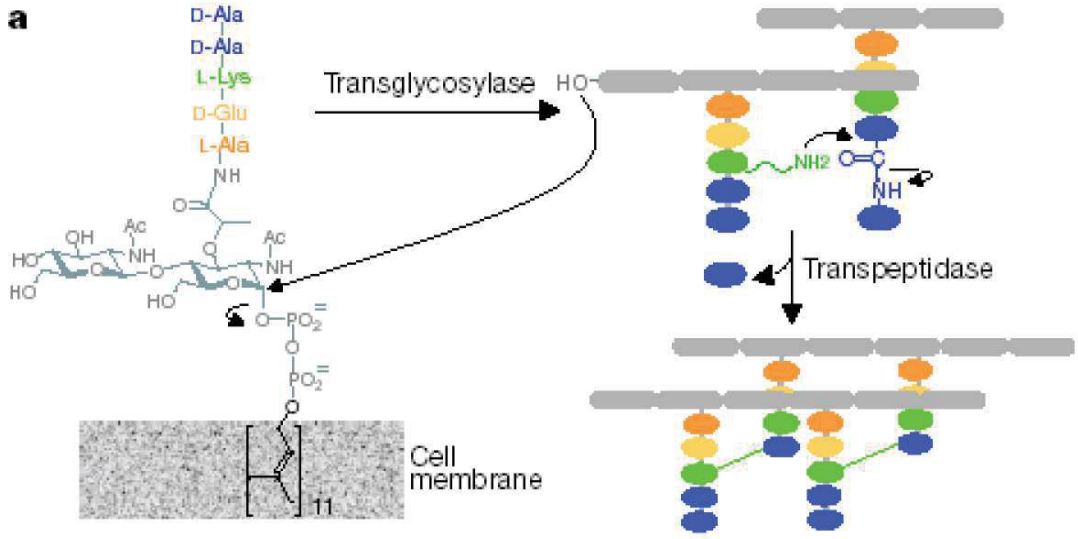


Fig a: Normal process of cell wall formation, involving transglycosylation and transpeptidation

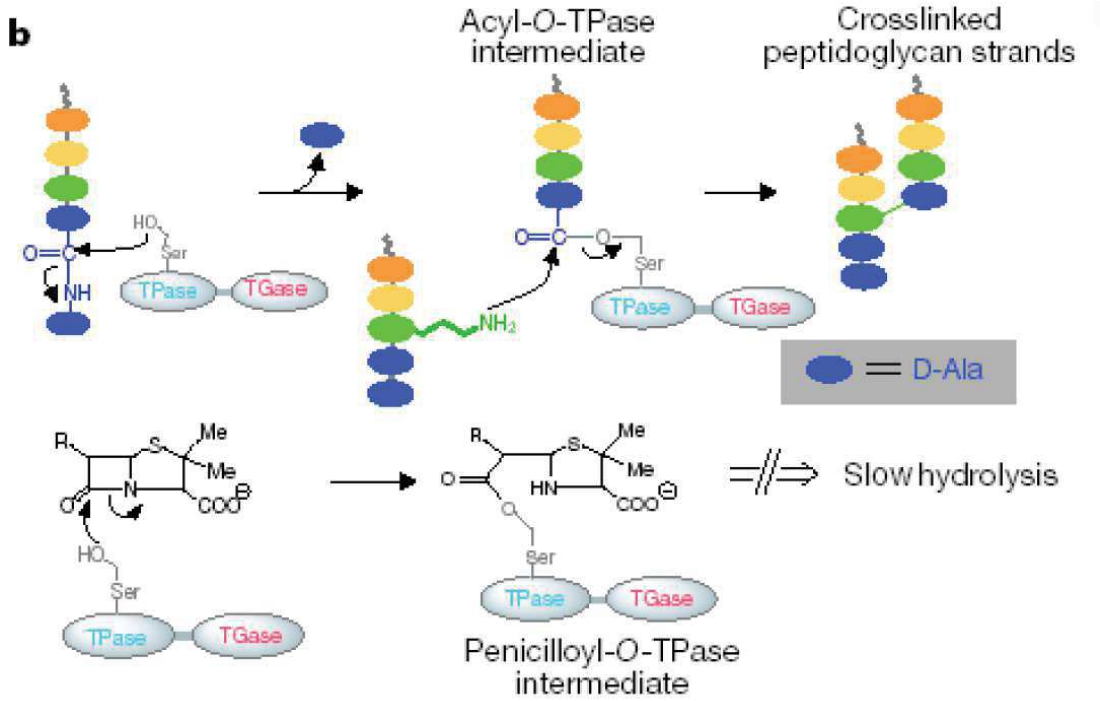


Fig. b: β-lactam ring of penicillin binds to serine residue of transpeptidase, leading to blockage of active site

b. Action of Vancomycin

- **Vancomycin** targets **transpeptidation** of peptidoglycan cell wall of bacteria
  - Vancomycin binds to pentapeptide ends of peptidoglycan (via van der Waals' forces)
  - Cross-links cannot be formed
  - Susceptibility to osmotic pressure → cell lysis

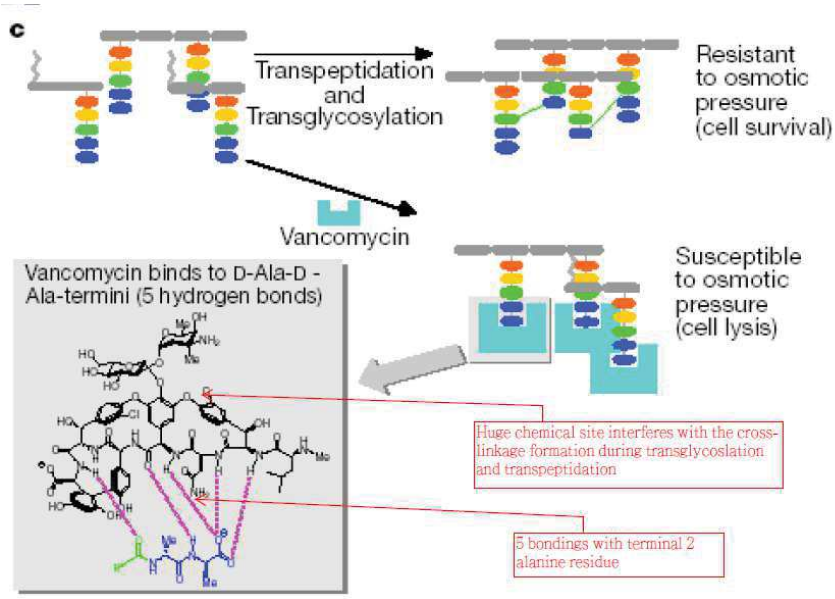
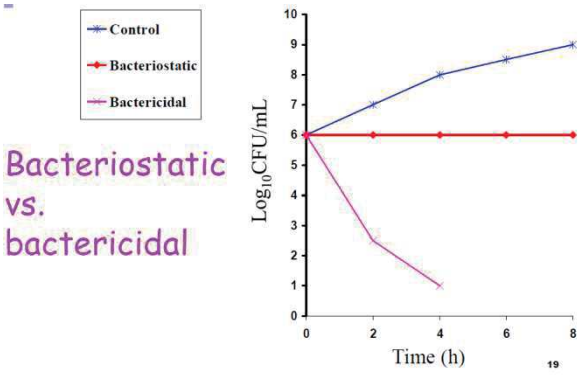


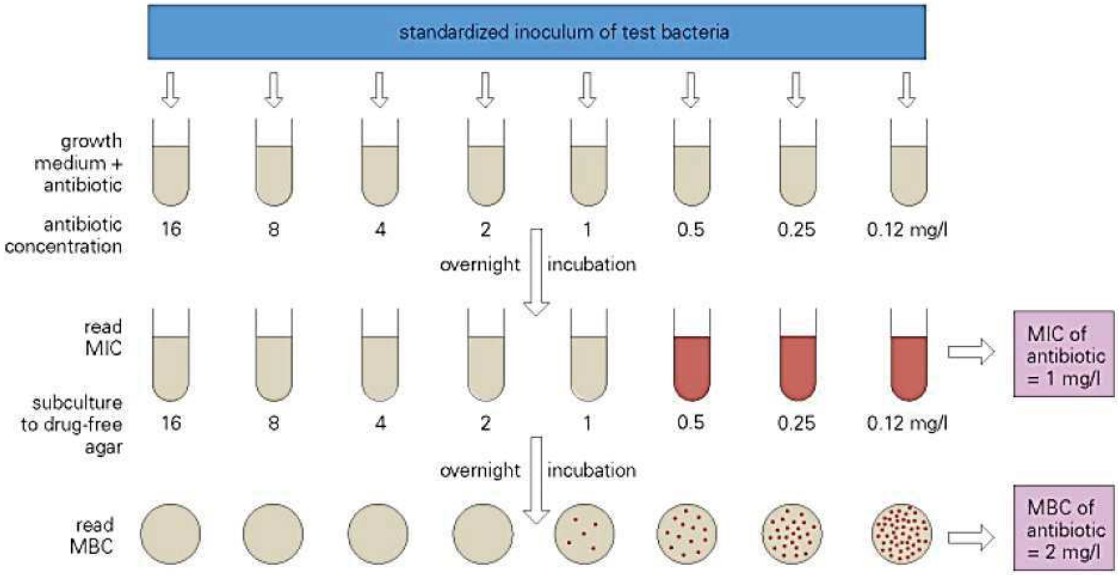
Fig. c: Vancomycin acts as a 'cap' on pentapeptide ends to prevent cross-linkage formation

2. Bactericidal and Bacteristatic Agents

- **Bactericidal**: agent whose action will <u>kill</u> the targeted microbe
  - Working definition: >99.9% killing
  - Associated with **minimum bactericidal concentration (MBC)**



- **Bacteriostatic**: agent whose action will <u>inhibit growth</u> of the targeted microbe but will not kill it
  - Working definition: prevent visible growth
  - Associated with **minimum inhibitory concentration (MIC)**
- <u>Arbitrary</u> designation (∵ categorization may differ against different organisms, at different concentrations and in different inoculum)
- 2015 meta-analysis shows that bacteriostatic/bacteriocidal categorization does NOT help treatment of pneumonia

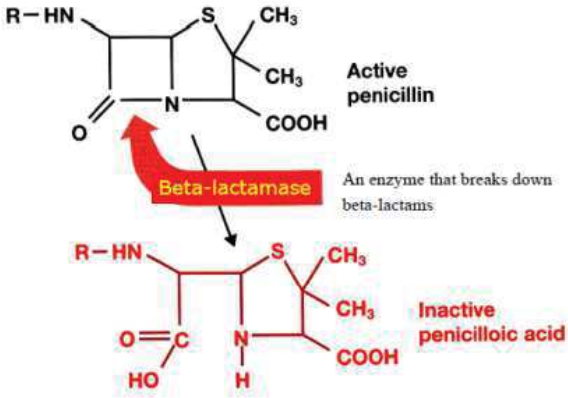


C. Mechanisms of Antibiotic Resistance

- Origin of resistance
  - Acquired by **mutation** or **horizontal gene transfer** (intraspecies or interspecies, via plasmid)
  - Intrinsic resistance: entire bacterial species resistant without additional genetic alterance (eg. *Pseudomonas aeruginosa*)
- Mechanisms:
  - **Decreased antibiotic uptake** (eg. porin mutation)
  - **Increased antibiotic export** (eg. efflux)
  - **Modification of antibiotic target** (eg. ribosomal protein methylation)
  - **Introduction of a new drug-resistant target** (eg. horizontal acquisition of *mecA* in methicillin resistance)
  - **Antibiotic hydrolysis** (eg. β-lactamase)
  - **Antibiotic modification** (eg. aminoglycoside modifying enzymes)

1. Penicillin Resistance

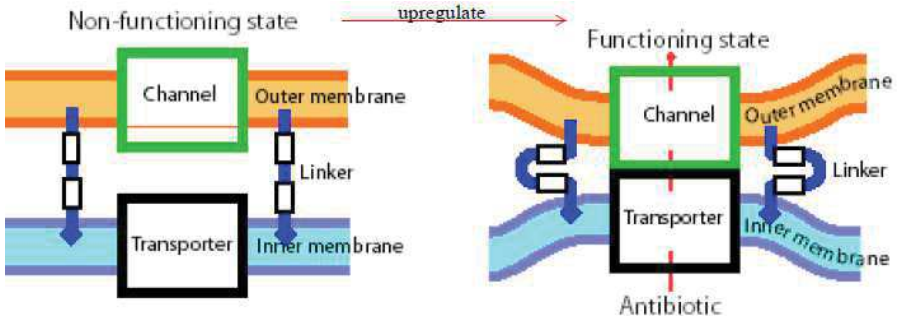
- By **penicillinase**:
  - Penicllinase (or more generally speaking β-lactamase) breaks down active β-lactam ring structure of penicillin
  - Secretion of penicillinase into extracellular space (for Gram +ve) or periplasm (for Gram –ve) confers resistance



  - Some β-lactamases are encoded in the bacterial genome (eg AmpC gene) that is triggered only during peptidoglycan turnover
    → May trigger false negative result to reaction test (∵ induction means AmpC gene may not always be expressed)

2. *Pseudomonas aeruginosa*

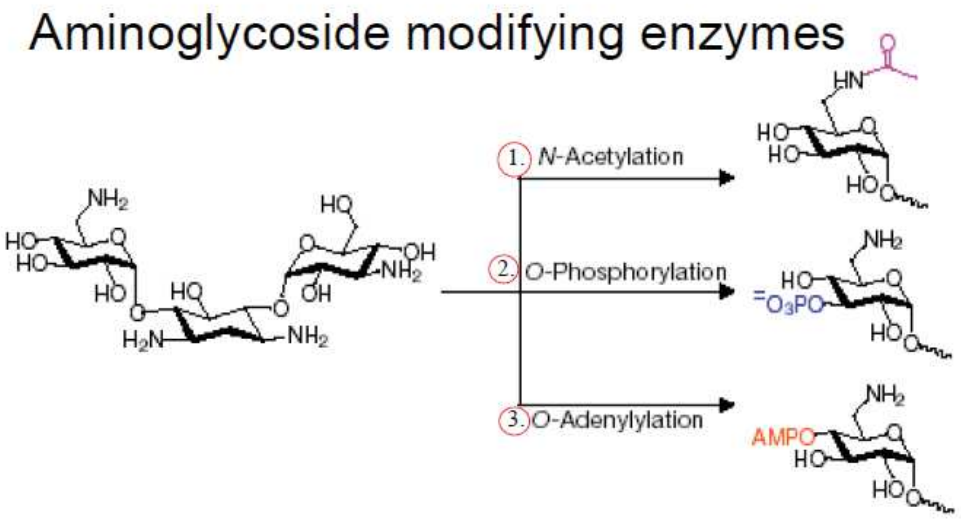
- Multi-drug resistance due to multidrug efflux pump
- Upregulation of efflux pump occurs only in presence of antibiotics
- Frequent resistance due to:
  - Mutations abolishing transcriptional repressor
  - Increased synthesis of proteins constituting the pump



- Other mechanisms:
  - Restricted outer membrane permeability
  - Chromosomally encoded β-lactamase

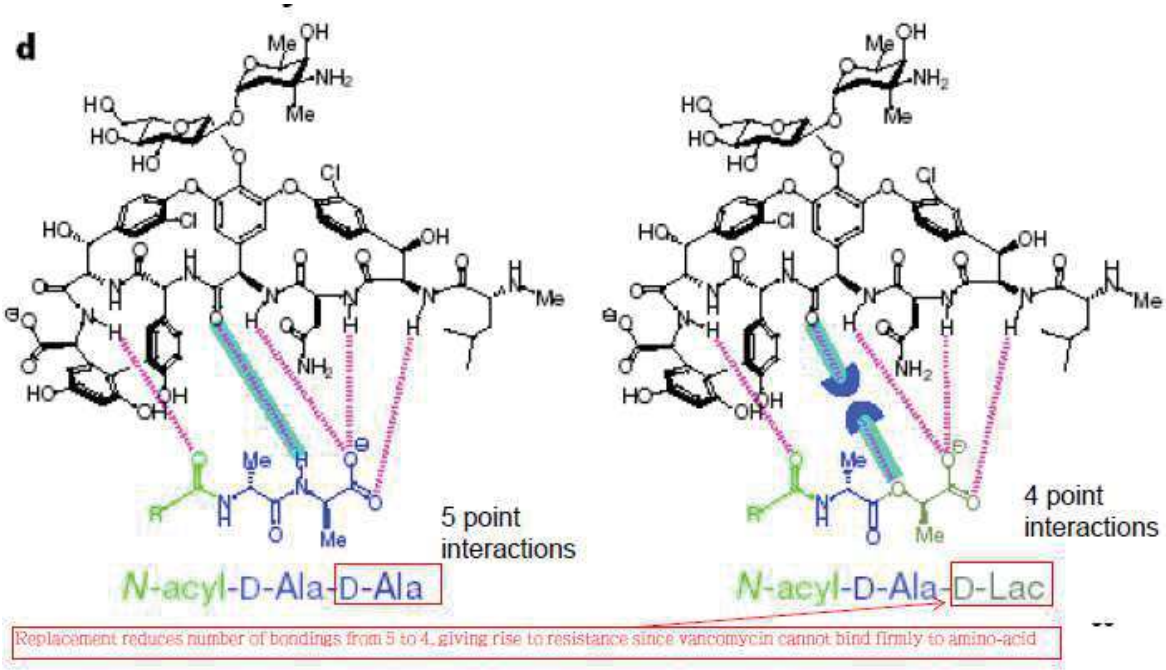
3. Aminoglycoside Modifying Enzymes

- Covalent modification of antibiotic **aminoglycosides** by enzymes confers resistance



4. Vancomycin-resistant Enterococcus (VRE)

- Modification of structure of pentapeptide ending (D-Ala → D-Lac) reduces affinity to vancomycin (∵ ↓ point interactions)

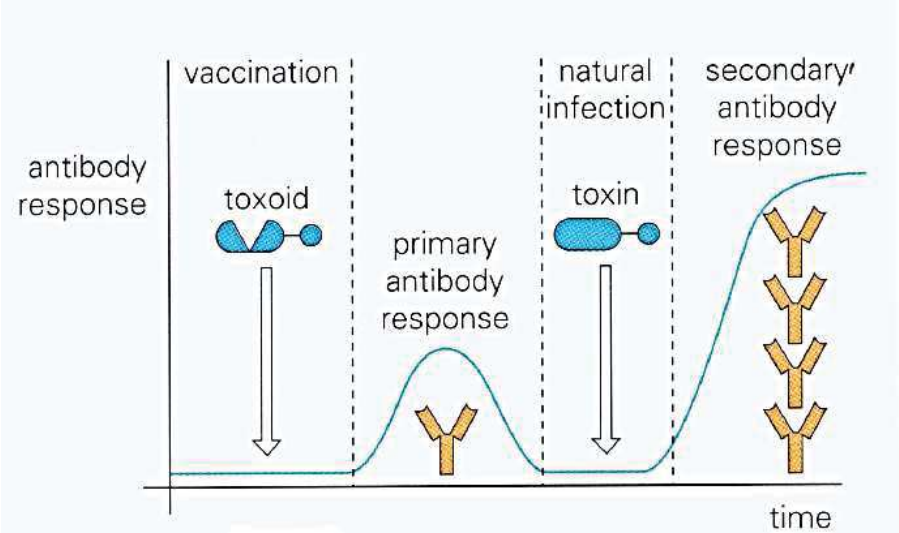


D. Immunization

- **Immunization**: process of <u>artificially inducing immunity</u> or providing protection from disease
- **Vaccine**: an antigenic preparation used to induce immunity
- Classification:
  - □ Live attenuated
  - □ Inactivated:
    - → Subunit vaccine
    - → **Toxoid**: modified bacterial toxin that has been rendered nontoxic but retains ability to stimulate formation of neutralizing antibodies (anti-toxins)

1. Active Immunization

- Induction of acquired immunity by 'priming' with antigen
  - Provide pre-existing immunity → prevent infection
  - Secondary response → only mild or subclinical infection → little or no damage
- Involves specific antibodies and/or T lymphocytes



a. Live Attenuated Vaccine

- Advantages:
  - Mimic natural infection
    - → Stimulate T-lymphocytes 'naturally'
    - → Induce **mucosal immunity** (ability to induce indicates vaccine's prowingness)
  - Protection of unvaccinated
    - → Infectious vaccine strain can infect those in contact with the one vaccinated
    - → Herd immunity will result
- Problem: vaccine strain may cause disease
  - Rare reversion to virulence in healthy recipients
  - Immunization of immunocompromised recipients before diagnosis of condition → onset of disease → person-to-person transmission of vaccine strain

b. Inactivated Vaccine

- Used when:
  - Difficulty in attenuation
  - Too risky to use live vaccine
  - Unknown stability (risk of reversion)
- Inactivation usually done by:
  - Heat (eg. *Vibrio cholera* vaccine)
  - Chemical
    - → Formaldehyde (eg. Hepatitis A virus, Diphtheria toxin and tetanus toxin)
    - → β-propiolactone (eg. influenza virus)
- Problems:
  - Relatively less immunogenic → multiple doses needed
  - Poorer stimulation of memory cell
  - Shorter duration of protection

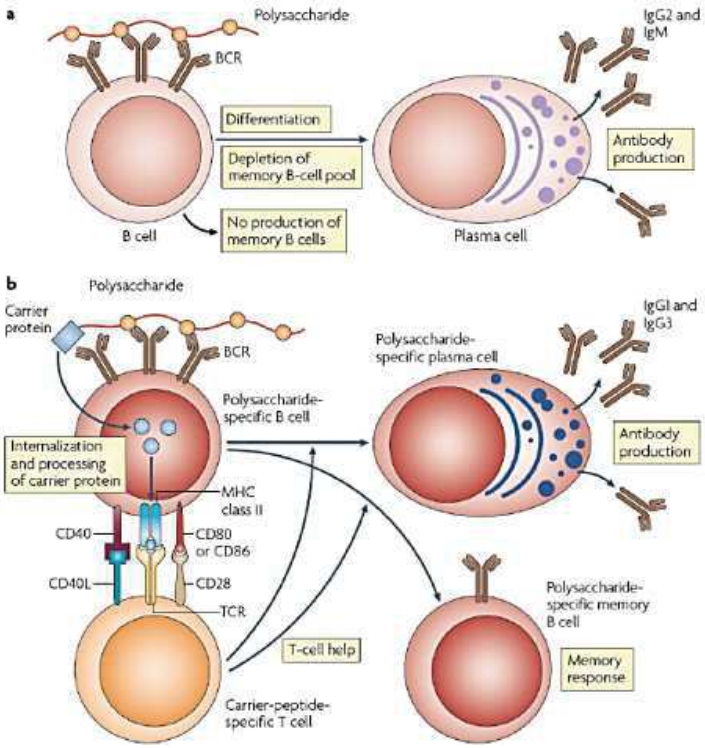
i. Improvement in Immunogenicity of Non-living Vaccines

(1) Adjuvant

- Substances used to boost immune response
- Non-specific immunostimulant
- Effective at inducing antibody responses but less active in inducing CMIR

(2) Protein Conjugation

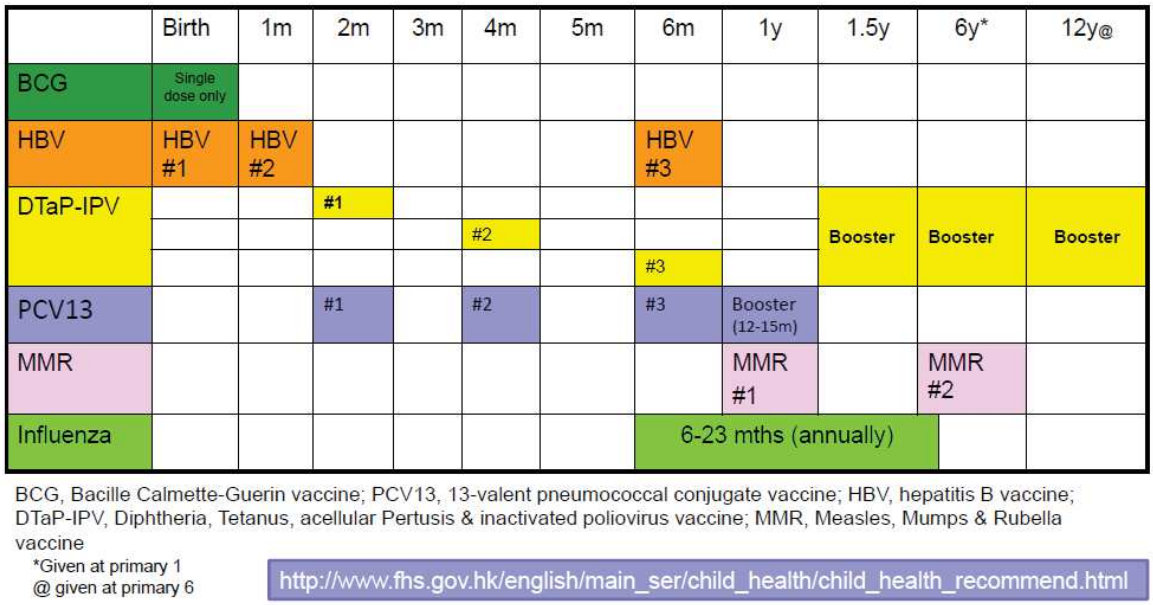
- Difficulty in producing infective vaccine against capsulated bacteria (eg. *H. influenza*, *S. pneumonia*, *N. meningitidis*) ∵ capsule is a major virulence factor
- Many polysaccharide antigens fail to stimulate T cells → only plasma cells are produced with no memory B cells → poor memory
- Conjugation of polysaccharide to a protein carrier → B cells present protein to $CD4^+$ T helper cells → T cell interact with B cells with cytokines → memory B cells produced
- Ineffective in children <2 years (∵ immature immune system)



- Eg. Pneumococcus conjugate vaccine 13-valent (PCV-13)

c. Recommended Childhood Immunizations in Hong Kong

- **Bacille Calmette-Guerin Vaccine (BCG)** for tuberculosis
- **Hepatitis B Vaccine (HBV)**
- **Diphtheria, Tetanus, acellular Pertissus and inactivated poliovirus vaccine (DTaP-IPV)**
- **13-valent pneumococcal conjugate vaccine (PCV-13)**
- **Measles, Mumps and Rubella vaccine (MMR)**
- **Influenza vaccines**



BCG, Bacille Calmette-Guerin vaccine; PCV13, 13-valent pneumococcal conjugate vaccine; HBV, hepatitis B vaccine; DTaP-IPV, Diphtheria, Tetanus, acellular Pertusis & inactivated poliovirus vaccine; MMR, Measles, Mumps & Rubella vaccine

*Given at primary 1
@ given at primary 6

http://www.fhs.gov.hk/english/main_ser/child_health/child_health_recommend.html

*Note difference in approach in HK and in US (23 injections up to 1 y/o) due to many factors (vaccine availability, efficacy, safety, disease burden, affordability or cost-effectiveness, availability of treatment…)

2. Passive Immunization

- **Passive immunization**: consists of providing temporary protection through administration of exogenously produced antibody or memory T cells
- Trans-placental transfer of Ab also one form of passive immunization
- As prophylaxis or replacement therapy
- Two types:
  - **Pooled human immunoglobulin** or **intravenous immunoglobulin (IVIg)**: sterile solution for parenteral administration containing Ab from unselected pools of donor blood (>1000 donors) → protection against all human diseases in the area
  - **Specific immunoglobulin**: specific preparations obtained from donor pools pre-selected for a high antibody content against a particular disease (eg. HepB Ig, VZV Ig, Tetanus Ig…)
- Provides immediate protection
- Useful for those unable to respond immunologically
- Problems:
  - Relatively short-lasting protection
  - Use of antiserum raised in animals can cause **serum sickness** (∵ allergy to antiserum proteins)
  - Risk of blood-borne infections (eg. HIV, HBV, HCV)

L66 Oxygen Exchange and Transport

A. Gas Exchange

- **Diffusion** as fundamental mechanism for gas exchange in organisms
- **Pulmonary gas exchange**: two diffusion processes
  - Ambient air homogenizes with alveoli gas through rapid diffusion in a gas-gas interface ($\because$ high total cross-sectional area $\rightarrow$ inability to sustain via bulk flow)
  - Gas exchange by diffusion in gas-liquid interface through membrane barrier in between alveoli and pulmonary capillary
- **Tissue gas exchange**: process of equilibration of gas content in tissue with blood gases
  - Dictates movement of O_2 and CO_2 in between capillary and tissue
  - Rate of diffusion coupled to metabolic requirement of tissue

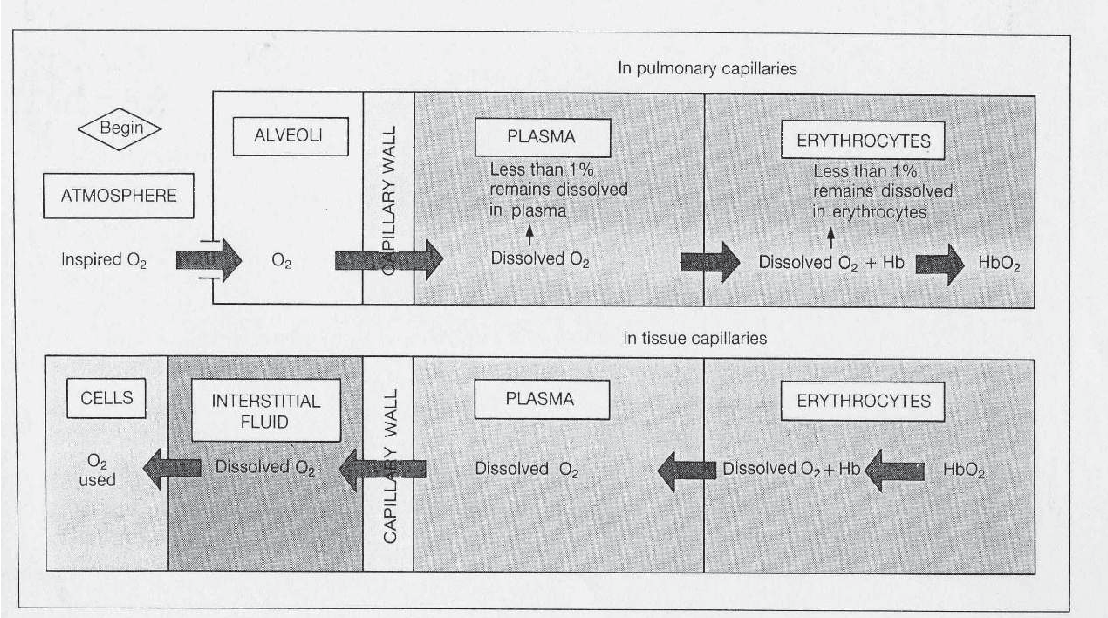


FIGURE 14-23 Oxygen movement in lungs and tissues. Movement of inspired air into the alveoli is by bulk flow; all movement across membranes is by diffusion.

1. Gas-to-liquid Phase Diffusion in Lungs

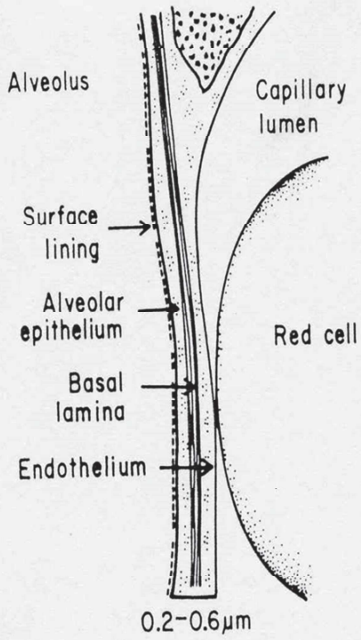


FIG. 1. The alveolar capillary membranes. To reach the red cell, O_2 traverses the surface lining layer, the alveolar epithelial cytoplasm, the basal lamina, the endothelial cell cytoplasm, and the plasma. In some locations, there is also loose interstitial tissue between the epithelium and the endothelium. (Approximate magnification ×20,000.) (Reproduced with permission from Ganong, W. F., *Review of Medical Physiology*, 8th Ed., Lange Medical Publications, Los Altos, CA, 1977.)

- Respiratory gases have to pass through alveolar wall, endothelium and RBC cell membrane before they can bind with Hb
- Thickness: 0.2-0.6μm → facilitates gas exchange
- Clinical significance:
  - **Pneumonia** leads to a thicker water layer (due to inflammation) → gas exchange impaired
  - **Emphysema** damages alveolar structure → ↓ total SA

a. Fick's First Law of Diffusion

- $\dot{V} = \frac{dV}{dt} = D\left(\frac{A}{T}\right)\Delta P$
  - V: volume of gas on the side with higher gas concentration
  - D: **diffusion constant** which is proportional to $\frac{solubility}{\sqrt{molecular\ weight}}$
  - A: area for diffusion
  - T: thickness
  - ΔP: partial pressure gradient
- Note **Henry's law**: $C = KP$
  - C: gas content in liquid
  - K: solubility of gas in liquid
  - P: partial pressure

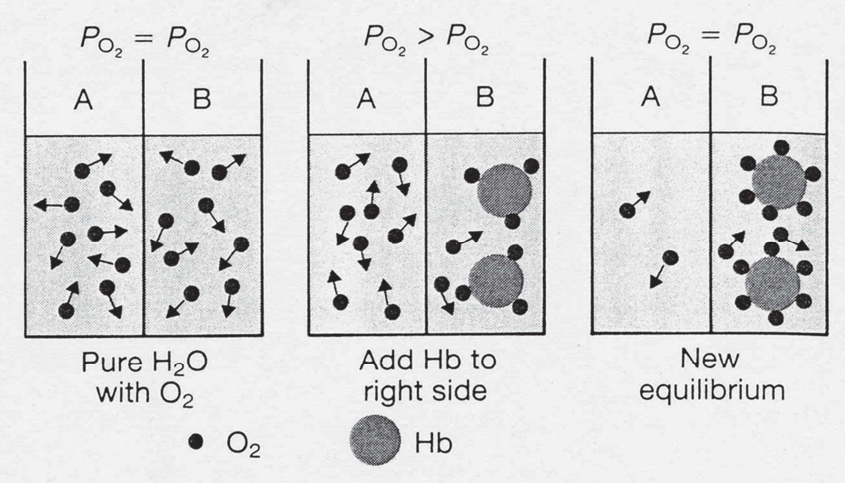
b. Diffusion Capacity of Lung (D_L)

- **Diffusion capacity of lung (D_L)** is defined as the **conductance** (flow per pressure gradient) of oxygen diffusion from alveolar into haemoglobin
  - $D_L = \frac{\dot{V_{O_2}}}{P_{A_{O_2}} - P_{C_{O_2}}}$
- D_L considers diffusion barrier and blood factors in one measurement:
  - Note that gas-liquid diffusion and haemoglobin binding are in series and that

    $\frac{1}{D} \propto \Delta P$

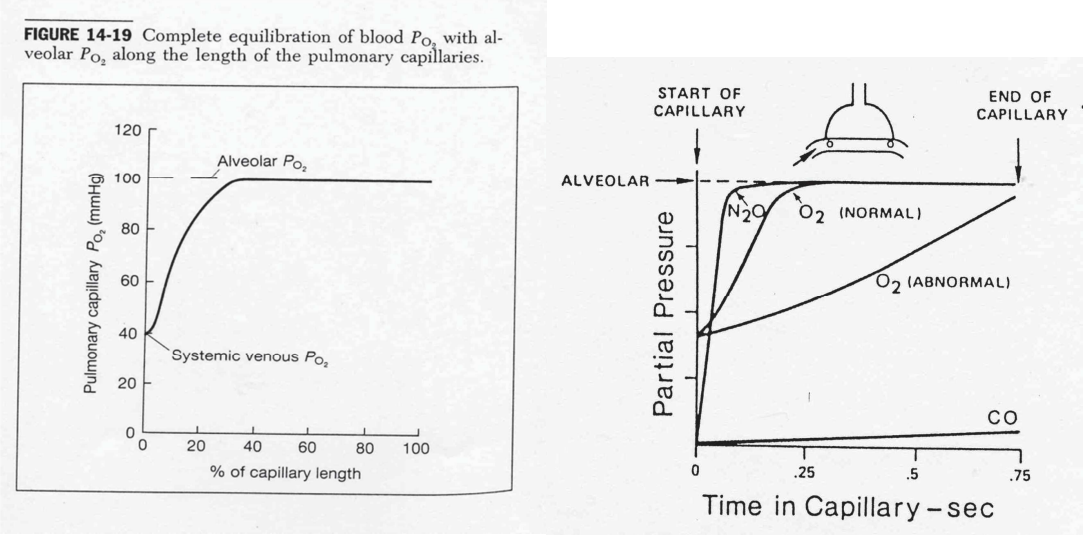
  - $\frac{1}{D_L} = \frac{1}{D_M} + \frac{1}{\theta V_C}$
    - → D_M: diffusion capacity of the membrane (physical property)
    - → θ: proportionality constant relating rate of Hb binding with capillary blood volume
    - → V_C: volume of capillary blood ($\propto$ amount of Hb present)
- In a healthy lung, D_L should be constant → main factor driving oxygen diffusion is pressure gradient ΔP
- Factors affecting D_L:
  - Respiratory surface area
  - Thickness of blood-gas barrier
  - Pulmonary blood volume
  - **Haematocrit (Hct)**: proportional amount of RBCs in blood

c. Facilitation of Pulmonary Gas Exchange by RBCs

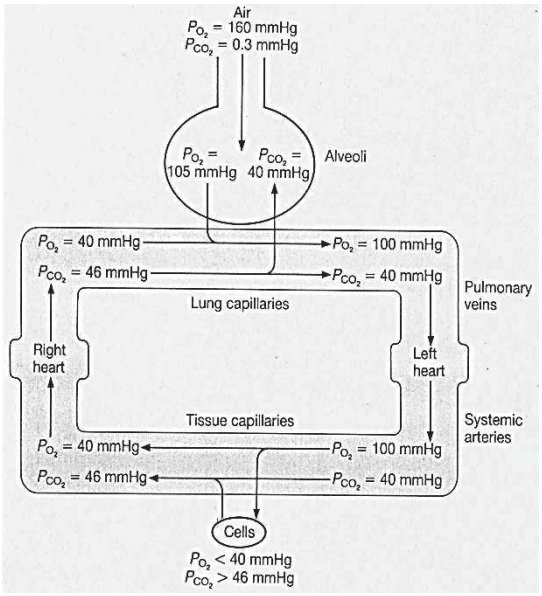


- Each **haemoglobin (Hb)** binds to four O_2 molecules in its four heme groups in 2α and 2β chains:
  - $Hb + 4O_2 \rightarrow Hb(O_2)_4$
- Hb helps remove O_2 from blood plasma $\rightarrow$ $\downarrow O_2$ partial pressure in blood $\rightarrow$ $\uparrow O_2$ pressure gradient $\rightarrow$ $\uparrow O_2$ carrying capacity and diffusion speed

d. Attainment of Equilibrium of Partial Gas Pressure



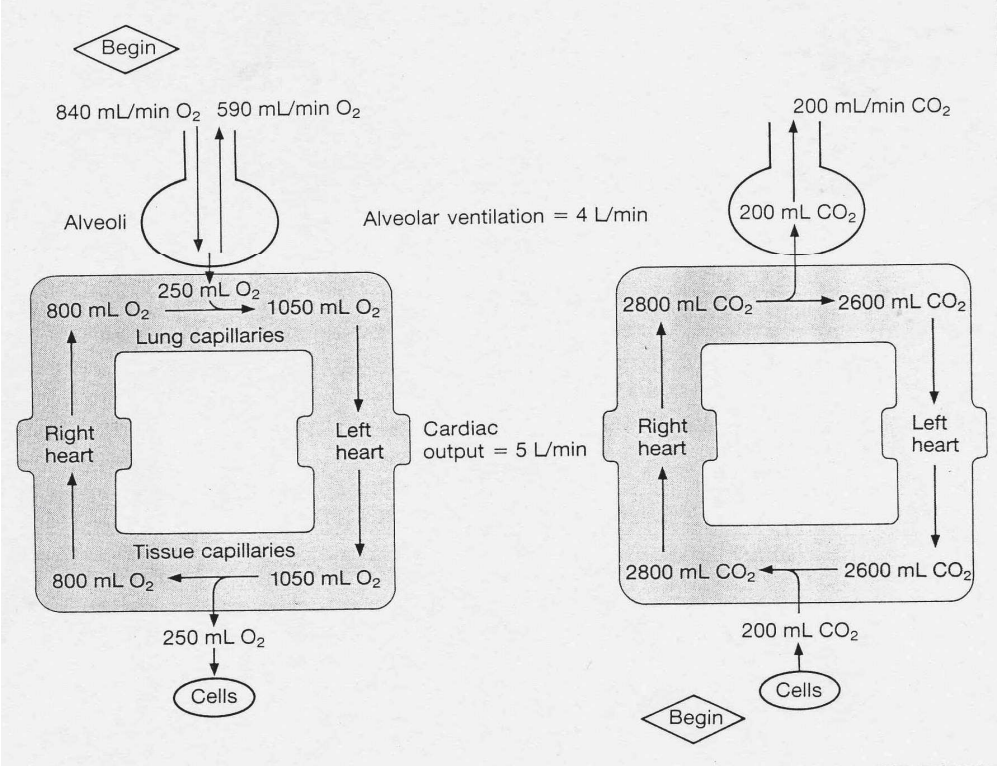
- For O_2, equilibrium is rapidly reached in the first 1/3 of the capillary length (0.25s)
  - Length required for equilibrium increases during <u>exercise</u> (∵ ↑ rate of blood flow) or <u>disease</u> (∵ ↓ D_L)
- O_2 exchange said to be NOT diffusion-limited
- Partial pressure of O_2 levels throughout circulation:
  - Alveolar (P_{AO2}): ~100mmHg
  - End-pulmonary capillary (P_{CO2}): ~100mmHg
  - Systemic arterial (P_{aO2}): ~95mmHg
  - Mixed venous (P_{VO2}): 40mmHg
- **Anatomical pulmonary shunt** (bronchial circulation) accounts for pO_2 difference between alveolus and systemic artery



2. Tissue Gas Exchange

- Tissue gas exchange explained by **cylinder model**: a cylindrical capillary exchanging materials with surrounding tissues
- Tissue pO_2 dependent on:
  - pO_2 in capillary blood
  - Distance from capillary (diffusion distance)
  - O_2 consumption in tissue
  - Diffusion capacity of tissue (D_t)
- Tissue acts as an **oxygen sink** with low pO_2 for O_2 to flow into it

3. O_2 Consumption



- **Fick's principle**: amount of substance X consumed or produced by tissues equals to difference between amount of X goes in and comes out of the tissue
- **Basic O_2 demand**
  = V_{O2} in – V_{O2} out
  = alveolar ventilation × O_2 content in air – V_{O2} out
  = 4L air/min × 0.21 – 590mL/min = 840 – 590 = 250mL/min
- **O_2 delivery** = 1000mL O_2/min
  - O_2 content in arterial blood (C_{aO2})
    = dissolved O_2 + Hb-bound O_2
    = solubility × pO_2 + Hb content × O_2 affinity
    = 0.03 mL L^{-1} $mmHg^{-1}$ × 100 mmHg + 147 g mL^{-1} × 1.34 g L^{-1}
    = 3 mL L^{-1} + 197 mL L^{-1}
    = 200mL O_2/L blood
  - O_2 delivery = O_2 content × cardiac output = 200 × 5 = 1000mL O_2/min
- Note that O_2 content in venous blood (C_{vO2}) = 150mL O_2/L blood = 200 – 150

B. Oxygen Transport

- Transport of O_2 depends on CVS formed from heart and vessels
- Importance: low solubility of O_2 in fluid → difficult to store O_2 in metabolic active tissues
- Facilitated by:
  - Reversible binding of O_2 and Hb in blood
  - Factors modulating O_2-Hb binding

1. O_2-Hb Dissociation Curve

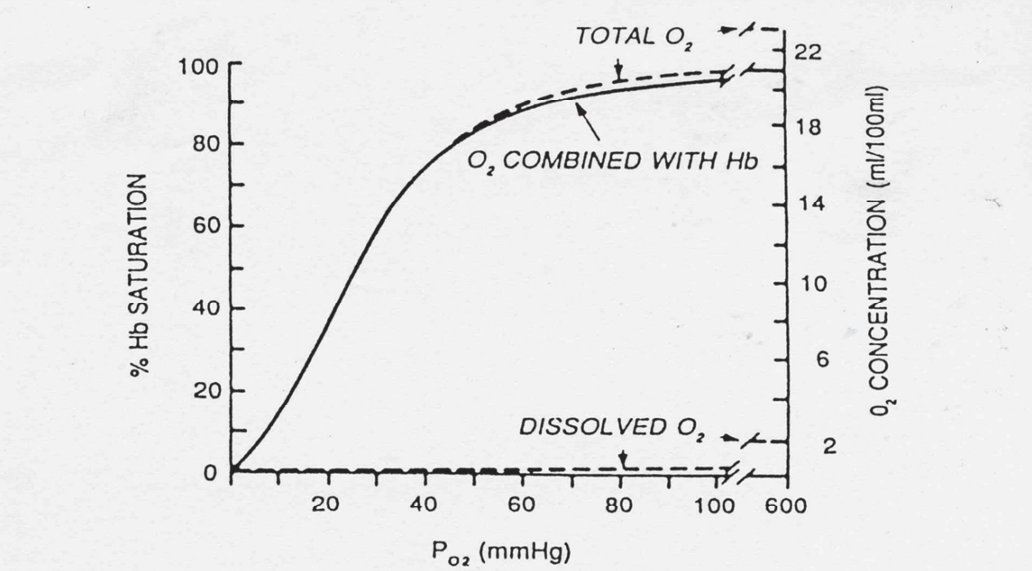


Figure 6.1. O_2 dissociation curve *(solid line)* for pH 7.4, P_{CO_2} 40 mm Hg, and 37°C. The total blood O_2 concentration is also shown for a hemoglobin concentration of 15 gm/100 ml of blood.

- **O_2-Hb dissociation curve**: graph showing change of O_2 saturation of Hb against pO_2
- Sigmoid shape due to **cooperative binding** (O_2 binding ↑ O_2 affinity)
- **Oxygen content**: total amount of O_2 in blood = dissolved O_2 + O_2 bound to Hb
- **Oxygen capacity** = Hb concentration × 1.34mL g^{-1} Hb
- **Hb saturation**: percentage of Hb saturation with O_2 ($\frac{O_2 \text{ bound to Hb}}{O_2 \text{ capacity}}$)
  - 97% in systemic arterial blood and 75% in venous blood
- O_2 delivery by CVS is 4× tissue O_2 consumption → O_2 content and Hb saturation difference in systemic arterial blood and mixed venous blood
- Curve often represented by pO_2 when Hb saturation is at 50% (i.e. **P_{50}**)

a. Modulation of the O_2-Hb Dissociation Curve

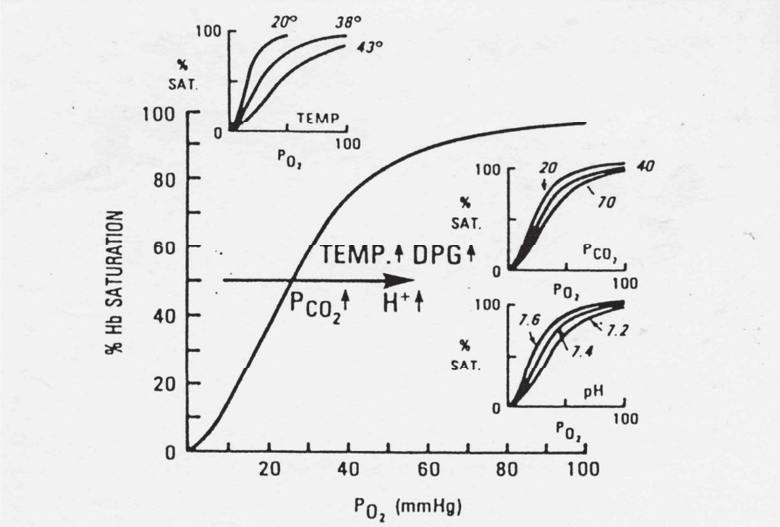


Figure 6.3. Rightward shift of the O_2 dissociation curve by increase of H^+, P_{CO_2}, temperature, and 2,3-diphosphoglycerate (DPG).

- Factors that shift curve to <u>right</u> (i.e. ↑ P_{50}):
  - ↑ CO_2 conc. (**Bohr effect**)
  - ↓ pH
  - ↑ temperature
  - ↑ 2,3-DPG concentration
- The above changes occur at tissue capillaries, facilitating O_2 unloading from Hb
- Increased pO_2 will also decrease Hb's affinity for CO_2 and H^+

i. Effect of 2,3-DPG on O_2-Hb Dissociation Curve

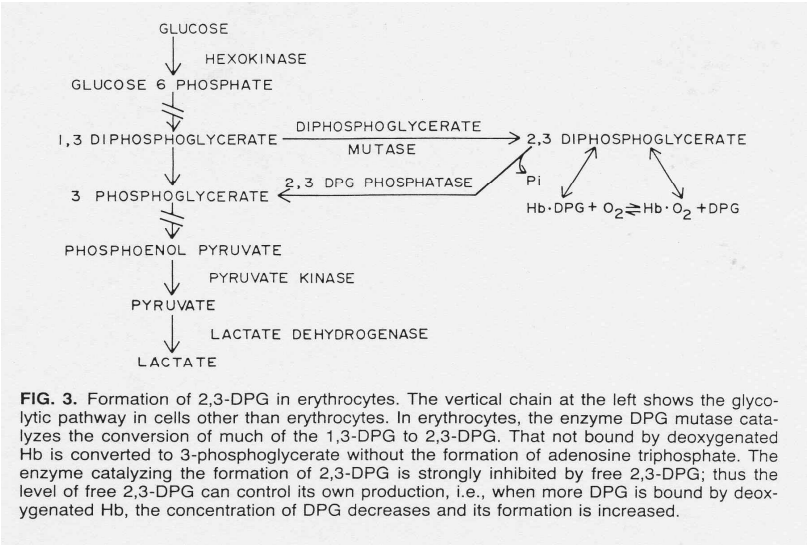


FIG. 3. Formation of 2,3-DPG in erythrocytes. The vertical chain at the left shows the glycolytic pathway in cells other than erythrocytes. In erythrocytes, the enzyme DPG mutase catalyzes the conversion of much of the 1,3-DPG to 2,3-DPG. That not bound by deoxygenated Hb is converted to 3-phosphoglycerate without the formation of adenosine triphosphate. The enzyme catalyzing the formation of 2,3-DPG is strongly inhibited by free 2,3-DPG; thus the level of free 2,3-DPG can control its own production, i.e., when more DPG is bound by deoxygenated Hb, the concentration of DPG decreases and its formation is increased.

- 2,3-DPG is a by-product of glycolytic pathway
- Mechanism:

1) 2,3-DPG has higher affinity to Hb than to HbO_2;
2) 2,3-DPG binds to Hb at tissue capillaries, leading to ↓ 2,3-DPG concentration;
3) 2,3-DPG binding reduces O_2 affinity of Hb (curve shifts to right);
4) ↓ 2,3-DPG concentration causes ↑ in 2,3-DPG production, total 2,3-DPG availability ↑ (curve shifts to right).

b. Effect of CO and Anaemia on O_2-Hb Dissociation Curve

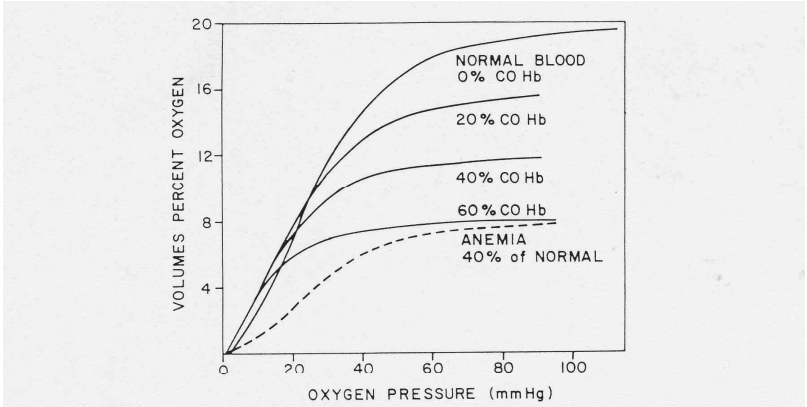


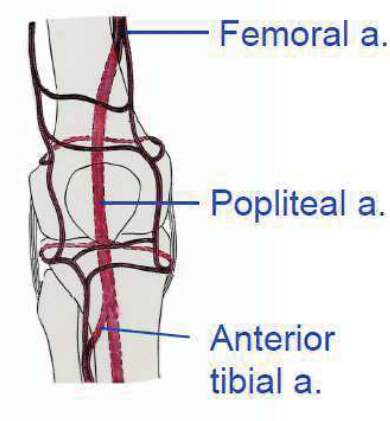
FIG. 4. Oxygen dissociation curves of human blood at pH 7.4 and 38°C, containing various percentages of carboxyhemoglobin *(solid lines)*, and of anemic human blood containing only 40% of the normal Hb content *(dotted line)*. (Reproduced with permission from Roughton, F. J. W., Transport of oxygen and carbon dioxide. In: *Handbook of Physiology, Sect. 3: Respiration,* edited by W. O. Fenn and H. Rahn, Vol. 1. Washington, D.C., American Physiological Society, 1964.)

- Hb affinity for CO 200× that of O_2 → HbCO formation ↓ Hb for O_2 binding → P_{50} and O_2 content ↓ (curve shifts to left)
  - □ No change in Hb saturation ∵ ↓ O_2 capacity
- >50% of CO binding → curve becomes hyperbolic i.e. ↓ cooperativity → O_2 cannot be released at tissues

L67 Vascular Supply to the Limbs

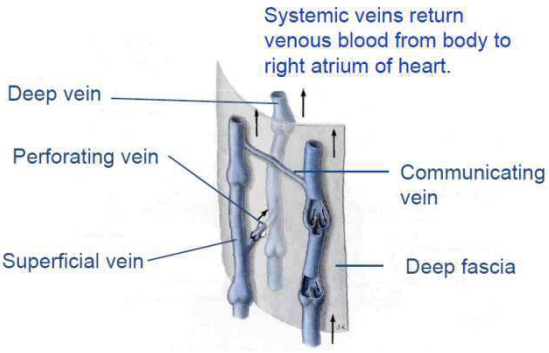
A. Anatomical Features of Vascular Supplies

1. Arterial Anastomoses

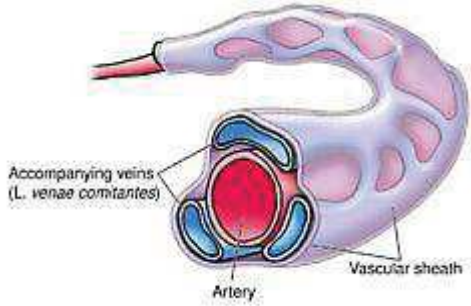


- **Arterial anastomoses**: a segment of artery joining two different arteries
- Provide <u>alternative channels</u> (**collateral circulation**) for blood to reach a given tissue or organ, especially if the main artery supplying a tissue or organ is slowly occluded
- Eg. **genicular arteries** linking **femoral**, **popliteal** and **tibial arteries** around the knee joint → collateral circulation for blockage of popliteal artery

2. Veins



- Most veins follow the course of an artery and are not specifically named (except large veins or those that do not accompany an artery)
- Classification of systemic veins:
  - **Deep veins** travel with arteries and found deep in muscles
    - → Share the same name with the artery
    - → <u>Drain</u> deeper structures such as muscles, bones and joints
  - **Superficial veins** running in superficial fascia and some are externally visible
    - → Drain subcutaneous tissues
    - → Clinical importance: **venipuncture** and **transfusion**; those in lower limb prone to **varicosities**
- **Deep** and **superficial veins** communicate by **perforating** or **communicating veins** piercing the **deep fascia**
  - **Deep fascia**: well-defined layer of connective tissue separating superficial tissues and deep tissues
- **Valves** help direct blood from superficial to deep veins
- **Venous tributary**: a smaller vein that drains into a larger vein



- Mechanisms of venous return:
  - Valves
  - Muscular pump (for **deep veins** found between muscles)
  - Pulsation of adjacent artery (for **venae comitantes**)
    → **Venae comitantes**: deep veins accompanying medium-sized arteries are usually paired

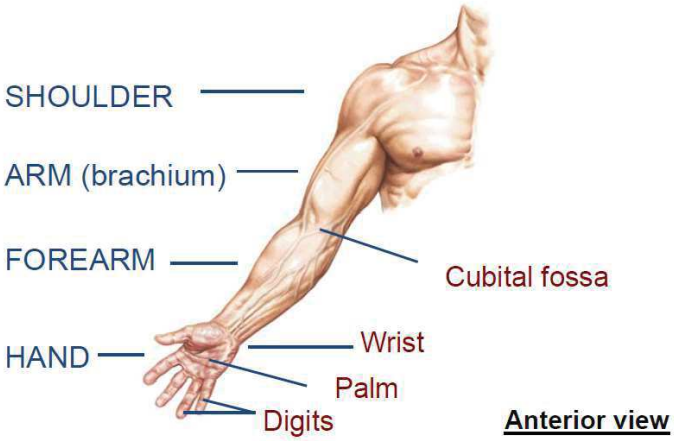
*Terminology: an artery supplies a region while a vein drains a region

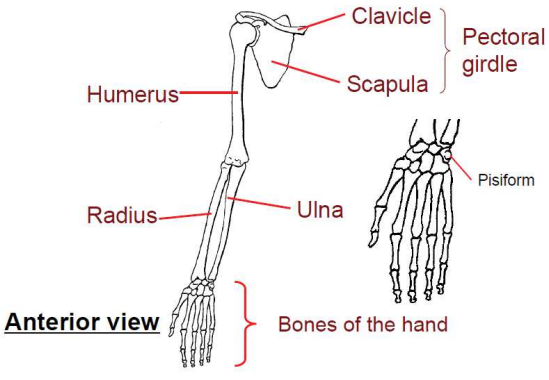
3. Lymphatics

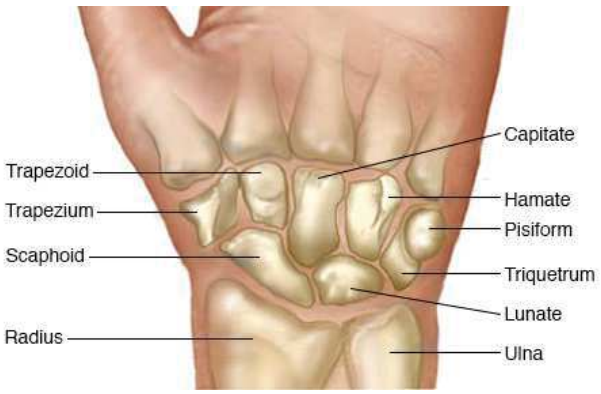
- **Lymphatic capillaries** drain lymph from tissues and return it to bloodstream
- **Lymphatic vessels** in limbs divided into:
  - **Superficial lymphatic collecting vessels** in the subcutaneous tissue travel along superficial veins
  - **Deep lymphatic vessels** accompany the deep blood vessels

B. Vascular Supply to Upper Limbs

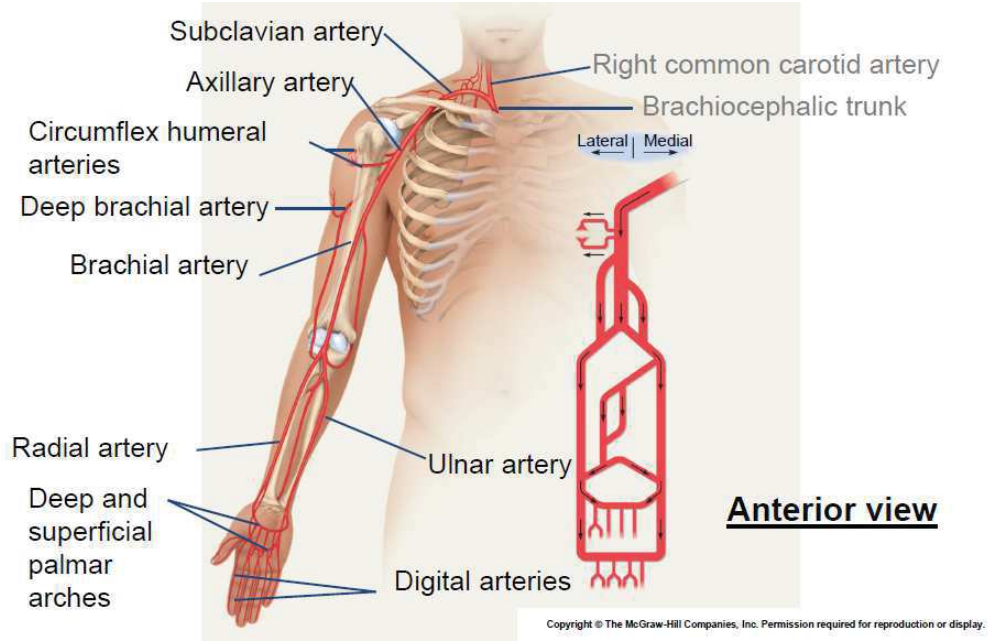
1. Anatomical Overview on the Upper Limb



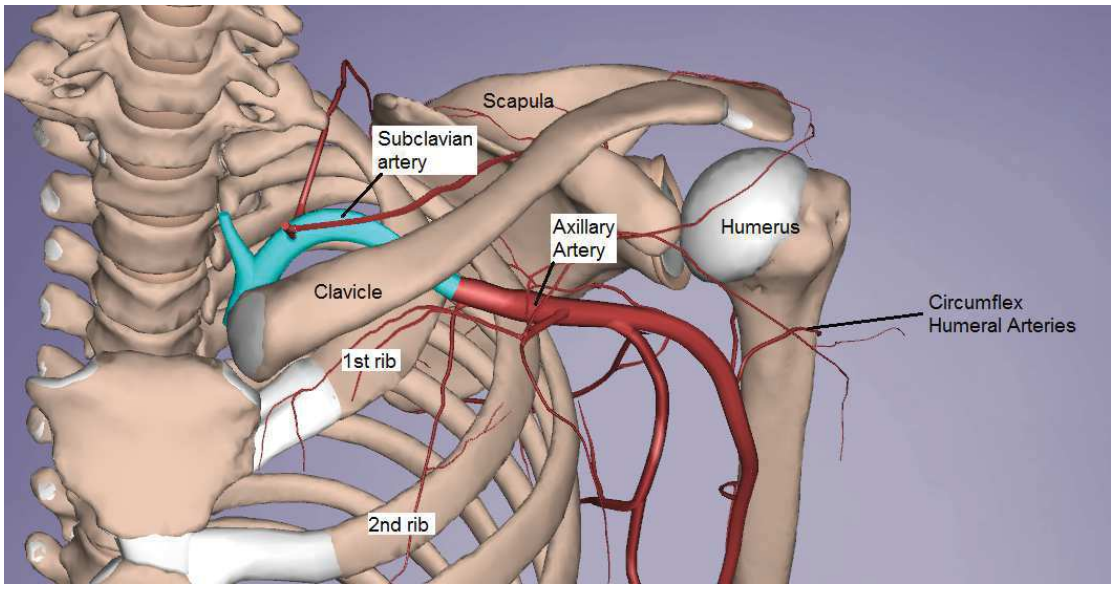




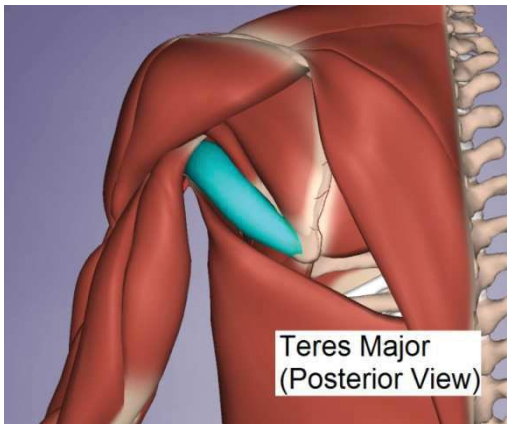
2. Main Arteries of the Upper Limb



a. Subclavian Artery



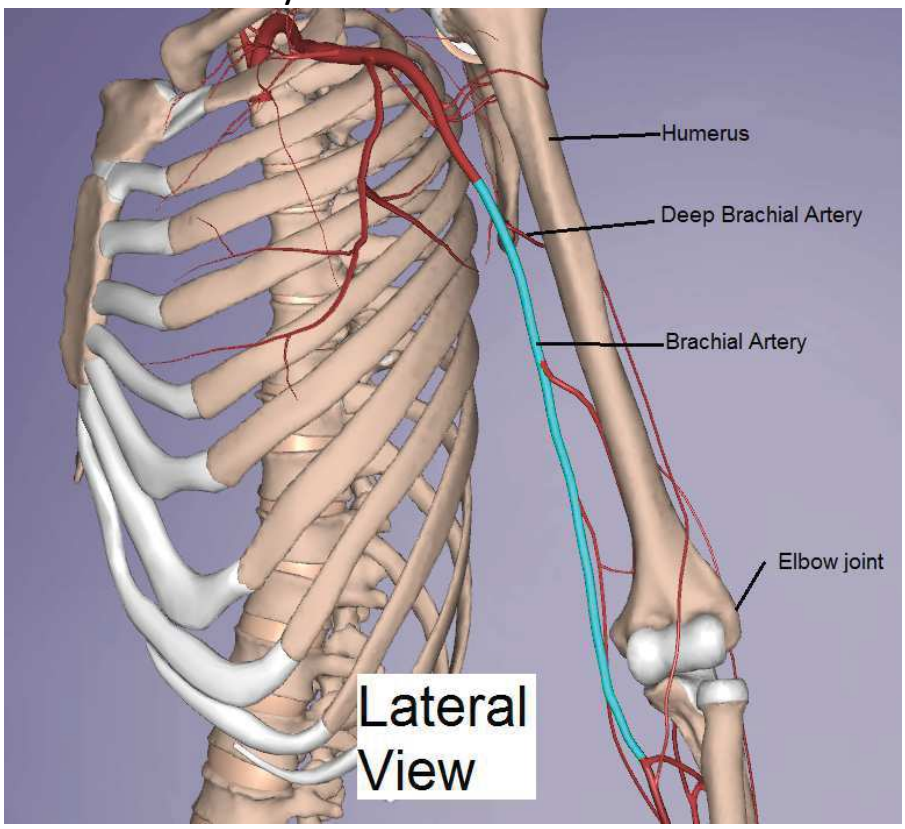
- **Left subclavian artery** arises directly from **aorta**
- **Right subclavian artery** is a branch of **brachiocephalic trunk**
- Becomes the **axillary artery** at lateral border of 1st rib
- Pulse can be palpated <u>posterior to midpoint of clavicle</u> where the artery passes over the 1st rib



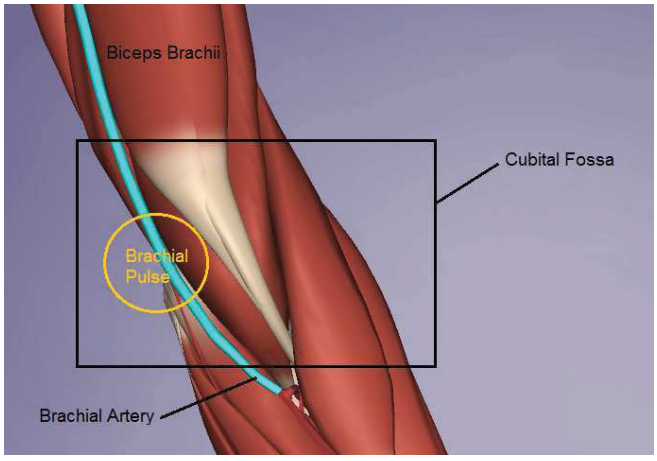
b. Axillary Artery

- Direct continuation of **subclavian artery**
- Located in the **axilla** (armpit)
- Begins at <u>lateral border of 1st rib</u> and ends at <u>lower border of **teres major**</u>
- Gives off branches to supply pectoral region, scapular region and shoulder and **anterior and posterior circumflex humeral arteries** (encircle surgical neck of **humerus**)

c. Brachial Artery



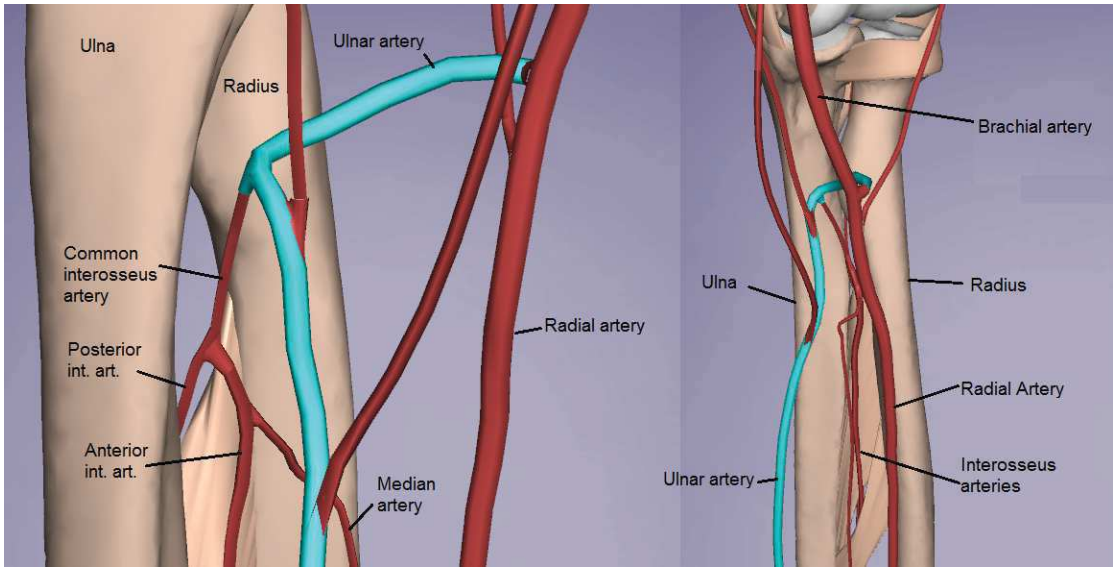
- Direct continuation of **axillary artery**
- Ends just <u>distal to elbow</u>
- Supplies muscles in the anterior compartment of the arm
- Pulse can be palpated in **cubital fossa**, medial to **biceps brachii tendon**
- Clinical significance: most common site of blood pressure measurement



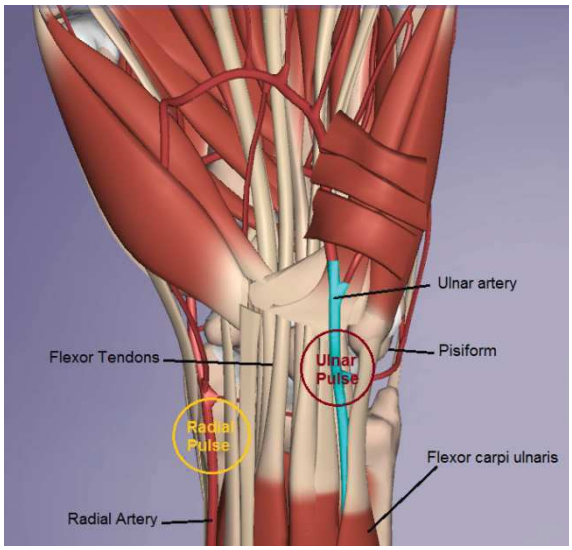
d. Deep Brachial Artery

- Also called **profunda brachii artery**
- Branches from **brachial artery**
- Supplies muscles in the posterior compartment of the arm
- Takes part in anastomosis around the elbow joint

e. Ulnar Artery



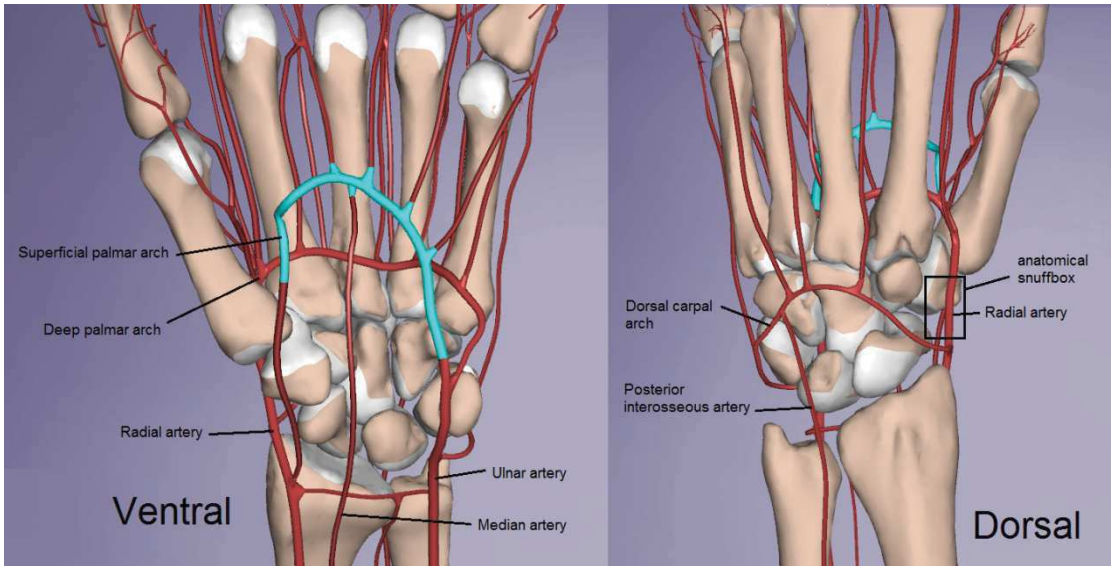
- Medial branch of **brachial artery**
- Supplies forearm muscles
- Gives off **common interosseous artery** which further branches into **anterior** and **posterior interosseous arteries** (on anterior and posterior surfaces of **interosseous membrane** respectively)
- Pulse can be palpated in front of wrist, lateral to **flexor carpi ulnaris tendon** and **pisiform bone**



f. Radial Artery

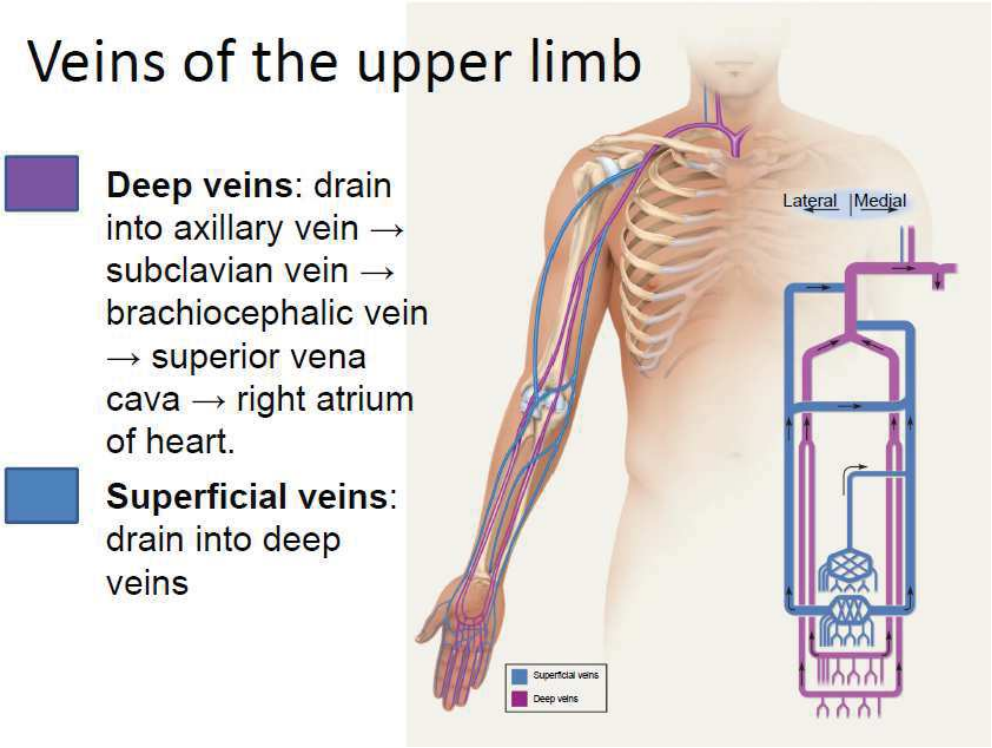
- Lateral branch of **brachial artery**
- Pulse readily palpable in front of wrist lateral to **flexor tendons** and in the **anatomical snuffbox** (area of depression on dorsum at base of thumb)
- Supplies lateral forearm muscles

g. Superficial and Deep Palmar Arches

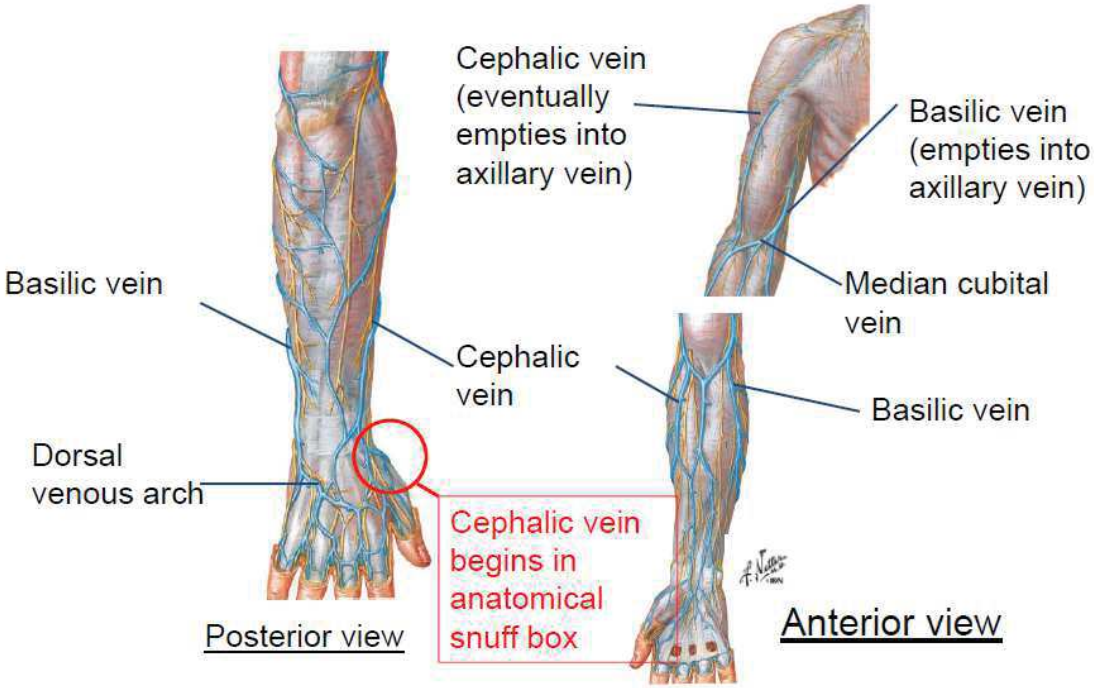


- Formed by anastomosis of radial and ulnar arteries
- **Superficial palmar arch**: direct continuation of ulnar artery
- **Deep palmar arch**: direct continuation of radial artery
- Together send branches to the digits

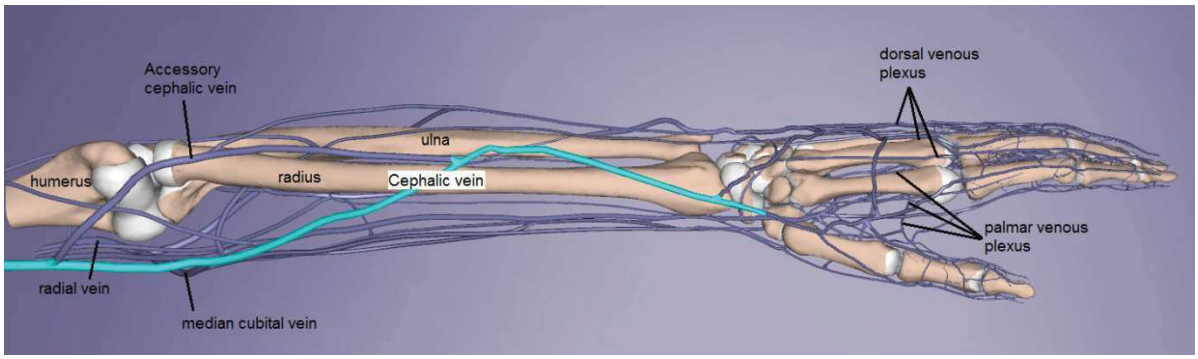
3. Veins of the Upper Limb



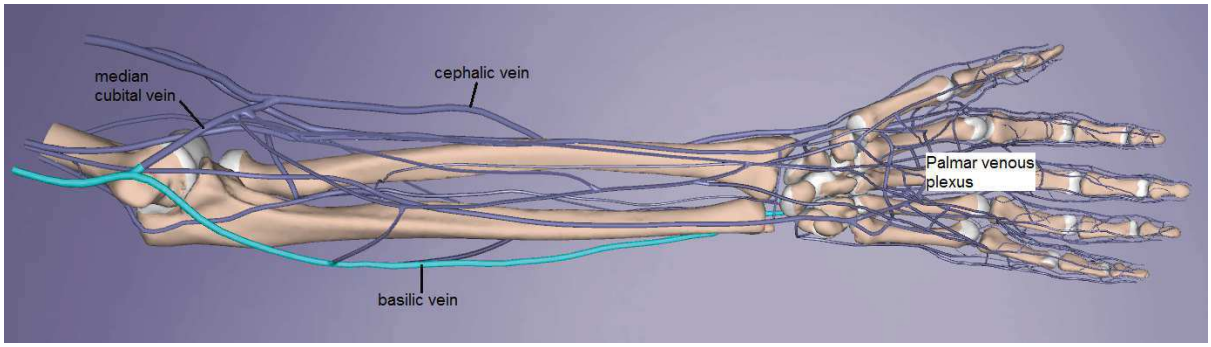
a. Superficial Veins of the Upper Limb



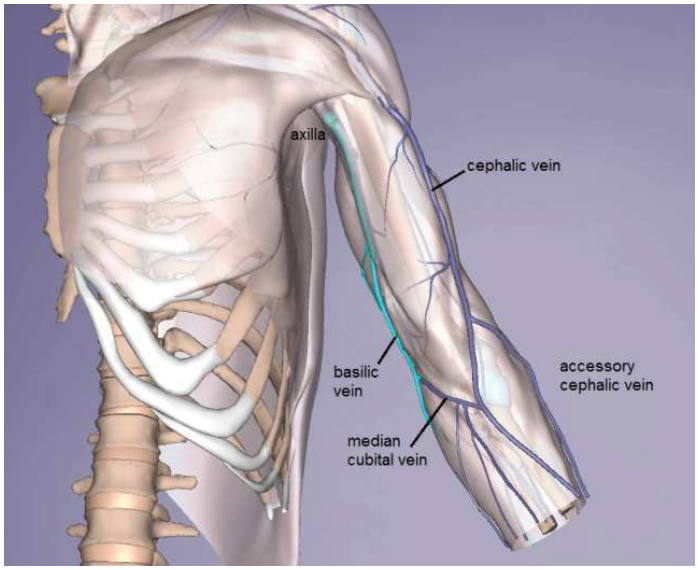
- **Dorsal venous network** on the back of the hand draining to **cephalic** and **basilic veins**

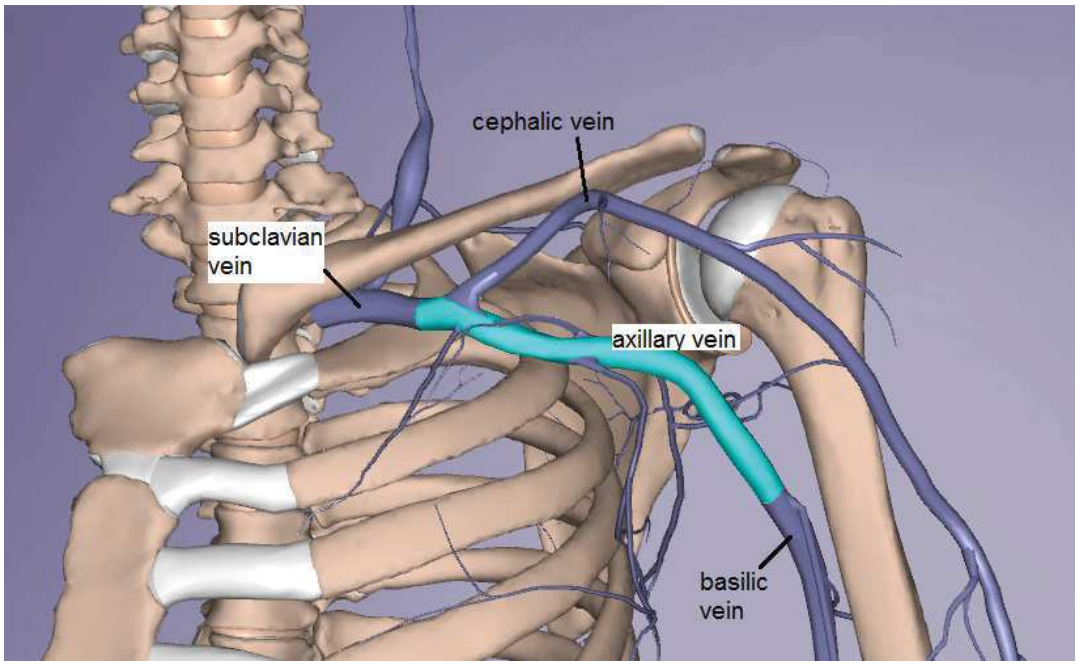


- **Cephalic vein**:
  - Visible at anatomical snuffbox
  - Runs up the lateral side of forearm and arm
  - Drains the lateral part of **dorsal venous network**
  - Drains into the **axillary vein** at axilla



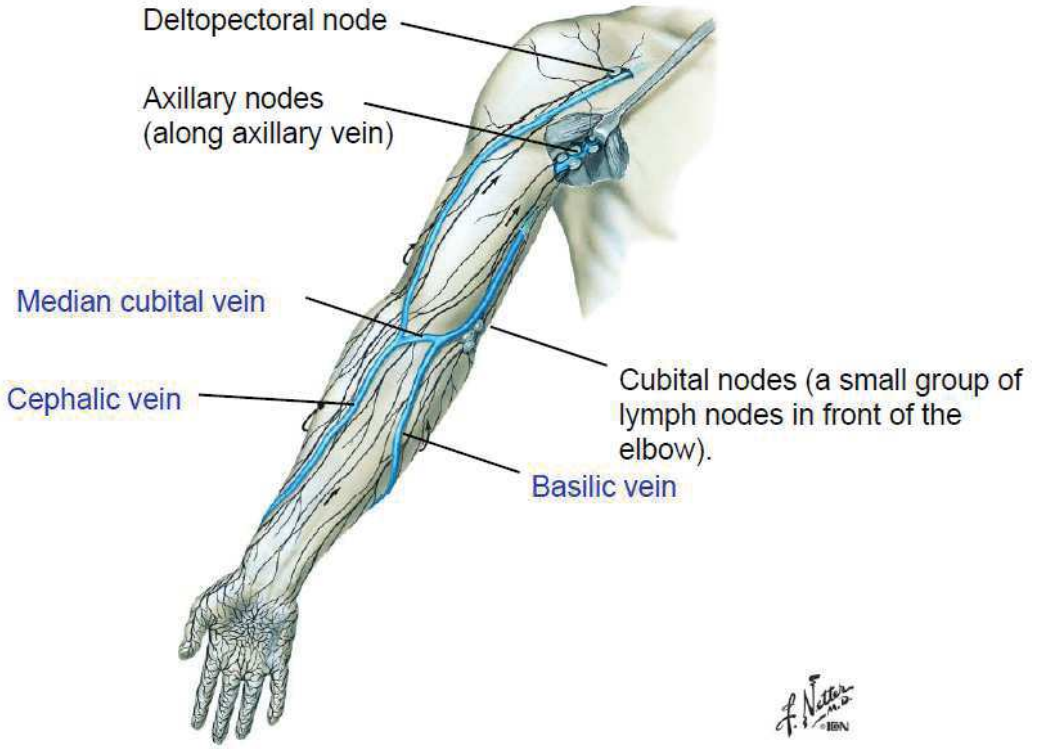
- **Basilic vein**:
  - Arises from medial side of dorsal venous network
  - Joins brachial veins to form axillary vein
- **Medial cubital vein**:
  - Anastomosis between cephalic and basilic veins at cubital fossa
  - Most common site for drawing blood





- **Axillary vein** drains into **subclavian vein**, **brachiocephalic vein** and then into **superior vena cava**

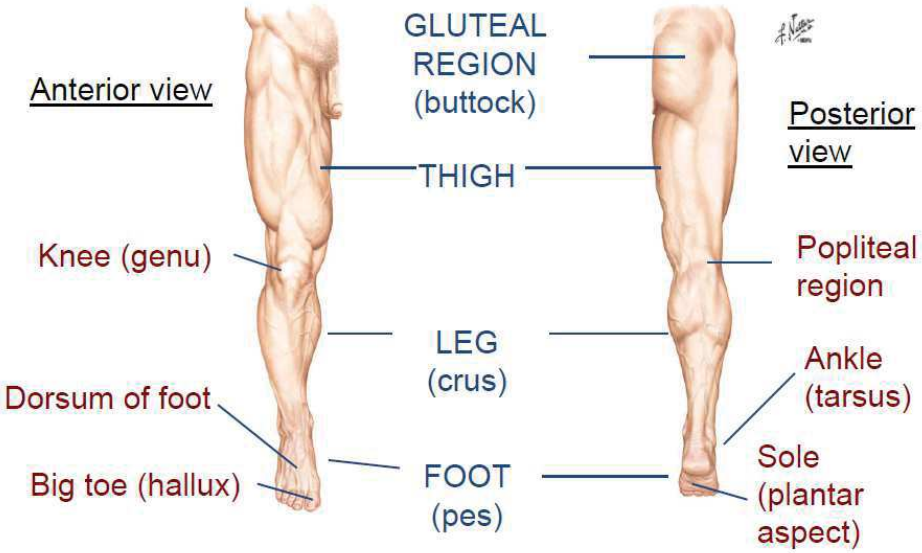
4. Lymphatic Drainage of the Upper Limb

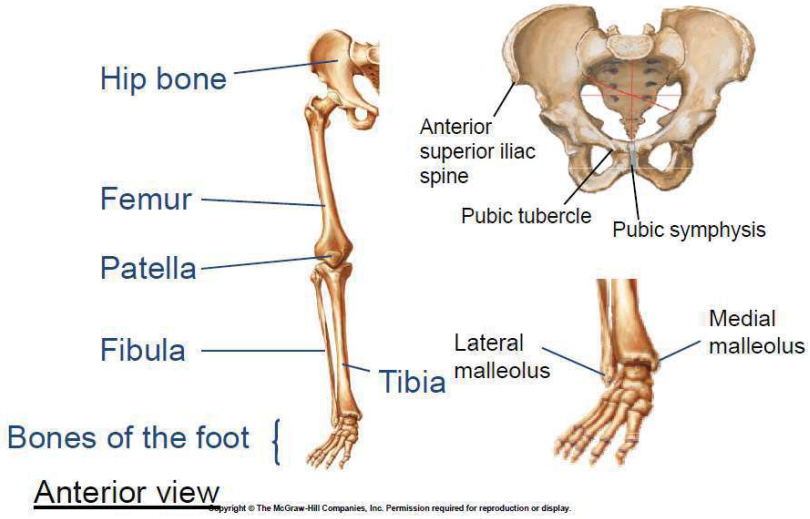


- **Axillary lymph nodes** drain upper limb, mammary glands, skin and superficial fascia of trunk above level of **umbilicus** and **hip**

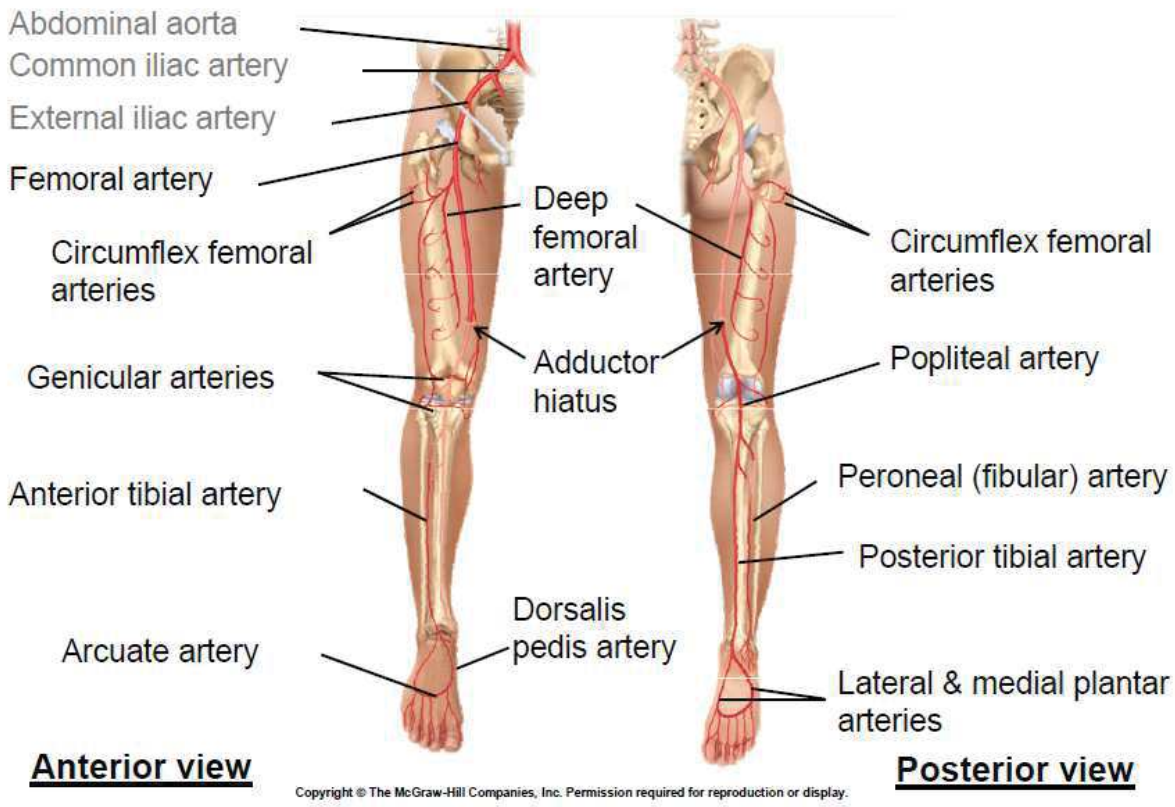
C. Vascular Supply to the Lower Limbs

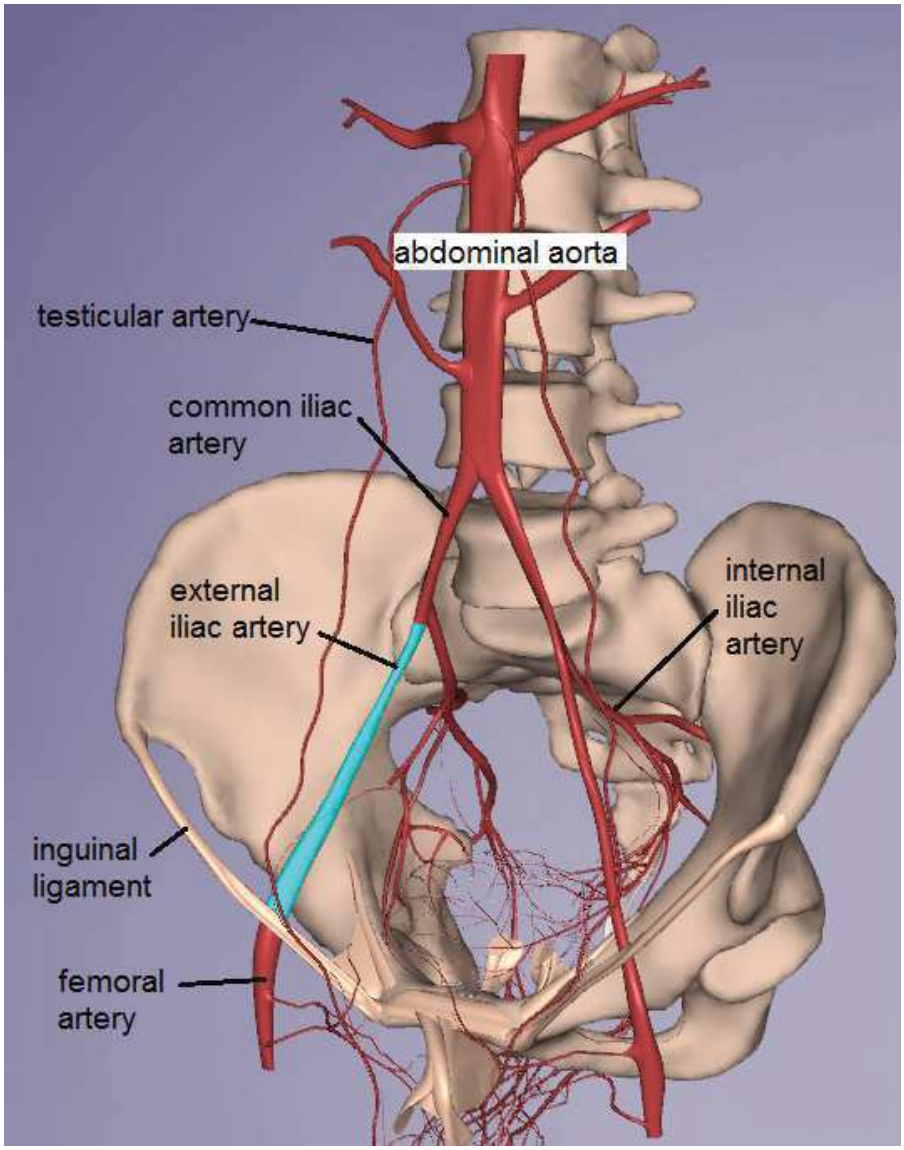
1. Anatomical Overview on the Lower Limbs





2. Main Arteries of the Lower Limb

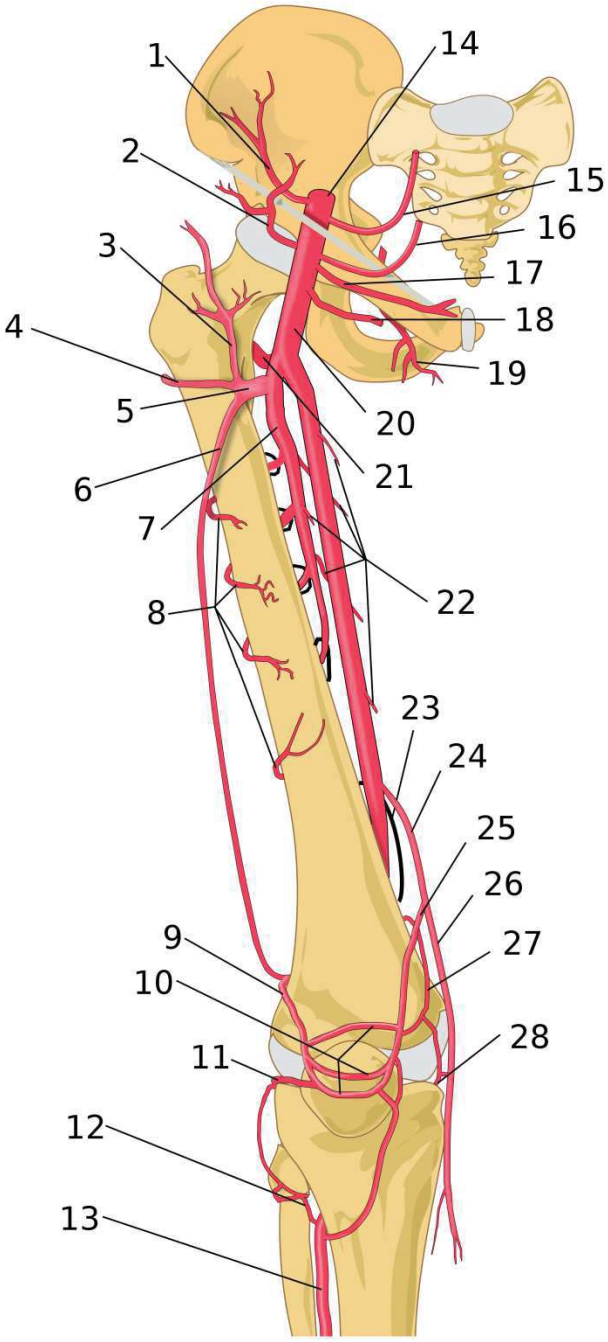




a. External Iliac Artery

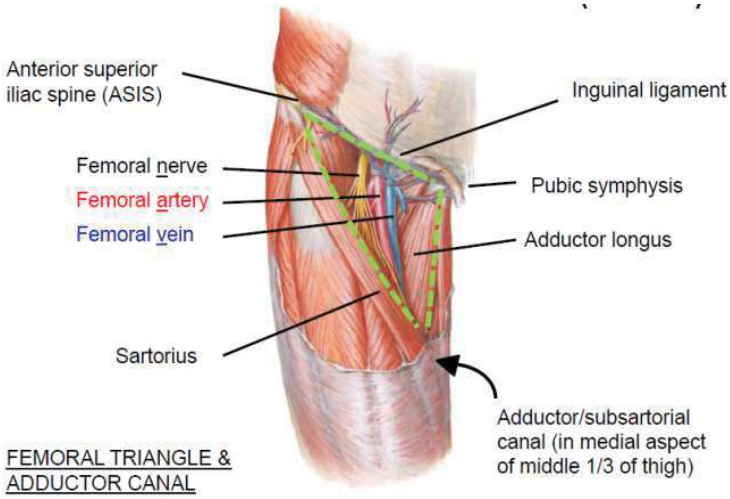
- **External iliac artery** arises from the **common iliac artery**
- Supplies the lower limbs via the **femoral artery**
- Becomes the **femoral artery** when pass through the **inguinal ligament**

b. Femoral Artery



1 - Deep circumflex iliac artery
2 - Superficial circumflex iliac artery
3 - Ascending branch of lateral femoral circumflex artery
4 - Transverse branch of lateral femoral circumflex artery
5 - LATERAL CIRCUMFLEX FEMORAL ARTERY
6 - Descending branch of lateral femoral circumflex artery
7 - DEEP FEMORAL ARTERY
8 - Perforating branches
9 - Superior lateral genicular artery
10 - Patellar anastamoses
11 - Inferior lateral genicular artery
12 - Circumflex fibular branch of anterior tibial artery
13 - ANTERIOR TIBIAL ARTERY
14 - EXTERNAL ILIAC ARTERY
15 - Inferior epigastric artery
16 - Superficial epigastric artery
17 - Superficial external pudendal artery
18 - Deep external pudendal artery (cut)
19 - Obturator artery (from internal iliac artery)
20 - FEMORAL ARTERY
21 - MEDIAL CIRCUMFLEX FEMORAL ARTERY (from deep femoral artery)
22 - Muscular branches
23 - ADDUCTOR HIATUS
24 - Descending genicular artery
25 - Articular branch of descending genicular artery
26 - Saphenous branch of descending genicular artery
27 - Superior medial genicular artery
28 - Inferior medial genicular artery

- **Femoral artery**: direct continuation of the **external iliac artery**
- Enters **femoral triangle** at **mid-inguinal point**
  - **Femoral triangle** bound by **inguinal ligament**, **adductor longus** and **Sartorius** and is covered by skin and fascia only
    → Clinical significance: can be easily accessible for cannulation
  - **Mid-inguinal point** is the mid-point of the imaginary line joining **anterior superior iliac spine (ASIS)** and **pubic symphysis**



- Passes through **Hunter's** (or **adductor** or **subsartorial**) **canal** in the medial aspect of the middle third of thigh
- Passes through **adductor hiatus** to enter **popliteal fossa** and become the **popliteal artery**
  - **Adductor hiatus**: a gap in **adductor magnus** muscle
- Note V-A-N arrangement from medial to lateral

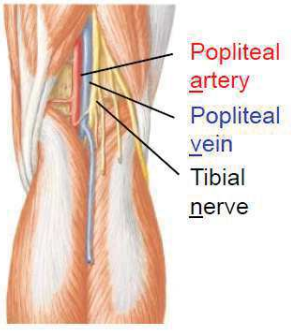
c. Deep Femoral Artery

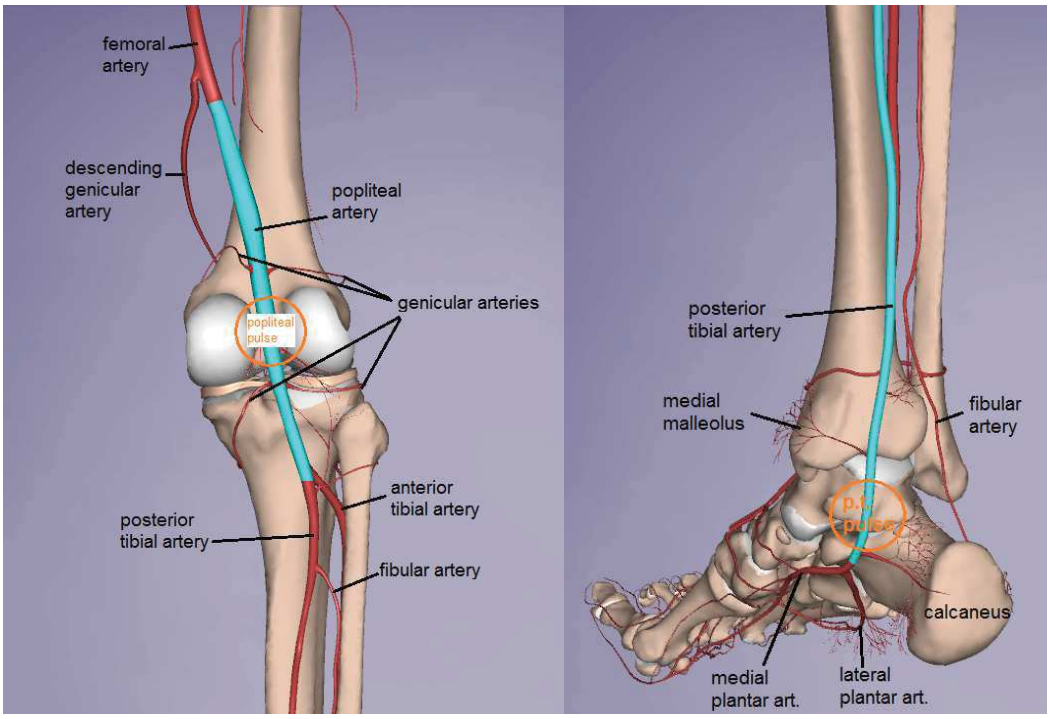
- Also called **profunda femoris artery**
- Branch of femoral artery
- Main arterial supply to thigh
- Branches: **medial** and **lateral circumflex femoral arteries** and 4 **perforating arteries**

d. Popliteal Artery

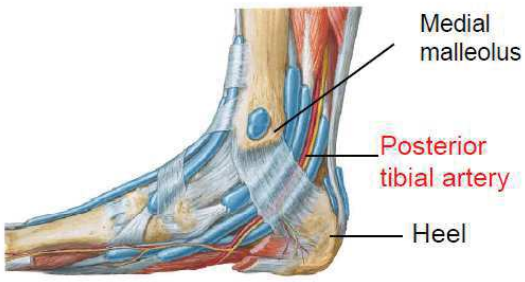
- Arises from **femoral artery** after it passes through **adductor hiatus**
- Divides into **anterior** and **posterior tibial arteries**
- Note A-V-N arrangement from deep to superficial
  - The 'N' is **tibial nerve** not **popliteal nerve**
- Pulse is more difficult to feel because it is the deepest structure in the popliteal fossa

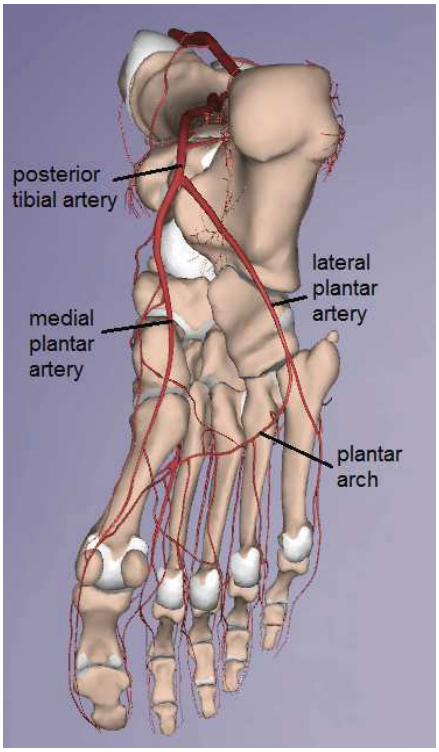
POPLITEAL FOSSA
(right)





Right ankle and foot
(medial view)

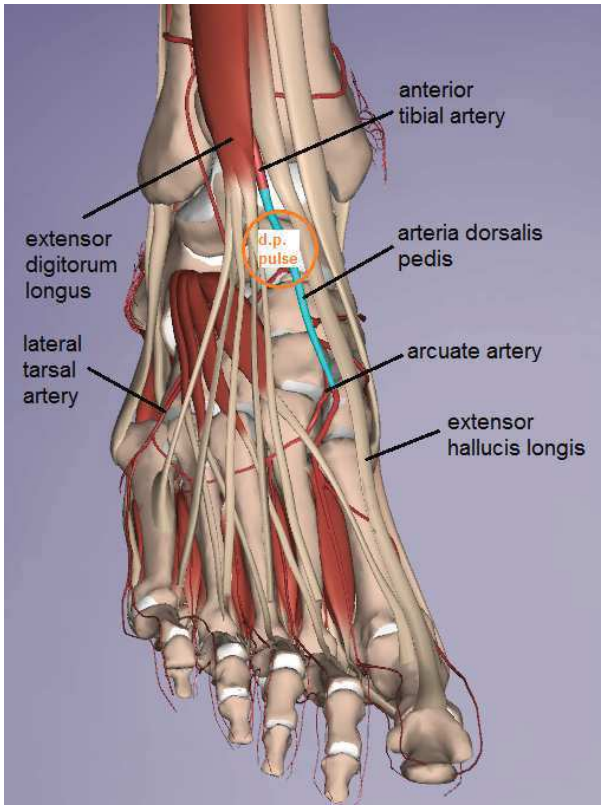




e. Posterior Tibial Artery

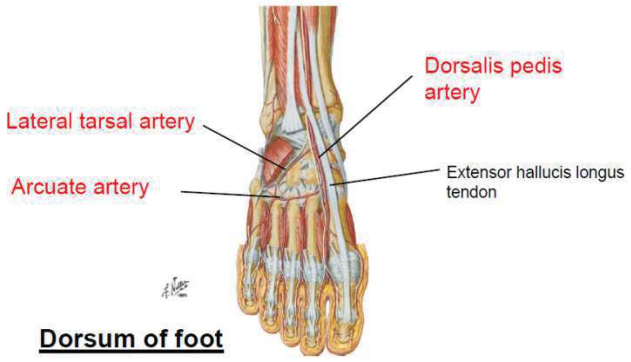
- Travels along the posterior side of the leg
- Supplies muscles of the posterior compartment of the leg together with its branch **peroneal (fibular) artery**
- Turns medially at the distal half of the leg to enter foot
- Divides into **medial** and **lateral plantar arteries** in the foot
- **Lateral plantar artery** forms the **plantar arch**
- Pulse: felt midway between tip of **medial malleolus** and medial margin of the heel

f. Anterior Tibial Artery



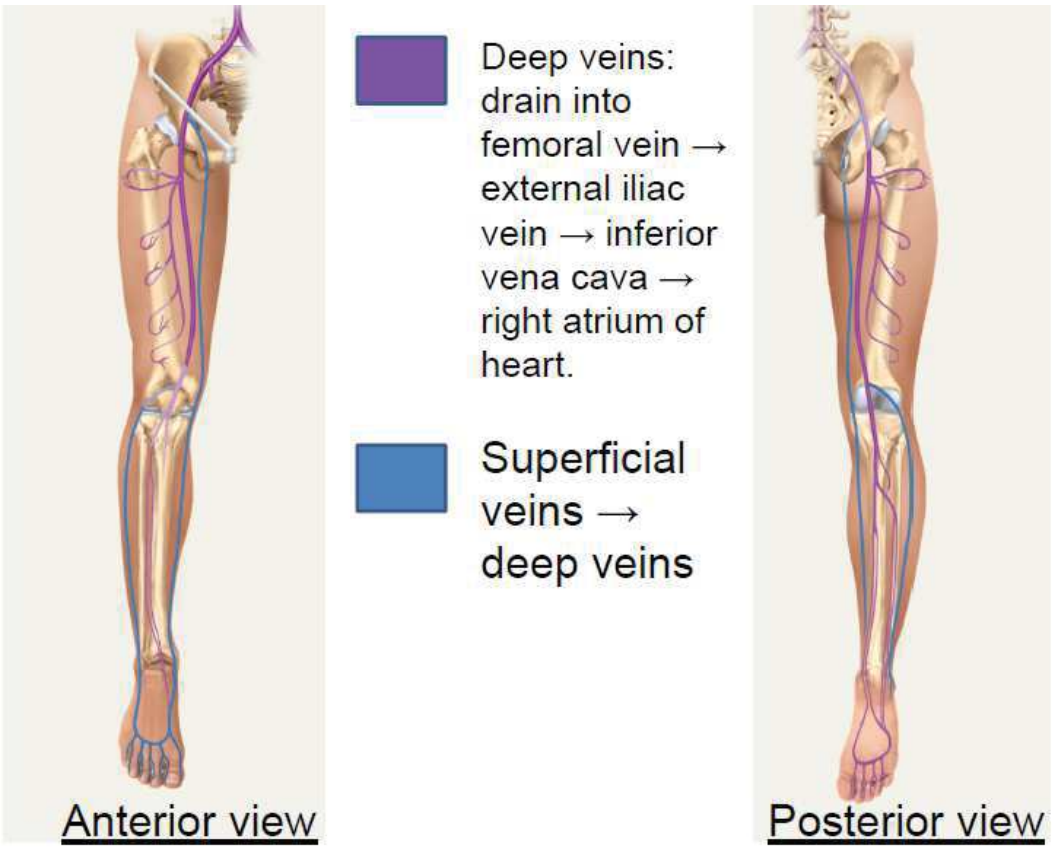
- Arises from **popliteal artery**
- Enters anterior compartment of leg through an opening in the **interosseous membrane**
- Continues into foot as **dorsalis pedis artery**
- Supplies muscles in anterior compartment of leg
- Pulse palpable in front of ankle midway between the two malleoli

g. Dorsalis Pedis Artery

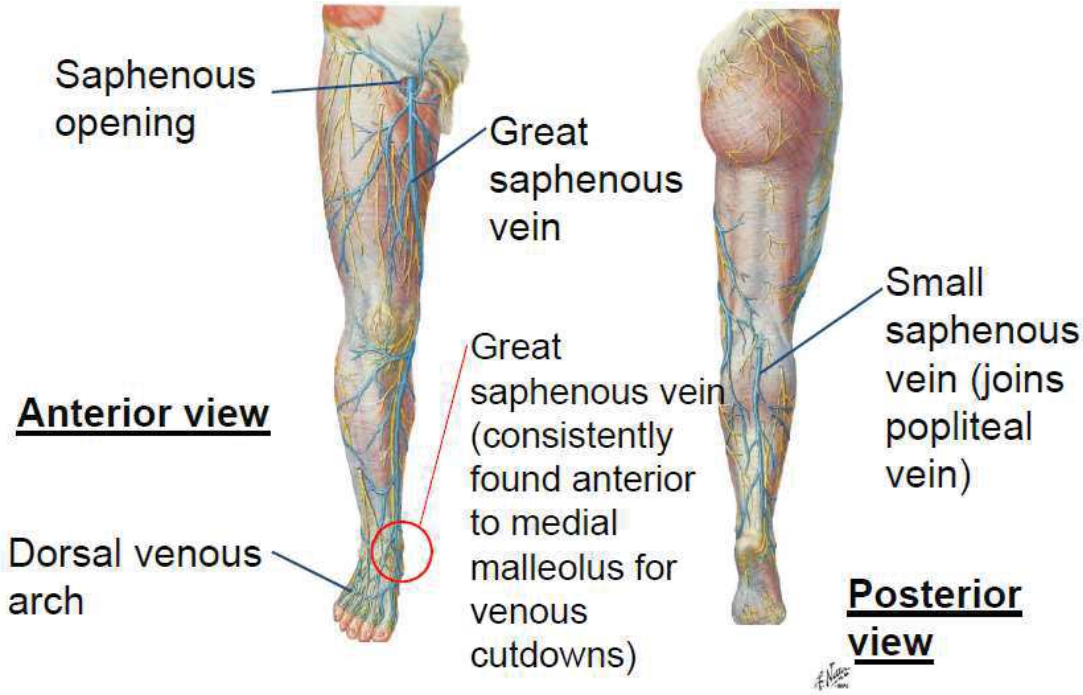


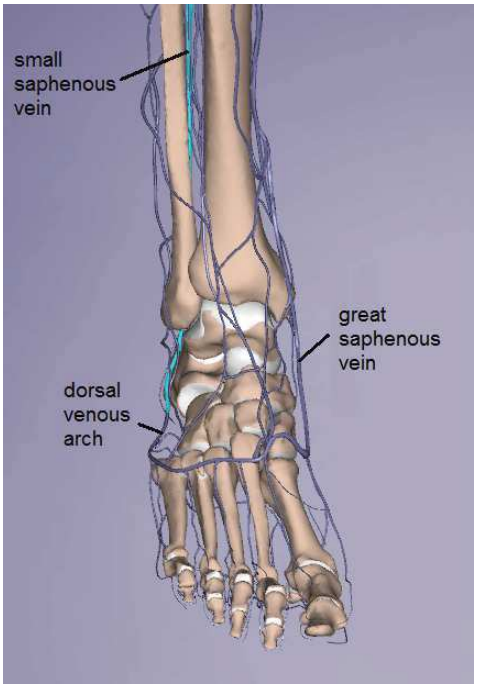
- Arises from **anterior tibial artery**
- Pulse readily palpable on dorsum of foot (pressing against **tarsal bones**) just lateral to tendon of **extensor hallucis longus** (extensor muscle for the big toe)

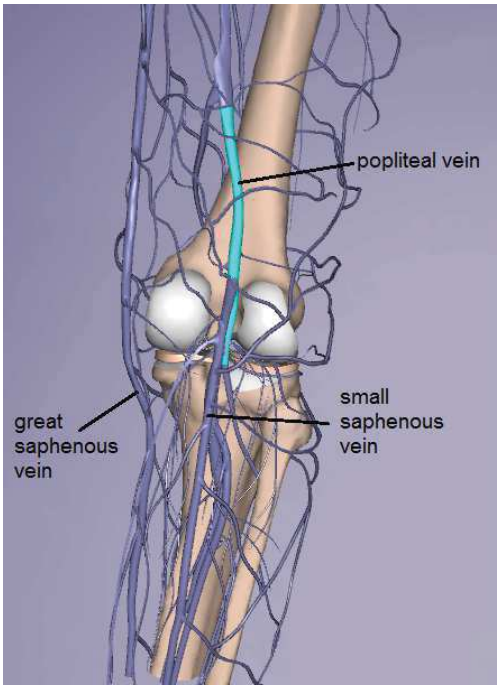
3. Veins of the Lower Limb



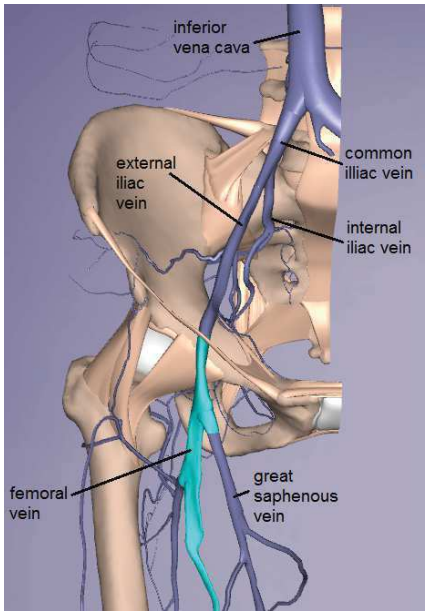
a. Superficial Veins of the Lower Limb

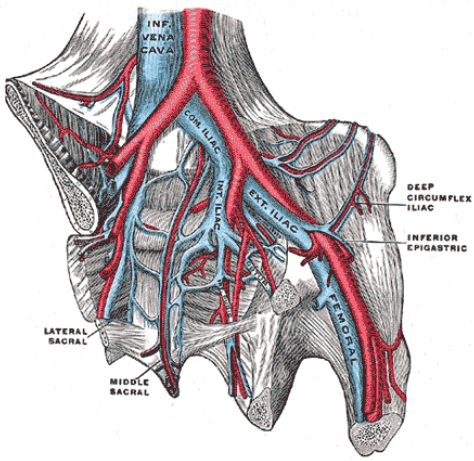




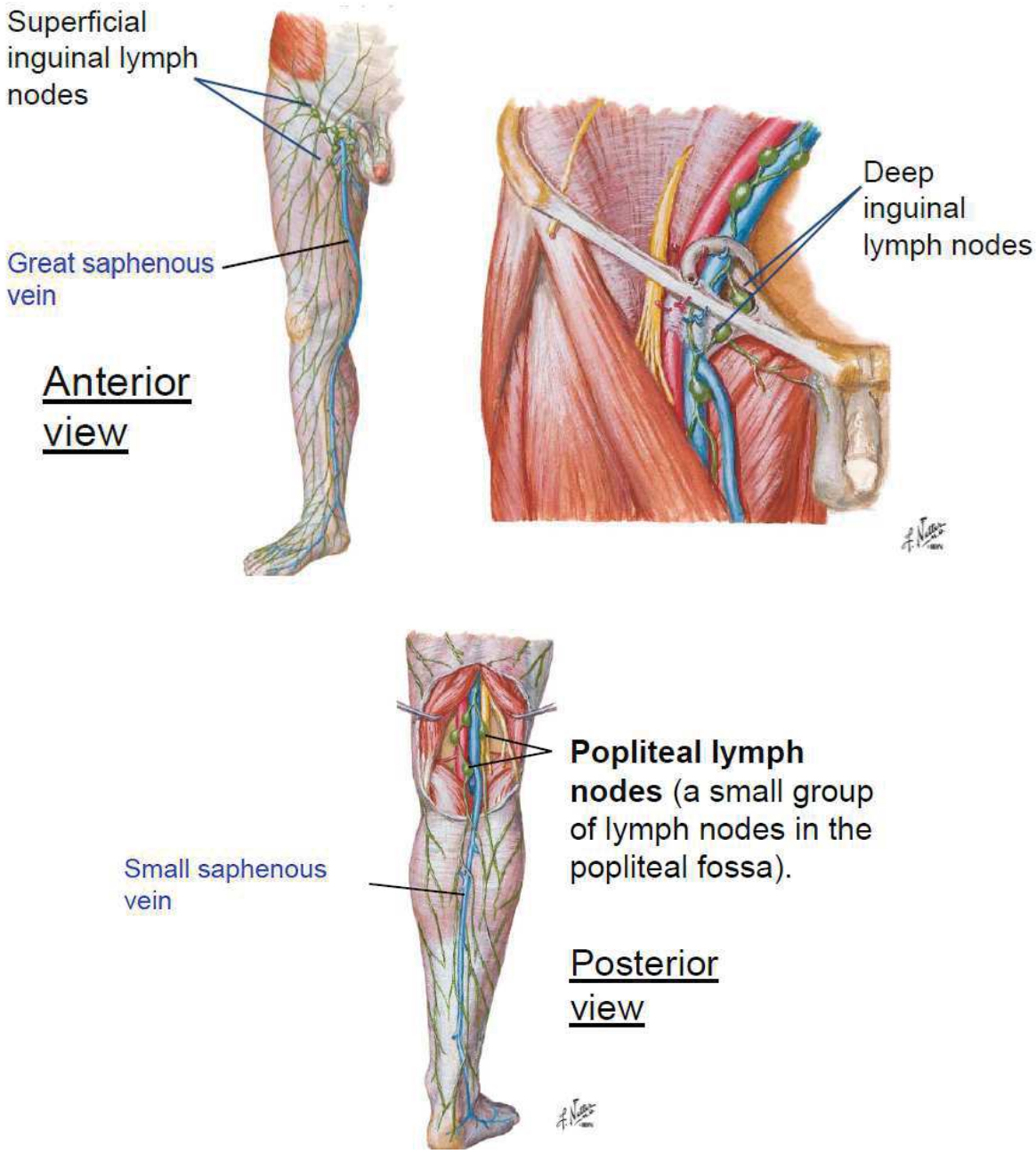


- **Dorsal venous arch** on dorsum of foot drains tissue on the foot
- **Great (greater/long) saphenous vein**:
  - Arises from medial side of the arch
  - Passes the region 2-3 cm anterior to medial malleolus
    - → Clinical significance: accessible for venous cutdowns
  - Travels up medial side of leg
  - Passes through **popliteal fossa** and then up medial side of thigh
  - Penetrates the deep fascia of the thigh through the **saphenous opening** below the inguinal ligament
  - Drains into **femoral vein**
- **Small (lesser/short) saphenous vein**:
  - Arises from lateral side of the arch
  - Runs up posterior portion of leg
  - Drain into **popliteal vein** at popliteal fossa
- **Popliteal vein** arises from small saphenous vein and drains into femoral vein at thigh
- **Femoral vein** arises from the small saphenous vein and drains into **external iliac vein** and then towards **common iliac vein** (then into **inferior vena cava**)





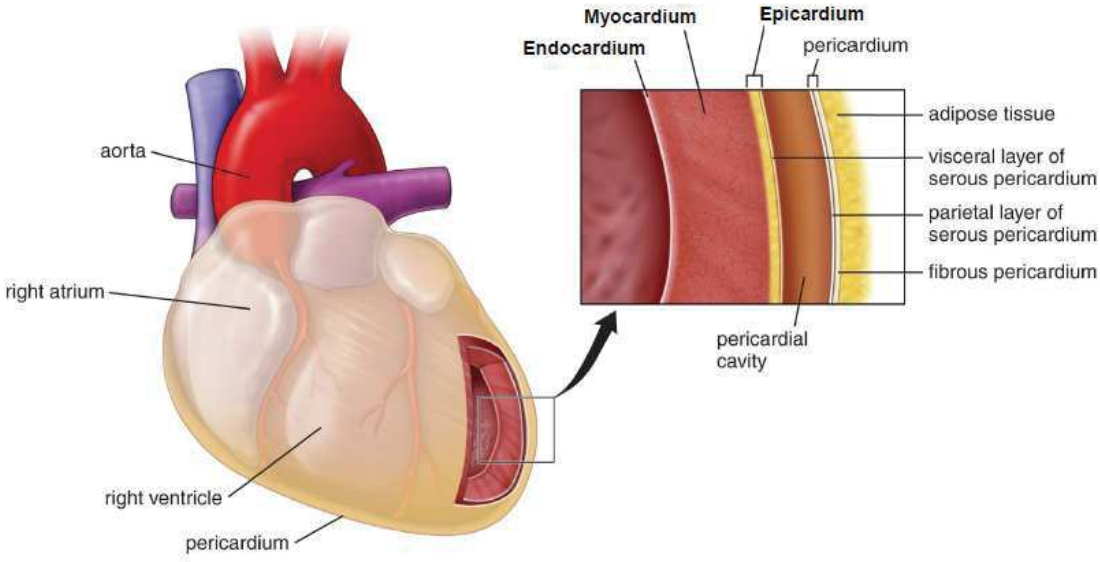
4. Lymphatic Drainage of the Lower Limb



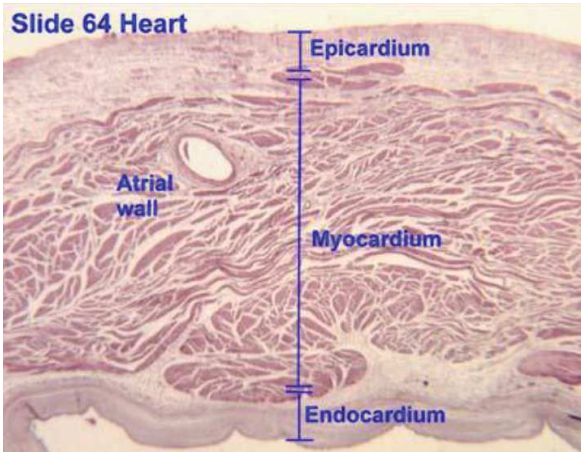
- **Inguinal lymph nodes** drain lower limb, skin and superficial fascia of the trunk below the **umbilicus**, external genitalia, mucous membrane of lower half of anal canal

L68 Histology and Functions of Blood Vessels

A. Structure of Heart Wall



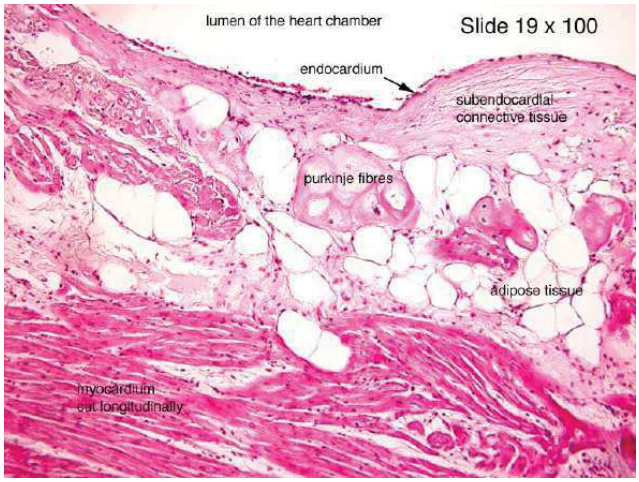
- **Endocardium**:
  - Lines chambers of heart and covers heart valves
  - Consists of:
    - → A layer of endothelial cells
    - → A sub-endothelial layer of c.t. (with veins, nerves and Purkinje fibres)
- **Myocardium**:
  - Consists of cardiac muscles
  - Highly vascularized
- **Epicardium**:
  - Also called **visceral pericardium**
  - Made up of fibroelastic c.t. with blood vessels, lymphatics, nerves and adipose tissues
  - Covered by a layer of **mesothelium** (simple squamous epithelium)



1. Cardiac Muscles

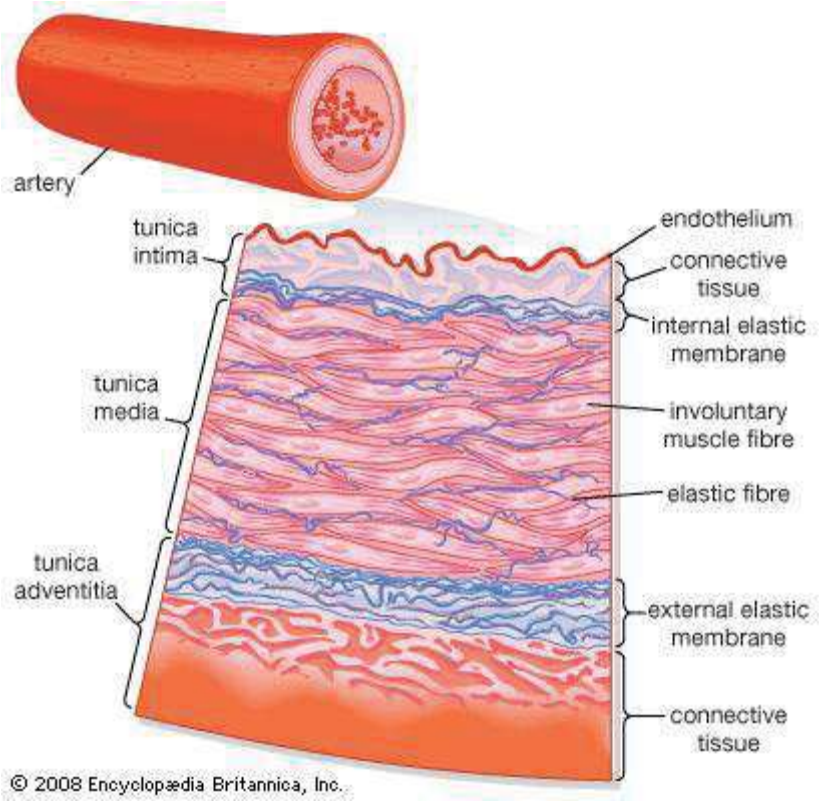
- Striated involuntary muscle
- Centrally located nucleus
- Branched muscle fibres
- **Intercalated disks** connects adjacent cardiomyocytes
  - Consists of 3 cell-cell junctions: gap junctions, adhering junctions and desmosomes → allow rapid transmission of electrical impulses
  - Form an **syncytium** for coordinated contraction

2. Purkinje Fibres

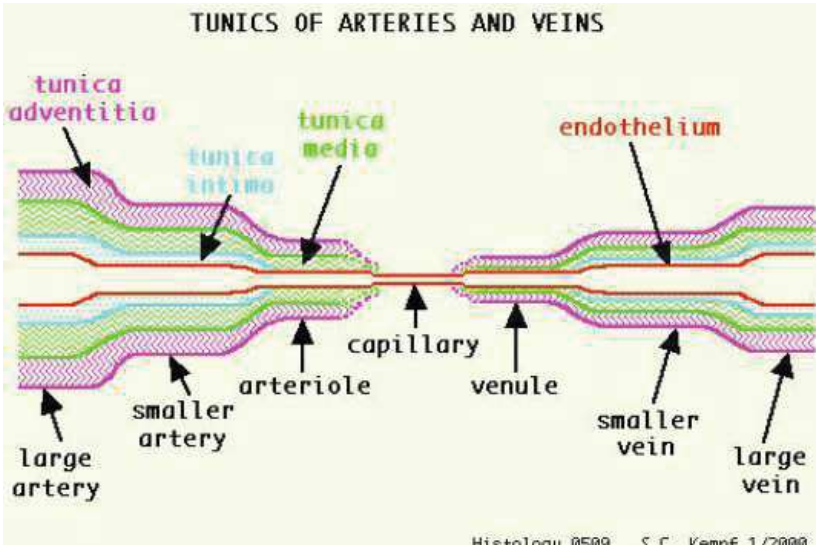


- **Purkinje fibres**: specialized cardiac muscle cells able to generate and conduct impulse (for synchronized contraction)
- Found in **sub-endocardium**
- Compared to cardiomyocytes:
  - Larger
  - Lots of glycogen and mitochondria
  - Fewer myofibrils
  - No T-tubules
  - No intercalated discs (connected by desmosomes and gap junctions)
- Histology: paler myocyte cells (conferred by higher glycogen content)
  - White pale region (glycogen) surrounded by pink cytoplasm (myofibrils)

B. Histological Layers of Blood Vessels



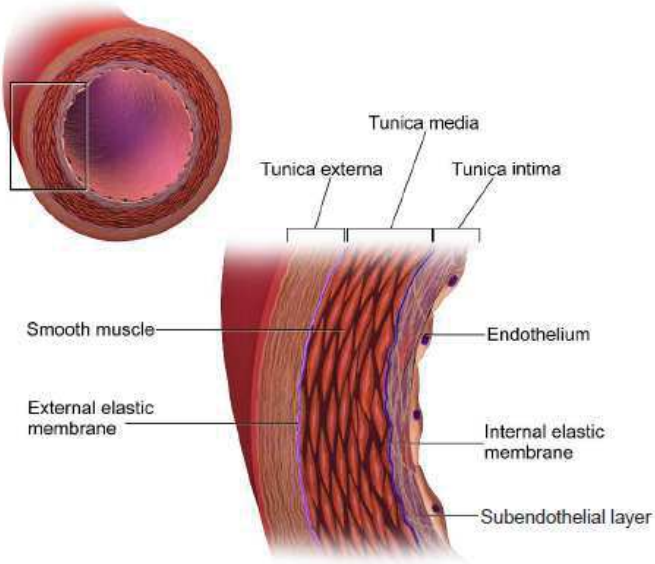
- Three layers:
  - **Tunica intima**
  - **Tunica media**
  - **Tunica adventitia**

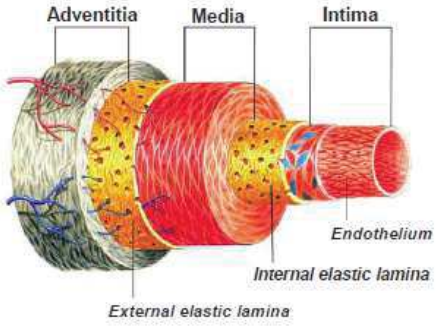


- Wall is thicker in larger vessels than in small vessels
- Arterial wall is thicker than venous wall
- Composition of wall changes gradually along blood vessels
- Endothelium is continuous throughout all blood vessels

1. Tunica Intima

- Three layers (from innermost):
  - **Endothelium**
  - Subendothelial c.t.
  - Internal elastic lamina
- **Endothelium**: simple squamous epithelium (on a basement membrane)
  - Regulates transport of substances from lumen into vessel wall
  - Controls local clotting through secretion of soluble factors (eg van Willebrand factor)
  - Enables migration of WBC from blood (via P-selectin → enables neutrophil migration into c.t.)
- **Subendothelial layer** of loose c.t. (with nerves and blood vessels)
- **Internal elastic lamina**: fenestrated membrane of elastic fibres separating intima from media
- Pathology: **arteriosclerosis** and **atherosclerosis**
  - **Arteriosclerosis**: thickening and hardening of arterial walls
  - **Atherosclerosis**: narrowing of arteries caused by buildup of fat and cholesterol in arterial walls
    → Restriction of blood flow
    → Formation of thrombus





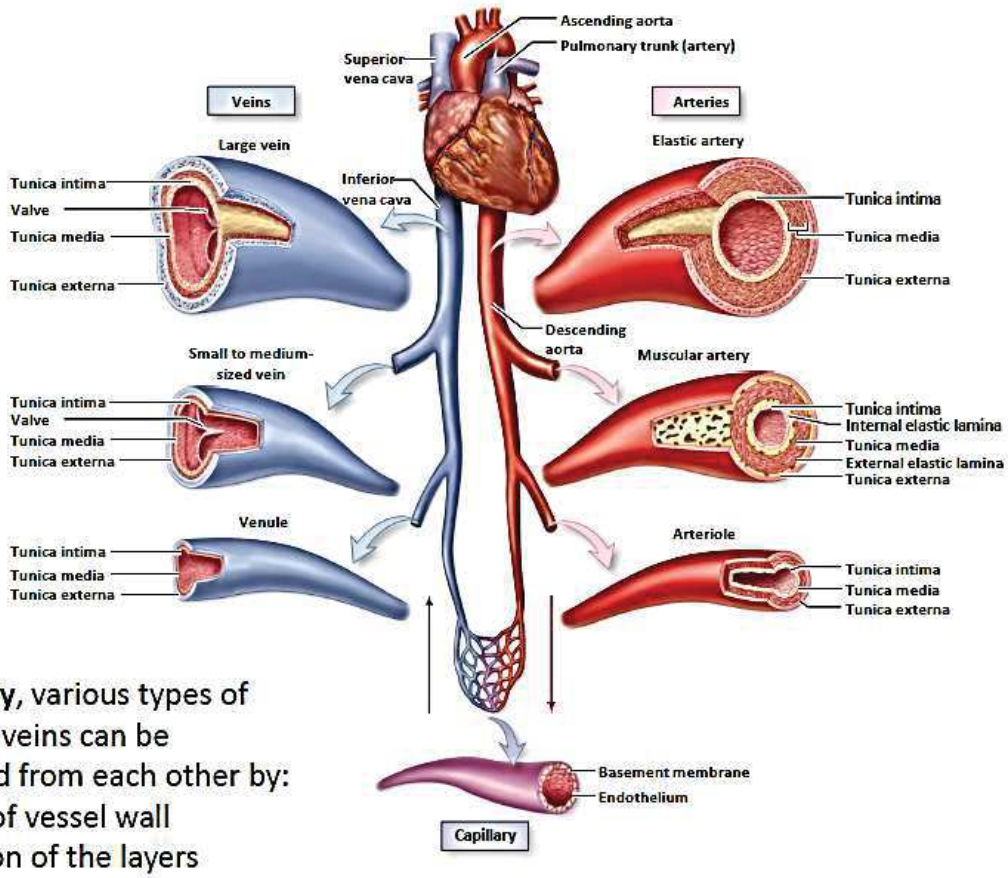
2. Tunica Media

- Two layers:
  - Smooth muscle layers
  - **External elastic lamina**
- **Smooth muscle layer**:
  - Circularly arranged smooth muscles
  - Elastic fibres (elastin)
  - Some collagen fibres
- **External elastic lamina** separating tunica media and tunica adventitia
- Pathology:
  - **Aneurysm**: 'ballooning' out of arterial wall
    → Smooth muscle weakness → extensive dilation of arteries
  - **Marfan Syndrome (MFS)**: genetic c.t. disorder with mutated fibrillin 1 gene (essential for formation of elastic fibres)
    → Separation between media and adventitia
    → Media shows mucoid deposition and fragmentation of elastic lamellae

3. Tunica Adventitia

- Three layers:
  - Loose c.t.
  - Smooth muscles, collagen and elastic fibres (large vessels only)
  - **Vasa vasorum**
- **Connective tissue** layer with many collagen and elastin fibres
- Continuous with surrounding c.t.
- **Vasa vasorum**: small nutrient arteries and veins providing blood supply to walls of larger blood vessels (∵ inadequate nutrient supply via diffusion)
- Importance: anchor blood vessel in c.t.

C. Different Types of Vessels

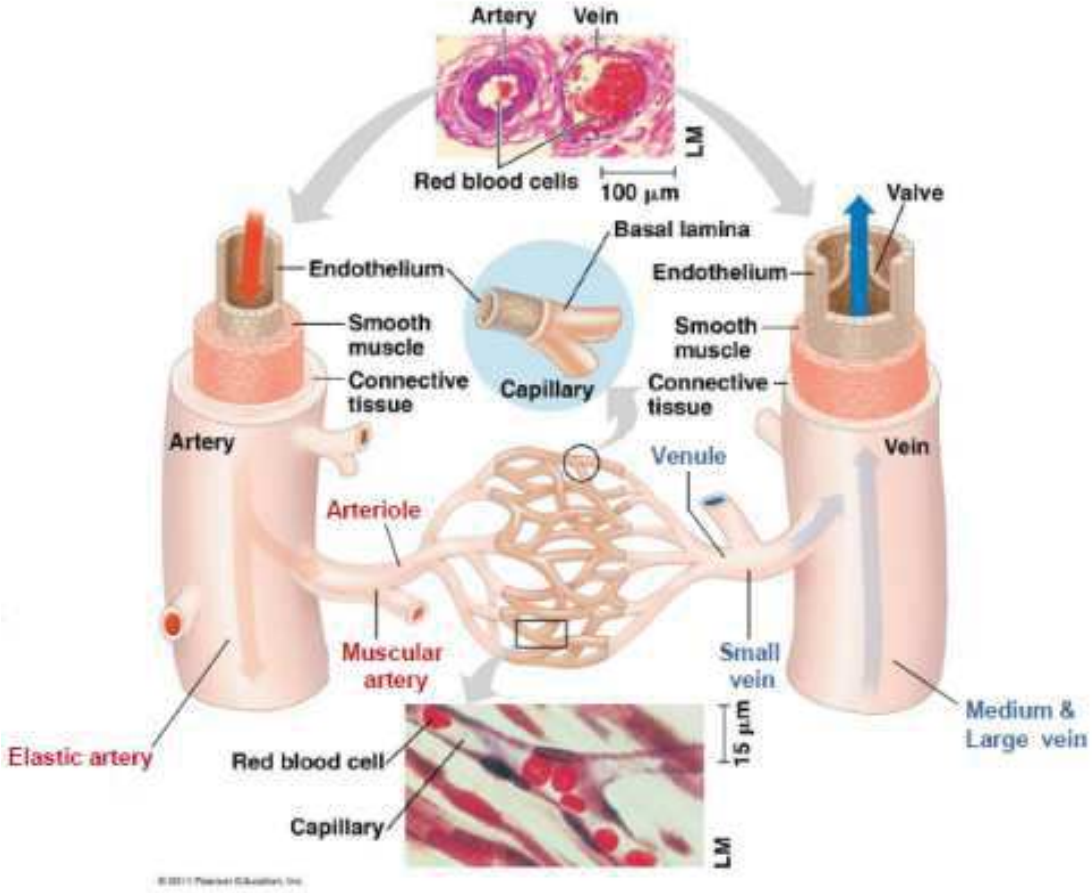


Histologically, various types of arteries and veins can be distinguished from each other by:

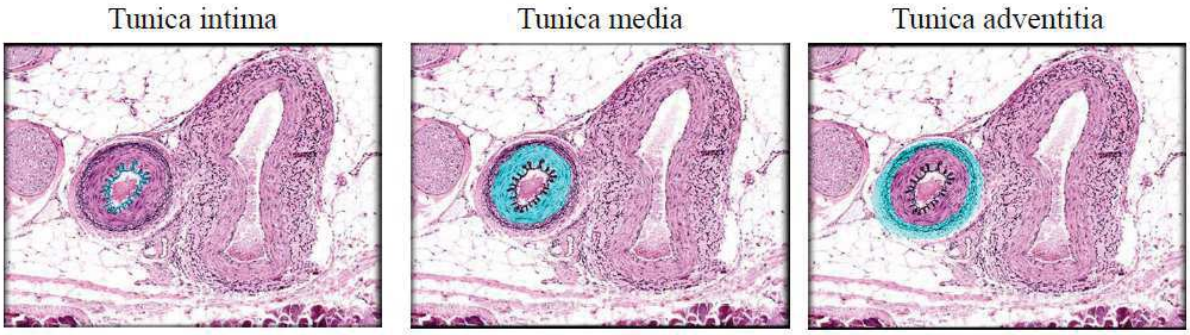
- Thickness of vessel wall
- Composition of the layers

	Arteries	Capillaries	Veins
Function	Carry blood **away** from the heart at **high** pressure	-Supply all cells with their **requirements** -Take away **waste** products	**Return** blood to the heart at **low** pressure
Structure of wall	-**Thick**, strong -Contain **muscles**, **elastic** fibres and **fibrous** tissue	**Very thin**, only one cell thick	-**Thin** -Mainly **fibrous** tissue -Contain far **less muscle** and **elastic** tissue than arteries
Lumen	-**Narrow** -Varies with heartbeat (increases as a pulse of blood passes through)	-**Very narrow** -Just wide enough for a red blood cell to pass through	**Wide**
Valves	(-)	(-)	(+) Prevent backflow
How structure fits function	-Strength and elasticity needed to withstand the pulsing of the blood, prevent bursting and maintain pressure wave -Helps to maintain **high blood pressure**, preventing blood flowing backwards	- No need for strong walls, as most of the blood pressure has been lost -Thin walls and narrow lumen bring blood into close contact with body tissue, allowing **diffusion** of materials between capillary and surrounding tissues. -White blood cells can squeeze between cells of the wall	- No need for strong walls, as most of the blood pressure has been lost - Wide lumen offers **less resistance** to blood flow

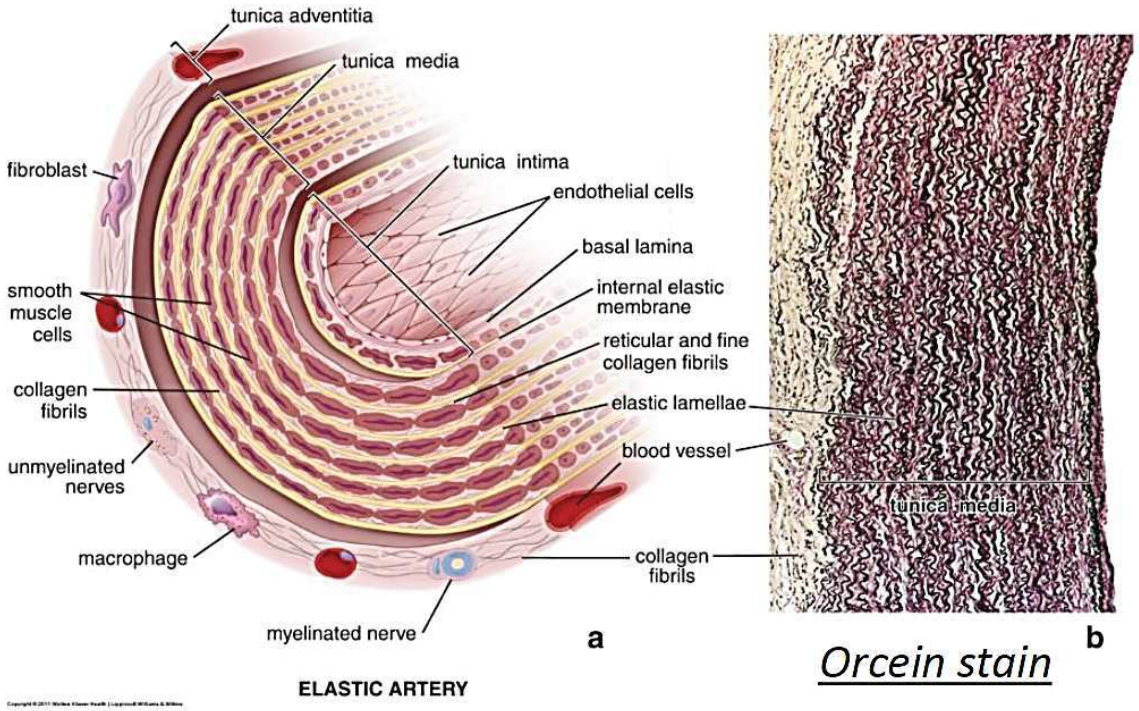
- Total thickness of wall varies with pressure, distance from heart and type (artery vs vein)
- Thickness of each layer also varies:
  - **Tunica adventitia** most distinguished layer in thin-walled vessels
  - **Tunica media** most distinguished layer in thick-walled vessels
  - 3-layered structure varies according to:
    - → Forces on vessel wall
    - → Size of vessel
    - → Function of vessels
- Types of blood vessels:
  - Arteries:
    - → **Elastic arteries**
    - → **Muscular arteries**
    - → **Arterioles**
  - **Capillaries**
  - Veins:
    - → **Venules**
    - → **Small veins**
    - → **Medium and large veins**


Artery
Vein
LM
Red blood cells
100 µm
Valve
Basal lamina
Endothelium
Endothelium
Smooth muscle
Smooth muscle
Connective tissue
Capillary
Connective tissue
Artery
Vein
Venule
Arteriole
Muscular artery
Small vein
Elastic artery
Medium & Large vein
Red blood cell
15 µm
Capillary
LM

1. Arteries



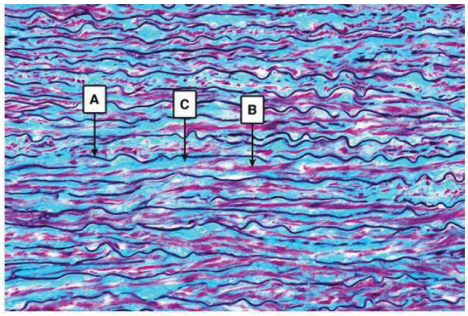
a. Elastic Arteries



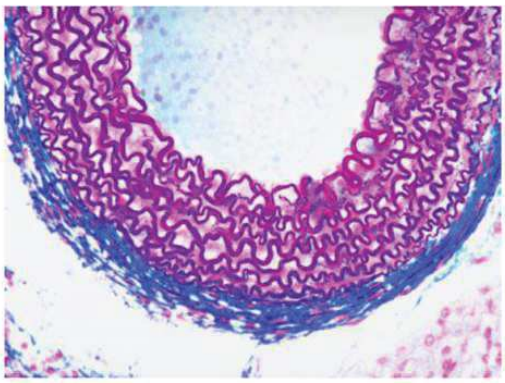
- Also called **conducting arteries**
- Examples: aorta, pulmonary artery
- Function: transport blood away from heart
- Feature:
  - Relatively thin wall: wall only amount to 10% of diameter
  - Elasticity
- Importance of elasticity:
  - Systole → expansion
  - Diastole → elastic walls recoil to maintain blood pressure → continue to drive blood through blood vessels

- Histological layers:
  - **Tunica intima**:
    - → Thick
    - → Consists of endothelium with a relatively thick loose c.t. sublayer
  - **Tunica media**:
    - → Consists of 50-75 concentrically arranged, fenestrated elastic laminae with smooth muscles and collagen fibre between them
    - → Internal and external lamina not readily recognizable
  - **Tunica adventitia**: thin loose c.t. with vasa vasorum

Tunica Media of the Aorta

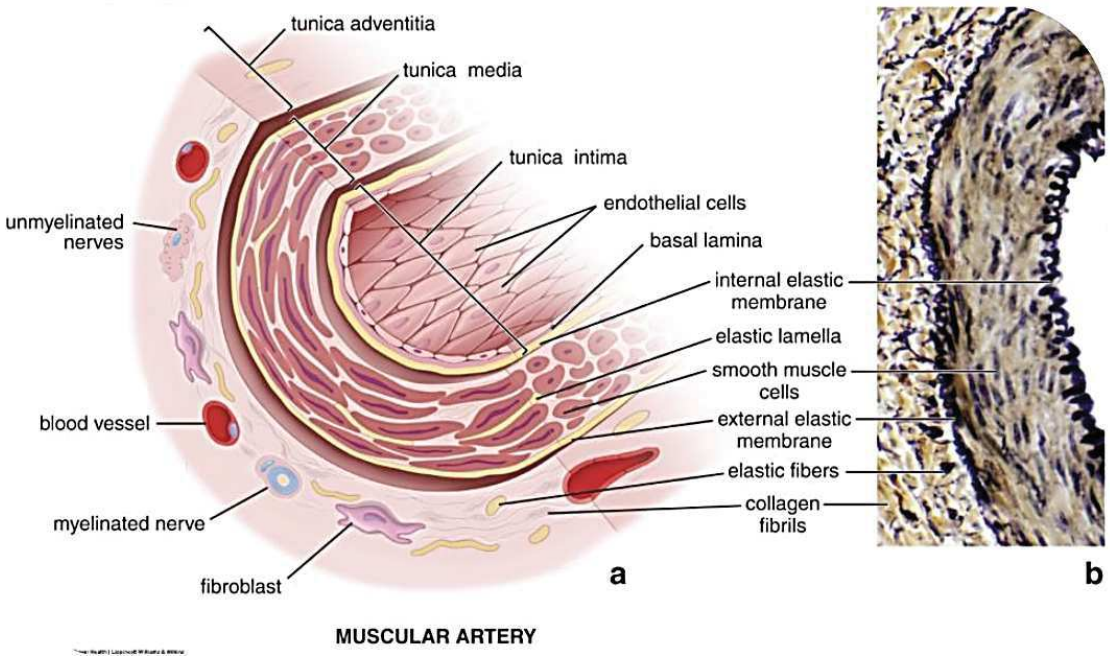


Verhoeff's Trichrome Stain
A – Elastic lamellae (**black**)
B – Smooth muscle (**dark red/purple**)
C – Collagen (**blue**)

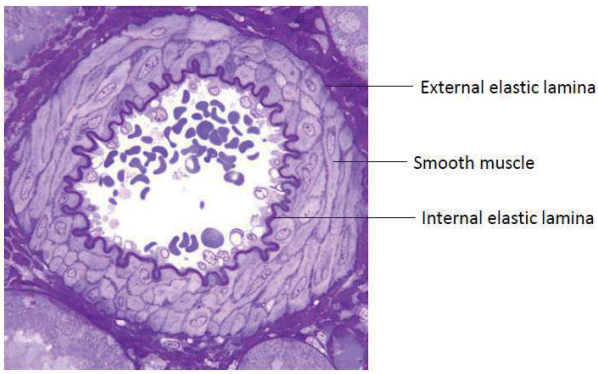


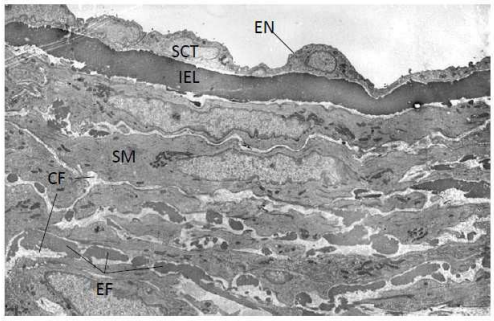
Masson Trichrome Stain
Blue – collagen
Dark red/purple – elastic fibers, nuclei
Red/pink – cytoplasm, muscle fiber

b. Muscular Artery



- Also called **distributing arteries**
- Examples: femoral artery, coronary arteries
- Function: <u>distribute</u> blood to various parts of body
- Features:
  - □ Relatively thick wall (25% of diameter)
  - □ Tunica media has a predominance of smooth muscles (→ regulate blood flow)
- Histological layers:
  - □ **Tunica intima**:
    - → Endothelium
    - → Flattened Subendothelial layer of collagen and elastic fibres
    - → Predominant internal elastic lamina

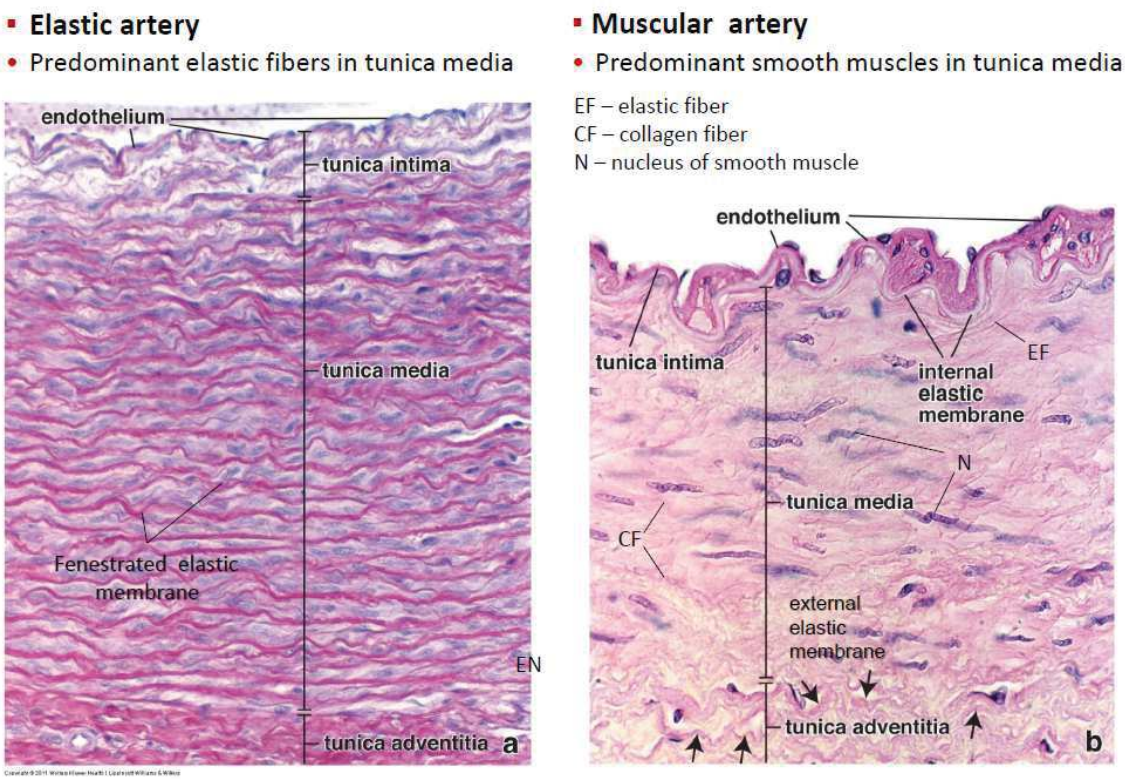




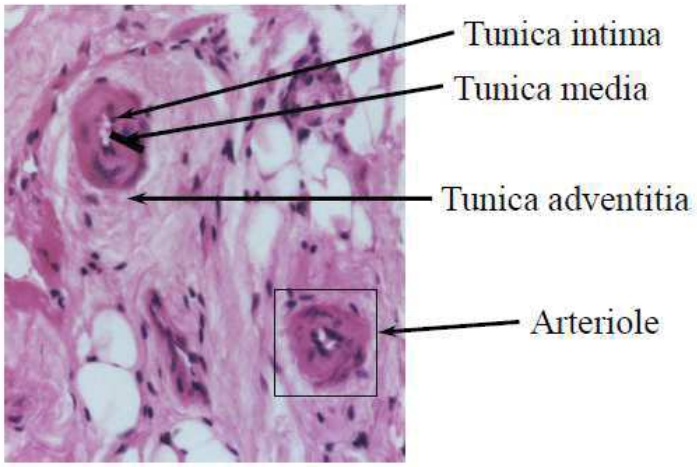
EN – endothelial cell
SCT – Subendothelial connective tissue
IEL – internal elastic lamina
SM – smooth muscle
EF – elastic fiber
CF – collagen fiber

- **Tunica media**:
  - → Thick smooth muscular layer with 10-40 circular layers of smooth muscle
  - → Much less elastin
  - → External elastic lamina present only in larger muscular arteries
- **Tunica adventitia**:
  - → Broad
  - → Mostly contains collagen, elastin and vasa vasorum

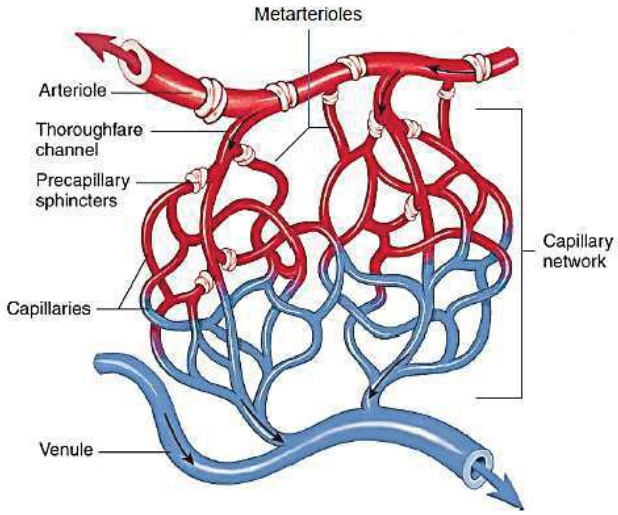
*Comparison between elastic and muscular arteries



c. Arterioles

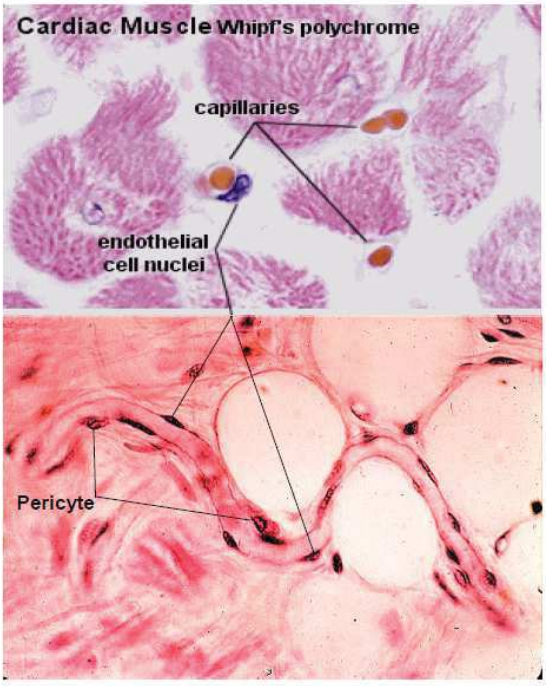


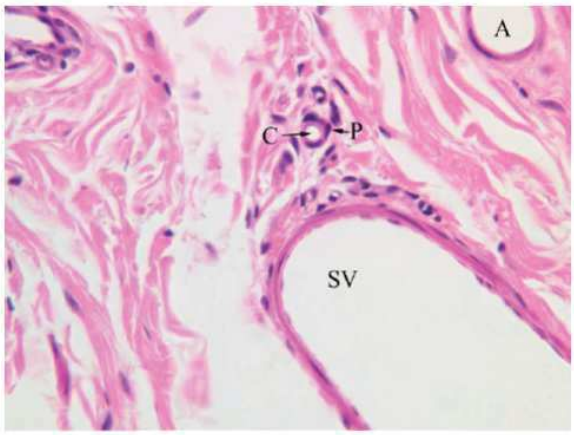
- **Arterioles**: small muscular arteries with diameter 0.04 – 0.4 mm
- Function: deliver blood and regulate blood flow to and in capillaries
- Retain general features of muscular arteries (but with much less layers of smooth muscles)
- Regulate blood flow through capillaries by **metarterioles**:
  - **Metarterioles**: vessels connecting small arterioles to capillaries
  - Metarterioles surrounded by **precapillary sphincter** smooth muscle at metarteriole-capillary junctions
  - Contraction and relaxation of precapillary sphincter
    → Control blood flow to capillary bed
    → Alter BP by altering peripheral resistance to blood flow (peripheral resistance vessels)



- Histological layers:
  - **Tunica intima**: very thin sub-endothelial layer of loose c.t.
  - **Tunica media**: 1-3 layers of smooth muscle (easiest way to recognize an arteriole)
  - **Tunica adventitia**:
    → Fairly prominent
    → Much thicker in venule than in arteriole
    → Continuous with surrounding c.t.

2. Capillaries

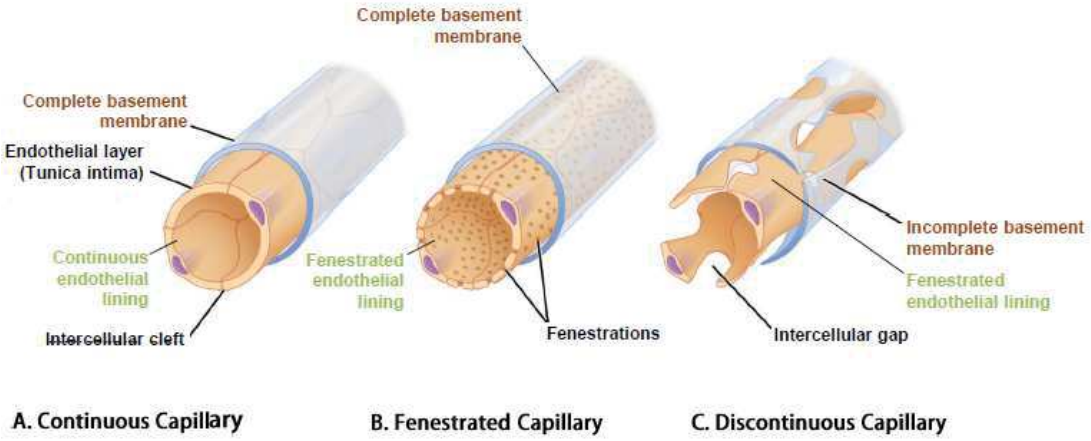




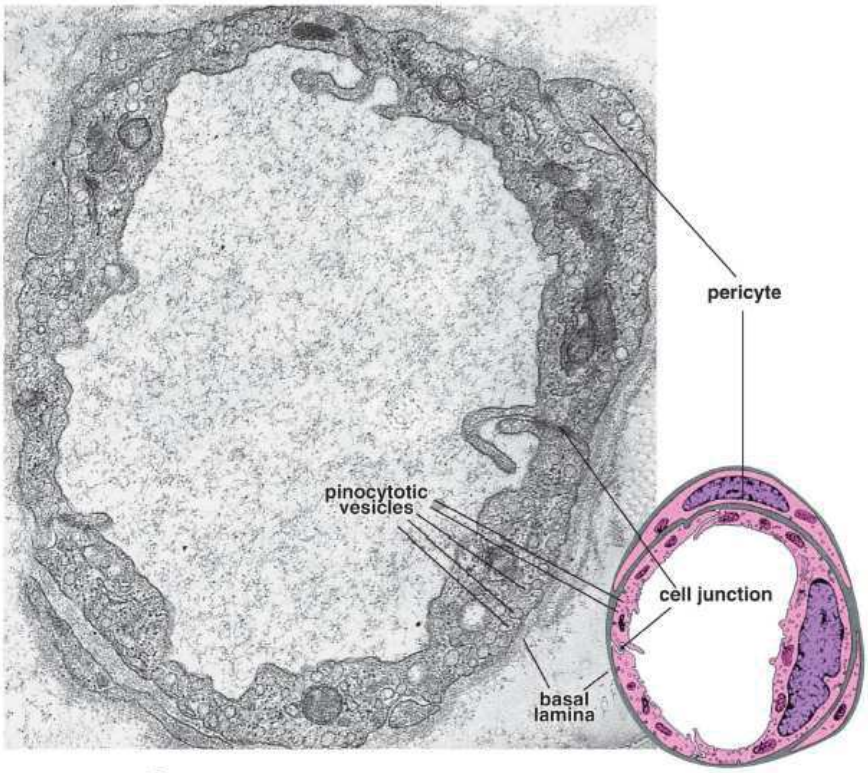
A – Arteriole
C – Capillaries
P – Pericyte
SV – Small Vein

- Usually 8-10 μm in diameter
- Connects arterioles to venules
- Wall consists of a single layer of endothelium surrounded by a basement membrane
- Scattered **pericytes** form discontinuous layer external to endothelium
  - Found between basement membrane and endothelium
  - Function: stem cells differentiating into smooth muscle cells or endothelial cells
- Function: allow exchange of nutrients and wastes between blood and tissues via passive diffusion and pinocytosis
- **Diapedesis**: WBC movement through intercellular junctions
  - Function: repair, fight infection and cancer metastasis

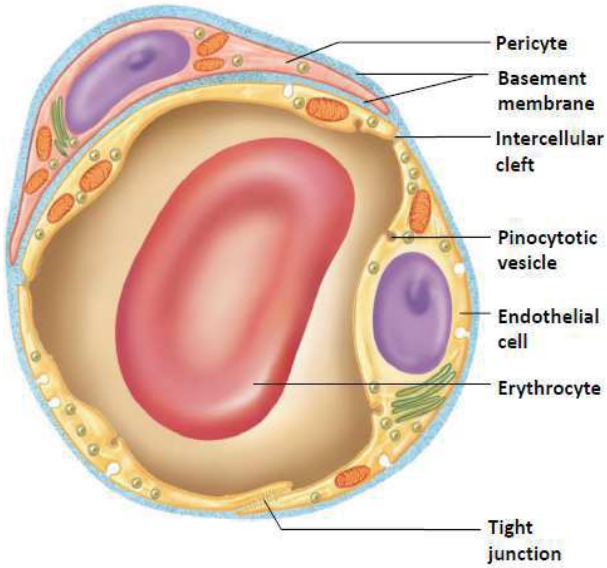
- Three types of capillaries:
  - **Continuous capillaries**
  - **Fenestratesd capillaries**
  - **Discontinuous capillaries (sinusoids)**



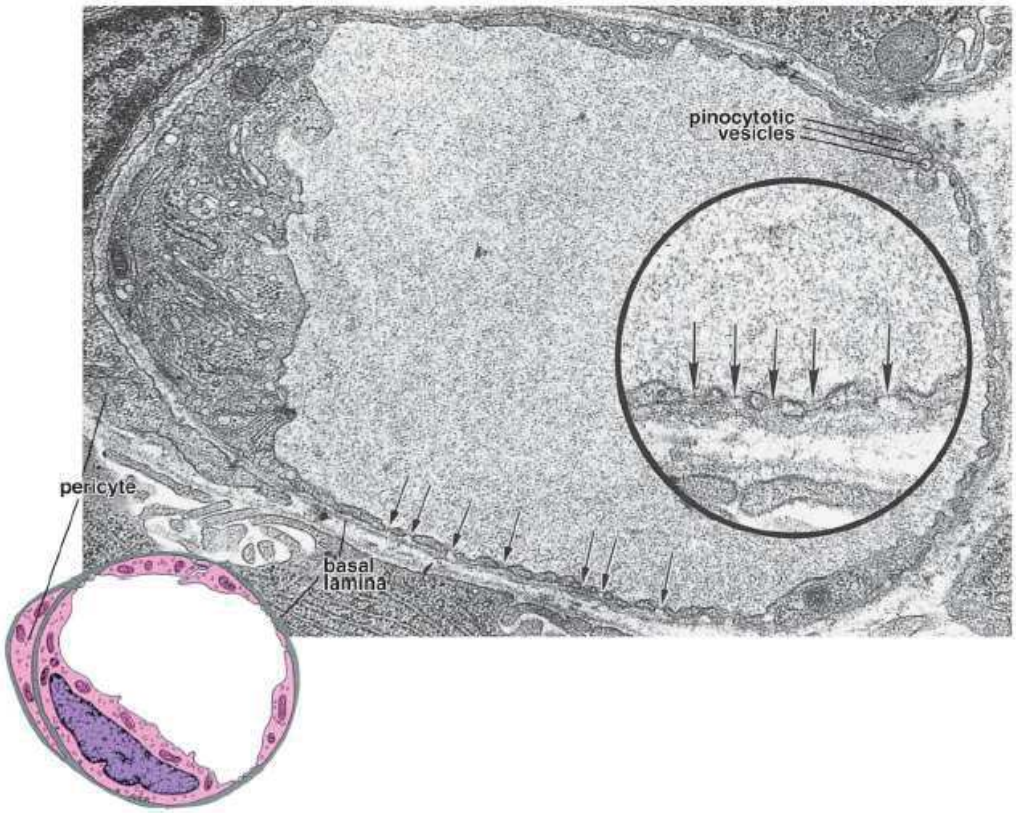
a. Continuous Capillaries



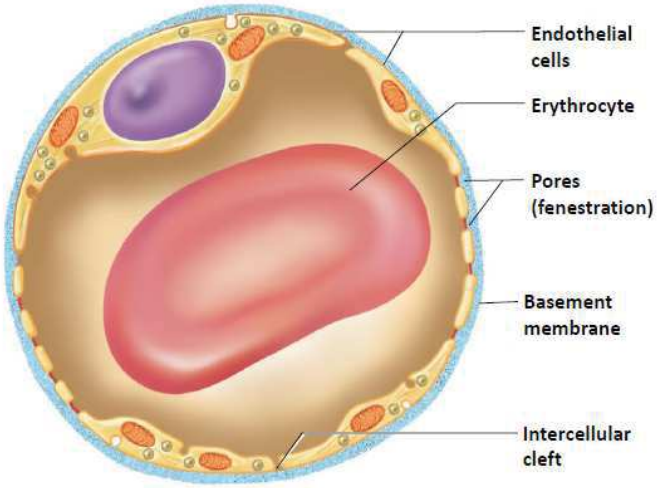
- Most common type of capillaries
- Found in muscle, fat, c.t., lungs and brain
- Features:
  - □ <u>No pore</u> or spaces between cells in walls
  - □ <u>Tight junctions</u> between adjacent endothelial cells
  - □ <u>Continuous basement membrane</u>
- Macromolecules pass through endothelial cells by **pinocytic vesicles**
- Cells move in and out by **diapedesis**
- Forms the **blood-brain barrier** in the brain (relatively impermeable)



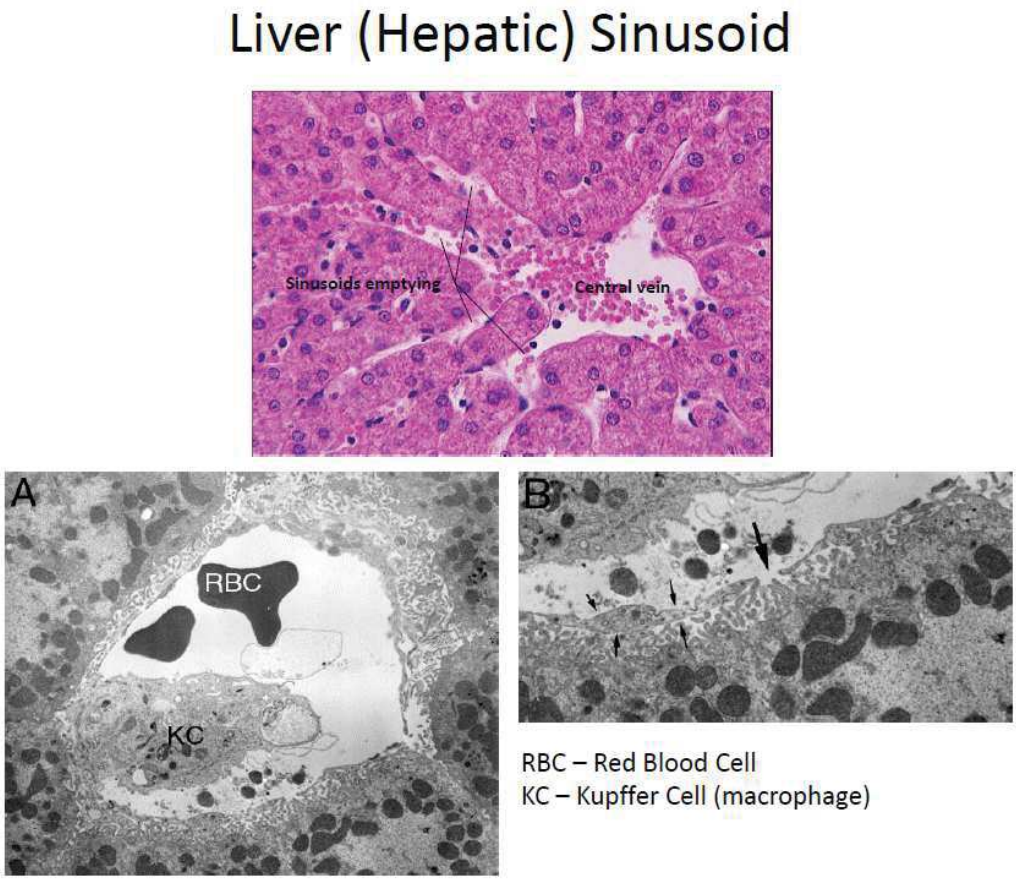
b. Fenestrated Capillaries



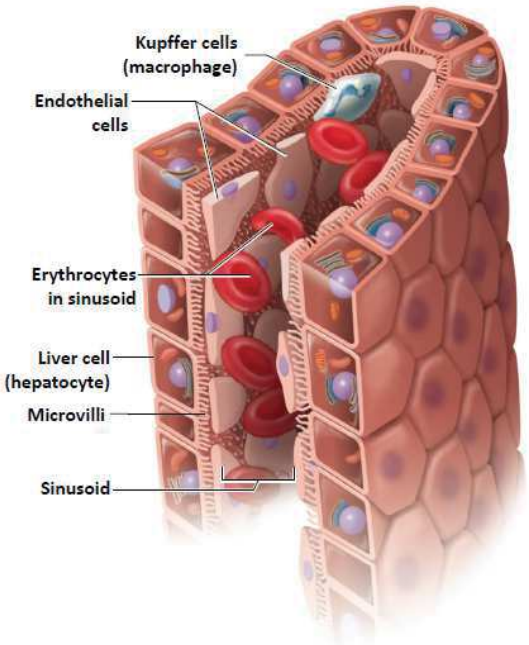
- Found in tissues where <u>rapid exchange of substances</u> occurs between tissues and blood
- Examples: kidney glomerulus, intestinal villi
- Have <u>pores penetrating endothelial cells</u> covered by a thin <u>diaphragm</u> (i.e. basement membrane, not found in kidney)
- <u>Continuous basement membrane</u>



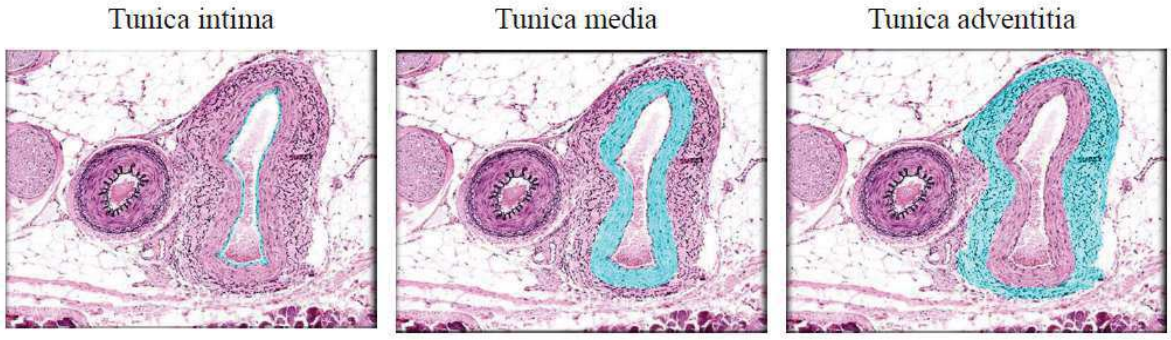
c. Discontinuous Capillaries (Sinusoids)



- Larger (30-40 μm) and more irregularly shaped
- Found in liver and haematopoietic organs (eg spleen and BM)
- Do not form a continuous lining between lumen and surrounding tissues (i.e. gaps found between adjacent endothelial cells without tight junctions)
- Basement membrane discontinuous or absent
- Endothelium poses no barrier to blood constituents
- Function: allow small and medium-sized proteins such as **albumin** to enter or leave blood
- Phagocytic cells (eg **Kupffer cells** in liver) are present



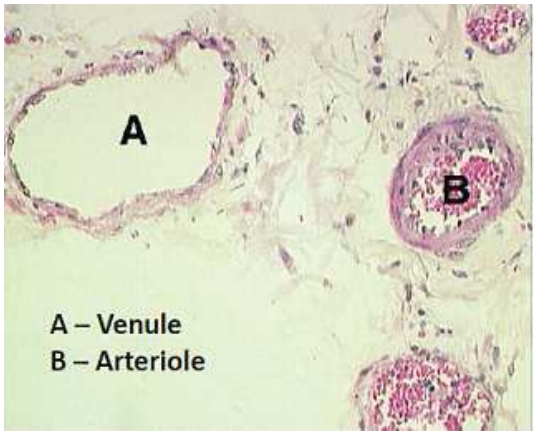
3. Veins



- Larger lumina (*sing.* lumen) and thinner walls than arteries
- Consists of 3 basic layers (tunica intima, tunica media, tunica adventitia)
- Less elastic and muscular components
- Collapsed wall (thinner and less elastic fibres)
- Medium and large veins with valves

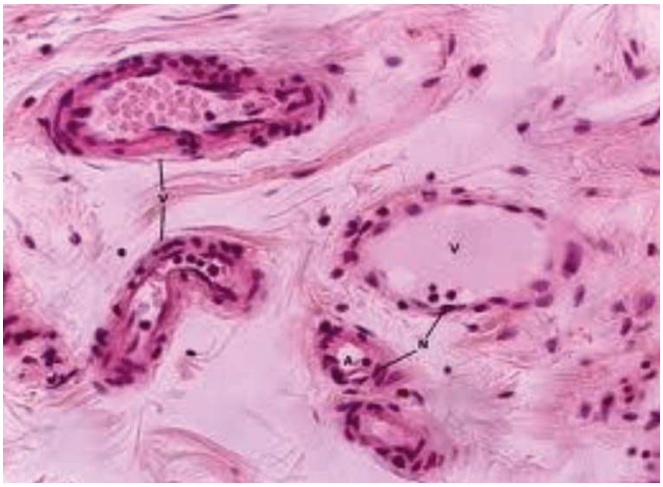
a. Venules

- Formed from confluence of capillaries (0.2-1 mm diameter)
- Histological layers: inconspicuous
  - **Tunica intima**: lacks subendothelial layer of c.t.
  - **Tunica media**: 1 or 2 layers of muscle fibres (↑ with size)
  - **Tunica adventitia**: fuses with surrounding tissues

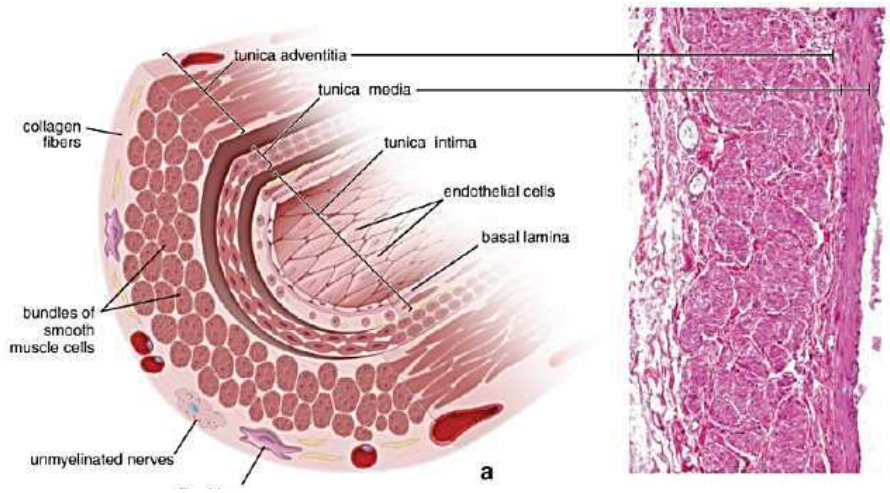


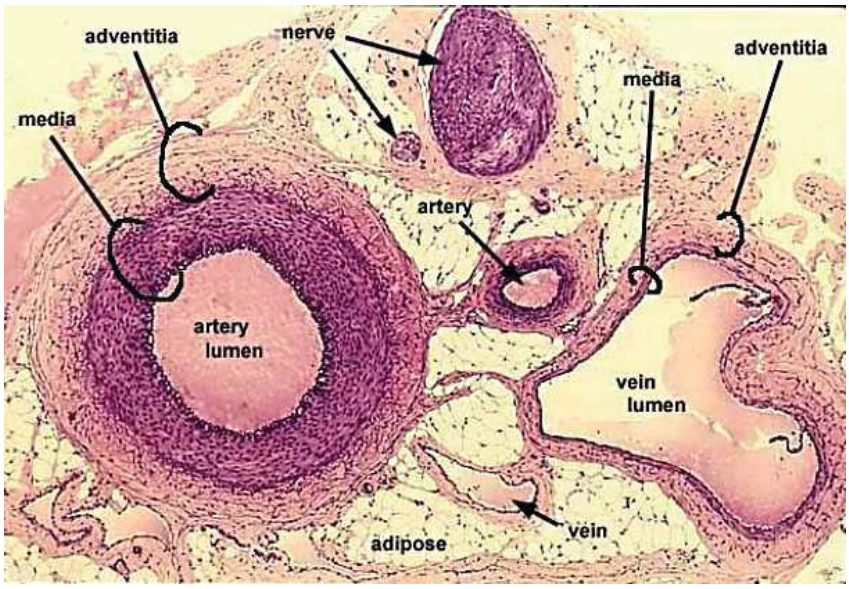
b. Small Veins

- 1-9 mm in diameter
- Histological layers:
  - **Tunica intima**: thin, very little supportive c.t.
  - **Tunica media**: contains 2-4 layers of circular smooth muscles
  - **Tunica adventitia**:
    → Broadest, well-developed layer
    → Rich in longitudinal collagen fibres



c. Medium and Large Veins





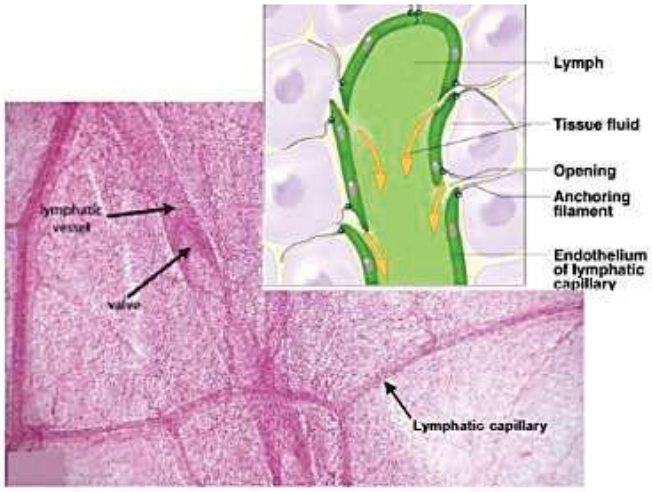
- All three tunics present
- Folds in endothelium forming **valves** (→ prevents backflow of blood)
- Histological layers:
  - □ **Tunica intima**: thin but well-developed
  - □ **Tunica media**:
    - → Muscular
    - → A few circular layer of smooth muscles
    - → Thin but can still regulate diameter (∵ low BP in veins)
  - □ **Tunica adventitia**:
    - → Thickest and best developed (predominant) layer
    - → Spirally arranged collagen and elastic fibres
    - → Longitudinal smooth muscles
    - → More vasa vasorum than arteries

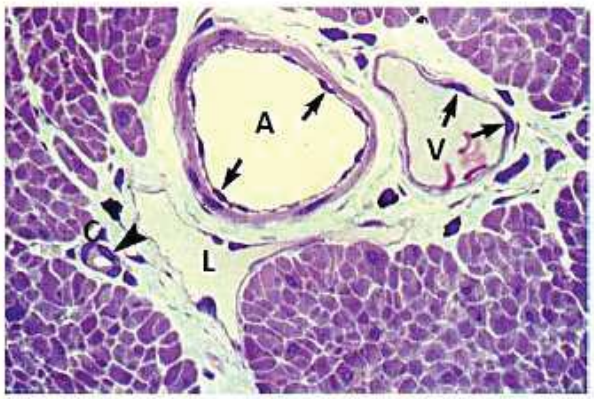
4. Lymphatic Vessels

- **Lymphatic vessels**: thin, endothelial-lined tubes that drains interstitial fluid

a. Lymphatic Capillaries

- Resemble blood capillaries but differ in the following aspects:
  - Begin as blinding-ending vessels
  - Incomplete or absence of basement membrane
  - Absence of pericytes
  - Larger, irregularly shaped lumina
  - No tight junctions
  - **Anchoring filaments**: contraction allows flow of tissue fluid into lymph vessels via opening between adjacent endothelial cells
  - No RBCs





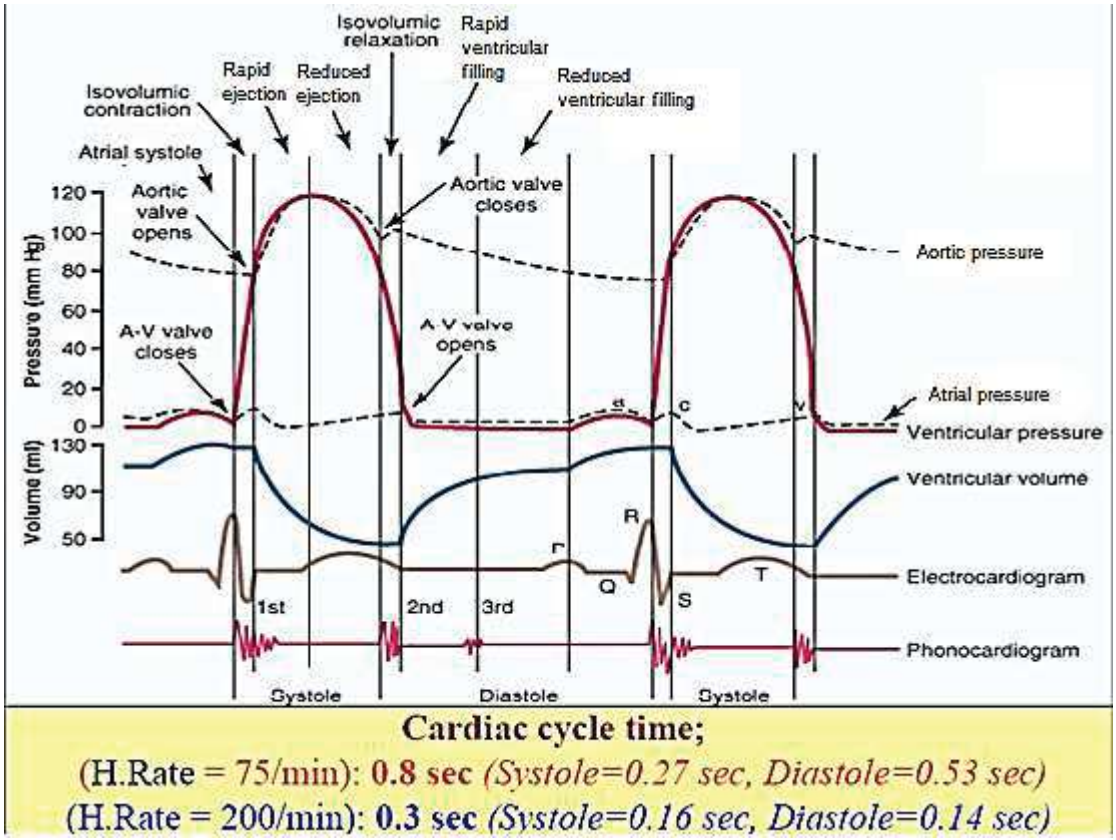
- Smaller vessels converge into larger vessels, returning tissue fluid back to veins
- Carries immune cells (eg lymphocytes)

b. Medium and Large Lymphatic Vessels

- Resemble venules and veins
  - Consists of three basic layers
  - With much less smooth muscles, elastic fibre and collagen fibres
  - Have valves
- Difficult to distinguish from small or medium-sized veins except absence of RBC in lumen

L69 The Cardiac Cycle

A. Phases of Cardiac Cycle



- At **resting heart rate** = 75 min^{-1},
  - 1 cardiac cycle = 0.8 s
  - 0 – 0.3s: **systole** (contraction)
  - 0.3 – 0.8s: **diastole** (relaxation)
- There are 7 phases in cardiac cycle:
  1) **Atrial systole**
  2) **Isovolumetric contraction**
  3) **Rapid ejection**
  4) **Reduced ejection**
  5) **Isovolumetric relaxation**
  6) **Rapid ventricular filling**
  7) **Reduced ventricular filing**
- Process identical and happens simultaneously in both sides of heart
  - Volume and atrial pressure changes very similar
  - Max RV pressure only about 25 mmHg (∵ weaker contraction)
  - Pulmonary arterial pressure correspondingly lower

1. Atrial Systole

- Depolarization initiated at **sinoatrial node (SA node)** → spreads throughout both atria → atria contract
- **a wave**: atrial pressure ↑ 5 mmHg
- Complete last 20-30% of ventricular filling through the open AV valves → small ↑ ventricular volume, pressure

2. Isovolumetric Contraction

- Ventricles contract → V-pressure > A-pressure → AV valve closed
- Semilunar valves still closed (∵ V-pressure < aortic pressure)
- No flow into and out of ventricle → ventricular volume cannot change
- Compression of blood → rapid ↑ ventricular pressure
- **c wave**: slight ↑ atrial pressure (∵ AV valve bulges backward into atrium)

3. Rapid Ventricular Ejection

- Ventricular pressure > aortic pressure → semilunar valve opens → blood rapidly ejected from ventricle into aorta
- Ventricular pressure remains slightly > aortic pressure throughout this phase
- Atrial pressure initially ↓ (**x descent**, ∵ AV valvular ring pulled downwards by ventricular contraction) → then gradually ↑ (**v ascent**, ∵ continuous venous return)

4. Reduced Ventricular Ejection

- Cytoplasmic Ca^{2+} level of myocardium begins to drop (∵ reuptake into SR) → ventricles stop contracting → ↓ ventricular pressure
- Aortic pressure also ↓ due to runoff of blood to periphery (but not as fast as ventricular pressure ∵ elasticity)
- Ventricular pressure < aortic pressure but outflowing blood has high KE → ejection continues at a decreasing rate
- When negative pressure gradient finally draws ventricular ejection to a halt, semilunar valve will close

5. Isovolumetric Relaxation

- Ventricles continue to repolarize and relax
- Both AV and semilunar valves closed → ventricular pressure ↓ rapidly

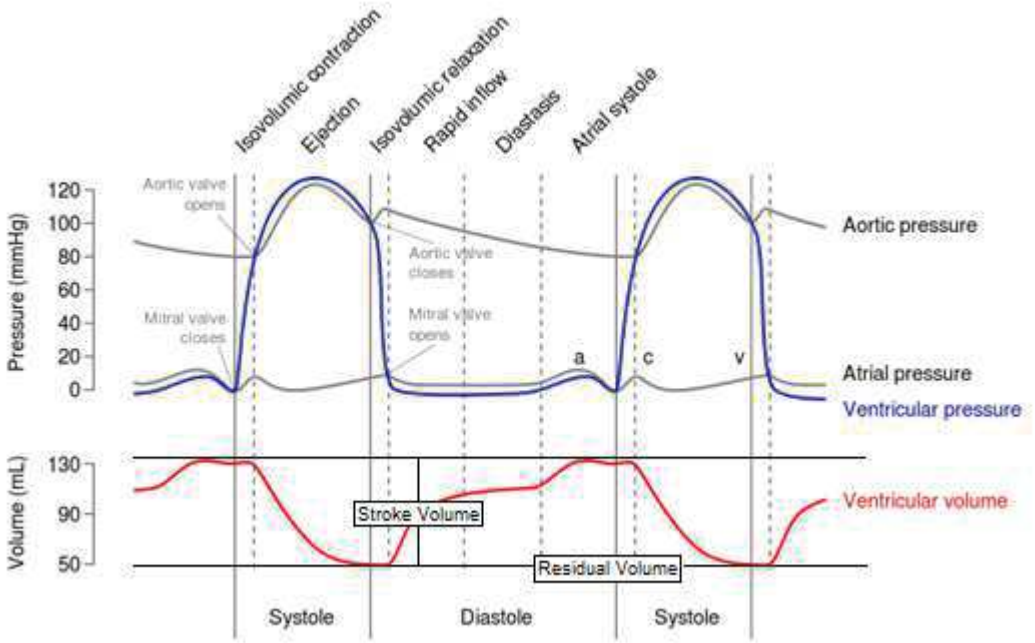
6. Rapid Filling

- Venous return filled atria throughout ventricular systole
- When ventricular pressure < atrial pressure → AV valves open → blood rushes into ventricle from atrium rapidly → ↓ atrial pressure (**y descent**)
- Occupies 1st 1/4 of diastole

7. Slow Filling

- Passive blood flow from major veins into atria and then into ventricles

B. Ventricular Volume



- **Stroke volume** = **End-systolic volume** of LV – **end-diastolic volume** of LV
- **Residual volume** = **End-diastolic volume** of LV

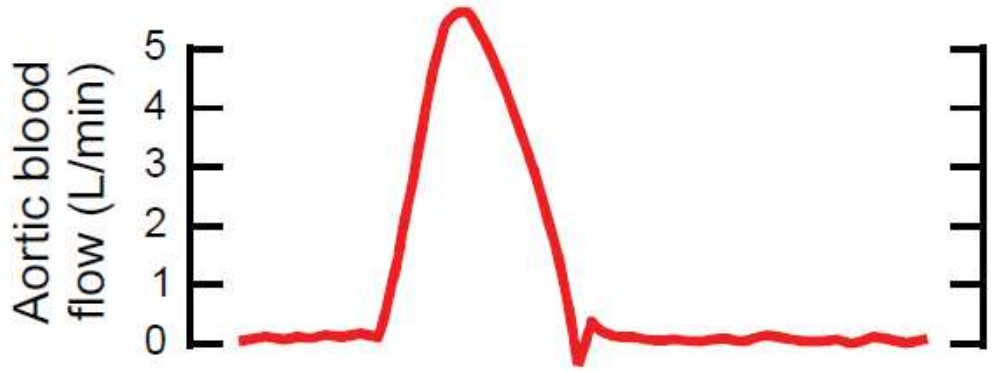
1) Atrial systole: ↑ to ~120-130mL
2) Isovolumetric contraction: -
3) Rapid/reduced ejection: ↓ to ~50mL
4) Isovolumetric relaxation: -
5) Rapid/reduced filling: ↑ to ~100mL

C. Function of Atria

- Atrial contraction is weak → only account for 20% of ventricular filling
- Main function: <u>passive conducting chamber</u>
  - Storage of continuous venous return when AV valve is closed
  - Stretching of atrial wall → ↑ atrial pressure → create pressure gradient for rapid/reduced filling when AV valve is open

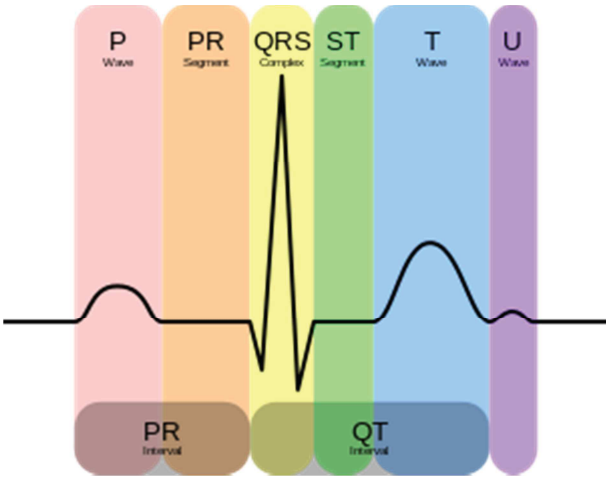
D. Aortic Pressure and Blood Flow

- Mainly determined by volume o blood in aorta → extent of arterial wall stretching
- Aortic pressure at maximum at the end of rapid ejection phase, then decrease gradually until next ejection phase
- **Dicrotic notch** or **incisura**: small amount of blood flows backward in aorta when aortic valve closes
  - Some KE of blood converted to PE as in ↑ pressure and wall tension
  - Can be observed in a small dip in aortic blood flow graph:



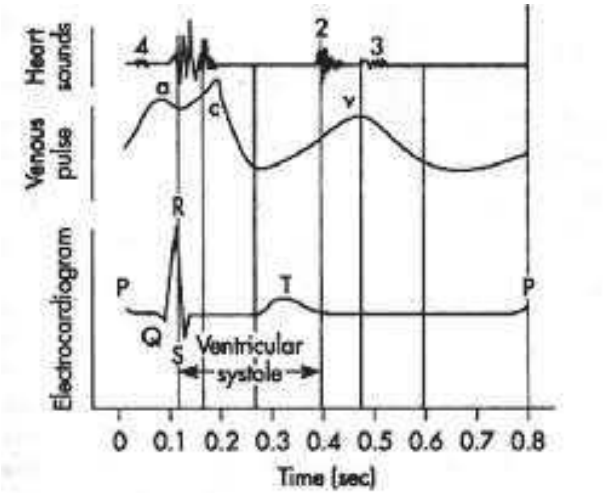
E. Electrocardiogram (ECG)

- Measured by placing electrodes on skin to measure current flow due to action potential in heart
  - Heart action potentials last for ~250ms (cf 10-20ms in nerves)



- **P wave** corresponds to <u>atrial depolarization</u> just before atrial contraction begins
- **QRS complex** corresponds to <u>ventricular depolarization</u> and begins just before isovolumetric contraction
- **T wave** corresponds to <u>ventricular repolarization</u> during reduced ejection

F. Heart Sounds



- **Heart sounds** are sounds from chest wall arising from events that cause vibrations in heart
  - Rigid structures vibrate better than soft structures (eg contracted heart muscles vibrate better than relaxed heart muscle)
- **First heart sound**: closure of AV valves and tensing of valves and muscles during contraction
  - Vibration arising from AV valve transferred to contracted myocardium and taut chordae tendinae (relatively rigid structures)
- **Second heart sound**: closure of semilunar valves
  - Occurs when ventricular muscle is beginning to relax → does not vibrate much
  - Vibration mainly in valve structure itself → shorter duration of sound
  - During inspiration → ↑ pressure difference → ↑ venous return to right heart → ↑ time required to empty right side of heart → pulmonary valve may close slightly after aortic valve → split second heart sound
- **Third heart sound**: rapid filling
  - Vibration of ventricles or valves due to impact of rapidly inflowing blood
  - Vibration is small (→ soft sound) because structures are relaxed
  - Normal in children
- **Fourth heart sound**: atrial contraction
  - Normally only heard if atrial pressure is abnormally high or ventricle is unusually stiff

L70 Control of Blood Flow

A. Factors Controlling Rate of Blood Flow

- Blood flow depends on:
  - **Pressure gradient** dictating the driving force
  - **Resistance** dictating the 'difficulty' for blood to flow
- **Poiseuille equation** describes the relationship between various factors controlling rate of fluid flow through a vessel

$$Q = \frac{\pi \Delta p r^4}{8\eta l}$$

  - Prerequisite: fluid is uncompressible and Newtonian
  - Q: flow
  - Δp: pressure difference along the length of vessel
  - r: radius
  - η: viscosity of fluid
  - l: length of vessel
- Flow = pressure/resistance $\rightarrow$ resistance = $\frac{8\eta l}{\pi r^4}$

1. Control of Blood Vessel Radius

- Radius is most important factor for controlling flow
- Importance:
  - Small change will have a large effect on blood flow ($\because r^4$)
  - Can be actively controlled
- Vascular resistance mainly resides in small muscular blood vessels:
  - **Pre-capillary resistance**: 2/3 of resistance originated from arterioles
  - **Post-capillary resistance**: 1/3 of resistance from venules
  - Smooth muscles arranged spirally around these vessels: contraction reduces radius and increases vascular resistance

a. Modification of Blood Vessel Radius

i. Passive Influences

- **Transmural pressure**: $P_{in} - P_{out}$
  - Blood vessel wall is stretchable $\rightarrow$ transmural pressure alters radius
- Radius depends on **transmural pressure** and **compliance** (stretchiness)
- Factors affecting compliance:
  - Thickness of wall: thick wall < thin wall
  - Composition of wall: collagen < elastin
  - Neurological influences: vasoconstricted < vasodilated
  - Pathology: eg atherosclerosis (↓), hypertension

ii. Active Influences

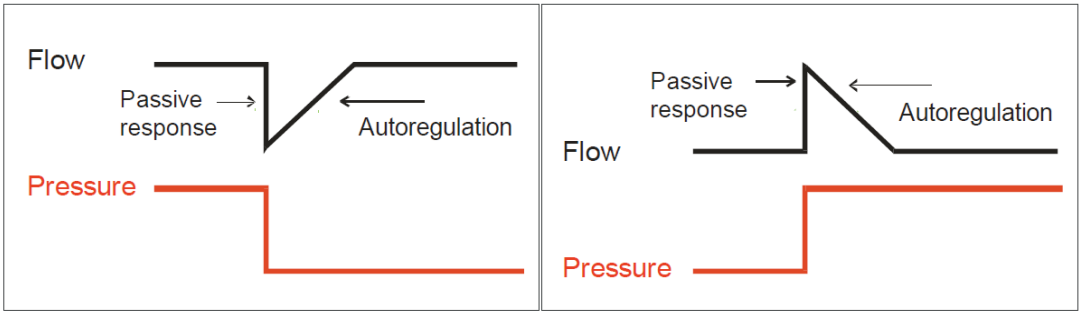
- **Active influences**: factors modifying extent of vascular smooth muscle contraction
- Two types of factors affecting vascular smooth muscle contraction:
  - **Extrinsic factors**: centralized initiation by neural or hormonal factors
  - **Intrinsic factors**: negative feedback to match blood supply to specific need of an organ or tissue
- Effect varies in different tissues and organs

(1) Extrinsic Factors

- **Extrinsic factors**: factors that modify blood flow so as to achieve an <u>appropriate distribution of blood flow between different organs and tissues</u> and enable organism to adapt to different situations:
- Two types of **neural control**:
  - Sympathetic control:
    - → **Sympathetic (constrictor) nerves** present in all arterioles
    - → Sympathetic nerves release **noradrenaline** → α receptors → contraction of vascular smooth muscles
    - → Removal of sympathetic tone → ↑ compliance → ↑ radius
    - → Alteration of sympathetic noradrenergic tone responsible for most day-to-day extrinsic control
  - Parasympathetic control:
    - → **Parasympathetic (dilator) nerves** present in very small number of blood vessels
    - → Parasympathetic cholinergic nerves release **acetylcholine** → cholinergic receptors → relaxation of vascular smooth muscles → ↑ compliance → dilatation
- **Hormonal control**: eg. adrenaline, angiotensin, ADH all constricts vascular smooth muscles
- Generally speaking,
  - **Neural control** responsible for distribution of blood flow between different organs
  - **Hormonal control** produces global responses with similar effects on most blood vessels

(2) Intrinsic Factors

- **Intrinsic factors**: negative feedback mechanisms to match supply of blood to needs of particular organ or tissue → **autoregulation**



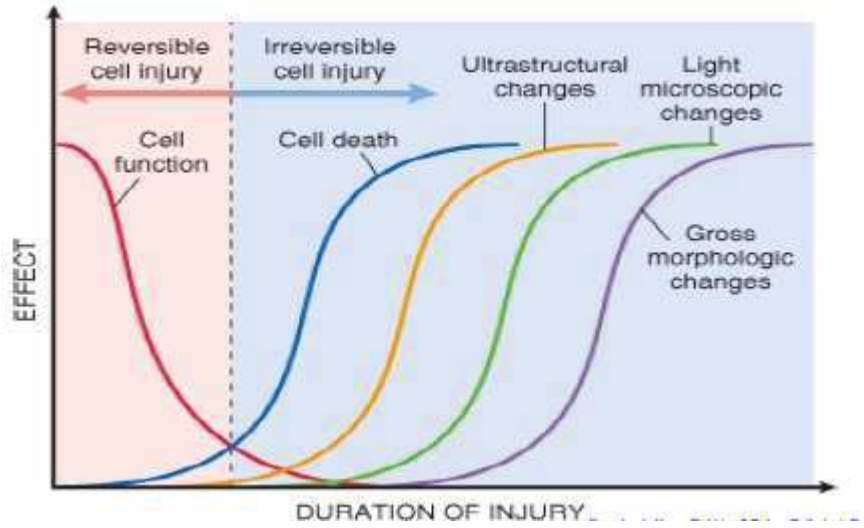
- **Myogenic autoregulation**: some vascular muscle responds to stretch by contracting back to its original size
  - **Myogenic contraction**: myocyte contraction generated by myocyte itself
  - Myocyte stretched → spontaneous release of Ca^{2+} by myocyte → membrane depolarization → myogenic contraction
- **Metabolic autoregulation**: waste chemicals stimulate vasodilation to facilitate metabolic demands
  - Decrease in blood flow → ↓ O_2 supply → ↑ metabolite production
  - ↑ metabolite production + ↓ removal → ↑ interstitial metabolite concentration
  - ↑ metabolite concentration → vasodilation → ↑ blood flow
  - Blood flow matched to metabolic demands
  - Examples: lactate, CO_2, adenosine (all vasodilators)

L71 Cell Injury and Cell Death

A. Causes of Cell Injury

- **Hypoxia**: lack of oxygen leading to cell injury
  - Causes:
    - → **Ischaemia**: inadequate blood supply (most common)
    - → Low blood O_2-carrying capacity
    - → Faulty intracellular oxidative enzyme
- Physical agents:
  - **Trauma**: physical harm from an external source
  - Change in atmospheric pressure
  - Temperature
  - Radiation
  - Electricity
- Chemical agents: poisons, drugs, alcohol
- Biological agents: bacteria, virus, fungus, parasite
- Immunological reactions: hypersensitivity states, autoimmunity
- Others: genetic defects, nutritional imbalance

B. Pathogenesis of Cell Injury

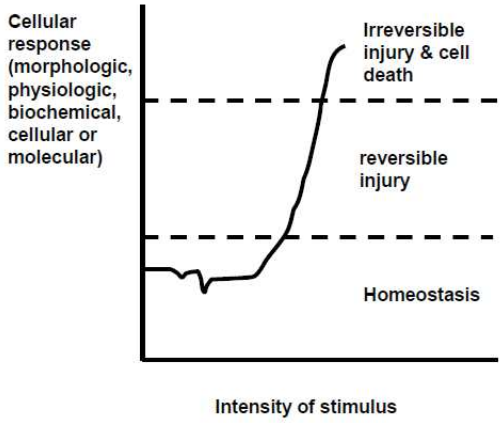


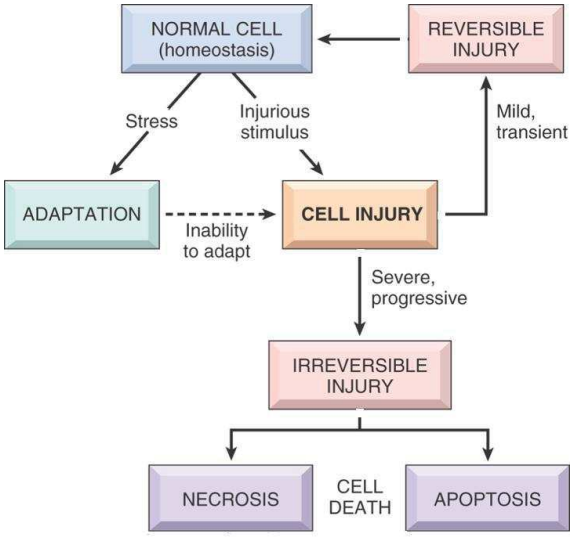
- Pathogenetic process:
  - All stresses and noxious influences exert effects first at the molecular or biochemical level:
  - Structural changes after biochemical disruption
  - Morphological changes detected by **histochemical** (stains, EIA etc.) or **ultralstructural** ('sub-LM' details) **techniques**
  - Morphological changes detected by LM or gross examination (hours to days)

- Vulnerable intracellular systems include:
  - Intracellular aerobic respiration
  - Cell membrane
  - Enzymatic and structural protein
  - Genetic apparatus

***Histology**: microscopic anatomy

C. Results of Cell Injury





- Results of cell injury depends on:
  - Type, duration and severity of injury
  - Type, state and adaptability of affectedcell
- Injury < adaptive response of cell → **reversible cell injury**
- Injury > adaptive response of cell → **irreversible cell injury** → cell death by **apoptosis** and **necrosis**

SUSCEPTIBILITY OF CELLS TO ISCHEMIC INJURY

High	**Neurons (3-5min)**
Intermediate	**Myocardium, hepatocytes, renal epithelium (30 min-2 hr)**
Low	**Fibroblasts, epidermis, skeletal muscle (many hours)**

D. Reversible Cell Injury

1. Intracellular Oedema

- Possible cause: cellular hypoxia → malfunctioning of Na^+/K^+/ATP pump → ion imbalance
- Features:
  - Derangement of cell membrane
  - Excessive influx of isotonic fluid into cell
  - Individual cell swollen with Na^+ and water ± vacuoles

2. Fatty Change

- Possible cause: inability to metabolize fat adequately due to:
  - Chemicals and toxins (esp alcohol)
  - Hypoxia
  - Starvation and wasting disease
  - Metabolic disorders (eg DM)
- Features:
  - Abnormal accumulation of fat within cells
  - Intracellular fat vacuoles ± displacement of nucleus
- Commonly found in liver, heart muscles and renal tubule

3. Hyaline Degeneration

- Deposition of glassy protein in tissue
- Feature: homogeneous glassy, pink alteration (can be found intracellularly or extracellularly)
- Eg. alcoholic liver disease, viral inclusion, **arteriosclerosis** (caused by benign hypertension)

4. Intracellular Accumulations

- **Lipofuscin**: oxidation product of unsaturated FA
  - Pigment of aging
- **Lysosomal storage disease**: defective lysosomal enzymes
  - May be caused by inborn error in metabolism)
  - Eg. glycogen accumulation
- **Haemosiderin**: iron storage proteins
  - May be caused by haemorrhage (∵ RBC lysis)
  - May be found in **haematoma** (bruise)

5. Sublethal Nuclear Damage

- Non-lethal damage to cell nucleus without any morphological changes
- May lead to:
  - Heritable disease (in germ cells)
  - Neoplasia (in somatic cells)

E. Irreversible Cell Injury

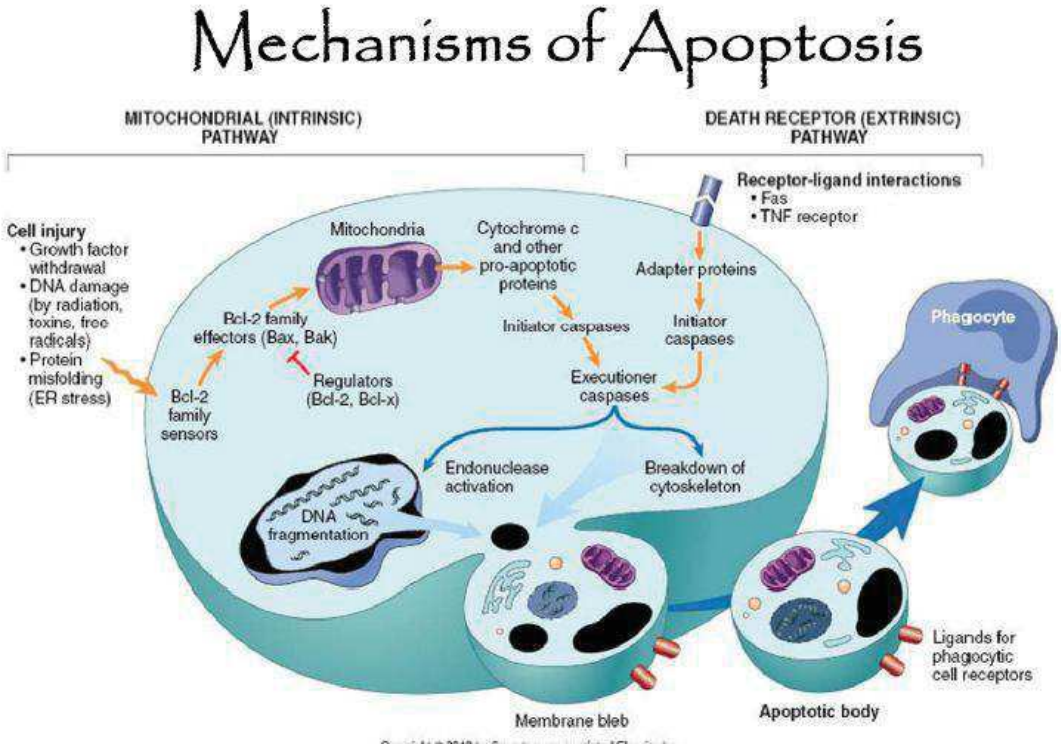
	Necrosis	Apoptosis
Cell size	Enlarged (swelling)	Reduced (shrinkage)
Nucleus	Pyknosis → karyorrhexis → karyolysis	Fragmentation into nucleosome-size fragments
Plasma membrane	Disrupted	Intact; Structure altered, (orientation of lipids)
Cellular contents	Enzymatic digestion; may leak out of cell	Intact; may be released in apoptotic bodies
Inflammation in adjacent tissue	Frequent	No
Physiologic or pathologic role	Always pathologic (culmination of irreversible cell injury)	- Often physiologic, elimination unwanted cells - may be pathologic after cell injury, especially DNA damage
Kumar, et al. Robbins & Cotran 8th Edition		

- Irreversible cell injury will result in cell death in two forms: **apoptosis** and **necrosis**

1. Apoptosis

- **Apoptosis**: programmed cell death of individual cells within living tissues
- No acute inflammation
- Tissue structure preserved
- May either be pathologic or physiologic:
  - Physiologic:
    - → Programmed cell destruction during embryonic development
    - → Normal cell turnover in adult organs
  - Pathologic:
    - → UV or ionizing radiation (eg radiotherapy)
    - → Cytotoxic T cells
    - → Cell-mediated immunity
    - → Drugs
    - → Tumour cell death

- Process:
  - Initiation via **mitochondrial (intrinsic)** or **death receptor (extrinsic) pathways**
  - Activation of 'executioner' **caspases**: proteolytic enzymes that is responsible for degradation of enzymes



1) Genetic change and **endonuclease** activity (to cleave DNA);
2) DNA fragmentation (200bp@);
3) Chromatin and cytoplasm condensation;
4) Cell body broken down into small **apoptotic bodies** (rounded or oval masses of intensely eosinophilic cytoplasm with dense nuclear chromatin fragment);
5) Phagocytosis by adjacent cells.

2. Necrosis

- **Necrosis**: death of sheets of cells involving disruption of tissue structure
- Two mechanisms:
  - **Autolysis**: structural disintegration due to digestion by lysosomal hydrolases of necrotic cells
  - **Heterolysis**: digestion by immigrant leukocytes
- Always pathological
- Nuclear changes:
  - **Pyknosis**: chromatin condensation → nuclear shrinkage
  - **Karyorrhexis**: cleavage of DNA (by endonuclease) → nucleus fragmentation
  - **Karyolysis**: dissolution of nucleus into cytoplasm
- Morphological types of necrosis: **coagulative**, **liquefaction**, **caseous**, **fat** and **fibrinoid** (each with its characteristic cause)
- Effects:
  - Loss of function
  - Release of cell contents
  - Acute inflammation
  - Effects of repair and regeneration
  - **Dystrophic calcification**: calcium deposition at degenerated or necrotic tissues
  - Infection

a. Coagulative Necrosis

- Most common type of necrosis
- **Infarction**: necrosis caused by deprivation of blood supply
- Usually in solid organs eg. myocardium, spleen, kidney
- Features:
  - Tissue architecture and cell outlines preserved with loss of nucleus
  - Conversion of cell to an acidophilic (red in H&E), opaque 'tombstone'
  - Presence of WBC to digest dead tissues

b. Liquefaction (Colliquative) Necrosis

- **Liquefaction (colliquative) necrosis**: ischaemic necrosis of brain
- Likely cause: bacterial infections
- Mechanism:
  - Powerful hydrolytic enzymes causes loss of tissue structure
  - Removal of cell debris → cystic spaces left
  - Eg. cystic infarct of brain

c. Caseous Necrosis

- Gross appearance: cream cheese appearance
- Histological appearance:
  - Disappearance of cellular outline
  - Tissue changed into amorphous mass surrounded by granulomatous wall
- Characteristic of tuberculosis (mechanism: granulomatous inflammation)

d. Fat Necrosis

- Fat necrosis of enzymatic origin
  - Cause: enzymatic
    - → Damage to lining to exocrine gland
    - → Release of enzymes → digestion of triglyceride in fatty tissues into fatty acids and glycerol
    - → FA reacts with Ca^{2+} to form soap
    - → Deposited as chalky white patches
  - Examples: acute pancreatitis, necrosis of pancreas
- Fat necrosis of traumatic origin
  - Cause: traumatic
    - → Lipid release from fat cells → chronic inflammatory and giant cell reaction → hard indurated mass
  - Examples: breasts (d/dx breast cancer, important non-tumor cause of breast lumps)

e. Fibrinoid Necrosis

- Tissue death accompanied by fibrin deposit (eg. collagen deposition)
- Examples:
  - **Rheumatoid nodule**: lumps felt at elbow or knees in **rheumatoid arthritis**
  - **Arthus reaction**: immune complex-mediated hypersensitivity reaction (deposition of Ag/Ab complex)
  - **Arteriolar lesions of malignant hypertension**

L72 Acute Inflammation

A. Acute Inflammation

- **Acute inflammation**: response of the body to injury
  - Goal: deliver defensive materials to a site of injury and restore homeostasis
  - Elements of blood leave the blood and enter tissues around damaged site
- Causes of inflammation:
  - Biological agents: bacteria, fungi and parasites
  - Physical agents: burns
  - Chemical agents: acids and dyes
  - Hypersensitivity

1. Clinical Signs of Acute Inflammation

- **Cardinal signs** of acute inflammation:
  - Redness
  - **Oedema**
  - Heat
  - Pain
- Gross features:
  - **Oedema**: swelling
    - → Due to fluid extravasation
    - → Finger leaves a depression after pressing into skin
  - **Erythema**: redness
    - → Due to vascular dilation
  - **Fibrinous exudate**: presence of thick strands of fibrin in exudate
  - **Purulent exudate**: presence of polymorphs and bacteria in exudate
- Histological feature:
  - Elevated neutrophil count in peripheral blood smear
    - → **Neutrophilia**: neutrophil count elevated from 4-8k/cm^3 to 12-20k/cm^3
  - Exudate with neutrophil and fibrin present in tissue

B. Inflammatory Cells

1. Neutrophils

- **Neutrophil** originated from bone marrow
- Cannot multiply and has a finite life span of 12-20 hours
- Primary function: **phagocytosis**
- Have cytoplasmic granules to complete phagocytosis:
  - **Primary** or **azurophilic granules** containing **myeloperoxidase** (forms hypochloric acid from H_2O_2 and Cl^-)
  - **Secondary** or **specific granules** containing antibacterial proteins (eg. lysozyme)
- Often phagocytosed by macrophages after phagocytosis

2. Lymphocytes

- Cells responsible for initiating the specific immune systems
- Morphology: small cells showng a dense nucleus and narrow rim of cytoplasm
- Three main types:
  - **B cells**: Ab production
  - **T cells**: initiation of CMIR and cytokine seretion
  - **NK cells**: non-MHC restricted cytotoxic cells

3. Eosinophils

- Morphology: cells with bilobed nucleus and show granules hat stain with eosin (orange colour)
- Eosinophil granules contain cationic proteins (eg major basic protein) for fighting parasitic infestation
- **Eosinophilia** (high eosinophil count in blood) is a manifestation of many allergic conditions

4. Monocytes and Macrophages

- **Monocytes** are produced in BM and leave blood to enter tissue to become **macrophages**
- Tissue **macrophages** have very long lifespan (months to years)
- Functions:
  - Phagocytosis
  - Antigen presentation
  - Secretion of inflammatory mediators
  - Induction of general effects of inflammation (eg fever)
- Can be activated by an antigen or stimulated to become **activated macrophages** (with enhanced secretion and killing ability)

C. Mediators of Inflammation

- **Mediators of acute inflammation**: serum proteins or chemicals which modulate inflammatory response
- Examples: histamine, complement, immunoglobulin, IL-1 and TNF-α

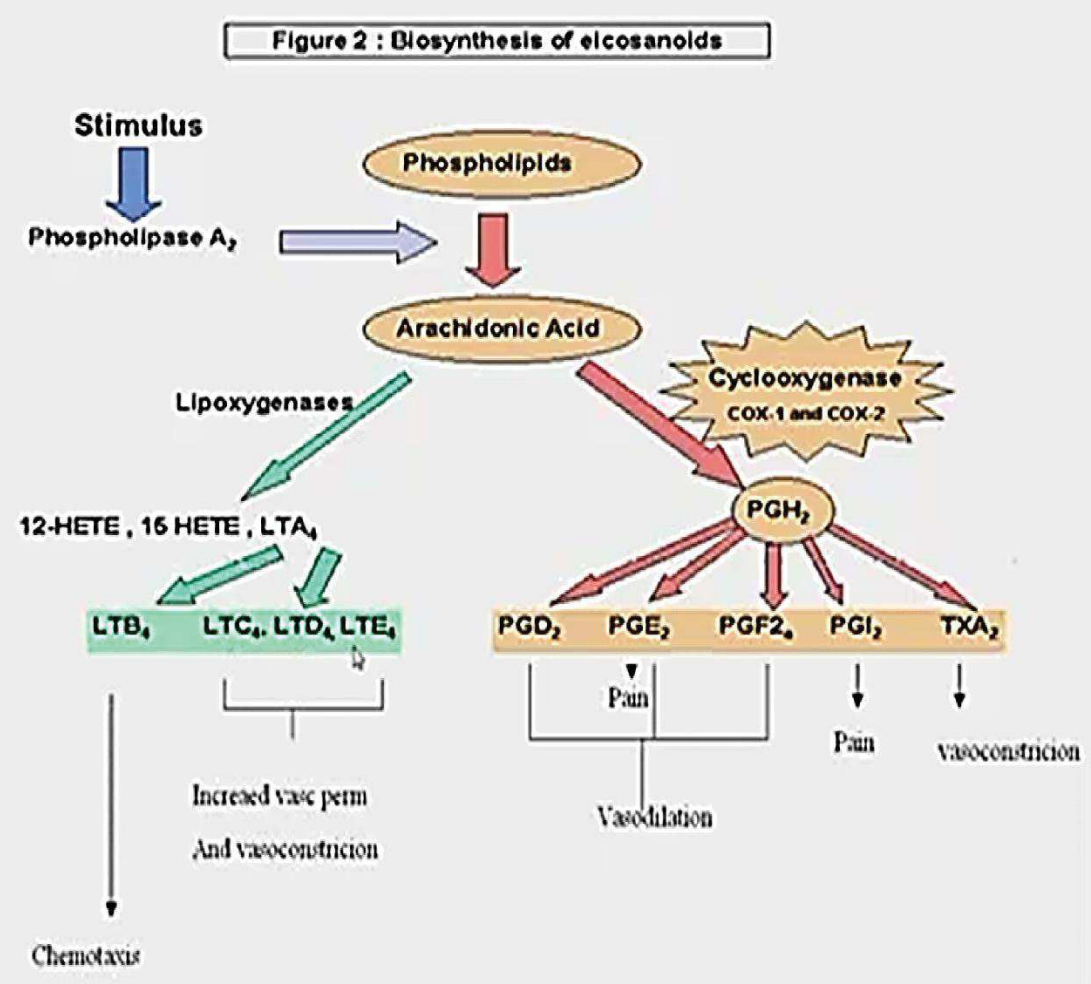
1. Cell-derived Inflammation Mediators

a. Vasoactive Amines

- **Vasoactive amine**: an amino-containing substance that is **vasoactive** (i.e. changes blood vessel permeability)
- **Histamine**: most important vasoactive amine composed of histidine residue
  - Released by **mast cells**
  - Dilates arterioles (but paradoxically contract large arteries)
  - Increases vascular permeability (via contraction of endothelial cells)

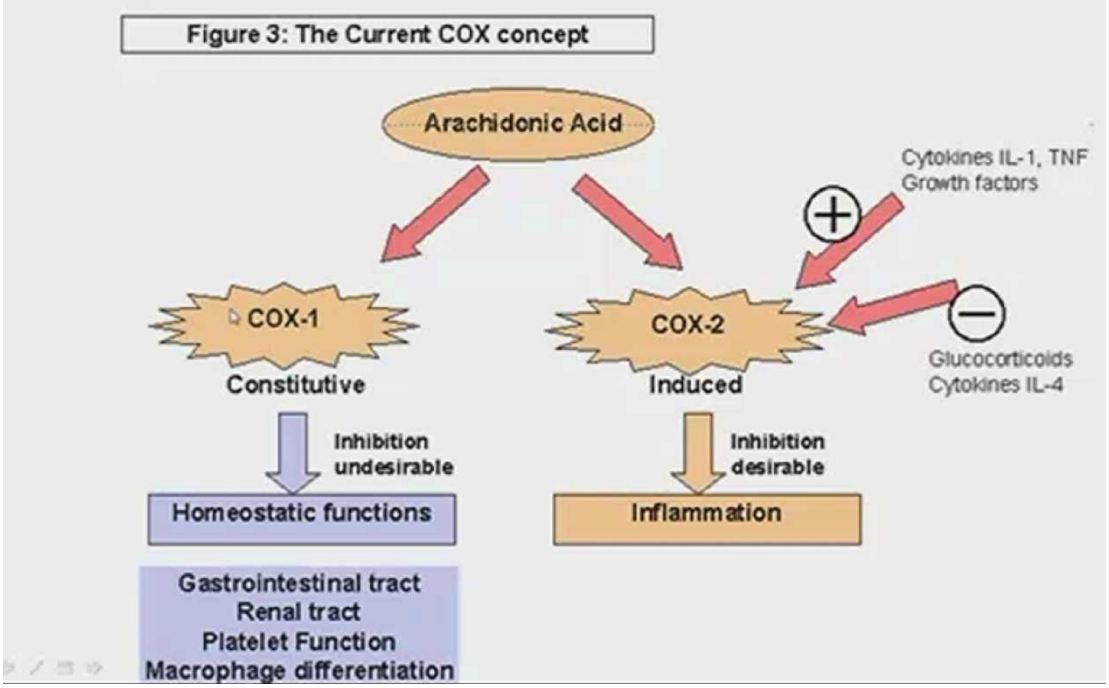
b. Eicosanoids

- **Eicosanoids**: lipid signaling molecules derived from **arachidonic acid**



- Inflammatory stimulus (eg trauma, cytokines) acts on phospholipase A_2 pathway which cleaves phospholipids on plasma membranes into arachidonic acid (20-C fatty acid)

- Arachidonic acid is metabolized into two different classes of lipid mediators:
  - **Leukotrienes (LT)**: oxidation products via **lipoxygenase (LOX)** pathway (active in leukocytes and macrophages)
    - → **Leukotriene B_4 (LTB_4)**: responsible for chemotaxis, ROS formation and lysosome enzyme release in neutrophils
    - → **Slow-reacting substance of anaphylaxis (SRS-A)** including LTC_4, LTD_4 and LTE_4: responsible for increase in vascular permeability and vasoconstriction
  - **Prostanoids**: oxidation products via **cyclooxygenase (COX)** pathway (active in almost all cells)
    - → **Prostaglandin D_2 (PGD_2)**: for vasodilation
    - → **Prostaglandin E_2 (PGE_2)**: for vasodilation and pain
    - → **Prostaglandin $F_{2\alpha}$ ($PGF_{2\alpha}$)**: for vasodilation
    - → **Prostacyclin (PGI_2)**: for vasodilation and pain (and inhibition of platelet activation)
    - → **Thromboxane A_2 (TXA_2)**: for vasoconstriction (and platelet aggregation)



- Note two types of COX pathways:
  - **COX-1**: constitutive pathway
    - → Responsible for physiological control of prostaglandin level
    - → Inhibition (eg by aspirin) is undesirable → impairment of some normal physiological functioning
  - **COX-2**: induced pathway
    - → Responsible for inflammatory or growth-related elevation of prostaglandin level
    - → Stimulated by growth factors and some cytokines (IL-1 and TNF)
    - → Inhibition (eg by corticosteroids and some other NSAIDs) desirable

i. Control of Fever by Prostaglandins

- Body temperature controlled and maintained at set-point by hypothalamus
- **Pyrogens** (such as endotoxins in Gram negative bacteria) triggers a cascade to elevate the thermoregulatory set-point:

1) **Pyrogens** (eg infectious agents, toxins, inflammatory mediators) stimulates monocytes/macrophages/endothelial cells or other cells to release **pyrogenic cytokines** (eg IL-1, TNF, IL-6, IFNs);
2) **Pyrogenic cytokines** stimulate **COX-2** pathway, leading to the release of **PGE_2**;
3) **PGE_2** acts on preoptic area of anterior hypothalamus to elevate thermoregulatory set-point;
4) Appropriate thermoconservatory responses are effected to elevate the core body temperature to the new set-point.

c. TNF-α and IL-1

- Produced by macrophages
- Functions:
  - Causes PG production in hypothalamus and thus fever
  - Increases expression of **adhesion molecules** on endothelium (→ facilitate neutrophil adhesion and migration)
  - Increase hepatic synthesis of **acute phase reactants**
    - → **C-reactive protein**: opsonin on microbes
    - → **Ferritin**, **haptoglobin**,…: binds iron to limit microbe iron intake

d. Platelet-activating Factor (PAF)

- **Platelet-activating factor (PAF)**: a shared pathway in thrombotic and inflammatory cascades
- Produced especially by cells involved in host defense (eg. platelets, endothelial cells, neutrophils, monocytes, macrophages)
- Functions:
  - Vasodilation
  - Oxidative burst
  - Chemotaxis
  - Arachidonic acid metabolism

e. Lysosomal Enzymes

- Normally used in digesting phagocytosed macromolecules
- Sometimes discharged to ECF (by **granulocytes**)
- Contains enzymes such as **hyaluronidase** leading to connective tissue damage
- Tissue damage further enhances release of inflammatory mediators

f. Oxygen-derived Free Radicals

- Function: raises oxidative stress in ECF
- Leads to tissue damage
- Further promotes release of inflammatory mediators

g. Nitric Oxide

- Released by macrophages, endothelial cells and some neurones
- Functions:
  - Vasodilation
  - Aids in leukocyte recruitment
  - Direct antimicrobial activity (at high conc.)
  - ↓ Platelet aggregation

2. Plasma Derived Mediators

a. Kinin System

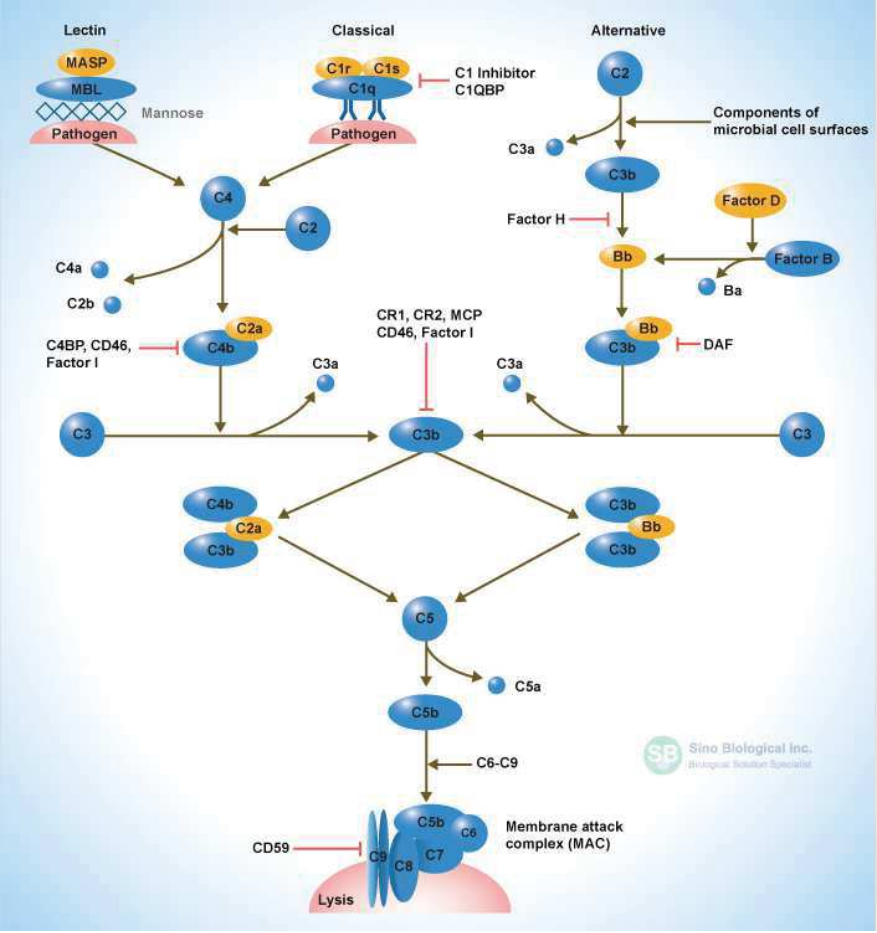
- **Kinin system**: poorly understood hormonal system involved in acute inflammation
- Includes **bradykinin** and **kallikrein**
- Causes vasodilation and increase in vascular permeability (by acting on phospholipase to increase arachidonic acid release and PGE_2 production)

b. Coagulation and Fibrinolytic System

- Refers to the physiological thrombotic and antithrombotic pathway
- Many coagulative and fibrinolytic mediators are heavily involved in inflammatory responses
- Inflammatory response generally have a pro-coagulant effect in order to localize infection by trapping microbes in thrombus

c. Complement System

- **Complement system**: a cascade that complement the action of immune system
- A type of innate immune response
- Can be activated by **classical pathway** or **alternative pathway**
- **Classical pathway**: initiation by IgG/epitope complex



1) IgG binds to epitope of pathogen;
2) **C1** binds to IgG and splits **C4** into **C4a** and **C4b**;
3) **C1-C4b complex** splits **C2** into **C2a** and **C2b**;
4) **C4b-C2a complex** called **C3 convertase** splits **C3** into **C3a** and **C3b**;
5) **C3b** binds to antigen as **opsonin** or combines with **C4b-C2a complex** to produce **C5 convertase**;
6) **C5 convertase** splits **C5** into **C5a** and **C5b**;
7) **C3a**, **C4a**, **C5a** functions as **anaphylotoxins** for degranulation of endothelial cells, mast cells or phagocytes, causing inflammatory responses such as chemotaxis, vasodilation and ↑ vascular permeability;
8) **C5b** will combine with **C6**, **C7**, **C8** and **C9** to form **membrane attack complex (MAC)** to punch holes in bacteria → water move into bacteria leading to cell swelling and lysis.

D. Process of Acute Inflammation

- Process of acute inflammation involves a **vascular phase** followed by a **cellular phase**

1. Vascular Phase

a. Vasodilation

- Tissue injury stimulates basophils, neutrophils and platelets to release vasodilators
- Smooth muscles lining arterioles relaxes, leading to vasodilation
- Capillaries become engorged with blood (active **hyperaemia**)

b. Increase in Vascular Permeability

- Normal capillary has endothelial cells kept together by tight junctions
- Cytokines and histamine stimulates endothelial cells to contract → gap junctions open up
- Fluid thus moves from blood to tissues → **oedema**
- **Transudate**: low protein content fluid (with low **specific gravity**) leaving capillary due to elevated hydrostatic pressure alone (with normal vascular permeability)
- **Exudate**: high protein content fluid (with WBCs) leaving capillary due to increased vascular permeability

***Specific gravity (SG)**: relative density (of fluid) compared to a certain reference (eg water)

2. Cellular Phase

a. Leukocyte Recruitment

- Normally, neutrophils and other WBCs move in the middle of vessel (axial flow)
- Cytokines and histamine increase expression of **adhesion molecules** (**selectins**) on endothelial cells causing neutrophils to stick
- Consists of four steps: **rolling**, **margination**, **emigration** and **chemotaxis**

i. Rolling

- **Rolling**: initial low-affinity adhesion between neutrophils and inflamed vascular endothelium resulting in rolling of cells along vessel wall
- Rolling caused by low affinity adhesion and driven by pushing force due to blood flow
- Involves interaction between **adhesion molecules** on neutrophil surface and on endothelial wall
- Can be amplified by mediators such as IL-1 and TNF-α

ii. Margination

- **Margination**: process whereby neutrophils adhere to endothelium
- Adhesion is only transient in normal conditions but will be prolonged when there is tissue damage
- Neutrophils adhere to endothelium via adhesion molecules on neutrophils and endothelial cells

iii. Emigration

- **Emigration**: polymorph pushes itself between two endothelial cells and basement membrane (repaired immediately)
- Also called **diapedesis** of neutrophils

iv. Chemotaxis

- Bacteria and injured cells secrete **chemotaxin** leading to directional movement of neutrophils
- Helps facilitate movement of polymorphs (and other leukocytes) to move to the injured site
- Examples: C5a, chemotaxin secreted by neutrophils and eosinophils

b. Opsonization

- **Opsonins** attaching to target cells to facilitate phagocytosis (by promoting phagocyte attachment)
- Most potent opsonin: immunoglobulins
- Other examples: C3b

c. Phagocytosis

- **Phagocytosis**: engulfing of target particle or cell by phagocytes

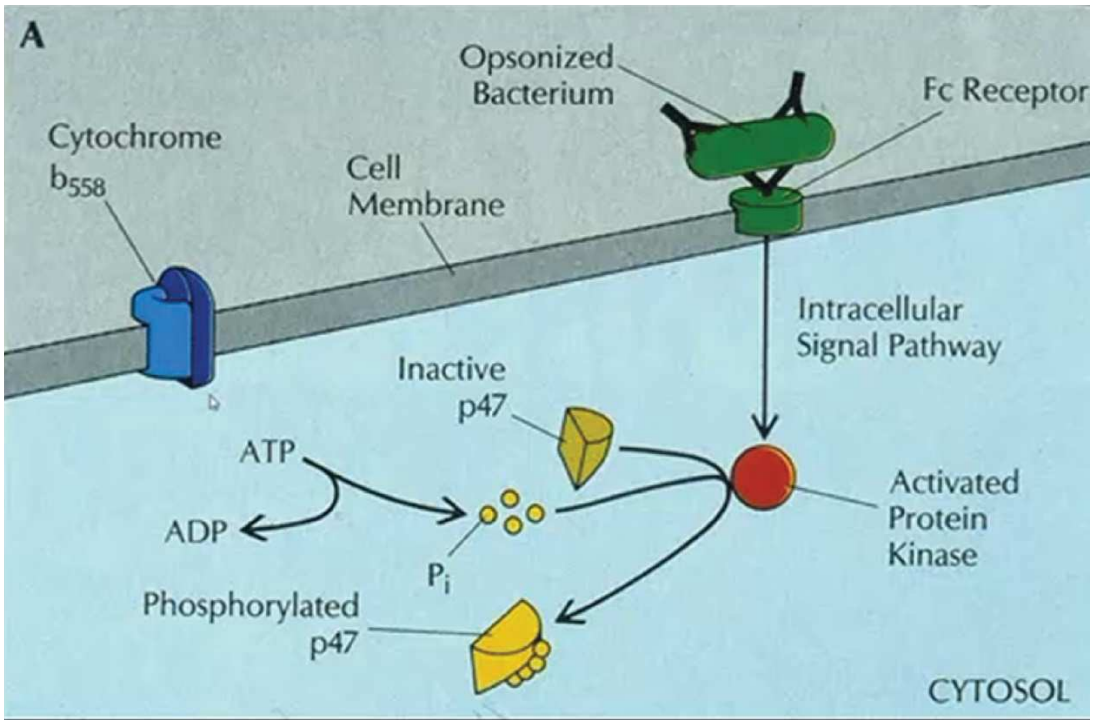
1) Ab binds to Ag;
2) Ab receptors (i.e. **Fc receptors**) binds to Fc region of Ab-Ag complex;
3) Formation of a cup-like process (**pseudopodia**) around target particle;
4) Particle internalized into a vesicle called **phagosome**;
5) Phagosome drawn towards Golgi region and fuses with **lysosome**;
6) **Intracellular destruction** takes place.

d. Intracellular Destruction

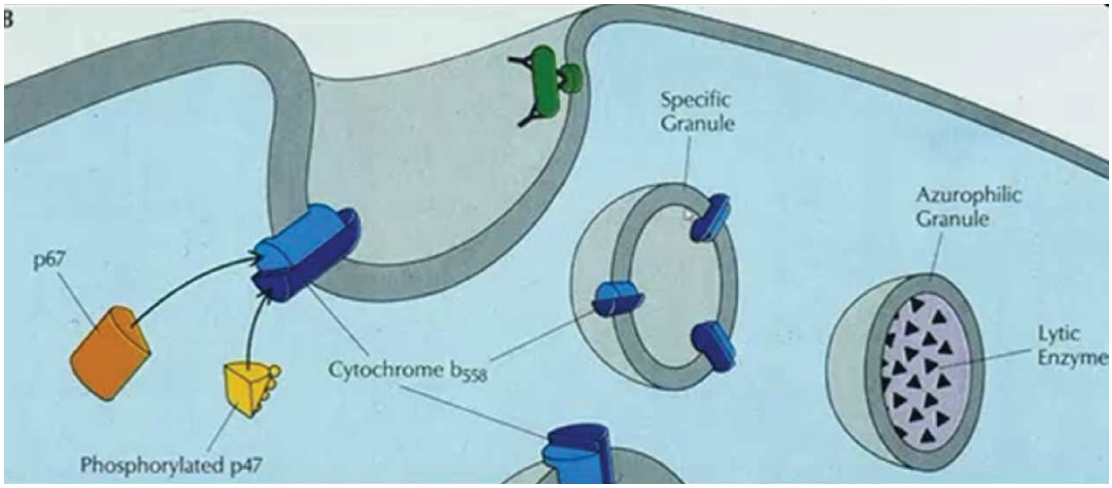
- Phagocytosed cell is destroyed by **intracellular killing** or **destruction**
- Primary granules of neutrophils function in intracellular killing of phagocytosed microbes
- **Phagosome** fuses with **lysosome** to form a **phagolysosome**
- Intracellular destruction may rely on **oxygen-dependent mechanisms** as well as **oxygen-independent mechanisms**

i. Oxygen-dependent Mechanisms

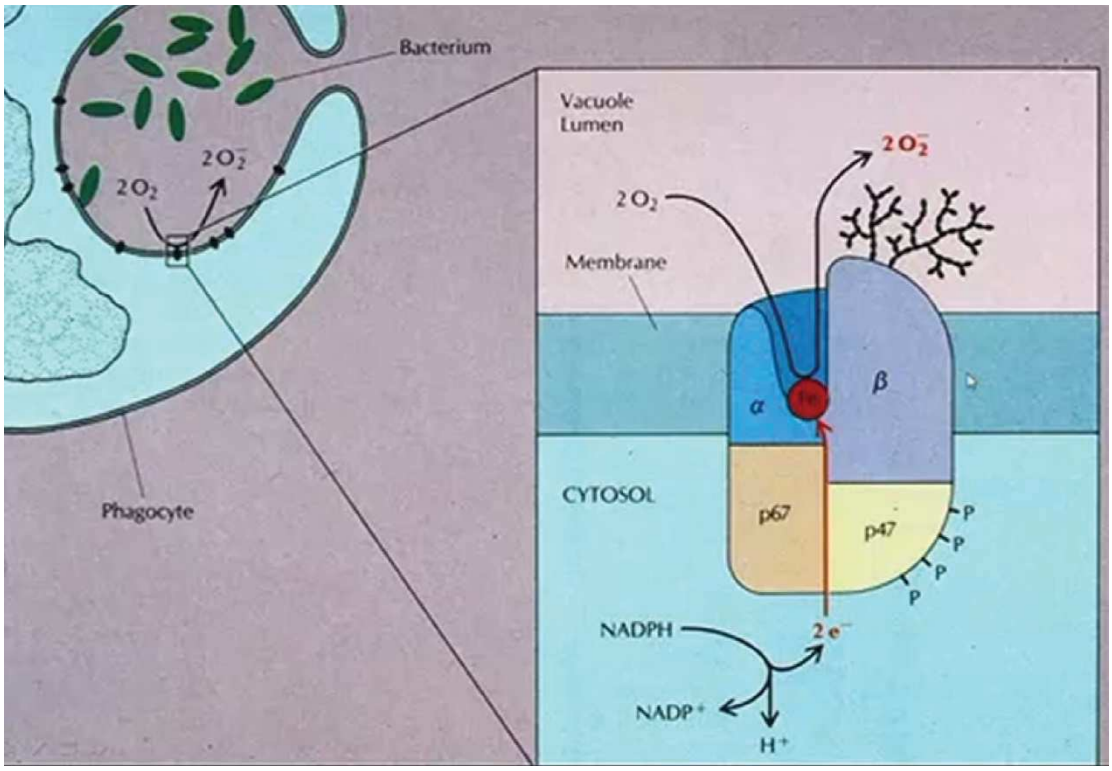
- When phagocytes ingest bacteria, they undergo a sudden increase in oxygen consumption called **respiratory burst** leading to formation of superoxide and other ROS for killing microbes



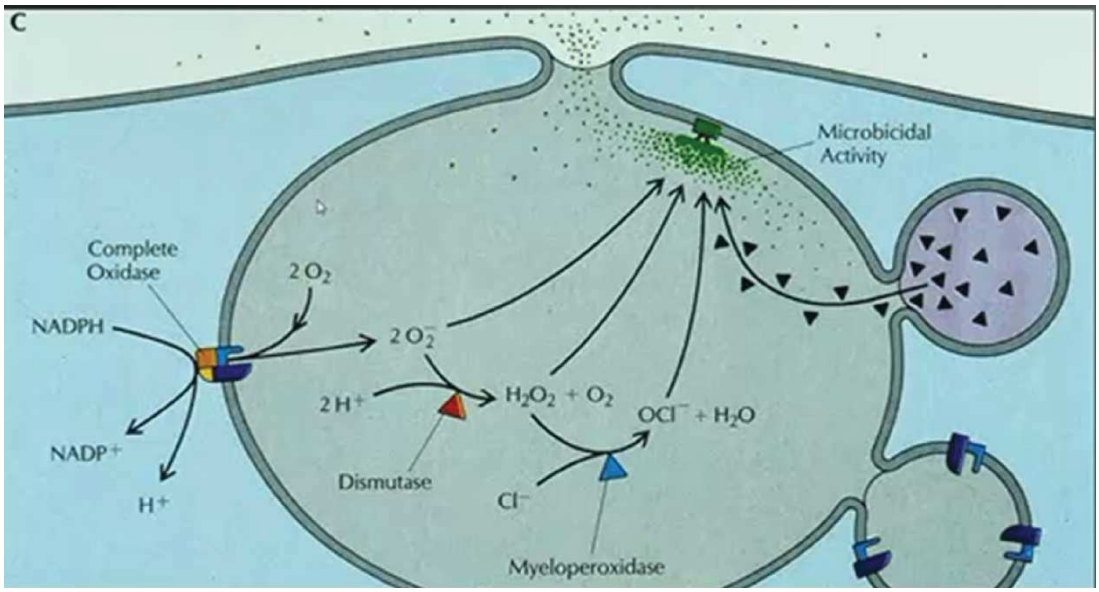
- Ab binding via Fc receptor triggers a signal transduction pathway that leads to phosphorylation of p47



- Phosphorylated p47, p67 and cytochrome b558 combine together to form **NADPH oxidase** in membrane lining **specific granules** (of neutrophil)



- The combined NADPH oxidase oxidizes NADPH and incompletely reduces oxygen gas into the reactive antimicrobial **superoxide ion (O_2^-)**



- The superoxide ion is further processed into **hydrogen peroxide** (via **dismutase**) and **hypochlorite ion** (via **myeloperoxidase**)

*Myeloperoxidase confers the green colour of pus

ii. Oxygen-independent Mechanisms

- Include use of various molecules especially enzymes in killing the microbe
- Example: **Bactericidal/permeability-increasing protein (BPI)**
  - BPI binds to LPS (endotoxin) of Gram negative bacteria
  - Inhibits the pro-inflammatory effect of endotoxin, thus reducing immunopathological damage due to LPS
  - Also lead to release of Ca^{2+} from bacteria cell membrane, activating phospholipase and thus degrading bacterial cell membrane → cell death

E. Clinical Significance of Acute Inflammation

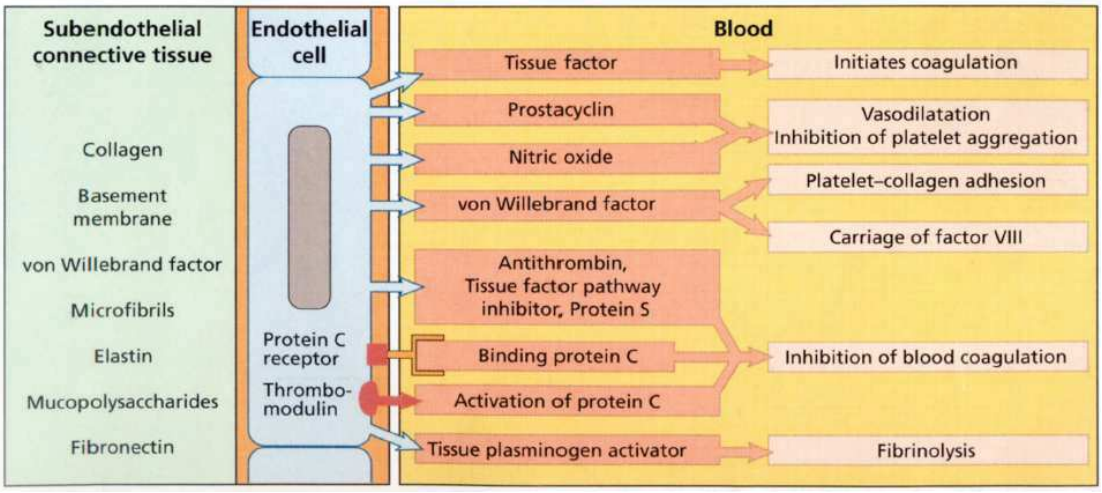
1. Lymphangitis

- **Lymphangitis**: secondary inflammation of lymphatic vessels
- Clinical manifestation: red streaks near a skin wound

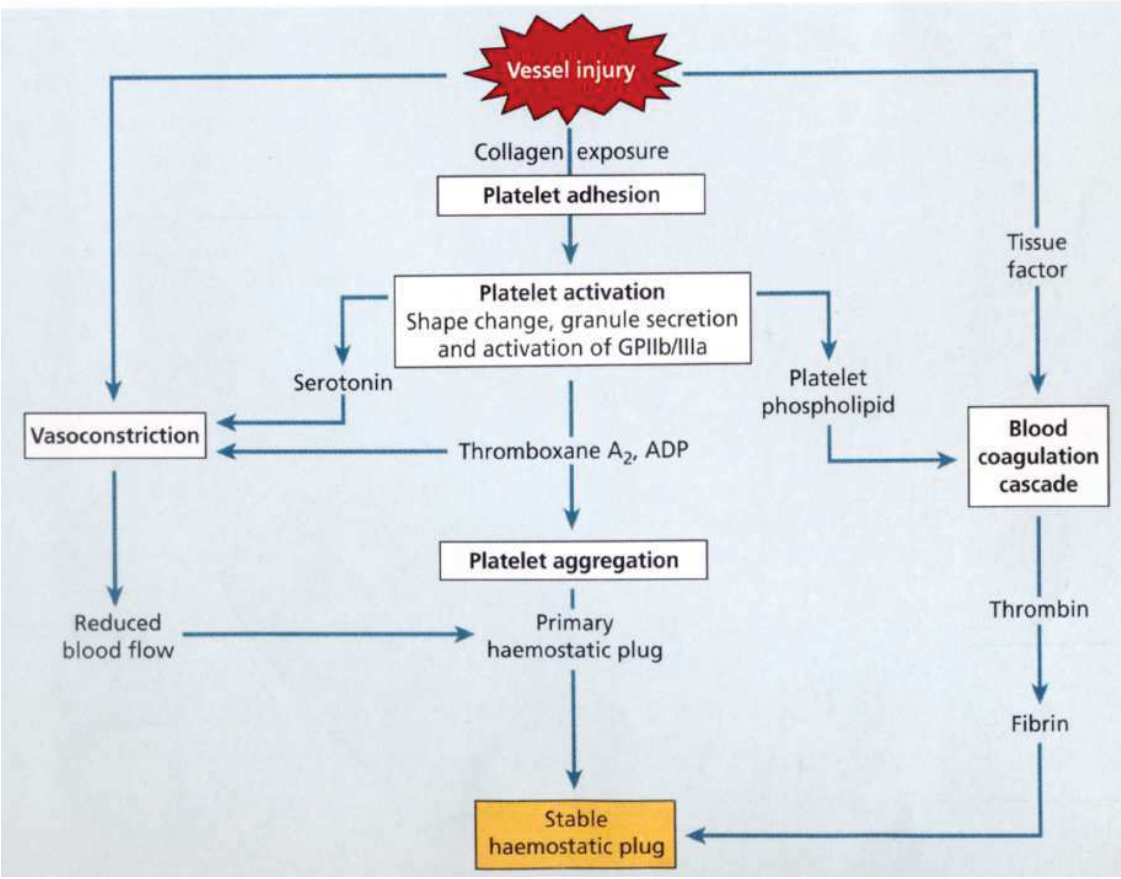
2. Possible Conditions Leading to Impairment of Inflammatory Response

- **Neutropenia** in cancer patients
- **Leukocyte adhesion deficiency (Type 1)** due to mutations in β chain of CD11/CD18 on leukocytes
- **Leukocyte adhesion deficiency (Type 2)** due to mutation in the enzyme needed to make neutrophil receptors on vessel wall
- **Chronic granulomatous disease**: defect in oxidative burst leading to inability in producing ROS → phagocytes cannot kill pathogen → formation of granulomatous tissue in multiple organs
- **Chediak-Higashi Syndrome**: mutations in protein needed for lysosomal membrane tethering → vesicles cannot be transported by microtubules to lysosomes properly → delayed fusion of phagosome with lysosome in leukocytes
  - Symptoms:
    - → Autophagocytosis of melanosomes in melanocytes → albinism
    - → Granular defects in NK cells and platelets
  - Mnemonic: CHINA – **C**hediak **H**igashi: **I**nfections, **N**europathy, **A**lbinism
- Complement deficiencies or excess activity:
  - **Systemic lupus erythematosus (SLE)** and other hereditary conditions can lead to deficiency in selected components of the complement cascade
  - Defects in enzyme that tethers complement regulators to cell wall → complement not regulated
  - Excess production can destroy RBCs → **paroxysmal nocturnal haemoglobinuria (PNH)**
- Anti-inflammatory agents blocking action of COX 1 ± 2
- **α1-antitrypsin deficiency** leading to non-destruction of proteases released by neutrophils → excess tissue destruction

L73 Blood Clot Formation and Lysis

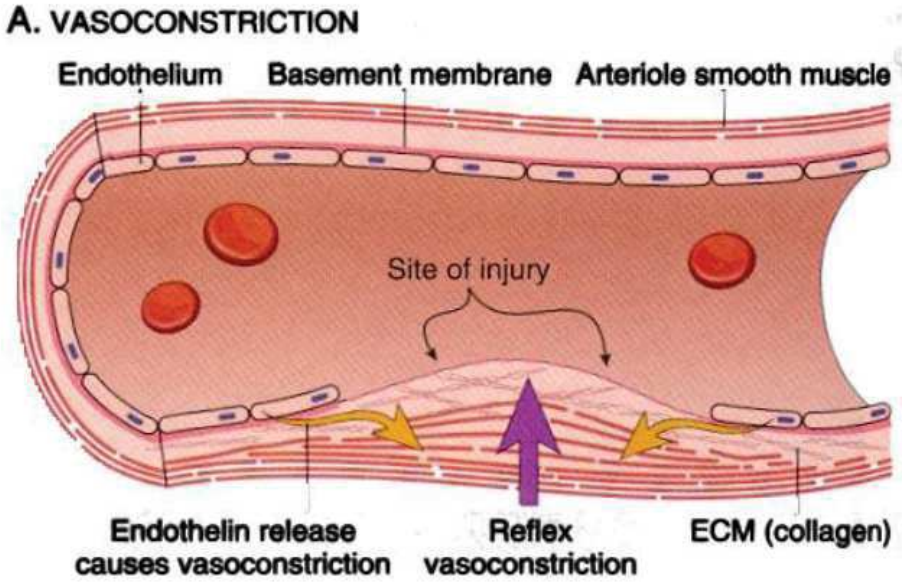


A. Haemostasis



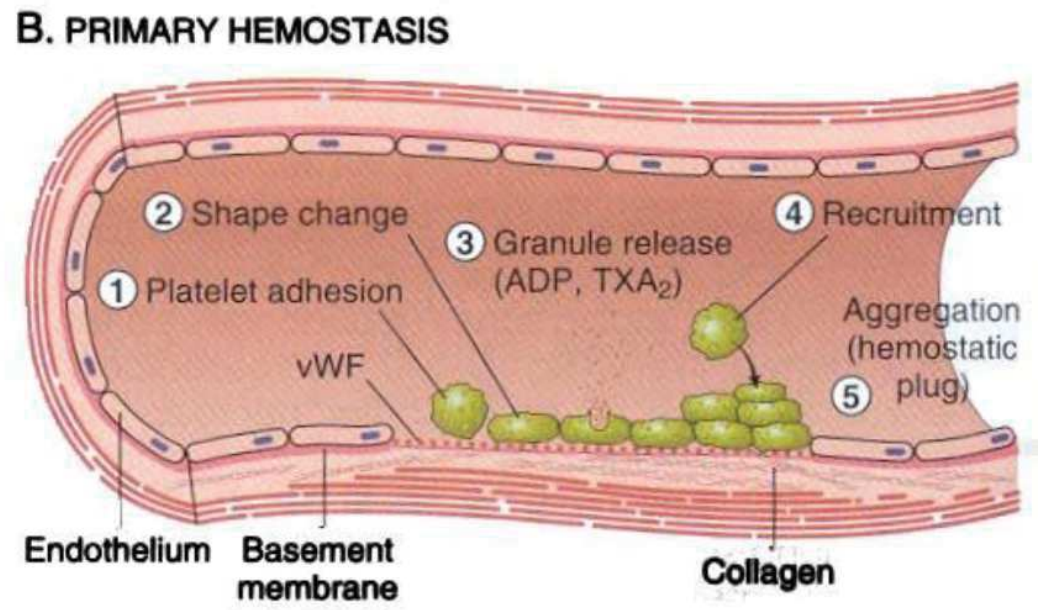
- **Haemostasis**: a series of response effected after vascular damage to arrest blood loss from vessels
- Three arms: **vasoconstriction**, **platelet responses**, **blood coagulation cascade**
- Involves interaction between blood vessel wall, circulating platelets and blood coagulation factors

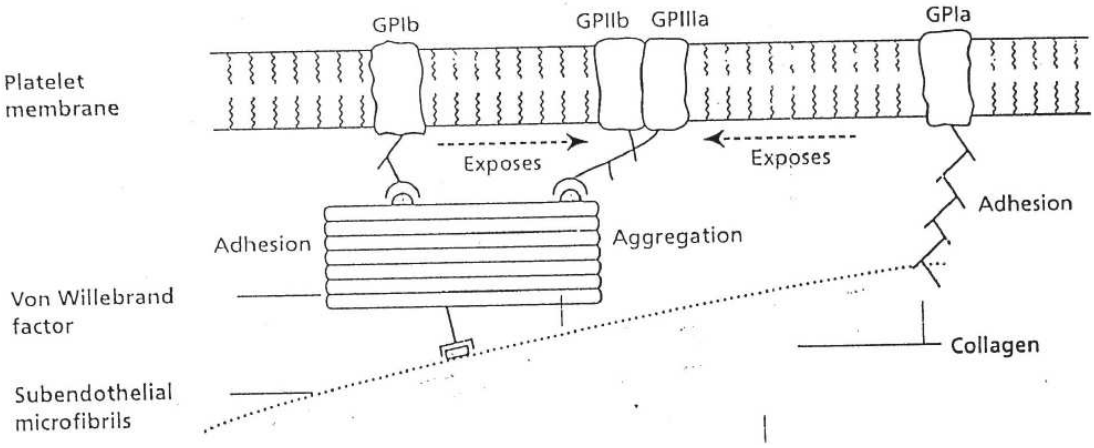
1. Vasoconstriction

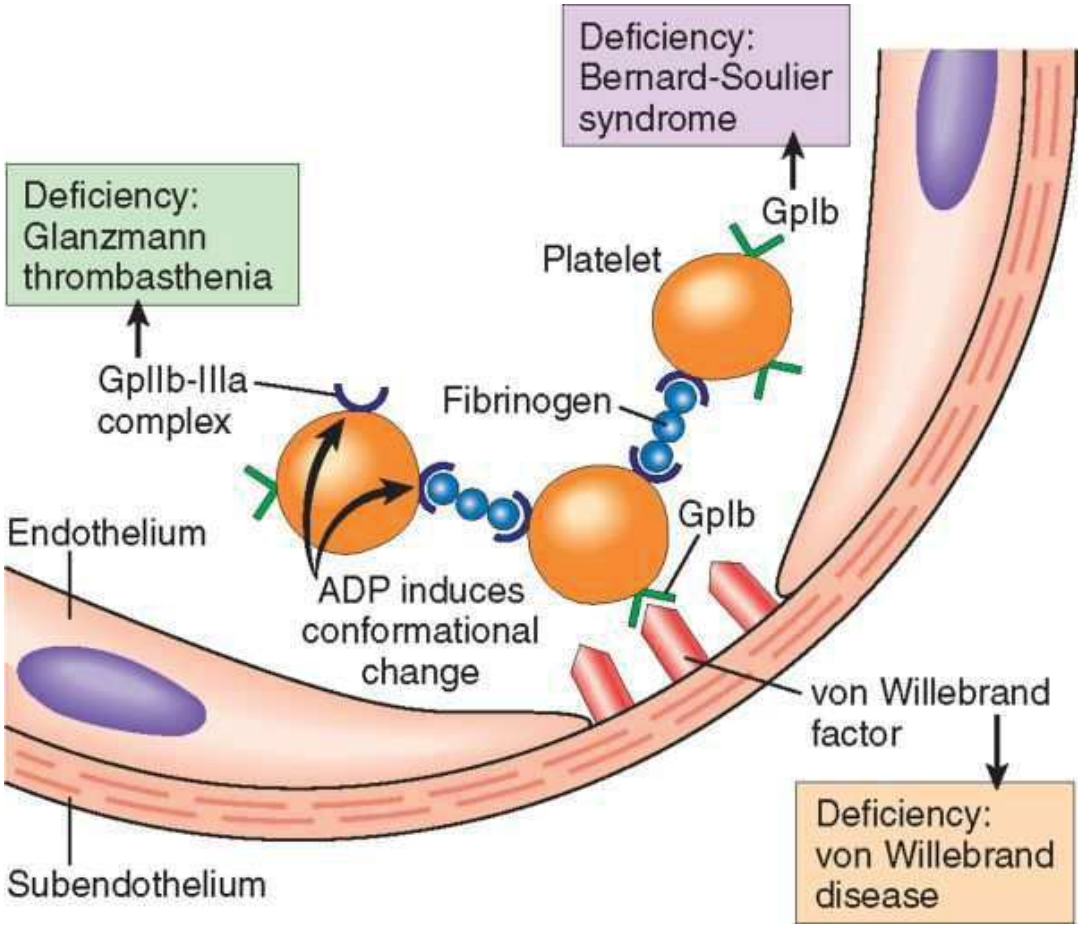


- Normal endothelial wall secretes **prostacyclin (PGI_2)** and **nitric oxide (NO)** which are vasodilators
- Damaged endothelium can no longer secrete these factors
- Vasoconstriction also stimulated by **thromboxane A_2 (TXA_2)** and **serotonin** from degranulation of activated platelets

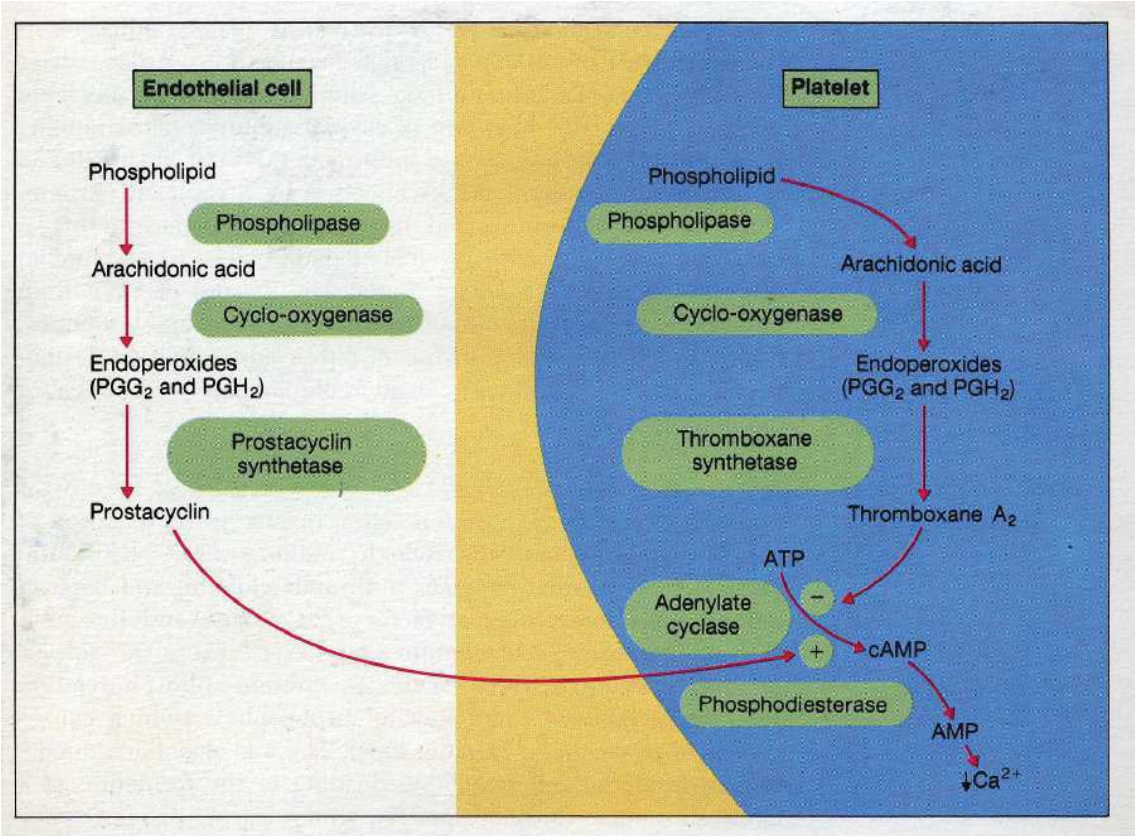
2. Platelet Responses



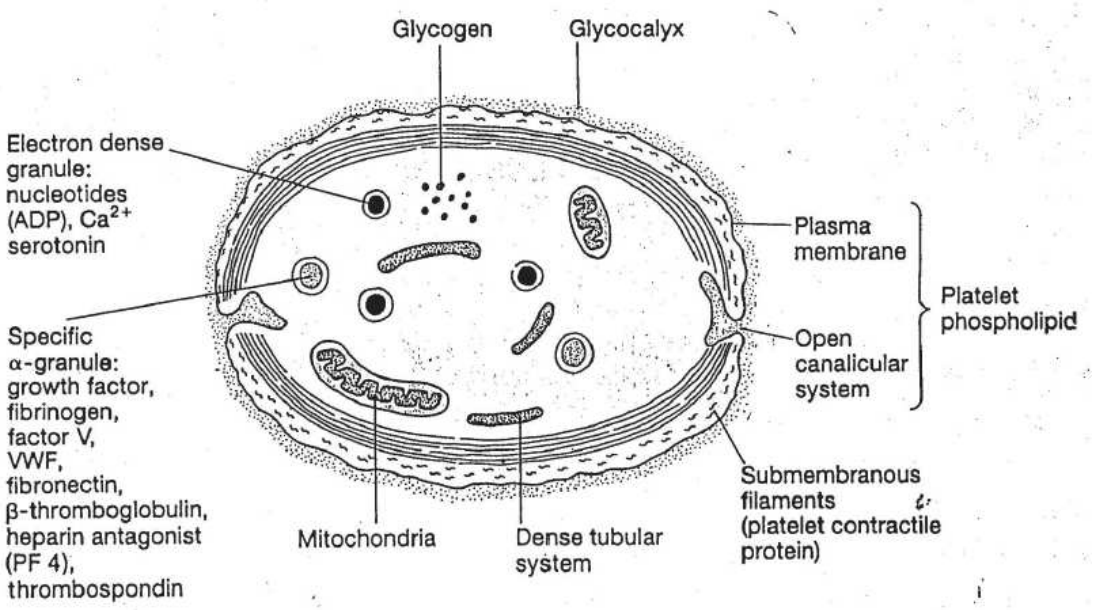




- Initiated by two events:
  - **Endothelial damage** → removal of endothelial inhibition of **thrombin** action (via **antithrombin III**, **thrombomodulin**, **protein C** and **protein S**)
  - **Endothelial damage** → collagen exposure → platelet adhesion mediated by plasma factor **von Willebrand factor (VWF)** → conformational change in **glycoproteins (GP)** on platelet membrane
    → **GPIb** binds subendothelium via VWF
    → **GPIIb/IIIa** binds other platelets



- **Thrombin** and collagen binding <u>activates</u> platelets → synthesis of **thromboxane A_2 (TXA_2)** → ↓ platelet cAMP level → ↑ Ca^{2+} level → platelet degranulation



- Platelet granules contain:
  - **ADP** stimulating platelet aggregation and swelling
    → Encourages adjacent platelet membranes to adhere with liberation of more ADP and TXA_2 (+ve feedback)
  - **Serotonin** stimulating vasoconstriction
  - **Thromboxane A_2 (TXA_2)** stimulating platelet activation, aggregation and vasoconstriction

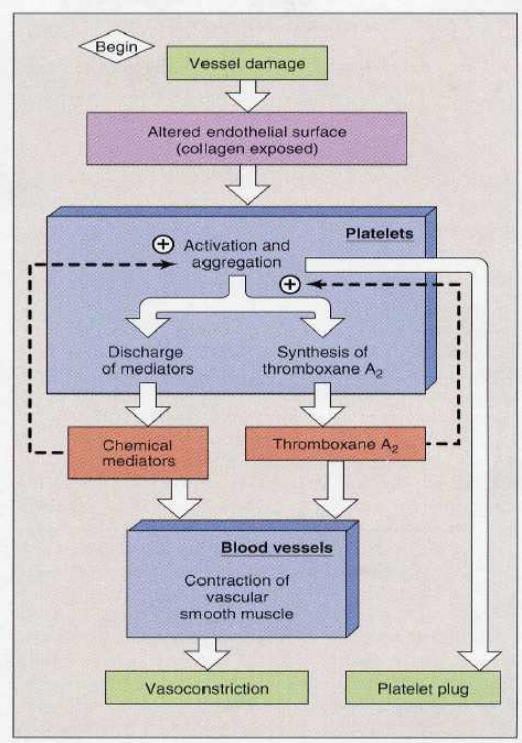


FIGURE 14–69
Sequence of events leading to formation of a platelet plug and vasoconstriction following damage to a blood-vessel wall. Note the two positive feedbacks in the pathways.

- Series of events with positive feedback mechanism stimulating activation and aggregation of platelets lead to formation of primary haemostatic plug (first minute or so following injury) → temporary control of bleeding
  - Seen as silvery lining on wound
  - Also provides a surface for fibrin formation
- **Platelet-induced clot retraction (PICR)**: reaction mediated by GPIIb/IIIa receptors to link cytoplasmic actin filaments to surface fibrin polymers → definitive haemostatic plug

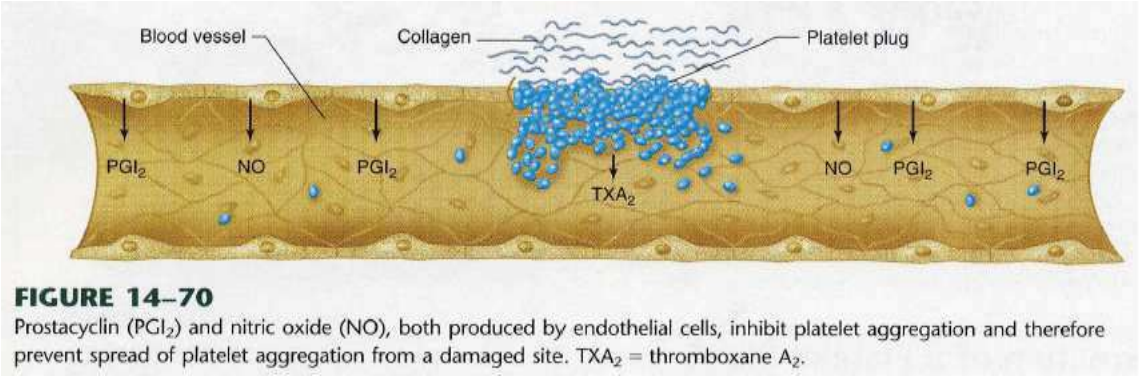
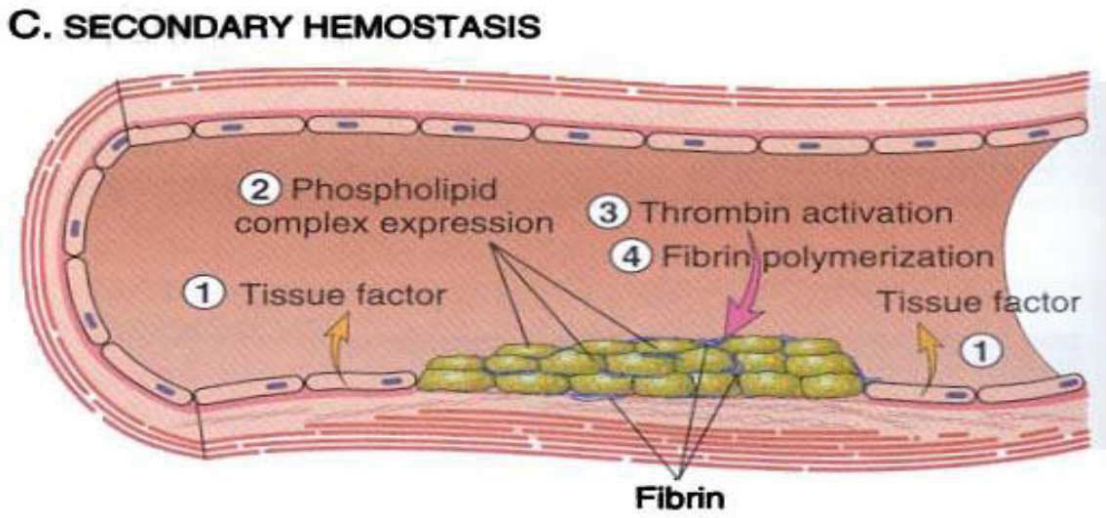
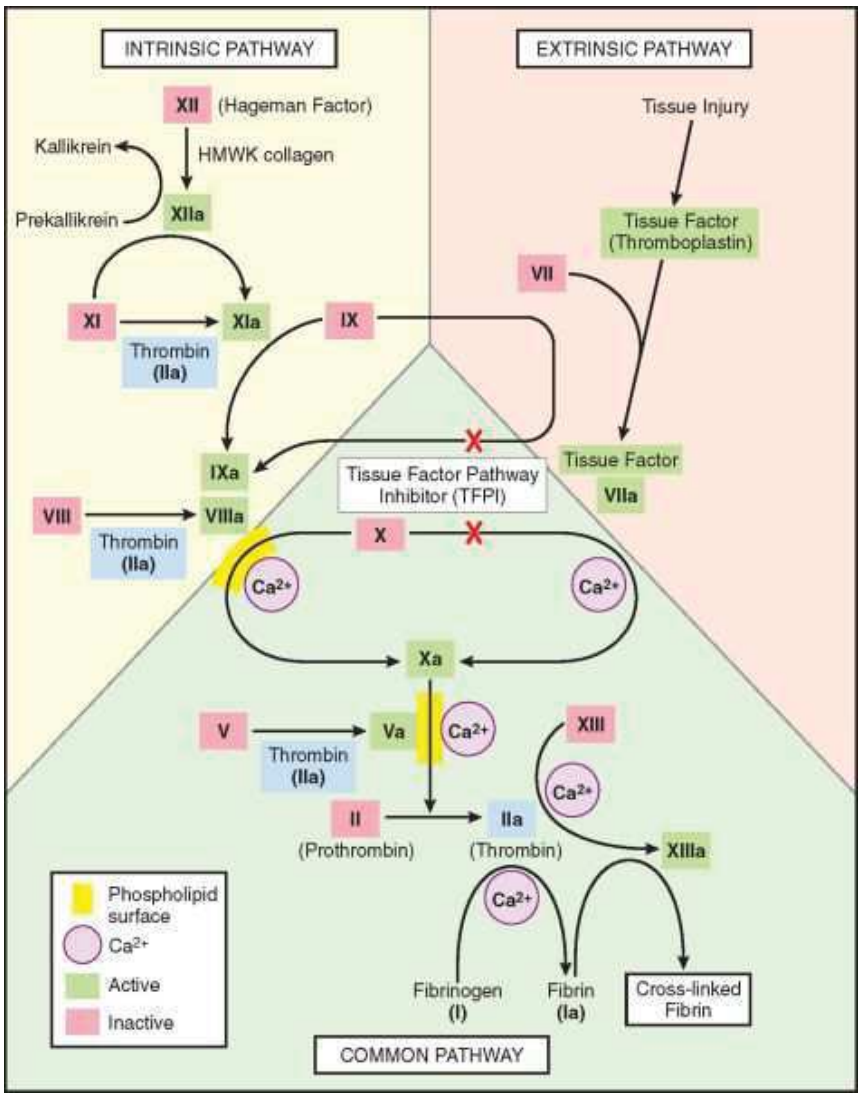


FIGURE 14–70
Prostacyclin (PGI_2) and nitric oxide (NO), both produced by endothelial cells, inhibit platelet aggregation and therefore prevent spread of platelet aggregation from a damaged site. TXA_2 = thromboxane A_2.

- Regulation: normal endothelium secretes PGI_2 (→ ↑ thrombocyte cAMP level) and NO to inhibit platelet aggregation and activation
  - Also prevents spread of platelet aggregation from damaged site

3. Blood Coagulation Cascade





- **Blood coagulation cascade**: responsible for fibrin deposition onto platelet plugs to achieve definitive haemostasis
- Activated by two pathways:
  - **Intrinsic pathway**: by contact with collagen in subendothelial c.t.
  - **Extrinsic pathway**: by **tissue factor (III)** (in endothelium)
    - → Dominant role in initiation of coagulation cascade
- Both pathways lead to conversion of **factor IX** into **IXa**
- Factor **IXa** then triggers another cascade (**common pathway**) leading to formation of **thrombin (IIa)** from **prothrombin (II)**

Table 18.1 The coagulation factors

Factor number	Descriptive name	Active form
I	Fibrinogen	Fibrin subunit
II	Prothrombin	Serine protease
III	Tissue factor	Receptor/cofactor*
V	Labile factor	Cofactor
VII	Proconvertin	Serine protease
VIII	Antihaemophilic factor	Cofactor
IX	Christmas factor	Serine protease
X	Stuart–Prower factor	Serine protease
XI	Plasma thromboplastin antecedent	Serine protease
XII	Hageman (contact) factor	Serine protease
XIII	Fibrin-stabilizing factor	Transglutaminase
	Prekallikrein (Fletcher factor)	Serine protease
	HMWK (Fitzgerald factor)	Cofactor*

*Active without proteolytic modification.
HMWK, high molecular weight kininogen.

- Exposed membrane phospholipid (platelet factor) serves as cofactor in two calcium-dependent reactions of coagulation protein complex formation:
  - Conversion of **X** into **Xa** by **IXa** and **VIIIa**
  - Formation of **thrombin (IIa)** from **prothrombin** by **Xa** and **Va**
- **Thrombin** then initiates a positive feedback mechanism to promote various reactions of the coagulative cascade (**amplification phase**)

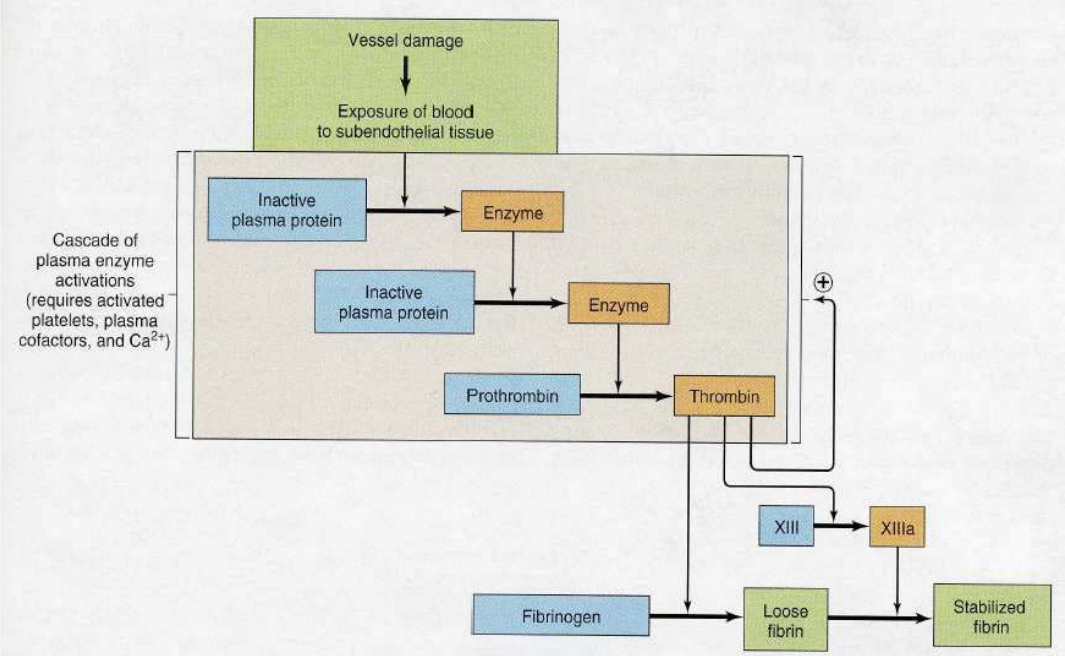


FIGURE 14–71
Simplified diagram of the clotting pathway. The pathway leading to thrombin is denoted by two enzyme activations, but the story is actually much more complex (as will be shown in Figure 14–73). Note that thrombin has three different effects—generation of fibrin, activation of factor XIII, and positive feedback on the cascade leading to itself.

- **Thrombin (IIa)** formed from common pathway then hydrolyzes **fibrinogen (I)** into **fibrin (Ia)** monomers
- **Fibrin (Ia)** monomers link spontaneously by hydrogen bonds to form a loose, insoluble **fibrin polymer**
- **Factor XIIIa** produced (from **factor XIII**) by **thrombin (IIa)** then participate in stabilization of **fibrin** to form a relatively strong haemostatic fibrin plug (by formation of covalent bond cross-links)

*Vitamin K critical for synthesis of clotting factors

∵bile salt helps suspend fat-soluble vitamin K in GI tract → impairment in bile secretion may also lead to clotting defects

a. Clinical Implications

- **Haemophilia**: lack of certain clotting factor(s) leading to inability to form clot effectively
  - **Haemophilia A**: lack of **factor VIII**
  - **Haemophilia B**: lack of **factor IX**
- Addition of **chelating agent EDTA** to blood removes Ca^{2+} from plasma → inhibits clotting

4. Roles of Different Vascular Components in Haemostasis

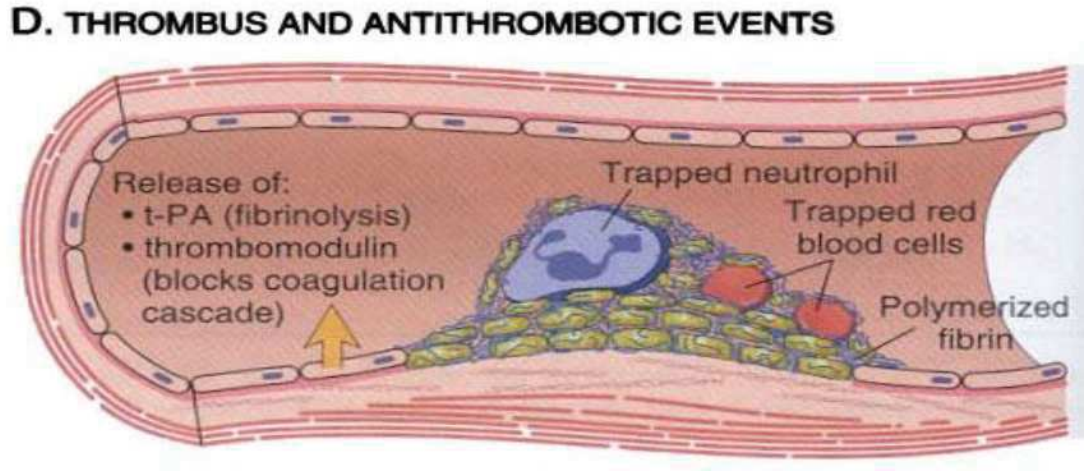
a. Endothelium

- Plays an active role in maintenance of vascular integrity
- Provides basement membrane that separates subendothelial c.t. from circulating blood
- Loss or damage to endothelial lining → haemorrhage + activation of haemostatic mechanisms
- Inhibits haemostatic response through synthesis of PGI_2 and NO → vasodilation + inhibit platelet aggregation

b. Platelets

- Main function: formation of mechanical plugs during normal haemostatic response to vascular injury
- Platelet adhesion and aggregation forms primary haemostatic plug for temporary arrest of bleeding
- Platelet release reaction releases ADP, TXA_2 and serotonin for vasoconstriction and platelet adhesion/aggregation
- Platelet also plays an important role in coagulating cascade (provides Ca^{2+} and phospholipid for coagulation)

B. Fibrinolysis



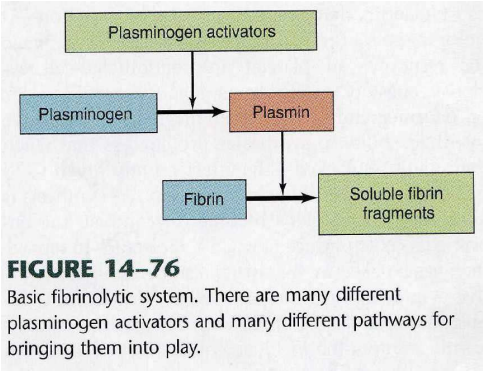


FIGURE 14–76
Basic fibrinolytic system. There are many different plasminogen activators and many different pathways for bringing them into play.

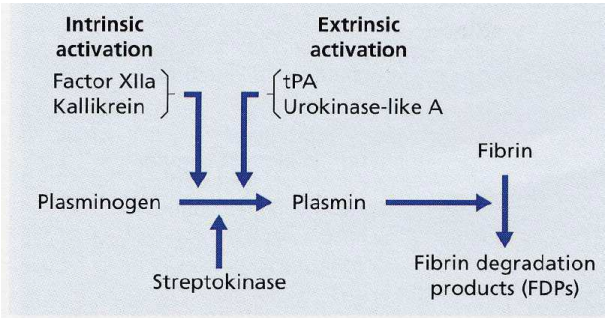
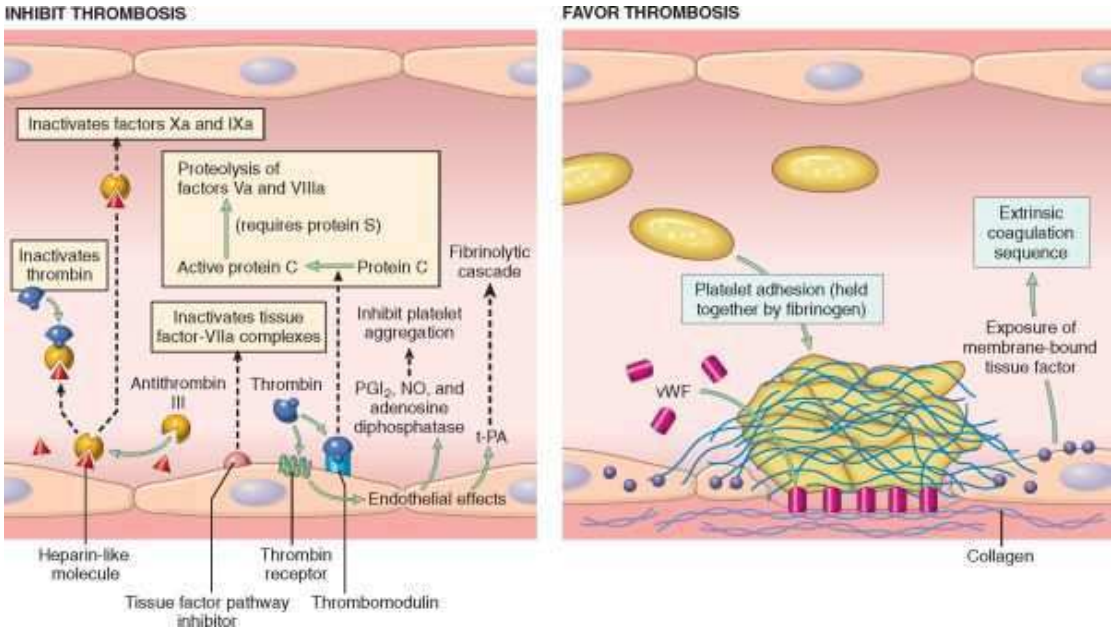
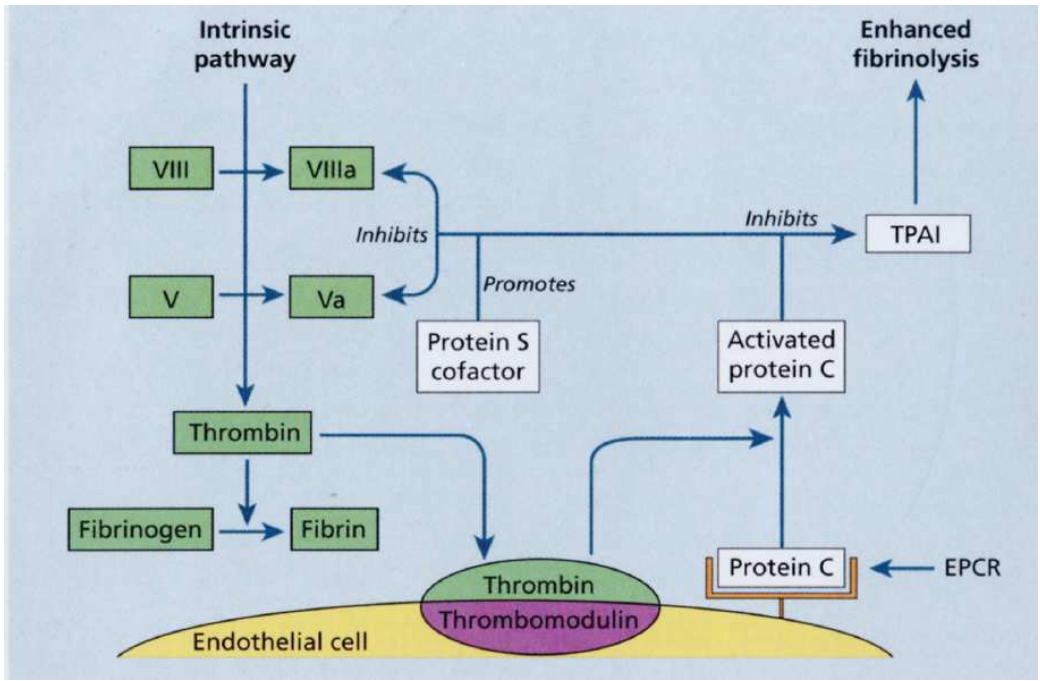


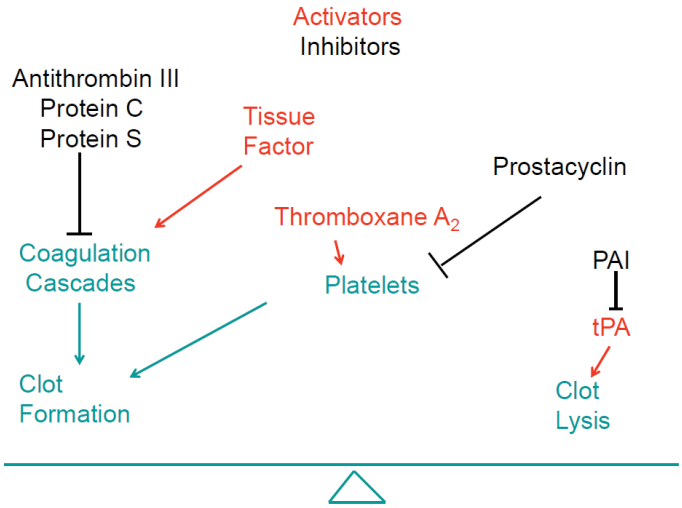
Fig. 18.11 The fibrinolytic system. tPA, tissue plasminogen activator.

- **Plasmin**: serine protease responsible for fibrinolysis
- Major activation of fibrinolytic system follows release of **tissue plasminogen activator (tPA)** from endothelial cells
  - **Tissue plasminogen activator (tPA)**: fibrin-binding serine protease
  - Fibrin binding ↑ conversion capacity of fibrin-bound plasminogen into plasmin → plasmin generation highly localized at fibrin clot
  - tPA released after stimuli such as trauma, exercise or emotional stress
  - Downregulated by **tissue plasminogen activator inhibitor (tPAI)**
- **Plasmin** capable of digesting **fibrin**, **fibrinogen**, **factors V** and **VIII**, other proteins
- Degradation products from peptide bond cleavage in fibrin and fibrinogen inhibits clotting:
  - Interfere with formation and action of thrombin
  - Interfere with polymerization of fibrin monomers

C. Physiological Maintenance of Balance between Haemostasis and Fibrinolysis







- Clotting cascade and fibrinolytic pathway are on opposite sides of a delicate balance in the body:
  - Clotting cascade too powerful → tendency for **thrombosis**
  - Clot lysis pathway too powerful → tendency for **haemorrhage**
- **Thrombomodulin** on endothelial cell membrane binds thrombin and activates **protein C**
- Activated **protein C** is a serine protease that cleaves peptide bonds in **factors Va** and **VIIIa** with the help of **protein S** as a cofactor
- Activated **protein C** also inhibits **tPA inhibitor (tPAI)** → fibrinolysis is enhanced
- Clinical significance: hereditary protein S or C deficiency → ↑ risk of thrombosis

L74 Atherosclerosis

A. Overview on Atherosclerosis

- **Atherosclerosis**: a predominantly intimal disease affecting large and medium-sized arteries characterized by presence of fibrofatty plaque or atherosclerotic plaque within **tunica intima**
  - Athero-: Greek for porridge
  - –sclerosis: Greek for hardening
- Atheroma has two main components:
  - Superficial fibrous cap made up of collagen and smooth muscle cells
    → Confers hardness
  - Central part of intracellular (within macrophages and smooth muscle cells) and extracellular (cholesterol and cholesterol esters) lipids, cellular debris, proteoglycan ground substance, collagen, elastin, plasma proteins, other leukocytes incl lymphocytes
    → Confers 'porridgeness'
- Precursor: **fatty streak**
  - Flat or slightly elevated spots or streaks occurring in everyone >1y/o
  - Histologically consists of lipid deposits in intima within macrophages and smooth muscle cells (SMCs) and sometimes a little extracellular lipids
- Results: occlusion of artery → ischaemia → infarction

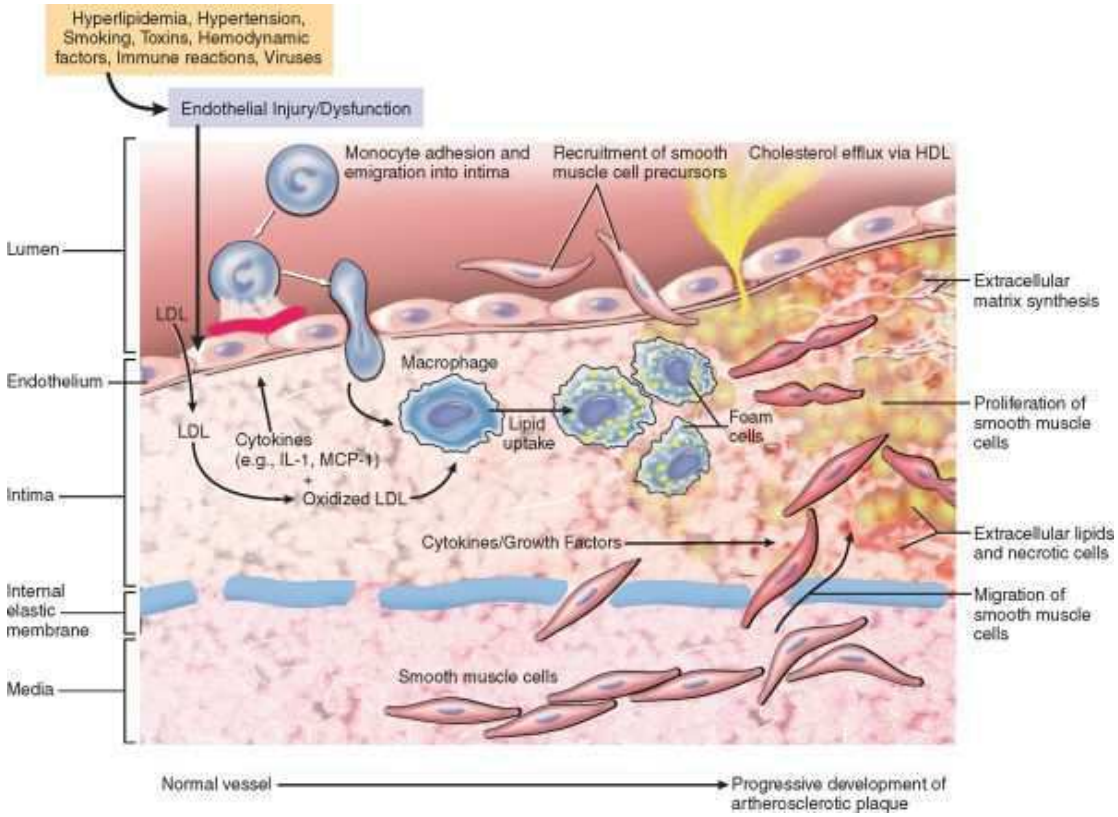
*Smooth muscle cells (SMCs) assume some of the properties of macrophages and are able to ingest lipids and become foam cells

**Often occur at bifurcation of artery ∵ more turbulent flow → ↑ endothelial damage

B. Mechanism of Atherosclerosis

- Exact pathogenesis of atherosclerosis not well understood
- Most popular single hypothesis is **reaction to injury**: endothelial injury is the driving force of formation of atheroma
  - May be caused by **homocysteine** or **nicotine** from cigarettes

1. Response-to-Injury Hypothesis



1) LDL enters endothelium and is trapped and accumulated (due to hyperlipidemia) in ECM (enhanced by endothelial injury caused by fluid shear stress or otherwise ∵ endothelial dysfunction increase pemeability);
2) LDL is oxidized into oxyLDL (inhibited by HDL);
3) Monocyte adhesion via adhesion molecules (eg. ICAM-1, selectins on endothelium and VLA-4, PCAM-1, integrins on monocytes) upregulated by endothelial dysfunction (→ abnormal expression of adhesion molecules (eg **ICAM-1**, **selectins**) + **endothelial cell growth factor (ECGF)** and IL-1 production) and oxyLDL (→ cytokine/growth factor production);
4) Monocytes proliferate and differentiate into macrophages in tunica intima under influence of cytokines;
5) Activated macrophages express **scavenger receptors** (eg CD36, SR-A) (not down-regulated, cf. LDLr) to phagocytose oxyLDL;

6) Non-down-regulation prevents macrophages from getting rid of oxyLDL and thus these macrophages die and become **foam cells**. Foam cells may release their lipid contents to form an extracellular lipid-rich debris. A fatty streak is formed;
7) Smooth muscle cells (SMCs) migrates from **tunica media** to **tunica intima** and proliferates under the influence of **homocysteine** and growth factors such as **ECGF** and **platelet derived growth factor (PDGF)**. They then synthesize ECM and form a fibrous cap on the atheroma. A mature atherosclerotic plaque is thus produced.
8) Endothelial dysfunction caused by atherosclerotic plaque may lead to platelet adhesion and aggregation and ultimately occlusion of artery.

- Interplay between various components contributes to build-up of atheroma:
  - OxyLDL further augments macrophage activation and release of cytokines and growth factors, causing further inflammatory cell recruitment
  - OxyLDL is cytotoxic to endothelial cells and SMCs and causes further endothelial dysfunction
  - ROS produced by activated macrophages also further drive LDL oxidation and elaborate growth factors that drive SMC proliferation
  - T lymphocytes also recruited into arterial wall to produce inflammatory cytokines (eg IFN-γ), stimulating endothelial cells, macrophages and SMCs to release growth factors for SMC proliferation and ECM synthesis

2. Complications of Atherosclerosis

- **Calcification**: foam cells may undergo calcification atrophy → hardening of atheroma
- **Ulceration**: atherosclerotic plaque may break and cholesterol embolus may be discharged
- **Thrombosis**: platelets may deposit on atherosclerotic plaque → thrombosis or even thromboembolism
  - Decrease in lumen size may lead to **recanalization** in thrombus
  - May also lead to **aneurysm** formation (∵ weakening of wall and thrombus formation)
- **Haemorrhage**: increase in BP may lead to loss of fibrous cap and haemorrhage into plaque

D. Risk Factors Leading to Atherosclerosis

1. Irreversible Risk Factors

- **Age**: incidence of clinically overt atherosclerosis (as measured by deaths from ischaemic heart disease (IHD)) increases with each decade up to age 85
- **Sex**: IHD deaths significantly higher in males up to age 75-85 when male and female incidence becomes the same
- **Genetics**: familial elevated risk of atherosclerosis may be due to familial hyperlipidaemia, familial hypertension and familial DM
  - Some families have higher IHD risks without any known risk factors

2. Reversible Risk Factors

- **Smoking**:
  - Nicotine causes endothelial damage
  - Enhances leukocyte adhesion onto vessel wall
  - Decreases ability of vessels to dilate
- **Hyperlipidemia**: excess lipid in blood
  - Elevated LDL level causes LDL accumulation in subendothelium
  - HDL helps remove free cholesterol from atherosclerotic plaque and thus reduces risk of atherosclerosis
- **Hypertension**:
  - Higher correlation with diastolic BP (↑ risk of IHD)
  - Causes mechanical injury to endothelium → endothelial dysfunction
- **Diabetes**:
  - DM II patients more likely have elevated LDL and reduced HDL
  - Increased glucose sticks to collagen to form **Amadori product** (or **advanced glycation end-product (AGE)**)
  - AGE recognized by macrophages (via AGE receptor) and platelets to stimulate cytokine release
  - Macrophages are then prompted to enter endothelium and become foam cells
- **Infection** and **inflammation**:
  - Endotoxins from Gram negative bacteria can damage endothelium
  - Cytokines from systemic inflammation stimulates blood coagulation, leukocyte adhesion and vessel constriction
  - **C-reactive proteins (CRP)** is now considered an important marker for atherosclerosis
- **Hyperhomocysteinaemia**:
  - Fasting hyperhomocysteinaemia is an independent risk factor for atherosclerosis
  - Homocysteine level normally kept low due to kidney conversion to methionine
  - Renal or liver disease and B6, 9 or 12 deficiency may lead to hyperhomocysteinaemia
  - Excess homocysteine upregulates FA synthesis and SMC migration
  - Homocysteine also autoxidizes and reacts with ROS to damage endothelium and stimulate thrombosis

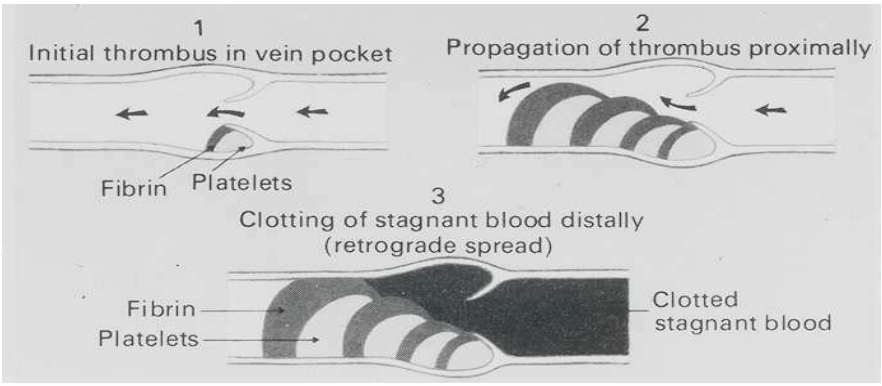
L75 Disrupted Circulation

A. Disorders of the Circulatory System

- Normal and transient:
  - **Congestion**: abnormal accumulation of blood in a certain part of body
  - **Haemostasis**: physiological blood clot formation
- Abnormal and may be permanent if unresolved:
  - **Thrombosis**: pathological blood clot formation in uninjured vasculature or after relatively minor injury
  - **Embolism**: intravascular mass causing obstruction to blood flow that is carried by blood to a site different from origin
  - Other obstruction eg. compression due to a heavy object
- Effect of obstructed circulation depends on:
  - Location and degree of vascular occlusion
  - Availability of collateral blood supply
    - → Most susceptible: tissues supplied by end-arteries without significant collateral supplies (eg brain, heart, kidney)
    - → Less susceptible: tissues with many anastomosis (eg GI tract)
  - Susceptibility of tissue to interruption of blood supply
- Effect of obstructed circulation:
  - Ischaemia
  - Prolonged → necrosis (infarction) and atrophy
  - Reperfusion after ischaemic necrosis → **haemorrhagic necrosis** (∵ lack of venous drainage)
  - **Gangrene** in *Clostridia* infection

1. Thrombosis

- **Thrombus**: a blood clot
- **Thrombosis**: pathological process whereby there is formation of a blood clot in uninjured vasculature or after relatively minor injury
- **Virchow's Triad**: three factors leading to pathogenesis of a thrombus
  - **Endothelial injury**:
    → Examples: shear stress, hypertension
  - **Abnormal blood flow**: either too fast (→ turbulent flow) or too slow (**stasis**)
    → Examples: **venous stasis**, **varicose veins**
  - **Hypercoagulability**:
    → Primary (genetic)
    → Secondary (acquired): may be due to excessive amount of certain cells, fat or proteins
    → Examples: obesity, smoking, genetic deficiency of antithrombin III
- Fate of thrombus:



  - **Propagation**: thrombus grow in length due to turbulent flow
  - Become organized and **recanalized**
  - Become organized and incorporated into the wall (∵ disorganized clot lysis → endothelium formation above thrombus)
  - Complete resolution
  - Dislodge and forms **embolism** in other parts of body

2. Embolism

- **Embolus**: a detached intravascular solid, liquid or gaseous mass that is carried by the blood to a site distant from its point of origin

a. Pulmonary Thromboembolism

- Probably the most common form of embolism
- Embolus derived from thrombosis of deep veins of lower limbs
- Predisposing factors:
  - Prolonged immobility: resumption of mobility leading to breaking off of thrombus
  - Dehydration

b. Fat Embolism

- Commonly found at autopsies
- Often associated with injuries to adipose tissues, long bones and stressful states
- May be quite asymptomatic
- Small size of fat droplets → can lead to extensive occlusion of vessels leading to haemorrhagic infarcts

c. Marrow Embolism

- Often seen together with fat embolism esp after long bone fractures
- Also quite commonly found after fractures of ribs during **cardiopulmonary resuscitation**

d. Air Embolism

- Associated with activities or work under high air pressure (eg. scuba diving, compression chamber workers, etc.)
- High pressure forces gases to dissolve in blood
- Sudden decompression → no time to escape slowly via lungs → bubbles form in blood → obstruction
- When >100mL of gases collects in right heart, pumping action of heart will lead to frothing → obstruction to blood flow → death

e. Amniotic Fluid Embolism

- Associated with high mortality
- Contents of fetal amniotic sac forced into maternal circulation during labour
- Fetal squamous cells and other materials in amniotic fluid believed to lead to an **anaphylactic** type reaction (i.e. severe allergy)
- Results in **disseminated intravascular coagulopathy (DIC)**:
  - Widespread activation of coagulation cascade → formation of multiple blood clots in small vessels throughout body
  - Platelets and clotting factors exhausted → normal clotting disrupted → multiple haemorrhage

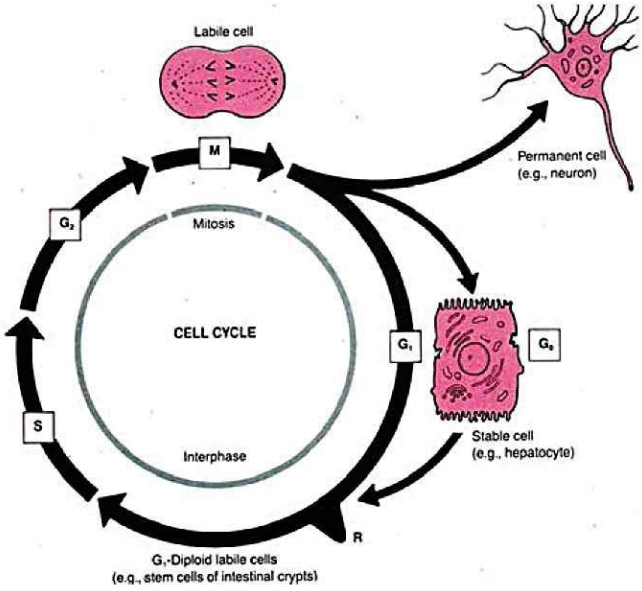
f. Other Embolism

- **Foreign object embolism**: variety of external material may give rise to embolism (eg. glass, bullets)
- **Tumour embolism**: embolism secondary to tumours
  - Tumour release tumour cells into blood stream via neovasculature
  - Most tumour cells destroyed by mechanical forces (shear) or immune response in blood
  - Surviving tumour cells may trigger coagulative cascade and cause formation of embolus
- **Septic embolism**: infected embolus break off from original site of infection ± coagulant secretion by bacteria

L76 Process of Healing in Health and Disease

A. Overview on Healing

- **Healing**: body's replacement of damaged tissue by living tissue
- Involves:
  - **Repair**: replacement of lost tissue by **granulation tissue** which matures to form **scar** (fibrous) tissues
    - → Inevitable when tissue damage is extensive or surrounding specialized cells do not possess capacity to proliferate (eg. muscle and neurones)
    - → **Granulation tissue**: repair tissue composed of proliferating capillaries, fibroblasts, myofibroblasts and macrophages
  - **Regeneration**: process whereby lost tissue is replaced by tissue similar in type through proliferation of surrounding undamaged specialized cells
- Somatic cells classified in terms of inherent regenerative capacities:
  - **Labile cells**: cells that continue to multiply throughout life
    - → Examples: epidermis, alimentary, respiratory and urinary tract epithelium and haemopoietic bone marrow
  - **Stable cells**: cells that normally cease multiplication when growth ceases but retain mitotic ability during adult life
    - → Examples: liver, pancreas, other endocrine organs
  - **Permanent cells**: cells that have lost their capacity to proliferate in infancy
    - → Examples: nerve cell, myocardium



B. Mechanism of Wound Healing

- Involves:
  - Regeneration of **parenchymal** (functional, cf. c.t.) cells
  - Migration and proliferation of parenchymal and c.t. cells
  - Synthesis of ECM proteins
  - Remodeling of c.t. and parenchymal components
  - Collagenization and acquisition of wound strength
- Exact mechanism not well understood
- Three important processes involved:
  - **Growth factor** secretion during inflammatory process
    - → Chemotaxis eg. **fibronectin**
    - → Angiogenesis eg. **vascular endothelial growth factor (VEGF)**
    - → Collagen synthesis eg. IL-1
    - → **Mitogenesis** (triggering of mitosis) eg. **epidermal growth factor (EGF)**
  - Cell-cell and cell-matrix interaction:
    - → Certain normal cell lines proliferate in culture eventually to form a confluent monolayer of cells, then cease proliferating
    - → Density-dependent regulation achieved through these interactions
    - → Regeneration ceases after defect caused by injury has been healed
  - ECM synthesis and Collagenization:
    - → Fibroblasts secrete ECM components (eg. proteoglycans, type III collagen)
    - → Eventually replaced by type I collagen to form a permanent scar
    - → Confers strength to the healed wound

C. Healing in Specific Tissues

- Main determinants of final outcome of any wound:
  - Type and extent of injury
  - Regenerative capacity of constituent cells
  - Extent of damage to ECM framework
- Combination of these factors dictates outcome:
  - Complete regeneration with restoration of normal function
  - **Fibrosis** leading to diminished functional capacity
- Organs may contain specialized cells and distinctive ECMs → organ specificity to healing response

1. Skin Wound Healing

- Two types:
  - Healing by **primary intention** in clean wounds or incisions with minimum of space between margins
  - Healing by **secondary intention** for open wounds

a. Healing by Primary Intention

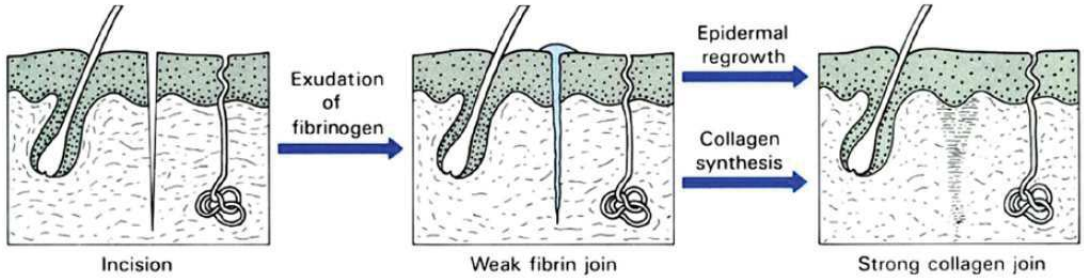
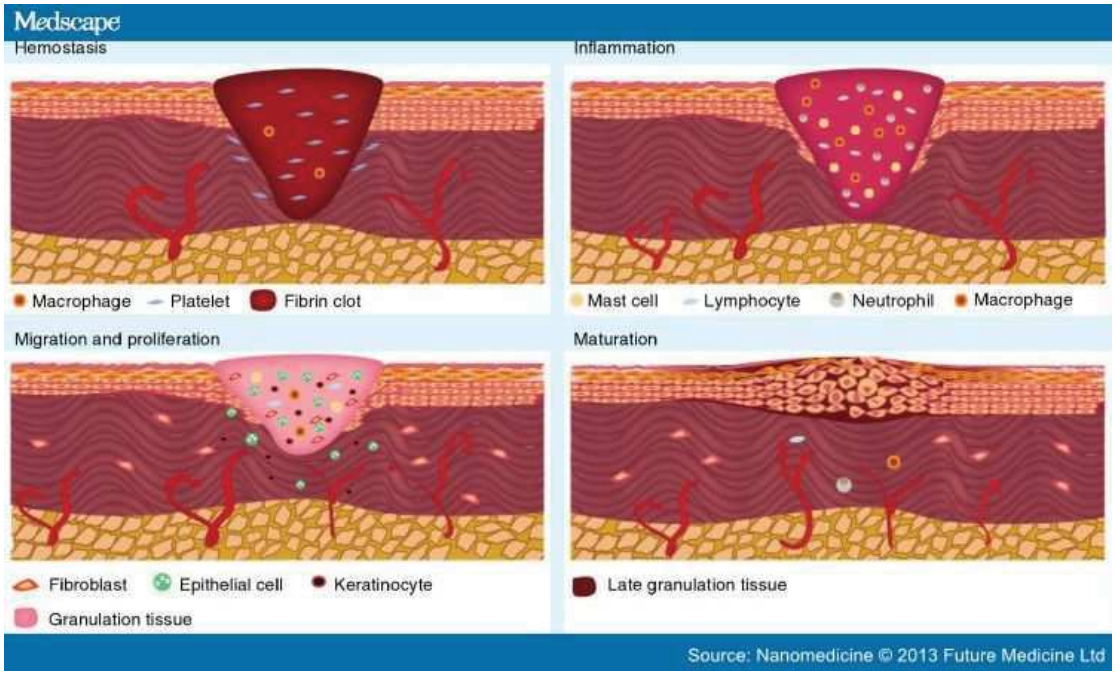
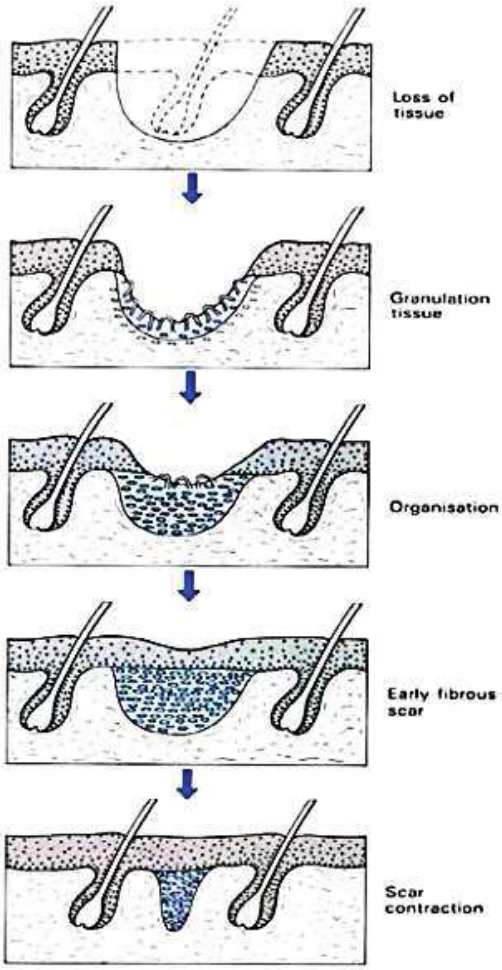


Figure 2 – Healing by primary intention



1) Escape of blood and **exudate** due to damaged vasculature;
2) Clotted blood and fibrin with dehydration forming a **scab**;
3) Acute inflammation during first 24 hours;
4) Proliferation and migration of basal epithelial cells of epidermis which undermine superficial blood clot. (Usually completed 24-36 hours after injury);
5) **Granulation tissue formation**: migration and proliferation of fibroblasts and endothelial cells (organization) seen between 48-72 hours;
6) Appearance of thin branching bundles of collagen fibrils coated with ground substance (**reticulin** fibres) 4-5 days after injury;
7) Progressive increase in mature collagen fibres during second week, forming a scar;
8) Loss of vascularity and shrinkage of scar.

b. Healing by Secondary Intention



- Differences from healing by primary intention:

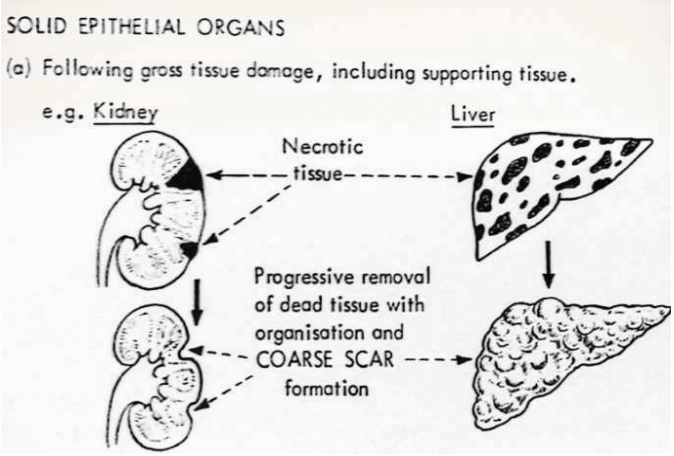
1) Greater tissue loss;
2) More inflammatory exudate and necrotic material to remove;
3) More granulation tissue therefore a bigger scar and more deformity;
4) **Wound contraction** necessary (by **myofibroblasts**);
5) Slower process;
6) Increased liability to infection.

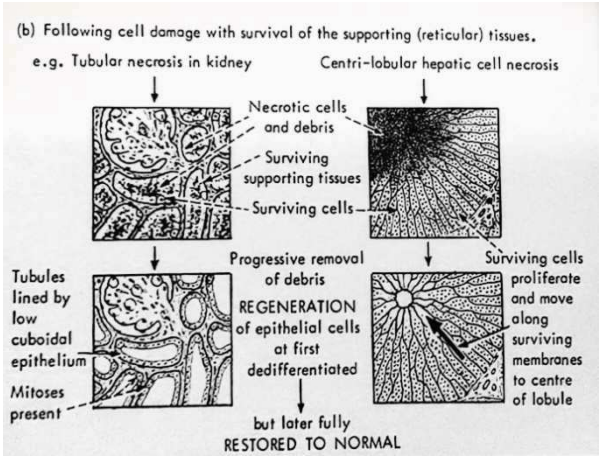
- Clinical application: doctors make a clean cut at vagina when vaginal dilatation not adequate for infant's head to pass through → healing by primary intention rather than secondary (faster healing)

2. Healing of Epithelial Ulceration

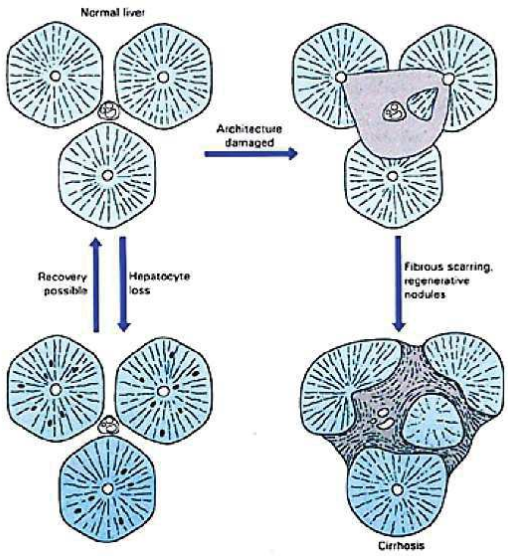
- Ulceration of internal organ epithelium is <u>similar</u> to skin wound healing
- Regeneration of epithelial cells normally occurs
- Extensive ulceration → difficulty in restoring normal architecture → scar tissue formation

3. Healing of Solid Epithelial Organs

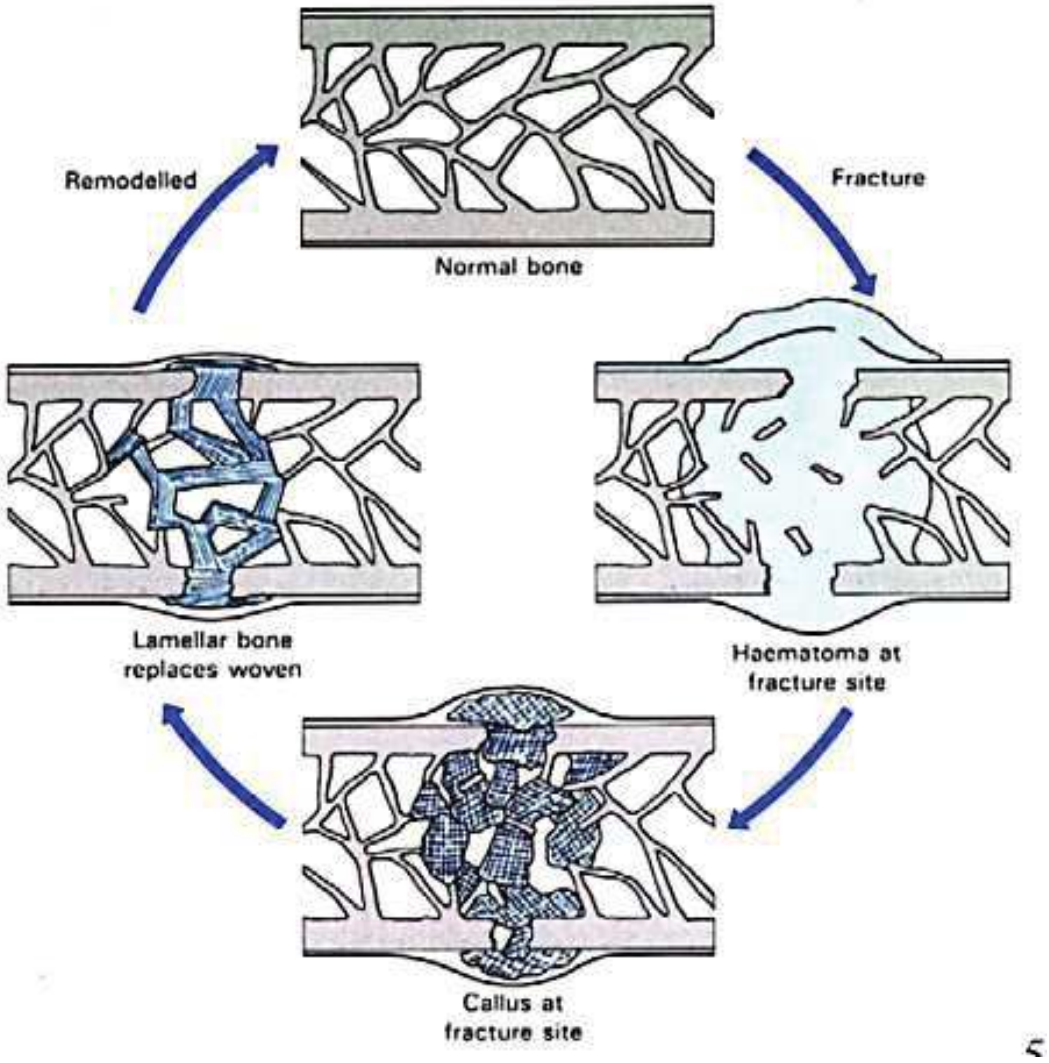




- **Gross tissue damage**: progressive removal of dead tissues leading to coarse scar (nodule) formation
- Cell damage with survival of supporting tissues: progressive removal of debris → regeneration of epithelial cells → full restoration to normal
- Example: liver:
  - Loss of hepatocyte without damage to architecture: recovery followed by full restoration of function
  - Damage to liver architecture: fibrous scarring + regenerative nodules → **cirrhosis**

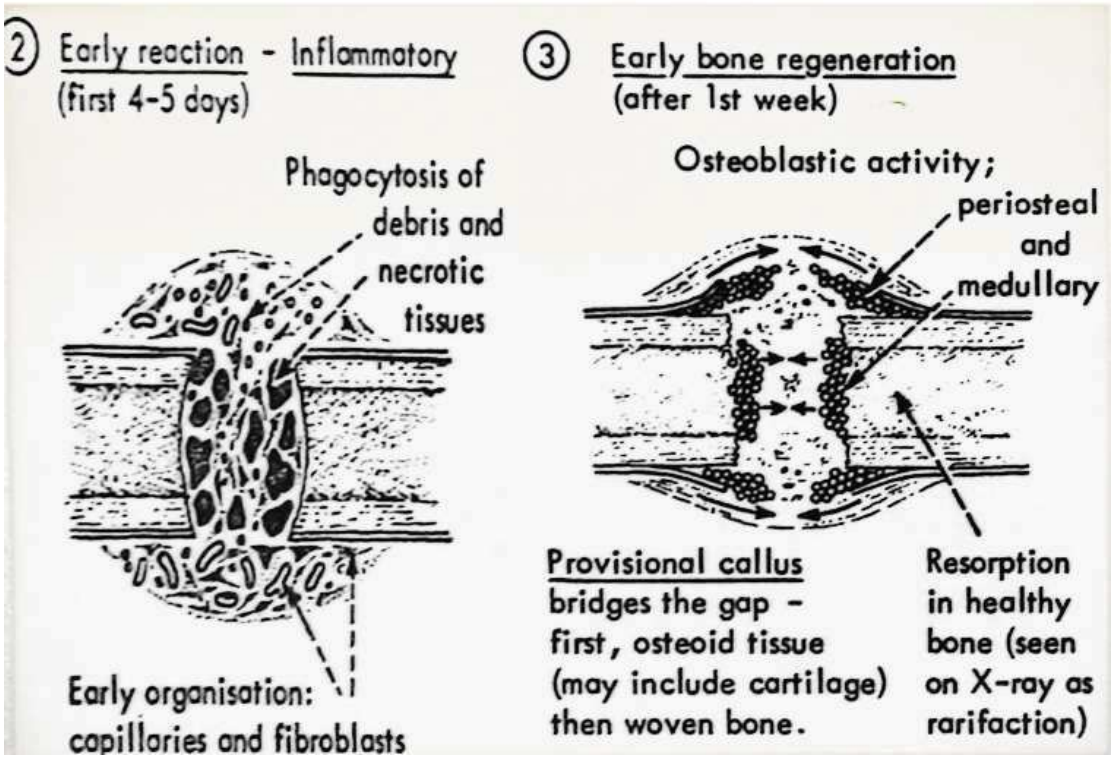


4. Healing of Bone Fractures



- Usually performed by proliferation of **callus**
- Divided into stages but entire area will not all be at same stage at the same time

1) Formation of a **haematoma**;



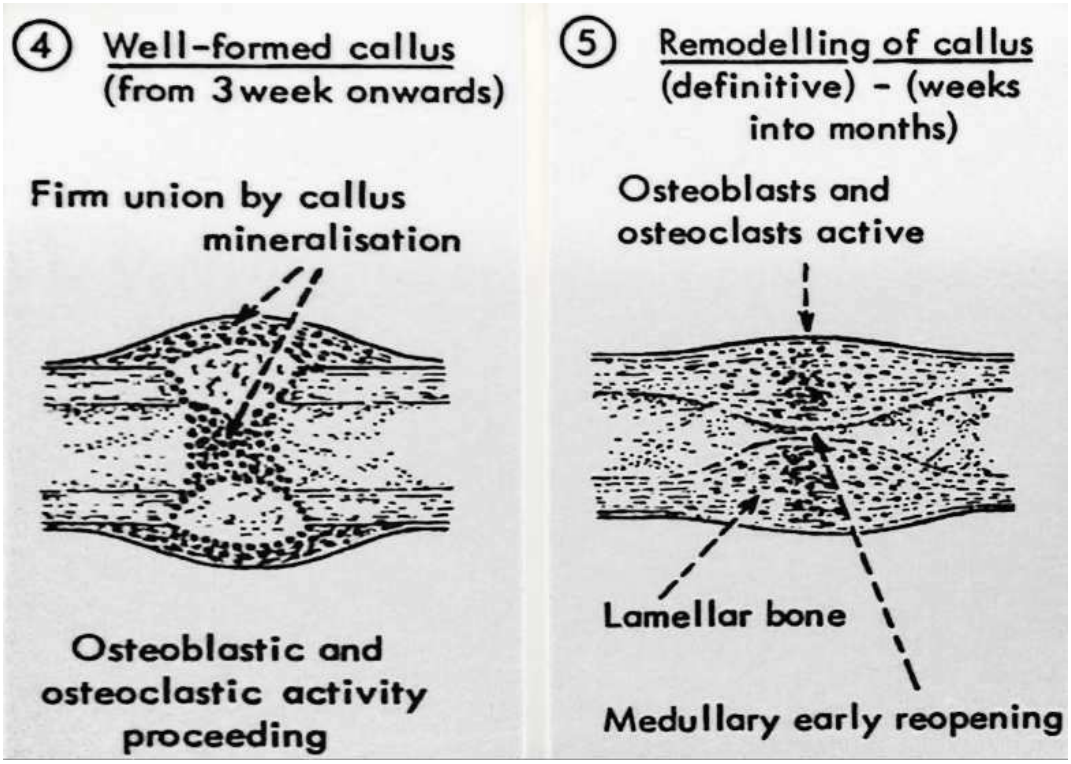
2) Traumatic **inflammation**: tissue damage excites an inflammatory response;
3) **Demolition**: fragments of bone detached from blood supply undergo **necrosis** and are removed by **macrophages** and **osteoclasts**;
4) **Granulation tissue formation**: ingrowth of capillary loops and mesenchymal cells derived from **periosteum** of **cancellous bone**. Integrity of periosteum is of great importance ∵ cells of its deep layer also have osteogenic property;

***Periosteum**: membrane around bone

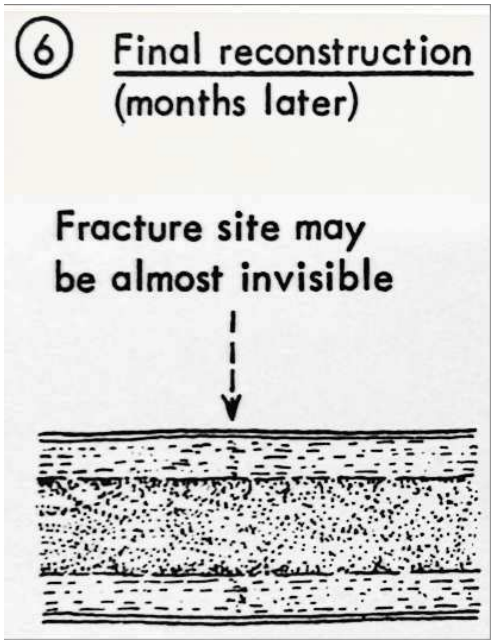
****Cancellous bone**: spongy bone

5) **Callus** formation: mesenchymal osteoblasts or **chondroblasts** next differentiate to form either new **woven bone** or **cartilaginous tissue** to unite fracture ends. Cartilage formation occurs in fractures in which there is movement and eventually undergoes calcification and death of cartilage cells;

***Woven bone**: random collagen arrangement (cf. **lamellar bone** with layered collagen arrangement)



6) Formation of **lamellar bone**: dead calcified cartilage disintegrates and is invaded by blood vessels and osteoblasts, whereas **woven bone** is removed by **osteoclasts**. **Provisional callus** is removed as osteoblasts further lay down **osteoid** to form **lamellar bone**. Collagen bundles are now arranged in orderly lamellar fashion and **Haversian Systems** are found;



*__Osteoid__: predecessor of bone ECM before mineralization consisting of organic substances (eg proteins)

**__Haversion Systems__ or __osteons__: functional unit of compact bone

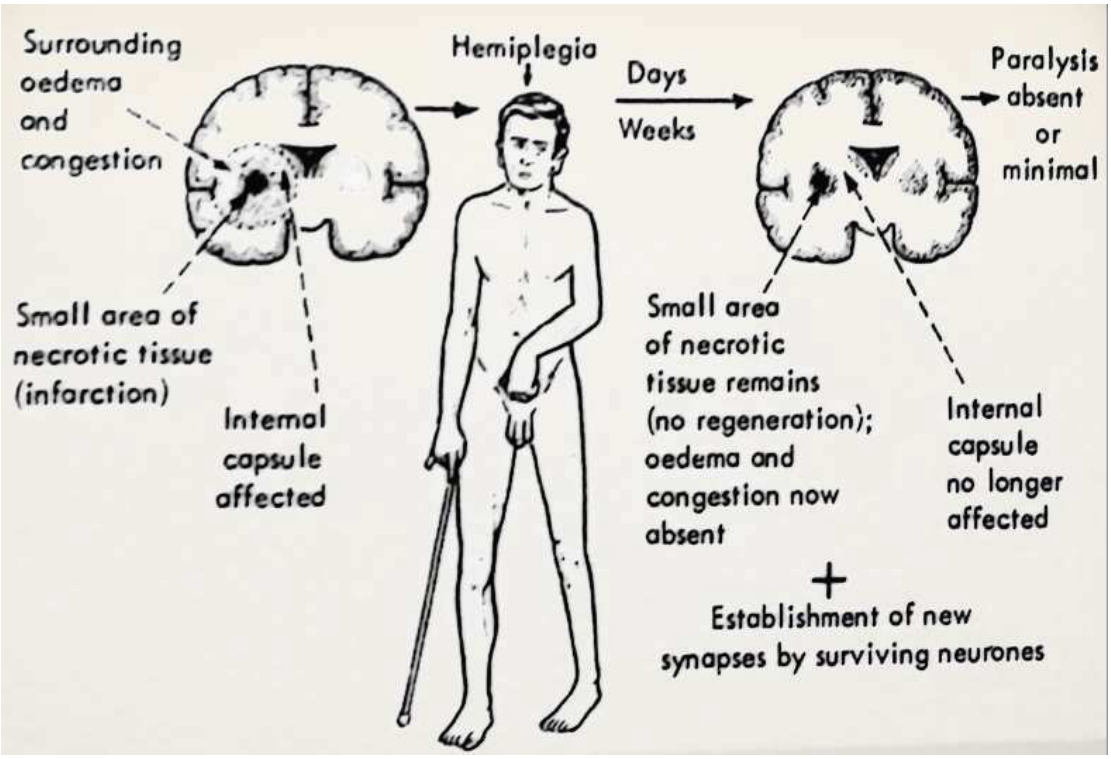
7) Remodeling: continued osteoclastic removal and osteoblastic laying down of bone which differs little from the original tissue.

- Complications of fracture healing:
  - Delayed union
  - Mal-union (angulation or shortening)
  - Fibrous union due to:
    - → Excessive movement leading to development of a false joint
    - → Infection (may also give rise to osteomyelitis)
    - → Ischaemia
  - Non-union if soft-tissues (eg, muscles or fat) are interposed between severed ends

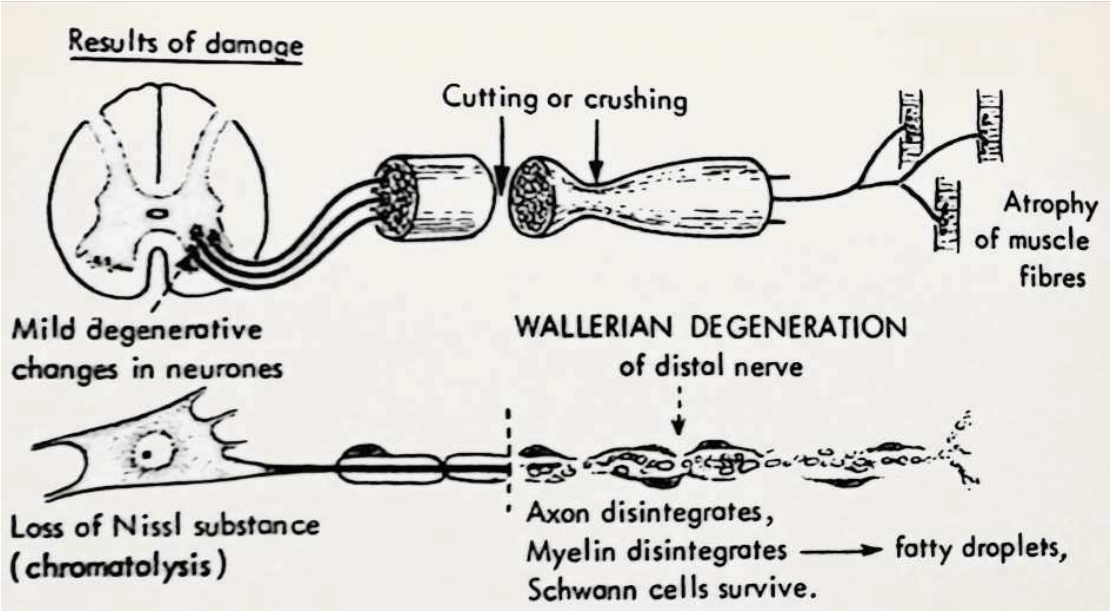
5. Repair of Cartilage, Tendon and Muscles

- Regeneration of cartilage is poor and repair occurs by growth of fibrous tissue (→ fibrocartilage formation)
- Regeneration of tendon is good but slow
- Regeneration of muscles depend on their type
  - Cardiac muscle shows no regenerative capacity (→ scar tissue formation → overall atrophy of myocardium → possible rupture of heart in second cardiac event following myocardial infarction)
  - Skeletal muscles regenerate by means of **satellite cells**
  - Smooth muscles has highest capacity to regenerate

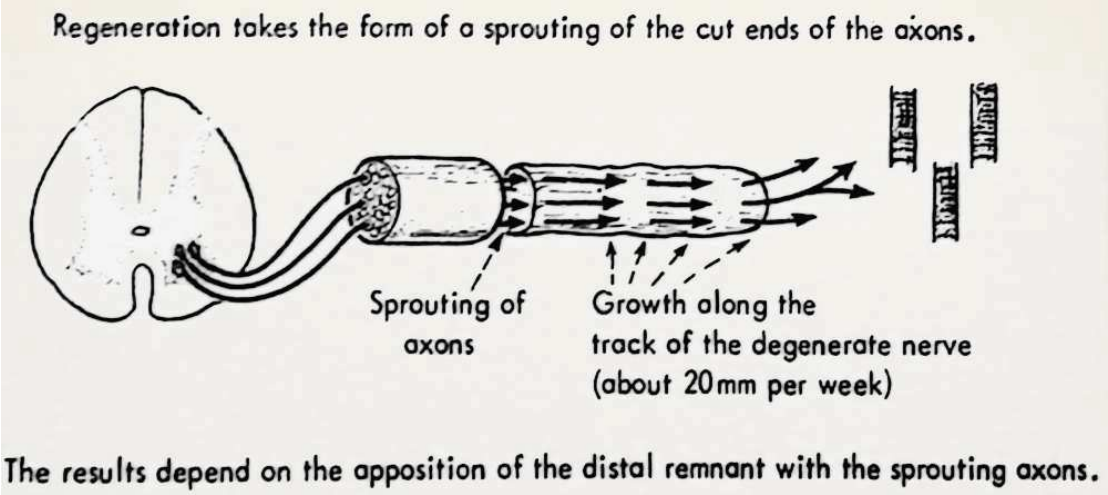
6. Repair of Nervous Tissues and Nerves



- Damage to neurones in CNS:
  - No regeneration
  - Repair follows same general pattern as in other parts of body
  - Macrophages present derived both from microglia and blood monocytes
  - Glial tissues formed by astrocytes instead of fibrous tissue



- Neurone processes in PNS have considerable regenerative capacity
- After axon injury, there is **Wallerian degeneration**: total axon degeneration and secondary breakdown of myelin sheath distal to the cut



- If site of injury is sufficiently distal, regeneration takes place:

1) Axon undergoes transient swelling and breakdown of ER (i.e. **Nissl bodies**);
2) **Schwann cells** proliferate within nerve sheath to form pathways along which axons may re-grow;
3) Axonal sprouts emerge from proximal end of interrupted axis cylinders and may grow into distal part of nerve.

- If injury is proximal or involves nerve soma → irreversible nerve cell death
- Lesion transects nerve → axonal sprouts of a set of nerve grow into lesion → of a painful **traumatic neuroma** + failure of complete axonal regeneration
- **Axonopathy** or **axonal neuropathy**: axonal degeneration due to diseases other than trauma
- **Giant axonal swelling**: observed in nerve toxicity related to n-Hexane, acrylamide and CS_2 exposure
  - Degenerating axons markedly swollen with accumulation of **neurofilaments** in axoplasm
  - Secondary degeneration of myelin sheaths follow
  - Usually start at distal end of axon and extend back to soma through **retrograde degeneration**
  - Subsequent regeneration depend on extent of damage incurred

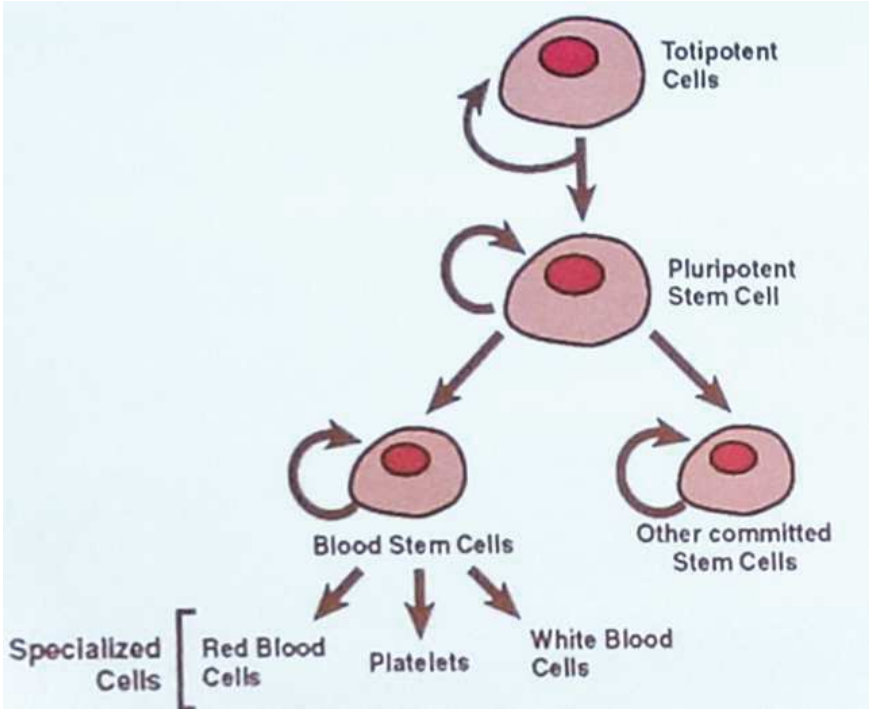
D. Factors Influencing Wound Healing

- Local factors adversely affecting healing:
  - Type of wounding agent: blunt, crushing, tearing etc
  - Infection
  - Foreign bodies in wound (chronic inflammation delay healing)
  - Poor blood supply
  - Excessive movement
  - Poor apposition of margins (eg. large haematoma formation)
  - Poor wound contraction due to tissue tethering (eg skin over tibia)
  - Infiltration by tumour
  - Previous irradiation
- General factors adversely affecting healing:
  - Poor nutrition → impairment of collagen formation (eg protein, vitamin C or zinc deficiency)
  - Excessive **glucocorticosteroid** production or administration
    - → **Glucocorticosteroid**: group of chemical (incl. cortisol) that will lead to glucose conservation in blood
    - → Systemic diseases such as DM, renal failure, **cachexia** (wasting syndrome)
- UV irradiation accelerates healing

E. Complications during Wound Healing

- **Infection**: delays or even stops healing
  - Tissue destruction due to infection is common
  - Simple incision will need to be healed by secondary intention if infected
- **Wound dehiscence**: ruptured wound sutures
  - Particularly important after **laparotomy** (surgery that opens abdomen)
  - Mortality of burst abdomen is high
  - Example: rupturing of intestinal suture may lead to peritonitis
- **Implantation**: abnormal growth of epithelial cells in sub-epithelium c.t.
  - Epithelial cells flowing into healing wound may proliferate to form **epidermoid cyst**
- Excessive tissue formation:
  - Excessive granulation tissue formation
  - **Keloid** formation: excessive scar formation (due to excessive formation of collagenous tissue) → raised area of scar tissue
- Pigmentary changes: healed chronic ulcers sometimes have a rusty colour due to deposition of **haemosiderin**
- Painful scars: **traumatic neuroma** formed when axonal sprouts grow into scar tissue causing painful swelling
- Weak scars: stretching may result from continuous strain on scar tissue
  - **Incisional hernia**: protrusion of organs out through an incision
  - Example: rupturing of abdominal suture due to coughing
- **Cicatrisation**: contraction by fibrous tissue in order to reduce scar size leading to tissue distortion
  - Late reduction in size of scar
  - Frequently produces great deformity
  - Example: healing in hollow viscera (eg urethra, oesophagus, intestine) leading to progressive **stenosis** (narrowing of lumen) with **stricture** formation (eg. duodenum cicatrisation)
- **Neoplasia**: very rarely **squamous cell carcinoma** may develop in scars

F. Stem Cells



- **Stem cells**: unspecialized cells that have the ability to divide indefinitely and continue to proliferate in spite of physiologic or artificial removal of cells from population
- Can give rise to different kinds of specialized cells (eg heart muscle cell, brain cell, BM cell etc)
- Sources of stem cells:
  - Adult mesenchymal: marrow, blood, fat, muscle, skin, conjunctiva
  - Umbilical cord blood
  - Embryonic: left-overs from IVF, cloned embryos
    - → Removed from inner cell mass at blastocyst stage four days after fertilization
- Stem cells in developing tissues give rise to multiple cell types that make up to heart, lung, skin and other tissues
- Discrete populations of adult stem cells in some adult tissues (eg. BM, muscle, brain) generate replacements for cells that are lost through normal wear and teaer, injury or disease
- Promises of stem cell research:
  - Cells as treatment for diseases eg. Parkinson's disease, DM and heart disease
  - Screening new drugs and toins
  - Understanding birth defects, development and gene control

- **Embryonic stem cells**:
  - Versatile and can be cultured more rapidly
  - More difficult to induce into specific tissues desired
  - Risk of rejection as foreign tissue
  - Danger of forming tumours
  - Danger of transmission of infection
  - Ethical issue: destruction of embryo
  - No successful example in treating human disease yet
- **Adult stem cells**:
  - Easier to coax into specialization
  - More finite lifespan
  - Less risk of uncontrollable growth tumours
  - Less risk of immune rejection
  - Examples:
    - → BM cells can differentiate into epithelial cells of liver, lungs, GI tract and skin
    - → Human fat cells from liposuction can differentiate into fat, cartilage, muscle and bone cells
  - Represent all clinical successes in use of stem cells:
    - → Examples: treatment of some forms of thalassemia and leukemia

L77 Chronic Inflammation

A. Chronic Inflammation

- **Chronic inflammation**: a prolonged process in which tissue destruction and inflammation proceed at the same time as attempts at healing or repair

1. Settings of Chronic Inflammation

- Two possible settings:
  - Following acute inflammation (inability of resolution)
  - Distinct process from outset (low-grade smoldering inflammatory response)

a. Chronic Inflammation following Acute Inflammation

- Acute inflammatory process has not been resolved in the normal way
- May be due to persistence of inciting stimulus, interference of normal healing process or repeated bouts of acute inflammation
- Mixture of acute inflammation, demolition, repair and regeneration
- Lesion produced called **chronic suppurative inflammation**
  - **Suppurative**: pus-forming
- Continuation of acute inflammation, ultimately resulting in repair with fibrous scarring
- Examples:
  - Unresolved Acute Inflammation
    - → **Empyema thoracis**: collection of pus in thoracic cavity
    - → **Chronic osteomyelitis**
    - → **Chronic abscess**
  - Repeated episodes of acute inflammation
    - → **Chronic pyelonephritis** (pyelo- = basin (pelvis) and nephr- = kidneys)
    - → **Chronic salpingitidis** (inflammation in fallopian tubes)
    - → **Chronic cholecytitis** (gall bladder inflammation)

b. Chronic Inflammation as Distinct Process from Outset

- Brief and minimal initial acute phase
- May begin as a low-grade smoldering response (slow, subdued inflammation) with none of classic symptoms of acute inflammation
- Includes some of the most common and disabling diseases in human
- May occur in settings such as:
  - Persistent infection by a certain microbe against which the body has only a limited resistance
    - → Many are intracellular organisms of low toxicity but evoke immunological reaction
    - → Eg. Hepatitis B, TB, **leprosy** (lepromatosis infection), **syphilis** **brucellosis** and some fungi
  - Insoluble particles in body
    - → Eg. Silica, asbestos, cholesterol
  - Persistent state of hypersensitivity
    - → Non-infective condition: eg. Allergic contact dermatitis
    - → Auto-immune conditions: eg. Rheumatoid arthritis
  - Unknown aetiology, eg. **Sarcoidosis** (abnormal collection of inflammatory cells forming nodules in the body)

2. Histological Hallmarks of Chronic Inflammation

- Major histological hallmarks:
  - Infiltration by mononuclear cells (vs PMNs in acute inflammation), principally macrophages, lymphocytes and plasma cells
  - Granulation tissue formation
  - Fibrosis and tissue destruction
  - Regeneration

a. Inflammatory Cells

- Persistence of macrophages is an important characteristics
- Other inflammatory cells such as plasma cells, lymphocytes and polymorphonuclear leukocytes are also present

i. Macrophages

- Central figure in chronic inflammation due to great number of biologically active products produced:
  - Can cause tissue damage, other WBC influx, fibroblast proliferation, vascular proliferation, collagen deposition, fibrosis
  - May thus lead to progressive tissue damage and loss of function

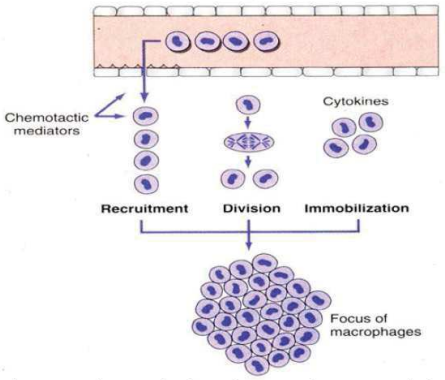


Figure 3 – Three mechanisms for macrophage accumulation

- Monocytes emigrate early in acute inflammation → transformation into macrophages in extravascular tissues
- Prompt removal of irritant in acute inflammation → disappearance of macrophages
- Failure to eliminate irritant → macrophage accumulation may persist for a prolonged period of time, possible causes include:
  - Continued recruitment of monocytes
  - Local proliferation of macrophages
  - Prolonged survival and immobilization at site of inflammation
- Activation of macrophages may form **epithelioid cells** or even **giant cells** in certain chronic inflammation specimens

ii. Plasma Cells

- Activated by macrophages
- Produce Ab against persistent antigen or altered tissue components at site of inflammation

iii. Lymphocytes

- Participate in both cell-mediated immunity and humoral immunity
- Can be activated by contact with antigens
- Activated lymphocytes:
  - Produce **lymphokines** → major stimulator of monocytes and macrophages
  - Cause **monocyte chemotaxis**, **macrophage activation** and **differentiation**
  - Products of activated macrophages in turn influence T and B cell functions

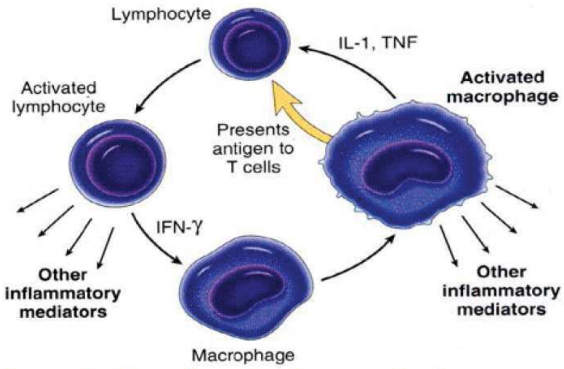


Figure 4 – Macrophage-lymphocyte interactions

iv. Polymorphonuclear Leukocytes (PMNs)

- Also called **polymorphs** or **neutrophils**
- Morphological feature: eccentric nucleus (on one side)
- Despite being hallmarks of acute inflammation, may still be present in chronic inflammation following on persistent acute inflammation (numerous PMNs found in pus)
- Eg. chronic osteomyelitis and actinomycosis (*Actinomyces* infection)

b. Granulation Tissue

- Proliferation of fibroblasts and small blood vessels
- Part of healing process
- Pus-filled cavity lined by **pyogenic membrane** (acutely inflamed granulation tissue) in **chronic suppuration**
- Vascularity of granulation → haemorrhage from thin-walled capillaries

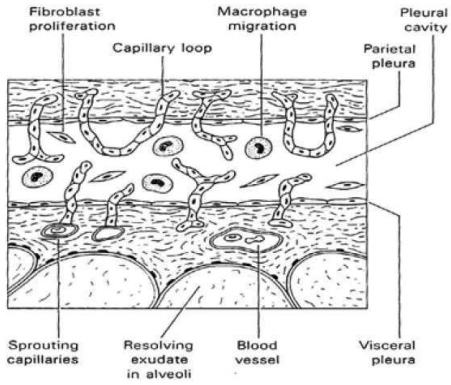


Figure 5 – Formation of granulation tissue

c. Fibrosis and Tissue Destruction

- **Tissue destruction**: an important hallmark for chronic inflammation
- **Fibrosis** may have important complications:
  - **Chronic rheumatic valvulitis** → **valvular stenosis** and **regurgitation**
  - **Chronic gastric ulcers** → **pyloric stenosis**
  - **Fibrous ankyloses** (stiffness of joint due to fibrotic abnormal adhesion) → severe limitation of movement
    → Frequently found in **rheumatoid arthritis**
- **Adhesion** may also result from tissue destruction (due to chronic inflammation)
  - Eg. serous cavity adhesion
  - Eg. Joint deformity

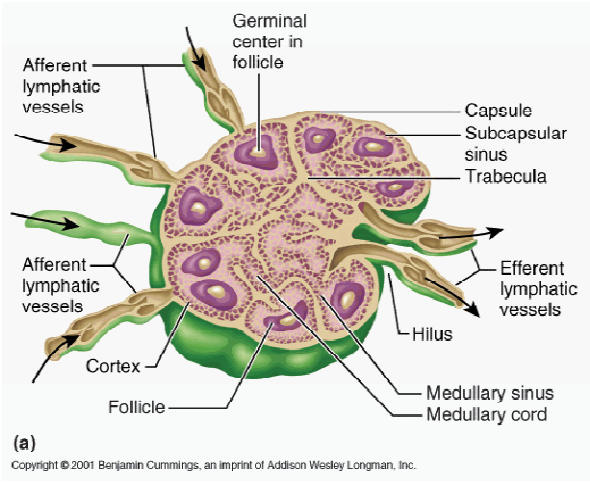
d. Regeneration

- Regeneration of destroyed tissue can result in **epithelial overgrowth**
  - Eg. at edge of a chronic gastric ulcer
  - Eg. invagination of gall bladder epithelium into muscle wall in **chronic cholecystitis** (inflammation of gall bladder)
  - **Endarteritis obliterans**: gradual occlusion of lumen of small arteries due to progressive proliferation of tunica media
    → Seen in bed of chronic peptic ulcers and following radiation damage

3. General Effects of Chronic Inflammation

a. Hyperplasia of Lymphoid System

- **Hyperplasia**: excessive increase in cell number
- Lymph nodes draining a chronic infective lesion will show hyperplasia (for sinus-lining cells and sometimes **germinal centres** and **medullary cords**)



b. Antibody Production

- Body effect an immune response with production of antibodies
- Demonstration of specific immunoglobulin is a useful diagnostic procedure
- Long-continued Ab production associated with **reactive systemic amyloidosis**: formation of **AA protein** (a type of acute phase protein) → abnormal aggregation in body parts

4. Granulomatous Inflammation

- **Granulomatous inflammation**: special type of chronic inflammation characterized by presence of **granuloma**
- **Granuloma**: small, 0.5-2 mm tumor-like collection of inflammatory cells especially modified macrophages called **epithelioid cells**
- **Epithelioid cells**: have abundant, pale-pink plump cytoplasm, resembling an epithelial cells
  - Derived from blood **monocytes**
  - Less phagocytic than macrophages
- Factors determining formation of granulomas:
  - Presence of <u>indigestible organisms</u> or <u>particles</u>
  - Presence of <u>cell-mediated immunity</u> to inciting agent: products of activated T enhance monocyte transformation (into epithelioid cells and multinucleate giant cells)

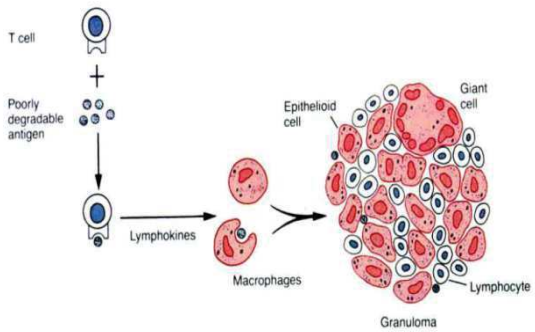


Figure 6 – Mechanism of granuloma formation

a. Types of Granuloma

- **Foreign body granuloma**: granulomatous inflammation incited by inert foreign bodies
  - Eg. talc, sutures, lipid/cholesterol, collagen
- **Immunological granuloma**: granulomatous inflammation incited by insoluble particles capable of inducting CMIR
  - Infective: eg. TB (**tubercle**: TB granuloma), leprosy, fungi, parasitic ova
  - Tumors: eg. **seminoma** (testicular cancer of germ cells)
- **Idiopathic granuloma**: eg. **sarcoidosis**

b. Causes of Granuloma

- **Bacterial**: eg. *Mycobacterium tuberculosis*, *Mycobacterium leprosum*, *Traponema pallidum*
- **Parasitic**: eg. *Schistosoma* (**schistosomiasis**)
- **Fungal**: eg. *Crytococcus*
- **Inorganic metal and dusts**: eg. silica dust (**silicosis**), beryllium (**berylliosis**)
- **Unknown**: eg. sarcoidosis

*TB tell-tale signs: tubercle (aggregate of epithelioid cells and Langhans' multinucleated giant cells), casous necrosis, acid-fast bacilli, cell-mediated hypersensitivity (type IV)

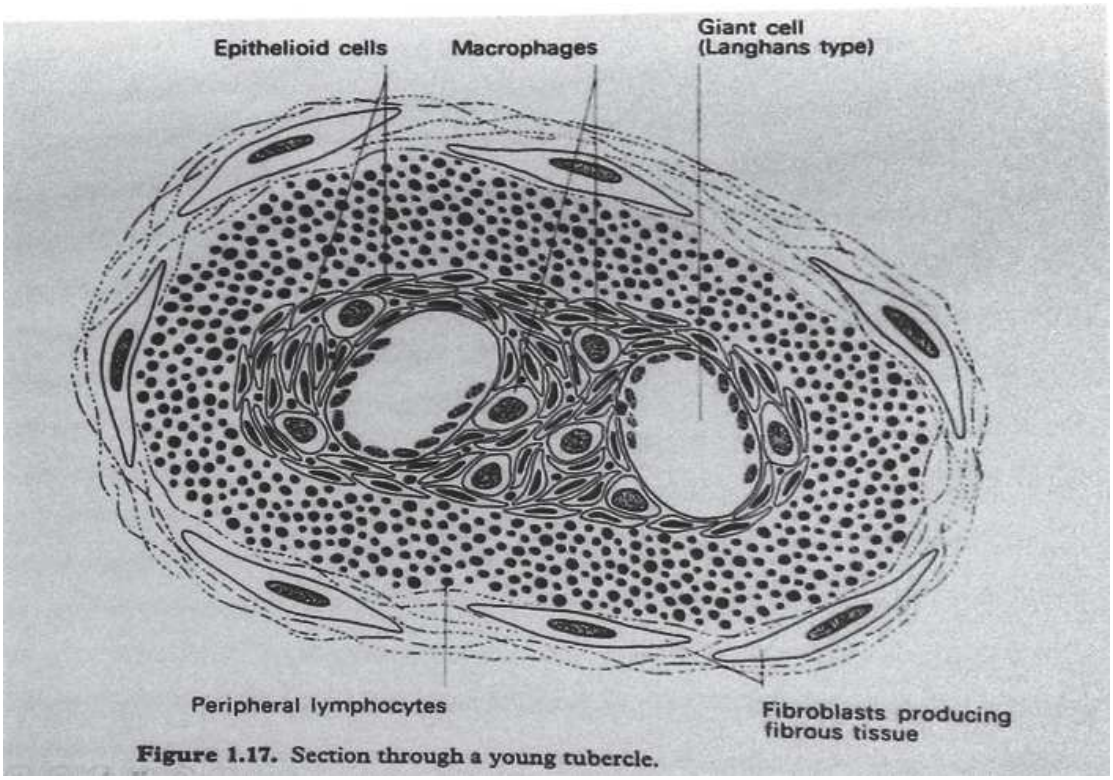


Figure 1.17. Section through a young tubercle.

c. Giant Cells in Granulomas

- Multinucleated giant cells may be present in granuloma
- Formed by **coalescence** (joining) and fusion of epithelioid cells
- May achieve diameter of 40-50 mm and contains up to 50 nuclei
- Examples:
  - **Langhans giant cells**:
    → Nuclei disposed around periphery in a horseshoe arrangement
    → Highly indicative of a tubercle
  - **Foreign body giant cells**
    → Nuclei scattered haphazardly throughout cell
    → Some present centrally
  - **Touton giant cells**
    → Found in **xanthomata** (deposition of lipid in body tissues)
    → Foamy peripheral cytoplasm with ring of nuclei surrounding central area of clear eosinophilic cytoplasm

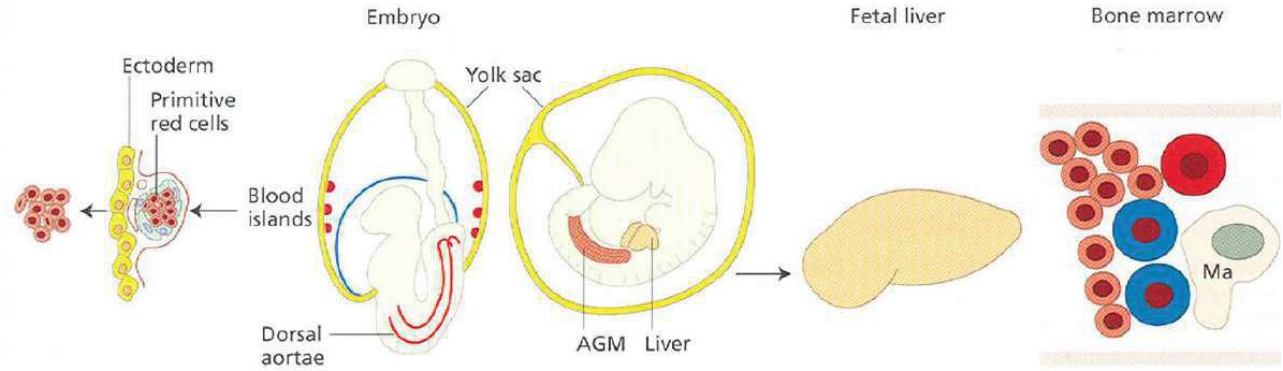
5. Differences between Acute and Chronic Inflammation

	Acute inflammation	Chronic inflammation
Aetiological onset	Brief and intense	Persistent and indolent
Duration	Days	Weeks to months
Nature	Exudative	Proliferative
Consequence	Resolution	Destruction and fibrosis

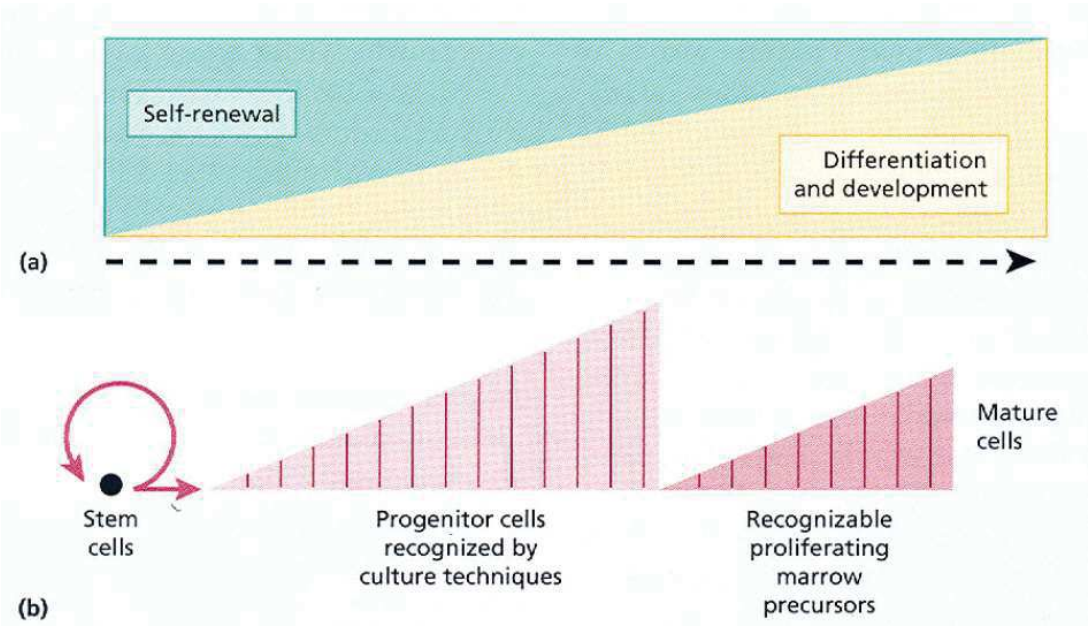
L78 Where does my blood come from and why do my eyes look different?

A. Haemopoiesis

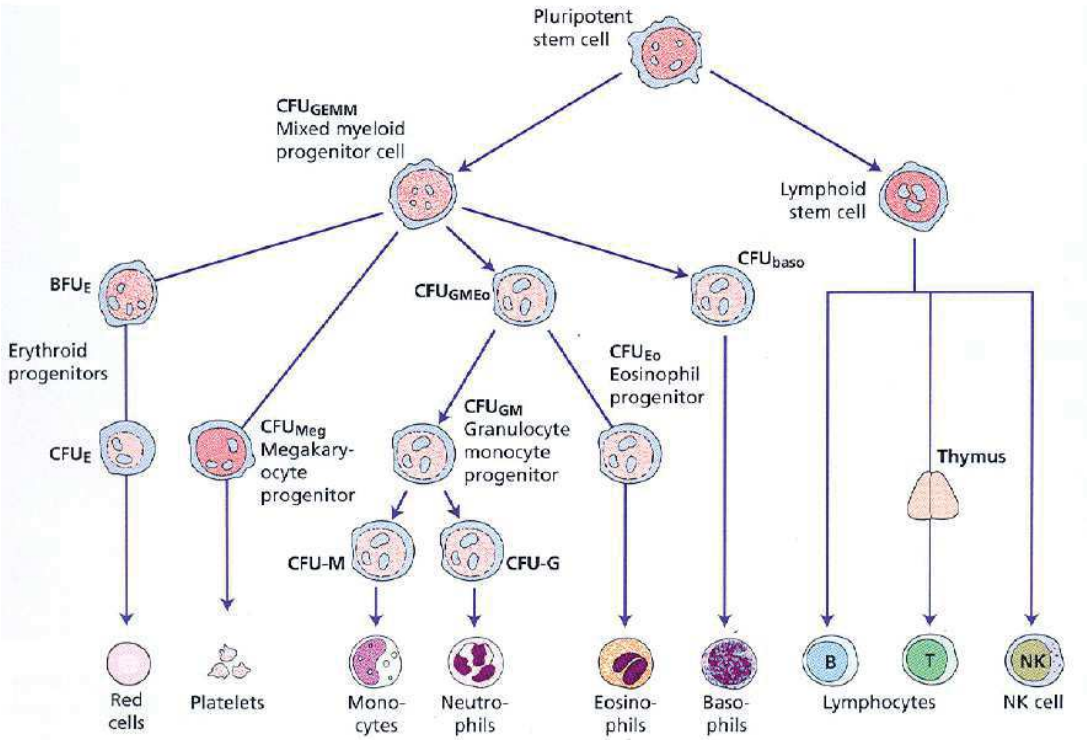
- **Haemopoiesis**: formation of blood cells
- 2M RBCs are formed every second



- Site of haemopoiesis changes with the developmental stage of body:
  - Embryonic stage:
    - → Yolk sac
    - → Placenta
    - → **Haemangioblasts** in aorta-gonad-mesonephros (classified into **haematopoietic** and **endothelial**)
  - Fetal stage: liver, spleen, bone marrow
  - At birth: bone marrow (of all bones)
  - Adult: ends of long bones, skull, sternum, ribs, vertebrae, pelvis



- Continued production of blood cells in BM sustained by **pluripotent stem cells (PSCs)**
  - 1 stem cells → 10^6 mature blood cells
- Achieved by unique properties of PSC:
  - Unlimited self-renewal
  - Multi-lineage differentiation potential
- PSCs generate **progenitor cells** upon reception of signals via **asymmetric replication**
  - **Progenitor cells**: cells which can divide but with limited self-renewal
    → Differentiate into erythroid, megakaryocytic and various WBC lineages
  - **Asymmetric replication**: one stem cell may generate two stem cells among which only one differentiate while the other keep on replicatin

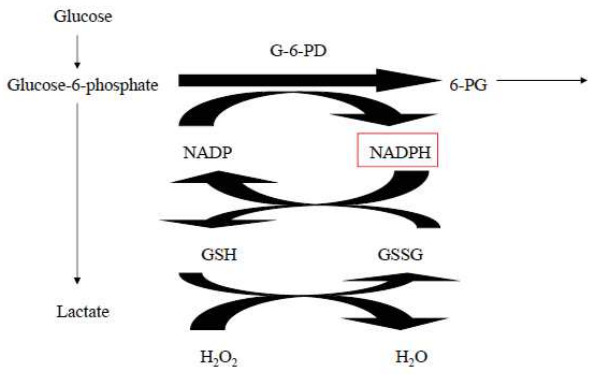


- Types of haemopoiesis:
  - **Erythropoiesis**: formation of RBCs
  - **Myelopoiesis**: formation of **myeloid cells** (i.e. granulocytes incl. monocytes)
  - **Lymphopoiesis**: formation of lymphocytes
  - **Thrombopoiesis**: formation of platelets
- **Extramedullary haemopoiesis**: pathological state of haemopoiesis outside BM
  - Cause: BM defect or disease → body compensate by extramedullary haemopoiesis
  - Possible sites: spleen, liver, lymph node, soft tissue mass
  - Diagnosis: biopsy of suspected site showing haemopoietic cells
  - Example: **myelofibrosis**
    - → Fibrosis of BM crowds out haemopoietic cells
    - → Resultant migration of haemopoietic cells causes extramedullary haemopoiesis

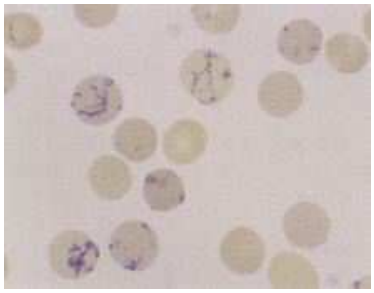
B. Erythrocytes and Anaemia

1. Erythrocytes

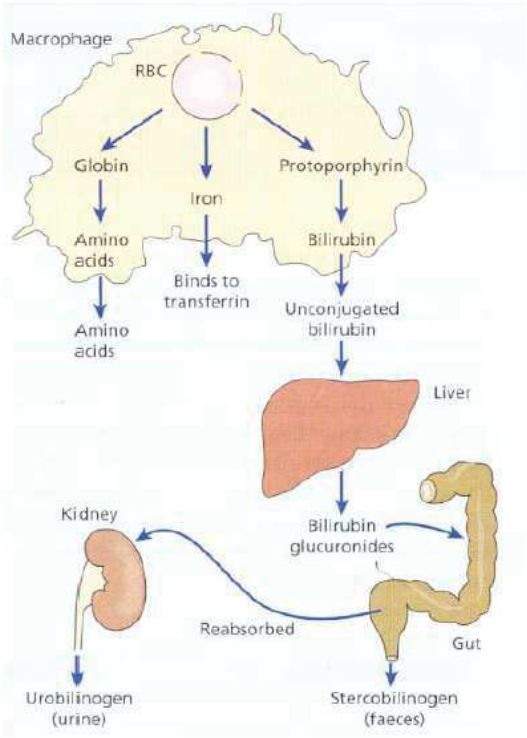
- **Erythrocyte**: a non-nucleated, biconcavely-shaped cell that contains **haemoglobin** with the function of carrying oxygen from lungs to tissues ad return with CO_2 to eliminate in the lungs
- Predominant structure of haemoglobin in adults is **HbA** ($\alpha_2\beta_2$)
- Erythrocytes impart characteristic colour to blood:
  - Bright red when oxygenated (i.e. arterial blood)
  - Dark red when deoxygenated (i.e. venous blood)
- Normal lifespan of RBC is about 100 – 120 days
- Survival depends on a number of factors:
  - Presence of intact cell membrane
  - Deformability of red cell membrane (allows erythrocytes (7-8μm in diameter) to pass through capillaries 2-3μm in diameter): conferred by interactions between specific proteins in the membrane and proteins in the cytoplasm)
  - Solubility of haemoglobin: crystallized or precipitated Hb damages membrane and affects deformability
  - Adequate supply of ATP (via glycolytic pathway)
  - Adequate supply of reducing power (via NADPH and NADH from hexose monophosphate shunt and glycolytic pathway)
- Importance of glycolytic pathway and hexose monophosphate shunt:
  - ATP: provides energy for Na/K pump to maintain cell volume, shape and deformability
  - NADH: reduces inactive oxidized (Fe^{3+}) Hb to active reduced (Fe^{2+}) Hb
  - 2,3-DPG (in side pathway): regulates oxygen affinity of Hb
  - NADPH (in HMS): important for synthesis of **glutathione (GSSG)** to eliminate ROS from RBC



- **Reticulocyte**: immature red blood cells in peripheral blood
  - ~1% of all RBCs
  - Larger and bluish in colour



a. Breaking Down of RBCs



- Breaking down of RBC:
  - Old RBC die in spleen, liver and marrow
  - Death of RBC mainly extravascular in tissue macrophages
  - Replaced through erythropoiesis in bone marrow
- Breakdown products are either recycled or excreted
- Leakage of free Hb plasma is bound to **haptoglobin**
- **Jaundice**: pathological state of hyperbilirubinaemia
  - **Bilirubin**: a breakdown product of the heme part of haemoglobin
  - Three types:
    - → **Prehepatic jaundice**: excess breakdown of RBC leading to inadequate capacity by the liver to process bilirubin
    - → **Hepatic jaundice**: faulty liver conversion of unconjugated bilirubin into conjugated bilirubin
    - → **Posthepatic jaundice**: blockage in excretion pathway of bilirubin (eg. bile duct blockage)
  - Symptoms: yellow tinge to skin (esp sclera of eyes)

2. Anaemia

- **Anaemia**: a state in which haemoglobin concentration in blood is low
  - Measured by machine with peripheral blood sample
  - Hb concentration measured as gram Hb per volume of blood (dL)
  - RBCs are lysed before the measurement
- NOT a disease but a manifestation of an underlying disease
  - Further tests on peripheral blood (eg blood count, reticulocyte count and smear examination) warranted

a. Causes of Anaemia

- Causes of anaemia: upset of balance between production and loss
  - Production defect: eg. bone marrow failure
    - → Inadequate RBC production
    - → Ineffective RBC production (eg. iron deficiency)
  - Over-destruction: eg. **haemolysis** (premature destruction of erythrocytes)
    - → Intravascular or extravascular (eg. in spleen) (or both) haemolysis
    - → Intrinsic (RBC membrane/enzyme/Hb defect) or extrinsic (Ab-mediated, physical damage or toxin) cause
    - → Erythropoietic rate will be raised in an attempt to normalize RBC level
    - → If compensatory mechanism fails → anaemia + hyperbilirubinaemia
  - **Sequestration**: trapping or isolation of RBCs such that they are not in effective circulation, eg. hypersplenism
  - Dilution: increase in plasma volume relative to RBC mass
- Most common cause in the world: iron deficiency anaemia
  - Acquired: **iron deficiency anaemia**
  - Hereditary: **thalessaemia**
    - → Diagnosed by haemoglobin study
  - Both are **microcytic anaemia**, i.e. RBC becomes smaller

i. Iron-deficiency Anaemia

- Most common form of anaemia
- Iron very important in body tissues (Hb, myoglobin, enzymes) but ability to absorb ingested iron from gut is limited (most from recycled iron) → delicate balance → easy development of iron deficiency
- Pathogenesis: deficient in iron → reduced erythropoiesis → reduction in blood RBC concentration
- Causes:
  - Chronic blood loss (most common, can be asymptomatic)
    - → GI bleeding
    - → Urinary tract bleeding (eg. **haematuria** (RBC in urine))
    - → Genital tract bleeding (eg. **menorrhagia** (abnormally heavy and prolonged menstrual period))
    - → Pulmonary tract bleeding (eg. **haemoptysis** (coughing up blood))
    - → Trauma (**NO ANAEMIA immediately after acute trauma** because RBC and plasma are lost at same proportion; anaemia occurs after plasma replenishment)
  - Increased demand (due to pregnancy or puberty → patient profile)
  - Poor intake/malabsorption (look for other signs eg. **cachexia**)
- Diagnosis: **serum iron profile**
  - Tests for serum iron, transferrin and ferritin level and transferrin saturation
  - Note ferritin level will be elevated anyway in inflammatory settings → irrelevant in hospital context

b. Clinical features of Anaemia

- Clinical features: mainly caused by body compensation/decompensation to tissue hypoxia
- **Pallor**: paleness in skin or mucosa caused by decreased HbO_2 levels
  - Observe superficial capillaries in conjunctiva → white instead of red
  - Note pallor of peripheral tissues may be due to vasoconstriction → must detect central area (eg. eye) with temperature about the same as core temperature
- **Palpitation**: own feeling that heart is beating (normally cannot be felt, usually caused by heart beating faster and stronger)
- **Shortness of breath**
- **Fatigue**
- **Organ failure**

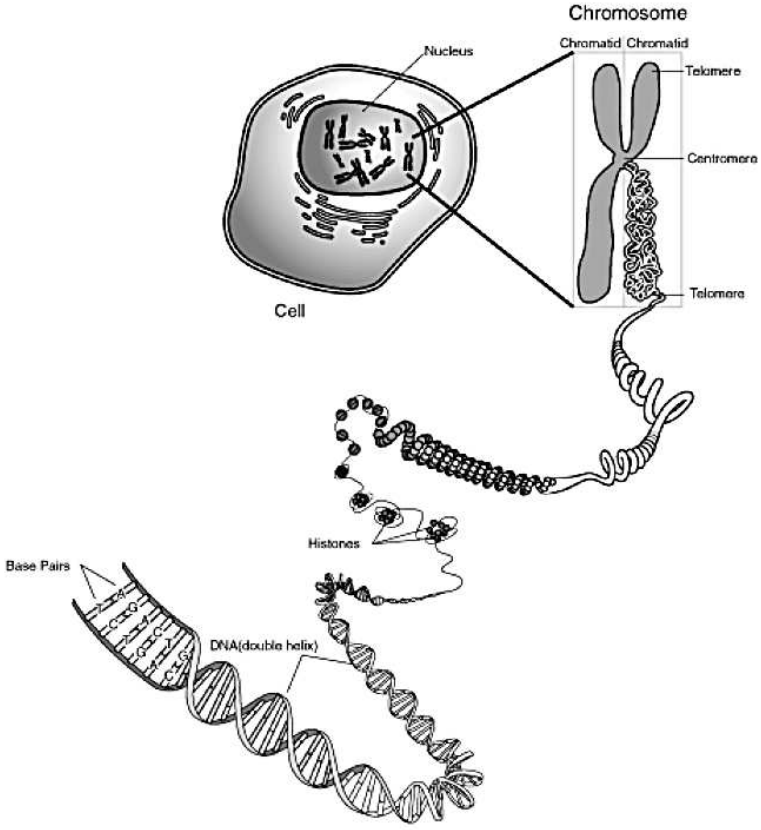
c. Approaches to an Anaemic patient

- History:
  - Age and situation of onset?
  - Triggering factor?
  - Similarly affected family members?
  - Symptoms related to underlying disease?
- Examination:
  - Signs of anaemia/haemolysis (pallor, jaundice)?
  - Signs of underlying disease?
- Laboratory tests:
  - Full blood count
  - Examination of peripheral blood smear
  - Reticulocyte count
- Lab tests that may be warranted after blood count, red cell indices and smear examination findings:
  - Iron and bilirubin levels
  - Ab detection on RBCs
  - Enzyme studies
  - Hb study
  - BM examination (if abnormal cells seen in blood smear or abnormal WBC/platelet count)

L79 Basic Human Genetics

A. Human Genome

- **Human genome**: complete set of genetic information of the human species (*H. sapiens sapiens*)
- Consists of 3×10^9 base pairs
- Features of human genome:
  - □ Stable
  - □ Usable (expression)
  - □ Organized
  - □ Accurately copied and passed to next generation
- Human genome consists of 23 pairs of **chromosomes**



1. Human Chromosomes

- Each human cell nucleus contains 22 pairs of **autosomes** and 1 pair of **sex chromosomes**
- Each chromosome consists of:
  - □ **Short arm (p arm)**
    → p for *petit* (*fr.* small)
  - □ **Long arm (q arm)**
  - □ **Centromere**: primary constriction
  - □ **Telomere**: a region of repetitive nucleotide sequence at the end of each chromatid for protection from damage or fusion
  - □ **Secondary constrictions**: constriction sites other than centromere (eg. nucleolar organizers)

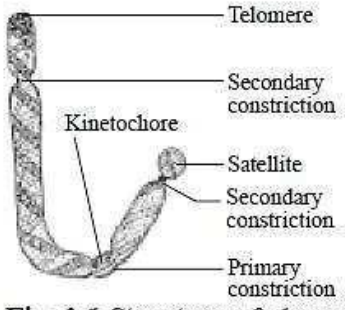


Fig. 3.1 Structure of chromosome

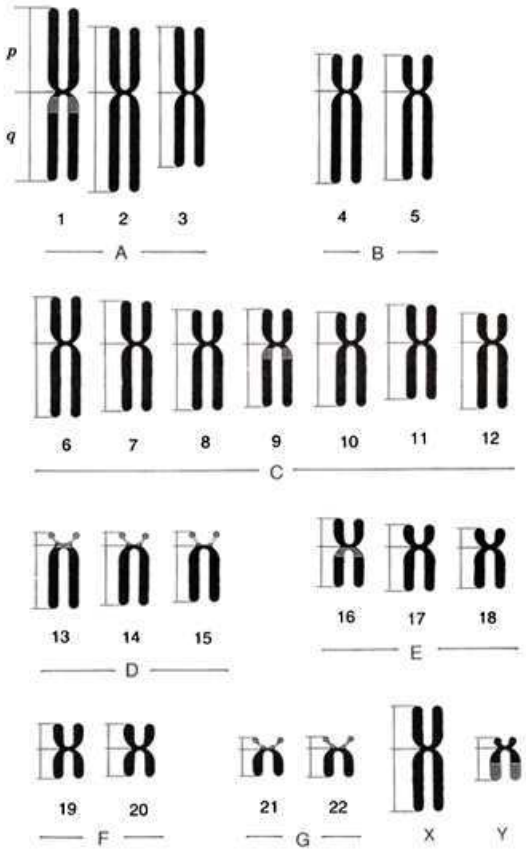
  - □ **Satellite chromosome**: parts separated from main body of chromosome by a secondary constriction
  - □ **Kinetochore**: protein structure at centromere for attachment of spindle fibres

a. Classification of Chromosomes

- Classification according to:
  - Centromere position
  - Arm length ratio
  - Presence of secondary constrictions
- **Metacentric**:
  - Centromere is median or near median
  - Chromosome consists of two sell-defined arms with length ratio from 1:1 to 2.5:1
- **Acrocentric**:
  - Centromere close to one end of chromosome
  - One arm substantially smaller with length ratio from 3:1 to 10:1
- **Telocentric**:
  - Centromere strictly terminal entity and chromosome one-ended
  - Always arranged with short arm on top

b. Grouping of Human Chromosomes

- Group A: chromosomes 1-3
  - c1-2: large metacentric
  - c3: large submetacentric
- Group B: chromosomes 4-5
  - c4-5: large submetacentric
- Group C: chromosomes 6-12, X
  - c6-12, X: medium submetacentric
- Group D: chromosomes 13-15
  - c13-15: medium acrocentric + **satellites**
- Group E: chromosomes 16-18
  - c16: short metacentric
  - c17-18: short submetacentric
- Group F: chromosomes 19-20
  - c19-20: short metacentric
- Group G: chromosomes 21-22, Y
  - c21-22: short acrocentric + satellites
  - Y: short acrocentric – satellites

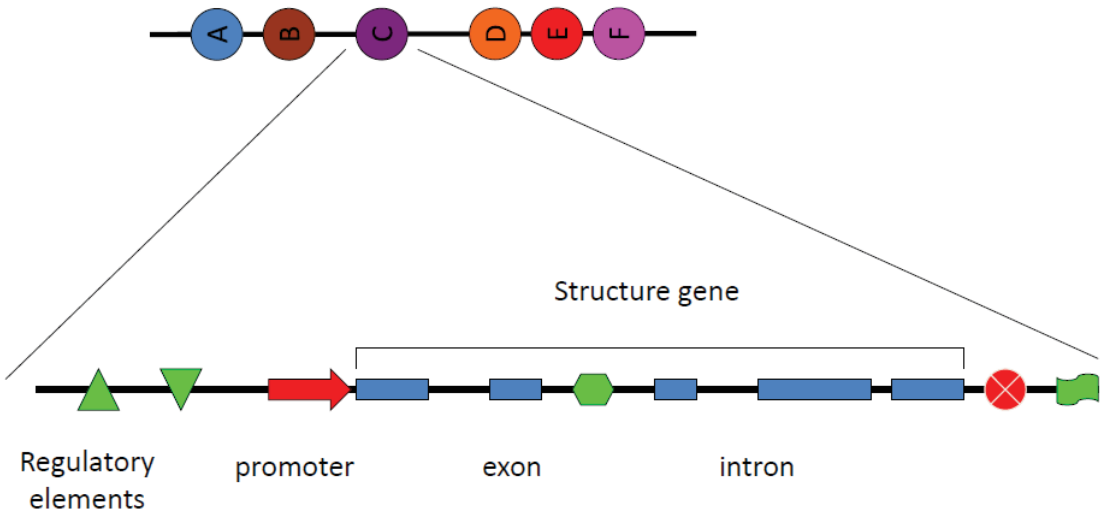


c. Karyotyping

- **Karyotype**: number, size and shape of chromosomes of a somatic cell arranged in a standard manner
- Method: **Giemsa staining (G banding)**
  - Source of cell: blood lymphocytes (add a **mitrogen**: **phytohaemagglutinin**), amniotic fluid, BM and skin
  - Colchicine added to arrest spindle formation → metaphase chromosomes accumulated
  - Differential staining of heterochromatic and less condensed regions:
    - → Heterochromatic regions tend to be more AT-rich and are stained darker in G staining
    - → Less condensed regions tend to be more GC-rich and are stained lighter in G staining
  - A chromosome-specific banding pattern is produced
  - Banding pattern used to identify chromosome abnormalities (eg translocation)

d. Genes

- **Gene**: molecular unit of heredity
- Number of genes in human genome: 20-25k
- Structure of a gene:



e. Human genetics

- **Gene locus**: specific position or location on a chromosome:
  - Gene is just a special type of locus
- **Allele**: different forms or states of the same locus or gene
  - Example: A, B, O in the ABO gene
- **Genotype**: genetic constitution or composition of an individual
  - Consists of pair of alleles at a given locus
  - Example: AA, AB, AO, BB, BO, OO in ABO gene locus
- **Homozygous**: genotype consisting of two identical alleles
  - Example: AA, BB and OO in ABO gene
- **Heterozygous**: genotype consisting of two different alleles
  - Example: AB, AO and BO in ABO gene
- **Hemizygous**: genotype consisting of <u>one allele only</u>
  - Example: any X gene in male
- **Nullizygous**: genotype consisting of <u>no allele</u>
- **Phenotype**: expression of a particular genotype
  - Observed result of interaction of genotype and environmental factors
  - Example: 6 genotypes at ABO locus correspond to 4 phenotypes
- **Dominant**: an allele that is expressed in heterozygous conditions
  - Example: A allele at ABO locus
- **Recessive**: an allele that is only expressed in homozygous conditions
  - Example: O allele at ABO locus
- **Co-dominant**: two alleles among which both are expressed in heterozygous conditions
- **Gene frequency**: relative frequency in a population of different alleles at a certain locus
- **Polymorphism**: a locus at which frequency of its commonest allele has frequency smaller than 95% (some say 99%)

L80, 82, 83 Genetic Diseases

A. Types of Genetic Disease

- **Chromosomal disorders**
- **Single gene disorders**
- **Polygenic or multifactorial diseases**
- **Somatic cell genetic disorders**

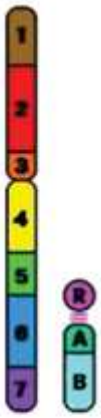
B. Chromosomal Abnormalities

- **Chromosomal abnormalities**: missing, extra or abnormal chromosomal DNA
- Types of chromosomal abnormalities:
  - **Numerical abnormalities**
  - **Structural abnormalities**
- Clinical consequences:
  - Spontaneous abortion
  - Birth defect

1. Numerical Abnormalities

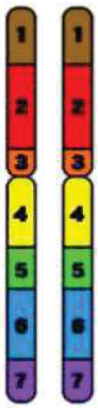
a. Deletion

- **Deletion**: a segment of chromosome is deleted
- Examples:
  - **Terminal deletion of short arm**: eg. removal of segment 1
  - **Terminal deletion of long arm**: eg. removal of segments 6, 7
  - **Deletion of long arm**: eg. removal of segment 5
  - **Ring chromosome**: eg. removal of terminal fragments of a chromosome and joining of two ends



b. Insertion

- **Insertion**: a portion of one chromosome has been deleted from its normal place and inserted into another chromosome
- Example: insertion of segment 5 into the locus between A and B



c. Duplication and Deletion

- A segment of DNA is transferred from one chromosome to its homolog, causing duplication in the homolog and deletion in the original
- Example: insertion of segment 5 into the position between 5 and 6 in its homolog

d. Translocation

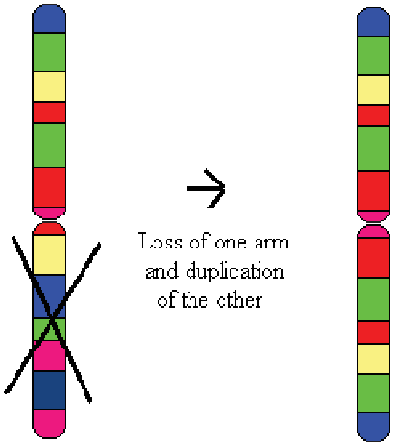
- A portion of one chromosome is transferred to another chromosome
- Examples:
  - **Reciprocal translocation**: exchange in material between two non-homologous chromosomes, usually harmless (except unbalanced ones)
    → c9-c22 translocation produces **Philadelphia chromosome**: Bcr-Abl gene elevates risk of leukemia
  - **Robertsonian translocation**: acrocentric chromosome joint long arms and become a new chromosome with only one centromere
    → Clinical significance: c14-c21 Robertson translocation may be phenotypically normal but may ↑ risk of unbalanced trisomy 21 in offspring

e. Inversion

- **Inversion**: a portion of the chromosome has broken off, turned upside down, and reattached, therefore the genetic material is inverted
- Examples:
  - **Paracentric inversion of short arm**: eg. inversion of segment 2
  - **Paracentric inversion of long arm**: eg. inversion of segments 5 and 6
  - **Pericentric inversion**: eg. inversion of segments 3 and 4

f. Isochromosome

- **Isochromosome**: a chromosome that has lost one of its arms and replaced it with an exact copy of the other arm
- Sometimes seen in Turner syndrome or tumor cells



2. Numerical Abnormalities

- Usually caused by chromosome non-disjunction in meiosis

a. Down Syndrome

- Cause: trisomy 21
- Symptoms:
  - **Upslanting palpebral fissures**: fissure between eyelids is at a higher position at the lateral side than the medial side
  - Redundant skin of inner eyelid
  - Low nasal bridge
  - Protruding tongue
  - Single palmar crease

b. Patau Syndrome

- Cause: trisomy 13
- Symptoms:
  - **Cleft lip (harelip)**: non-fusion of two sides of the upper lip
  - **Polydactyly**: extra digits
  - **Cyclopia**: single eye

c. Sex Chromosome Disorders

- **Klinefelter syndrome**:
  - Karyotype: 47, XXY
  - Symptoms: hypogonadism, sterility
- 47, XYY: normal phenotype
- 48, XXYY: developmental delays and ↓ testosterone
- **Turner syndrome**:
  - Karyotype: 45, X or 46, X (abnormal deletion)
  - Symptoms: broad chest, sterility, **amenorrhoea** (lack of menstruation)

C. Single-gene Disorders

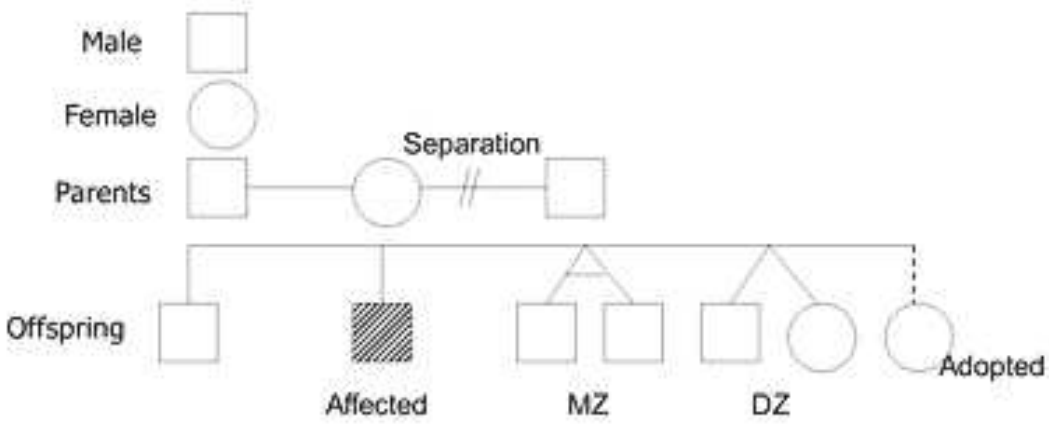
- **Single-gene disorder**: disease caused by a mutation at a single gene
- Also called a **Mendelian disease**
- Different genes may be involved in different patients
- >7000 identified in humans
- Close relationship between disease gene and disease status i.e. genotype and phenotype closely correlated
- Gene may be autosomal or X-linked
- Disease may be dominant or recessive
- Disease is usually rare (due to negative selection pressure)

1. Types of Mutations

- **Missense mutation**: a mutation leading to difference in only one amino acid
- **Nonsense mutation**: a mutation leading to premature ending of translation
- **Frameshift mutation**: a mutation leading to a shift in reading frame, possibly causing premature ending of translation

2. Inheritance of Single-gene Disorders

a. Pedigree Tree



MZ: Monozygotic twins; developed from same fertilized ovum; 100% genetic sharing

DZ: Dizygotic twins; developed from 2 separate fertilized ova: 50% genetic sharing

b. Types of Inheritance

- **Autosomal dominant**: eg. **Huntington's disease (HD)**
- **Autosomal recessive**: eg. **cystic fibrosis (CF)** and **sickle cell anaemia**
- **X-linked dominant**: eg. **vitamin D-resistant rickets (X-linked hypophosphataemia)**
- **X-linked recessive**: eg. **haemophilia A**

c. Segregation Ratios

- **Segregation ratio**: ratio of affected to normal individuals among offspring of a particular type of mating

Mode of inheritance	Mating type	Segregation ratio Affected:Normal
Autosomal dominant	Affected x Normal	1:1
Autosomal recessive	Carrier x Carrier	1:3
X-linked dominant	Normal father x Affected mother	1:1 (heterozygous) or 1:0 (homozygous)
X-linked recessive	Normal father x Carrier mother	1:3

d. Hardy-Weinberg Law

- In a large population under random mating:
  - Allele frequencies in offspring, denoted as p and q, are the same as those in parental generation
  - Genotype frequencies in the offspring will follow ratios $p^2 : 2pq : q^2$ regardless of genotype frequencies in parents
- Basis:
  - Alleles in one generation is a simple random sample of alleles in previous generation
  - Alleles transmitted from two parents are independent $\rightarrow$ probability of a certain combination of alleles is equal to product of population of frequencies of the alleles
  - Double counting in heterozygous conditions

e. Kinship Coefficient

- **Identical-by-descent (IBD)**: a term used to describe two (or more) alleles that share all sequence variants due to co-ancestry
- **Kinship coefficient (*r*)**: probability that two alleles, one from each individual, drawn at random at any autosomal locus, will be **identical-by descent (IBD)**
  - Also called **coefficient of genetic relationship**

Type of Relationship	r
MZ Twins	1/2
Parent - offspring	1/4
Full sibs (including DZ Twins)	1/4
Half Sibs	1/8
Aunt/Uncle – Nephew/Niece	1/8
First Cousins	1/16

- **Consanguinity** i.e. inbreeding
  - With genotype frequency p and kinship coefficient r
    Possibility of getting an autosomal recessive disorder = $rp + (1 - r)p^2$
  - Consider heredity of alleles as a random event identical to drawing a random allele from each of the parent
  - rp = probability of mutated gene present in one parent being IBD
  - $(1 - r)p^2$ = probability of mutated gene present in both parents but not IBD

3. Examples of Single-gene Disorders

a. Huntington's Disease

- Inheritance: autosomal dominant
- Symptoms: unsteady gait, involuntary movements, slurred speech, difficulty in swallowing, personality changes, depression, impaired judgment
- Caused by mutations in the **Huntingtin** gene:
  - Gain of function mutation
  - Unstable CAG repeat coding for polyglutamine tract
  - Repeat length >35 → unstable, >40 → HD
  - More repeats → earlier onset
  - Pathogenesis: intracellular mutant protein toxic to neurones

b. Cystic Fibrosis

- Inheritance: autosomal recessive
- Symptoms: delayed growth, fatigue, diarrhoea, coughing and wheezing, recurrent respiratory infections, infertility in males, high sodium in sweat
- Caused by mutation in **cystic fibrosis transmembrane conductance regulator (CFTR)** gene:
  - Loss of function mutation
  - CFTR is a chloride ion channel important in sweat, digestive juices and mucus secretion
  - Genetic mutation → non-functional or unstable proteins

c. Sickle Cell Anaemia

- Inheritance: autosomal recessive
- Symptoms: haemolytic anaemia
- Caused by mutation in gene coding for **haemoglobin**:
  - Glutamic acid residue in normal Hb (HbA) is mutated into valine in sickle-cell Hb (HbS)
  - Hydrophobic valine leads to changes in intermolecular forces
  - HbS polymerizes at low O_2 concentration → RBC changes shape or may even lyse

d. Haemophilia A

- Inheritance: X-linked recessive
- Symptoms: heavy bleeding and easy bruising
- Caused by mutations of the **F8** gene coding for **factor VIII**:
  - Loss of function mutation
  - Mutation of F8 genes may result in loss of function of factor VIII
  - Factor VIII ineffective in coagulation cascade → difficulty in clotting

D. Multifactorial Diseases

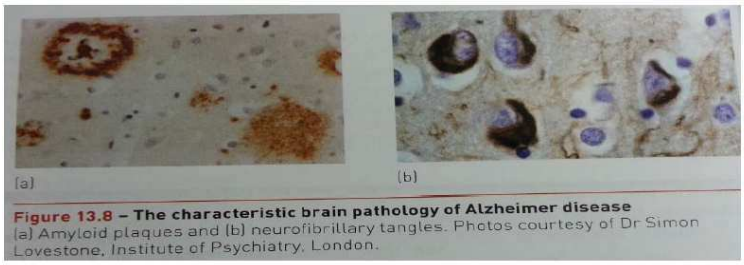
- **Multifactorial disease**: a genetic variant only increases risk of disease
- Examples: diabetes (IDDM and NIDDM), asthma, schizophrenia/manic depression, epilepsy, Parkinson's disease, infectious diseases, Alzheimer's disease
- Relationship between a particular gene and disease is less clear cut
- Mutations predispose to a disease (or even protect against)
- Much more common than Mendelian disorders

	Disorder type	
	Mendelian	Multifactorial
Population frequency	Low	High
Number of genes required to produce the phenotype	One	Many
Penetrance of individual genes	High	Low
Environmental influence on disease occurrence	Low	High
Method of patient ascertainment	Family history and/or Patient symptoms	Medical screening
Method of detection	Linkage analysis (successful detection of disease genes)	Linkage analysis (less successful) Association studies Genome association

•Environmental variation usually irrelevant
•Usually rare
•Occurs in isolated pedigrees

•Environmental variation usually important
•Often common
•Occurs in general population

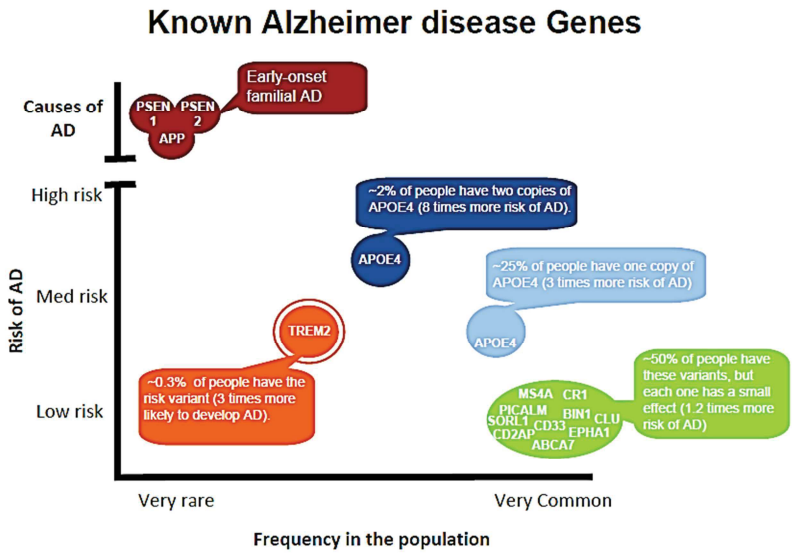
*__Alzheimer's disease__: a chronic neurodegenerative disease characterized by memory loss and **dementia** (loss of ability to think)



Amyloid plaques

Neurofibrillary tangles

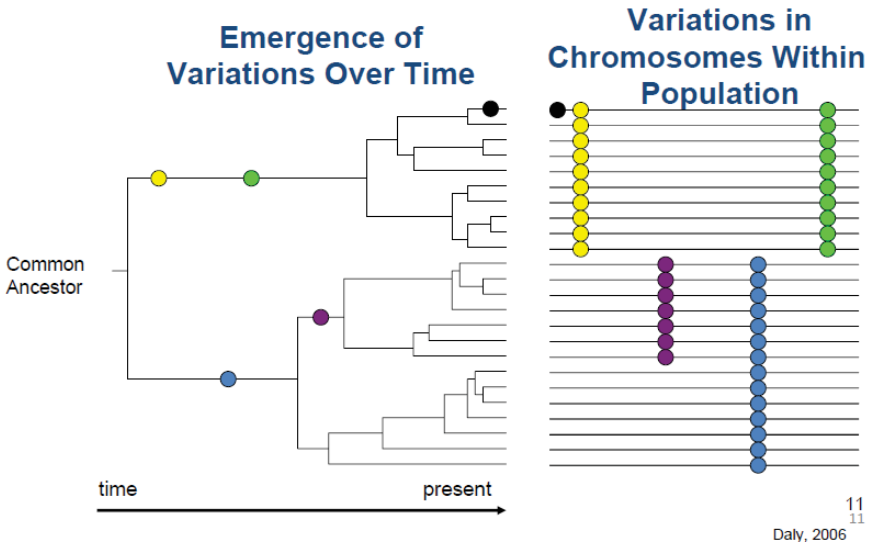
Genetic composition of Alzheimer's disease:



1. Genetic Architecture of Multifactorial Diseases

- Number of loci and frequency of alleles influencing disease risk
- Two main models:
  - **Common disease/common variant (CDCV)**: disease risk only determined by common alleles in a few genes of low **penetrance**
  - **Polygenic model**: many low frequency alleles (at many genes with high penetrance) influencing disease risk (emphasis on interaction)
- Oligogenic model: a reduced number of genes have a predominant effect, modified by the genetic background
- Currently unclear which model fits more
- Studied by **genetic epidemiology**

a. Origin of Allele Frequencies

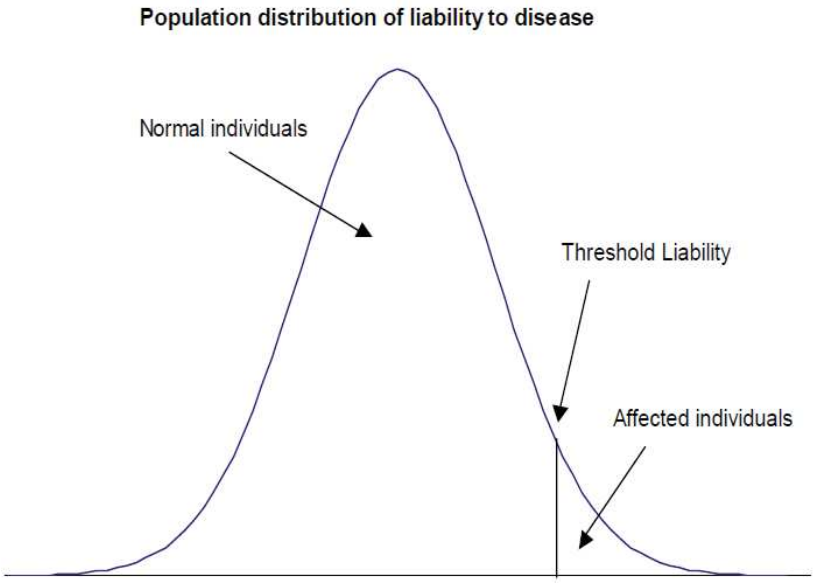


- Allele frequencies related to time
- On average, common alleles are older than rare alleles
- Common alleles (>0.1%) predates separation of human population, present in most populations of the world
- Rare alleles tend to be more population restricted

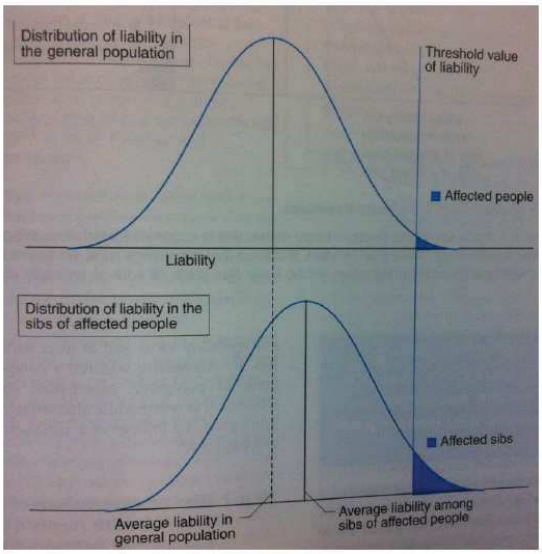
2. Quantitative Traits

- **Quantitative traits**: traits that are measured on a continuous numerical scale
- Examples: BP, BMI, blood cholesterol, general intelligence (G)
- Many are relevant to health and disease
- Trait in son is not completely determined by trait in father, always regress to mean instead
- Can often be approximated by **normal distribution** as in the **bell curve**

a. Liability-threshold Model



- Liability-Threshold model:
  - Each individual has an underlying liability for disease
  - Liability is determined by the number of multifactorial genes for the trait
  - Underlying quantitative liability is polygenic and normally distributed in the population
  - Disease develops if an individual's liability exceeds a certain threshold value



- Provides a link between quantitative genetics and complex multifactorial diseases
- Bell curve shifts to the right among individuals with familial history (due to higher average liability) → greater proportion exceeding threshold → disease tends to run in families

3. ACE Decomposition

- **ACE decomposition**: separation of effects of genetic (multiple genes), common family environment and individual environment contributing to a certain quantitative phenotype
- Mathematical presentation: $Var(Y) = Var(A) + Var(C) + Var(E)$
  $1 = h^2 + c^2 + e^2$ (as proportion of Var(Y)
  - Y: quantitative phenotype
  - A: additive genetic effects
  - C: common family environment
  - E: individual environment

a. Heritability (h^2)

- **Heritability**: proportion of phenotypic variance due to <u>additive genetic effects</u>
  - $h^2 = Var(A)/Var(Y)$
- Characteristics:
  - Population-specific
  - May change with changes in the environment
  - High heritability does not preclude effective prevention or intervention (especially if intervention is not part of naturally occurring environmental variation)
- Note: heritability is NOT a measure of how a condition is caused physiologically or biochemically in a person but a broad, population-oriented term used to describe the proportion of differences between people in a population at a specific time is caused by genetic differences

b. Twin Concordance Rates

- **Twin concordance rate**: proportion of monozygotic and dizygotic twins having the same trait
- Note that monozygotic twins share ALL of genetic material while dizygotic twins share HALF
- Considering the difference between concordance rates among MZ and DZ twins can help us find h^2
- Mathematical presentation:
  - MZ twin concordance = $h^2 + c^2$
  - DZ twin concordance = $1/2h^2 + c^2$
  - Thus, $h^2 = 2$ (MZ twin concordance – DZ twin concordance)

	MZ twin	DZ twin	Heritability
High Blood pressure	0.6-0.8	0.05-0.5	**0.60**
Asthma	0.12-0.89	0-0.05	**0.72-0.8**
Type 1 Diabetes	0.25-0.35	0.03-0.05	**0.72**
Type 2 diabetes	.50	0.37	**0.26**
Rheumatoid Arthritis	0.15	0.04	**0.32**

Heritability estimates de genetic contribution to the phenotype
Strictly genetic trait: Concordance MZ = 100%, DZ = 25-50%

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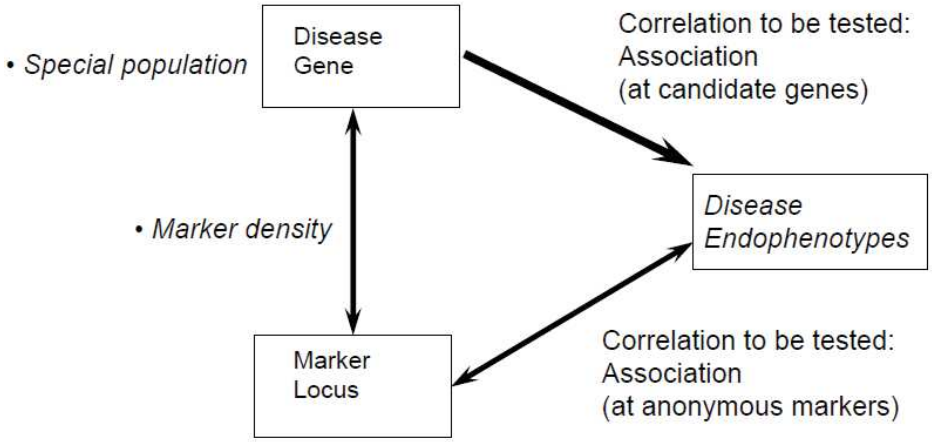
4. Recurrence Risk

- **Recurrence risk**: risk that a disease will occur elsewhere in a pedigree, given that at least one member of the pedigree exhibits the disease
- Recurrent risk ratio $(\lambda_S) = \frac{\text{risk for family member of proband}}{\text{risk of general population}}$
- Difference in recurrence risks of Mendelian and multifactorial traits:
  - Multifactorial traits have higher recurrence risks if disease is more severe (because the patient has more related genes) but not for Mendelian disorders
  - A family with more affected children has higher recurrence risks (because it means that they are closer to threshold) but not for Mendelian disorders
- Examples of values of λ_S:
  - Mendelian disorders:
    - → Cystic fibrosis: 500
    - → Huntington's disease: 5000
  - Complex disorders:
    - → Autism: ~110
    - → Multiple sclerosis: ~25
    - → DM I: ~15
    - → DM II: ~3.5
    - → Schizophrenia: ~10

5. Genetic Analysis of Multifactorial Disorders



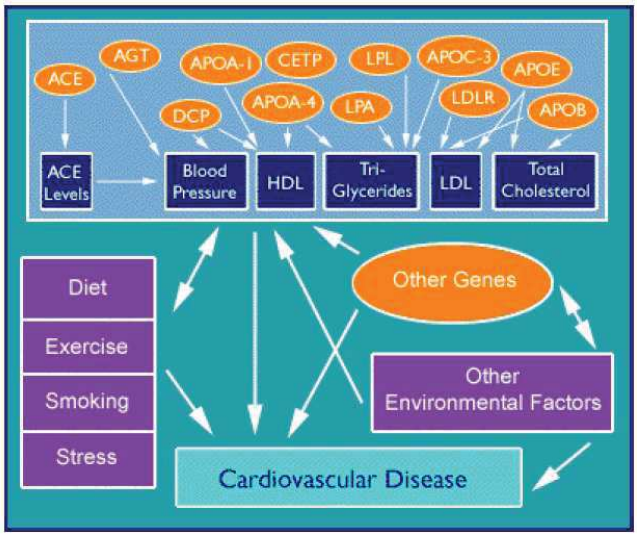
- **Genetic mapping**: determines the relationship between diseases and genes
- Two general approaches:
  - **Candidate gene approach**: relationship between genetic variants at genes of known function (relevant to the disease studied) and disease incidence is studied
  - **Disease-marker approach**: investigation of correlation between presence of a certain allele in certain marker locus and the disease incidence
- Relationship between phenotypic similarity and genotypic similarity among individuals is examined
- Practical aspects of a study lead to underestimating of this similarity (genotyping and phenotyping errors)→ mapping more difficult
- Background information that must be known:
  - How 'genetic' is the disorder
  - Evidence of **familial aggregation**
  - Evidence that familial aggregation is due to common genes rather than shared environmental factors (by **heritabililty analysis**)

a. Phenotypic Definition and Genetic Analysis

- Phenotypic definition is critical in genetic analysis
- Mendelian disorder: variations in phenotype can suggest differences in underlying gene causing disease → **genetic heterogeneity**
- Complex disorders:
  - Narrow phenotype definitions used to reduce genetic heterogeneity
  - Common disorder may have **Mendelian subtypes**
- Strategies to strengthen genotype-phenotype relationship:
  - Sample patients with standard criteria
  - Focus on more severe clinical forms, incl. earlier onset
  - Examine 'intermediary' phenotypes (i.e. within body) which might be closer to gene action (eg. blood enzyme and metabolite levels, HIR/CMIR, MRI structural analysis)

b. Candidate Gene Approach

- A bunch of genes related to the disease is shortlisted and screened for relationship with the disease
- Example: approach to cardiovascular disease

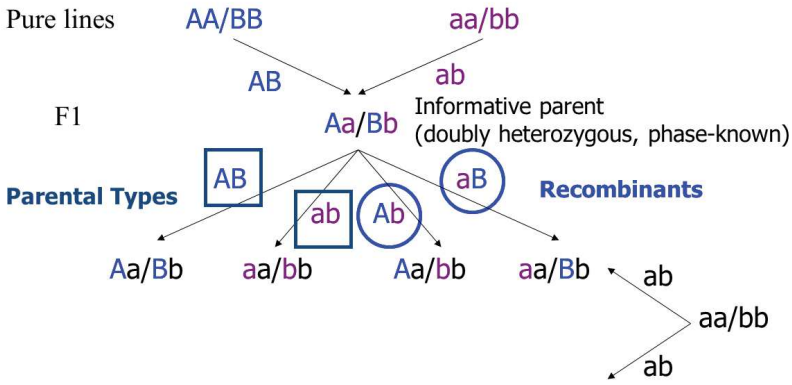


c. Disease-marker Association Approach

- A marker locus is associated with a disease if distribution of genotypes at the marker locus in disease-affected individuals differs from distribution in general population
- A specific allele may be positively associated (over-represented in affected) or negatively associated (under-represented)
  - Microsatellite markers (eg. CACACA…)
  - Single-nucleotide polymorphisms (SNPs) (eg. C/T)

L81 Pedigree and Gene Linkage

A. Recombination Fraction



- **Recombinant**: an individual having a different combination of alleles from either of the parents
- **Recombination fraction (θ)**: proportion of gametes that are recombinant for two loci
  - For two loci on different chromosomes, Mendel's 2nd law applies → $\theta = 1/2$
  - For two loci on same chromosome (**syntenic**), more parental types than recombinants → $\theta < 1/2$
    → $\theta \neq 0$ ∵ presence of chromosomal crossover
    → In this case, θ = probability of having an odd number of crossover points between the two loci
  - $\theta \rightarrow 0$ as distance between two **syntenic** loci → 0
  - Two loci with $\theta < 1/2$ said to be in linkage

B. Genetic Mapping and Recombination

- Genetic distance (in Morgans) between two loci can be described as the average number of cross-over events that occur between them per meiosis
- **Haldane map function**: assuming independence of cross-over points,

$$\theta = \frac{1 - e^{-2m}}{2}$$

  - m = genetic distance (Morgans, M)
- Mathematical derivation:
  - For a particular interval with length m, let p_k be probability of having k exchange points in the interval

    $\theta = p_1 + p_3 + p_5 + \cdots$ (1)

    $m = p_1 + 2p_2 + 3p_3 + \cdots$ (2)

  - Applying Poisson distribution, $p_k = e^{-m}\left(\frac{m^k}{k!}\right)$ clearly fits (2)
  - Substituting the Poisson expression into (1),

$$\begin{aligned} r &= me^{-m} + e^{-m}\left(\frac{m^3}{3!}\right) + e^{-m}\left(\frac{m^5}{5!}\right) + \cdots \\ &= e^{-m}\sinh(m) \\ &= e^{-m}\left(\frac{e^m - e^{-m}}{2}\right) \\ &= \frac{1 - e^{-2m}}{2} \end{aligned}$$

- Implication: genetic loci can be mapped relative to each other by examining the recombination fractions between them

C. Genetic Markers and Linkage Mapping

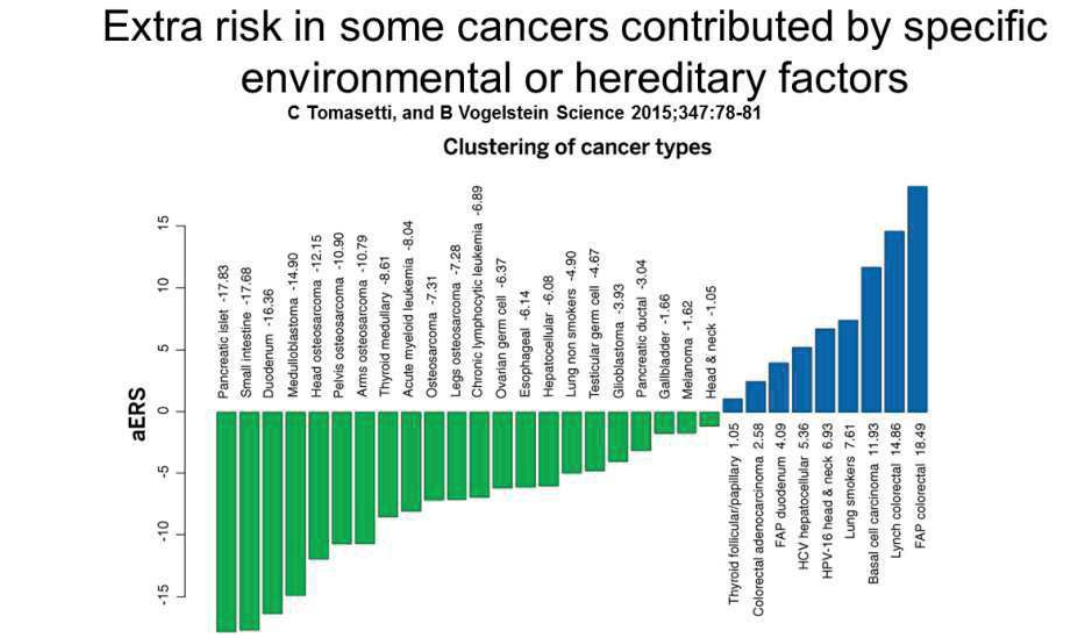
- Use of linkage to map disease-related genes requires availability of measurable naturally occurring DNA sequence variations (polymorphisms) of known chromosomal locations
- Determination of alleles present at a polymorphic marker in an individual (i.e. genotyping)
- Common molecular genetic markers used include:
  - Restriction fragment length polymorphisms (RFLPs)
  - Variable-length short-sequence repeats (SSRs)
  - Single-nucleotide polymorphisms (SNPs)
- Linkage studies:
  - Collect a sample of families with disease
  - Genotype subjects in the families using a standard set of genetic markers (eg. 400 microsatellites or 10k SNPs) fairly evenly spaced throughout the genome
  - Consider genetic markers in each genomic region for evidence of linkage to the disease
- 1112 disease genes discovered from 1981 to 2000

L84 Genetic Basis of Development of Cancer

A. Genetic Basis of Cancer Development

- Carcinogenesis requires mutations in multiple genes
- Epidemiological evidence: both incidence rate and mortality rate increase dramatically with age
  - Slope of log-log plot suggests that six independent mutational events are required for development of colorectal cancer
    ($y = kx^n \rightarrow \ln y = n\ln x + n\ln k$)
  - Slope of 0 if a single mutational event that can occur at any time is required
  - Typically, 3-7 events are required dependent on the cancer type
- Theoretical consideration based on intrinsic error rate:
  - Assumption: all cancers are caused by intrinsic error in replication, all gene mutations are independent events
  - Endogenous mutation (background) rate due to uncorrected DNA replication
  - 6×10^9 nucleotides replicated per cell division
    x) ~10^{14} cell divisions undergone to reach adulthood
    = 6×10^{23} nucleotides replicated $\rightarrow$ impossible to be error-free
    (error frequency $\fallingdotseq$ $10^{-9} – 10^{-11}$ per incorporated nucleotide)
  - Typical mutation rate per gene per cell division $\fallingdotseq$ 10^{-7}
  - 100 cancer-associated genes out of total of 21,000 genes
  - Assume only 1 mutagenic events needed,
    probability = $10^{-7} \times 100/21000 \times 10^{14}$ = ~5×10^4 (too high)
  - Assume 6 mutagenic events needed,
    probability = $(10^{-7} \times 100/21000)^6 \times 10^{14}$ = ~1.2×10^{-40} (too low)
    cf. 1/4 men and 1/5 women would develop cancer by age 75 in HK

- Tomasetti and Vogelstein (2015) showed a strong relation (0.804) between number of stem cell replications and cancer risk → 65% of differences in cancer risk can be explained by random mutations



Adjusted "Extra Risk Score" (aERS) calculated for each cancer:
R (Replicative)-tumors (green) – Cancers with -ve aERS; risk mainly due to stochastic factors presumably related to DNA replication errors i.e. somatic mutations; not preventable?
D (Determinative)-tumors (blue) – Cancers with +ve aERS; have known links to specific environmental (e.g. smoking, sunlight, viral infection) or hereditary (e.g. known germline mutations) risk factors; extra risk preventable and early detection important!

- Limitations: some common cancer (eg. breast, prostate) not included

B. Multistage Model of Cancer

- Cancer develops along a **multistage model** because earlier mutations can:
  - Enhance cell proliferation creating a larger target population of cells for subsequent mutations (↑ probability of multiple mutations)
  - Increase overall mutation rate (eg. interferes with correcting mechanism of DNA replication)

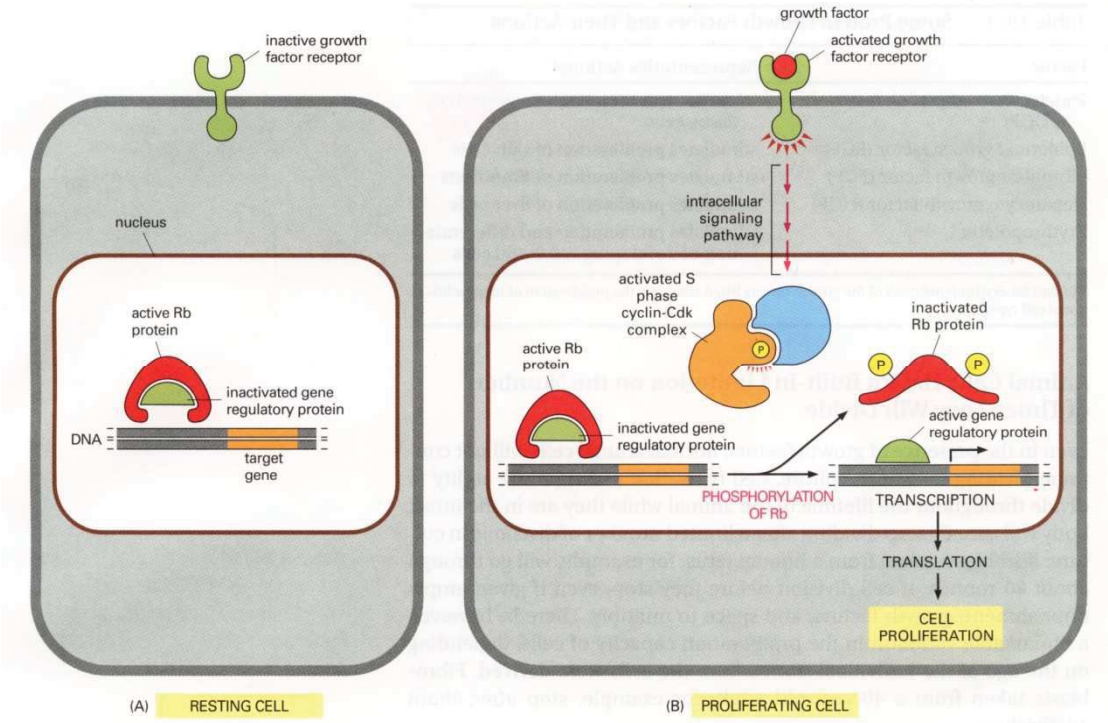
1. Genetic Basis of Multistage Model

a. Enhancing Proliferation

i. Activation of Oncogenes

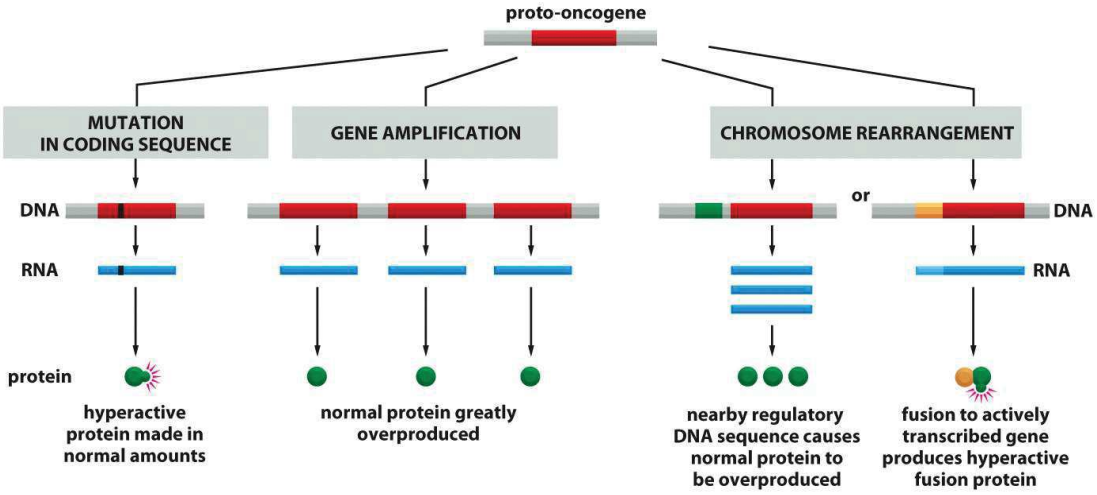
- Mutations may activate **oncogenes** or **proto-oncogenes** that positively promote cell proliferation
- Examples:
  - Growth factor receptors eg. **epidermal growth factor receptor (EGFR)**

  - Signal transduction components eg. **HRAS1**

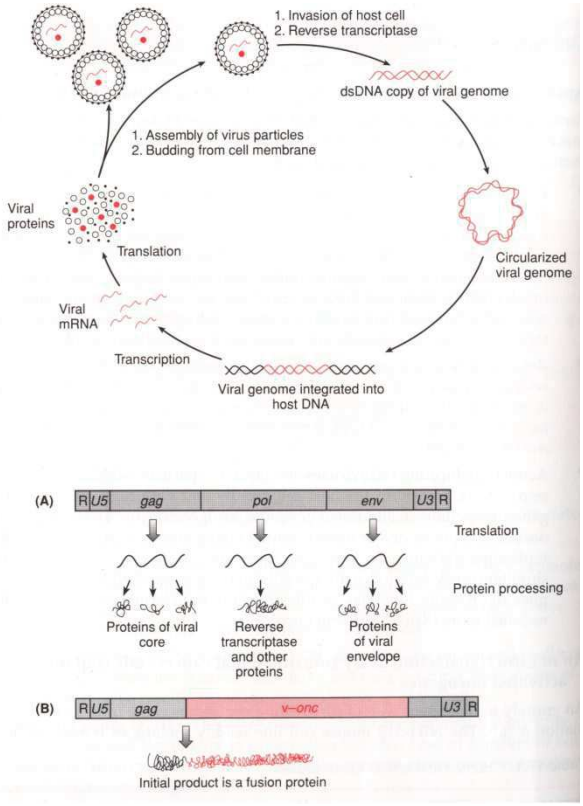


  - **FOS**: (DNA-binding transcription factor)

- Usually components within growth (or mitogenic) signaling pathways



- Activation (or **gain-of-function** effect) can result from:
  - Point mutation
  - Small deletion or insertion
  - Viral integration



- □ Translocation: may result in **fusion genes** that have neoplastic effects (eg. **Bcr/Abl** gene from c9/c22 philadelphia chromosome)

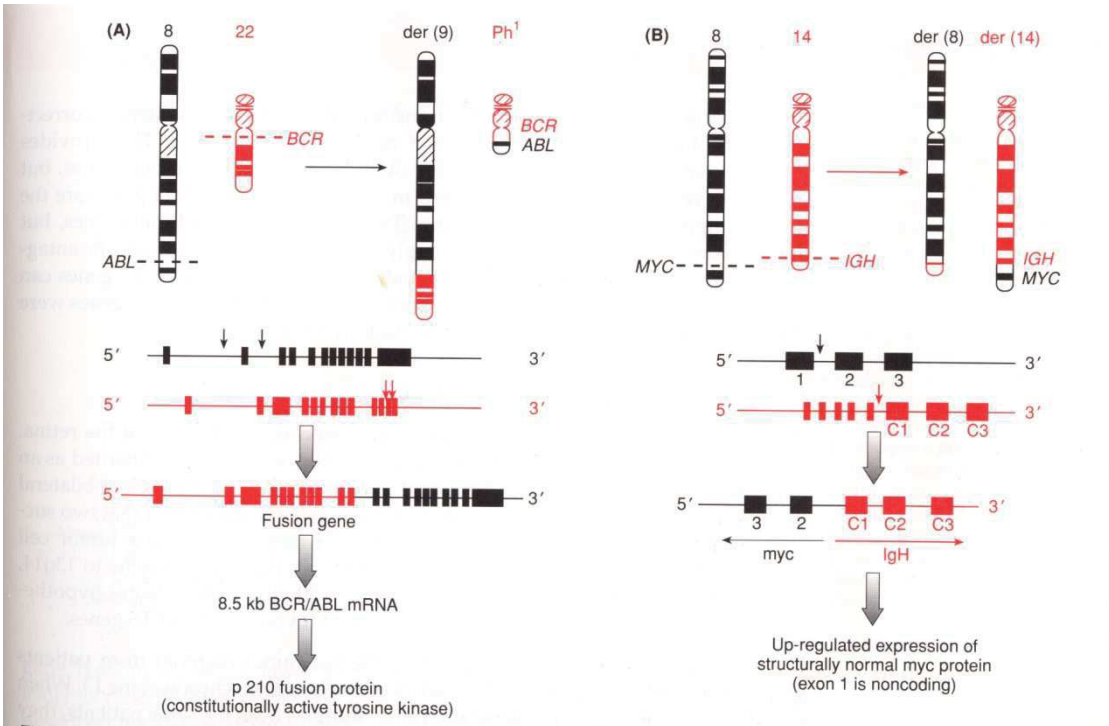


Figure 17.7: Chromosomal translocations which activate oncogenes.

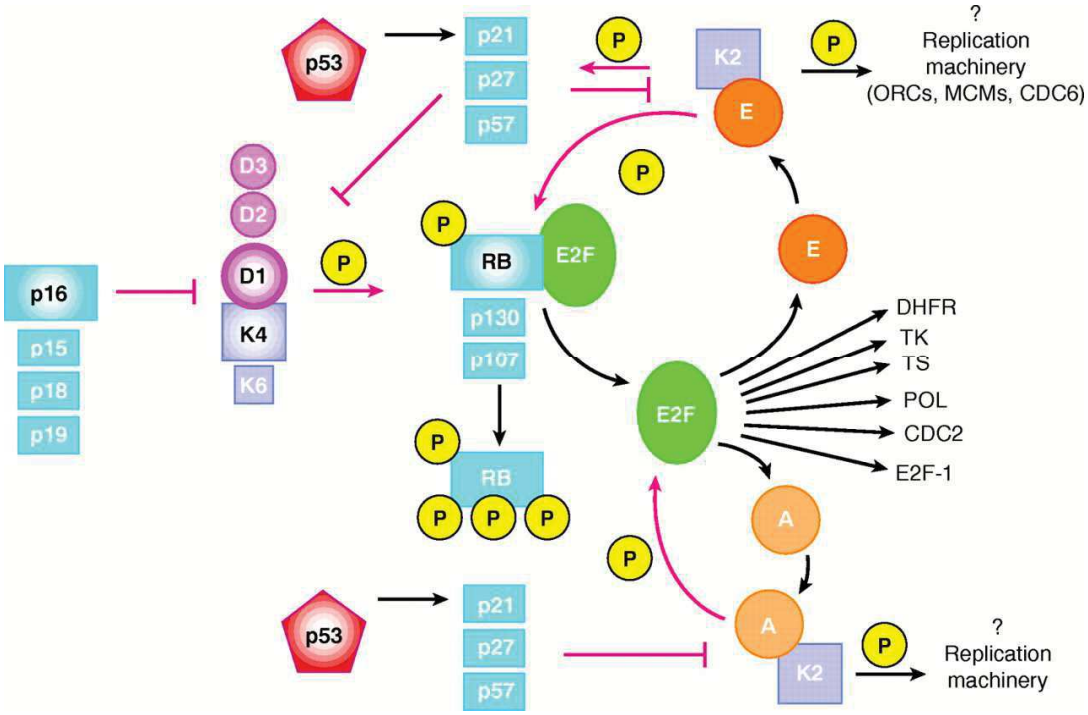
(A) Activation by qualitative change in the t(9;22) in chronic myeloid leukemia. The chimeric *BCR–ABL* fusion gene on the Philadelphia chromosome encodes a tyrosine kinase which does not respond to normal controls [see Chissoe *et al.* (1995) for more detail]. **(B)** Activation by quantitative change in the t(8;14) in Burkitt's lymphoma. The *MYC* gene from chromosome 8 is translocated into the Ig heavy chain gene. In B cells this region is actively transcribed, leading to overexpression of *MYC*.

- □ Gene amplification:
  - → As **double minute chromosomes** (small fragments of extrachromosomal DNA) i.e. duplication of many extrachromosomal copies
  - → As duplication of intra-chromosomal copies (shown in homogeneously staining region)

▶ Mutation of <u>one allele</u> is enough to generate a biological effect

ii. Mutation of Tumour-suppressor Genes

- Mutations of **tumour-supressor genes** may lead to loss of suppression on cell proliferation



- Examples:
  - RB coded by *RB* inhibits cell cycle entry
  - p16 coded by *CDKN2A* inhibits cell cycle entry
  - p53 coded by *TP53* inhibits cell cycle entry, stimulate senescence and apoptosis
- Loss-of-function can result from:
  - Missense mutations at critical residues
  - Nonsense mutations leading to protein truncation
  - Deletions
  - Chromosomal loss
- Both alleles need to be inactivated to lead to altered cellular function
  - Note for some TS genes (eg. *APC*) mutations of one allele are enough to cause a phenotype because of **haplo-insufficiency** or **dominant negative effect** (i.e. mutant protein interfering with normal protein function)

b. Elevation in Mutation Rate

- Mutation in **DNA repair (mutator)** genes that function in DNA repair pathways may raise mutation rate
- Examples:
  - **Human MutS Homolog 2 (hMSH2)** in DNA mismatch repair pathway
  - **Ataxia Telangiectasia Mutated (ATM)**: a kinase required for repair of DNA double-strand breaks by homologous recombination (failure → p53 activation)
- Both alleles need to be inactivated to reveal physiological effect

2. Multistage Model for Development of Colorectal Cancer

- Developed by Vogelstein (John Hopkins)
- Fits observation that increased longevity and diet changes led to increased incidence
- CC easily accessible by colonoscopy and staged:
  - Normal epithelium
  - Hyperproliferative epithelium (or dysplastic aberrant crypt foci)
  - Adenoma (early <1cm; intermediate >1cm –foci; late >1cm +foci of carcinoma)
  - Carcinoma

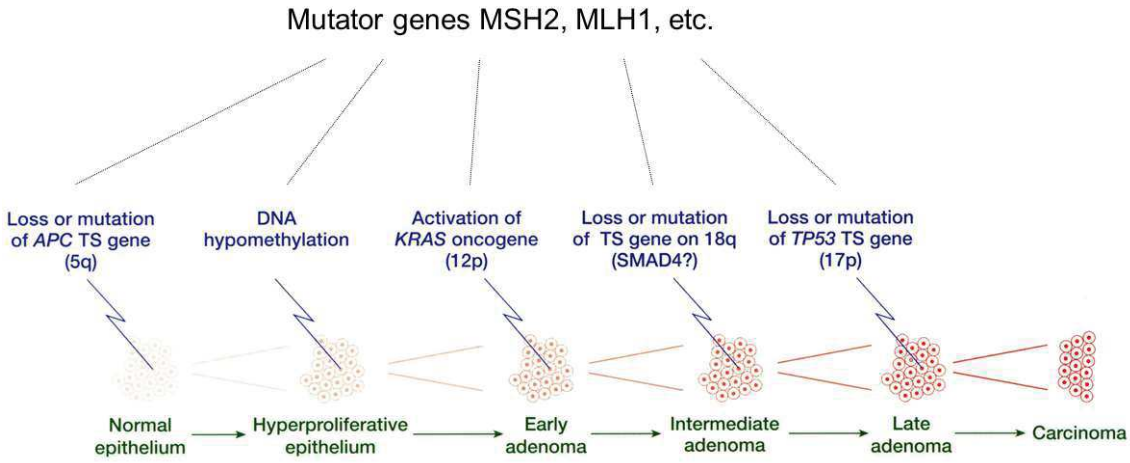


Figure 17.17: A model for the multi-step development of colon cancer.

See text for further details. This is primarily a tool for thinking about how tumors develop, rather than a firm description. Every colorectal cancer is likely to have developed through the same histological stages, but the underlying genetic changes are more varied. According to Fearon and Vogelstein (1990) the figure illustrates a particularly common sequence of events. Smith *et al.* (2002) have questioned the validity of this model because in their series of 106 tumors, only seven had mutations in all three of *APC*, *KRAS* and p53.

- Genetic changes at every stage analyzed and multistage model proposed:

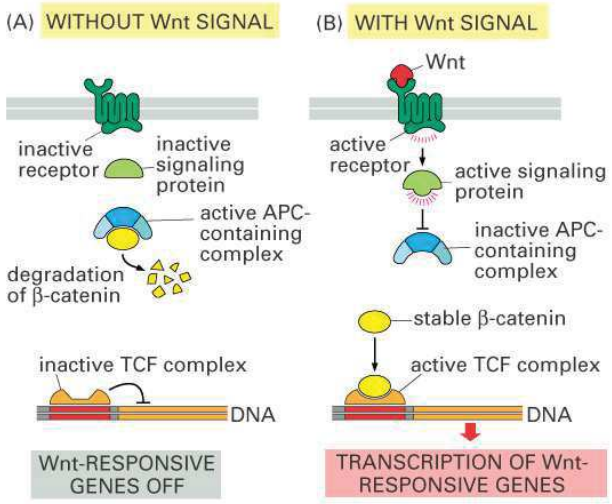


Figure 21-50 Essential Cell Biology, 2/e. (© 2004 Garland Science)

- □ Gatekeeper: mutation of one copy of **adenomatous polyposis coli (APC)** gene (TS) leads to **familial adenomatous polyposis (FAP)**
  - → **Wnt** is an important signaling pathway in triggering transcription of many genes
  - → Misregulation will lead to unchecked proliferation
  - → Without stimulus, Wnt receptor activates APC-containing complex to lyse β-catenin
  - → With stimulus, APC-containing complex is inactivated, leading to β-catenin accumulation in cytoplasm → transcription is enhanced
  - → Mutated APC gene will remove suppressing effect on cell proliferation
- □ **KRAS** (oncogene) mutated in 50% of <u>intermediate and late adenomas</u> but only about 10% of early adenomas
- □ Deletion of 18q (**SMAD4** likely to be TS gene involved) in 50% of late adenomas and carcinoma but relatively rare in early and intermediate adenomas
- □ Very high frequency of mutation in **p53** (TS) gene in carcinomas but not adenomas
- □ Mutations of mutator genes (eg. **MSH2**, **MLH1**) identified in colorectal tumors at different stages (also in **hereditary nonpolyposis colorectal cancer (HNPCC)** cases)

*Scanning for genetic mutations in human cancer genomes

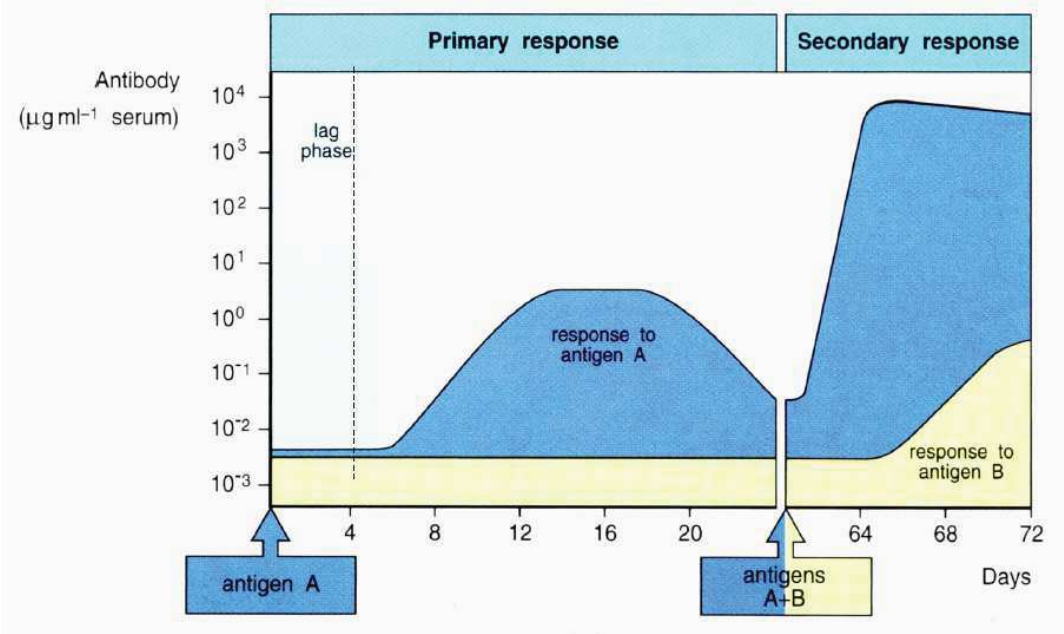
- ▶ >50 genetic alterations found per tumour
- ▶ Challenge is to distinguish passenger mutations (no positive or negative selective advantage but are retained by chance during clonal expansion) from cancer-causing gene mutations

L86 Immune System in Health and Disease

A. Aspects of Immunity

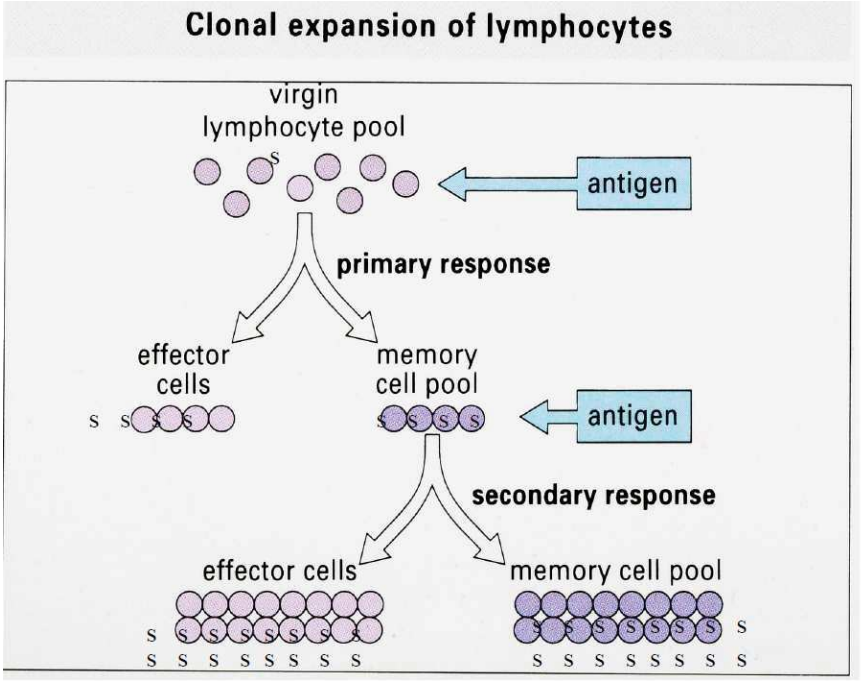
- Scope of immunity:
  - Immunity against infectious agents
  - Immunity against 'non-self': allergy, transplantation immunity
  - Immunity against 'self': autoimmunity, anti-tumour immunity
- **Pathogens**: infectious organisms
  - **Bacteria**:
    - → Prokaryotic (lacks cell nucleus)
    - → Extracellular or intracellular
  - **Fungi**:
    - → Eukaryotic (with cell nucleus)
    - → Unicellular or multicellular
    - → Examples: yeasts and molds
  - **Parasites**:
    - → Host-dependent
    - → Examples: worms and protozoa
  - **Viruses**:
    - → Replicate only in living cells
    - → Examples: RNA virus, DNA virus
- Types of immunity:
  - **Innate (natural) immunity**:
    - → Components: macrophages, NK cells
    - → Early, rapid but limited in strength
    - → Non-specific
  - **Adaptive (acquired) immunity**:
    - → Components: B and T lymphocytes
    - → Takes time but powerful
    - → Specific with memory

B. Immunological Memory



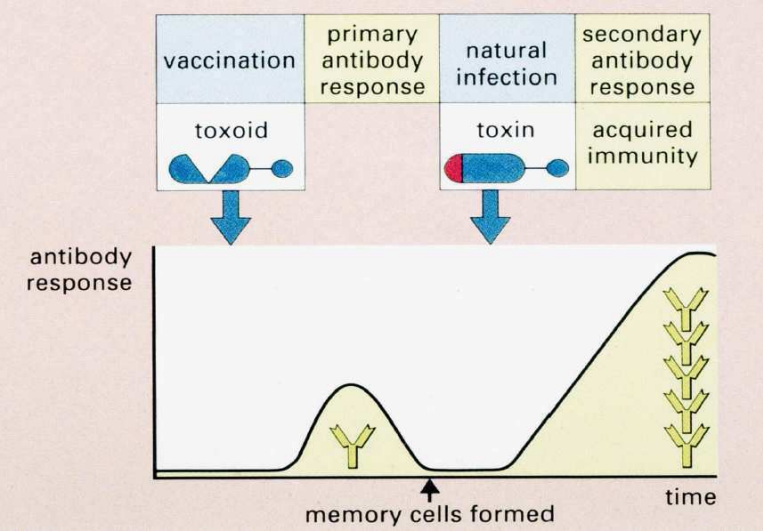
- General principle of immunological memory:
  - First infection: slow response → pathogens proliferate → disease symptoms
  - Second infection: fast response → pathogens killed → no disease symptoms
  - With exceptions eg. HIV infection
- Memory and specificity: key features of adaptive immunity

1. Theoretical Basis of Immunological Specificity and Memory



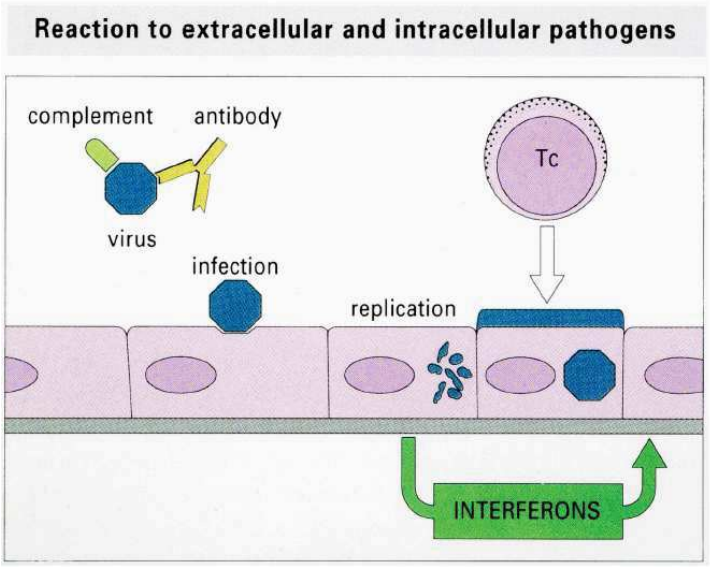
- **Burnet's clonal selection theory**:
  - Each lymphocyte produces one type of Ag receptors only
  - Antigen selects and stimulates cells carrying receptors specific for the antigen
  - Primary response: foreign antigen → Ag detected by specific lymphocyte → specific lymphocyte proliferates to form a large population of **effector cells** (eg. plasma cells) and **memory cells**
  - Secondary response: foreign antigen → Ag detected by existing specific **memory lymphocyte pool** → proliferation into effector cells and an even larger memory cell pool
  - ∵ Large population of specific lymphocytes → stronger secondary response

2. Principle of Vaccination



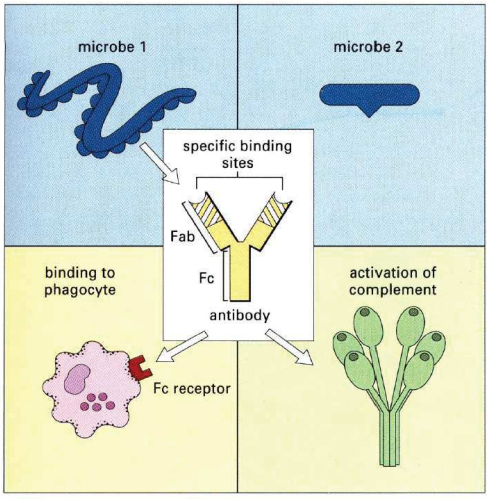
- **Vaccination** involves injection of foreign antigen into body to mimick primary infection without causing disease
- Next time the body comes across with the same antigen, secondary response is initiated with rapid disposal of pathogens → no symptoms

C. Types of Immunity

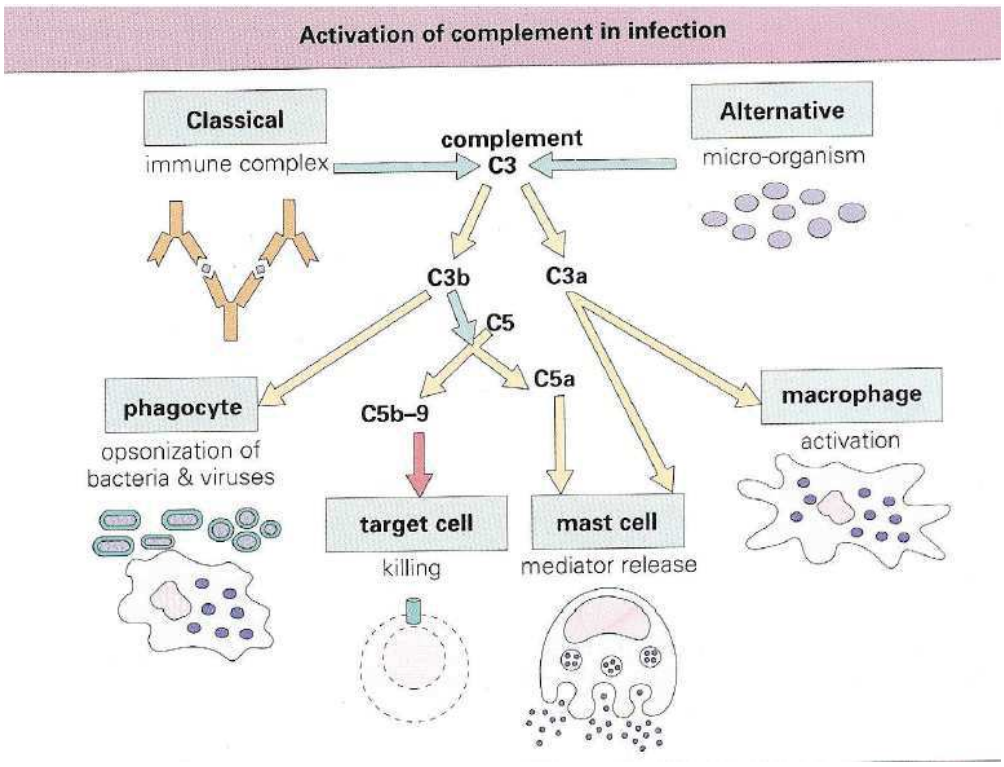


1. Humoral Immunity

- **Non-specific components**:
  - Complement system
  - Interferons α/β (from virus-infected cells)
  - Acute phase proteins



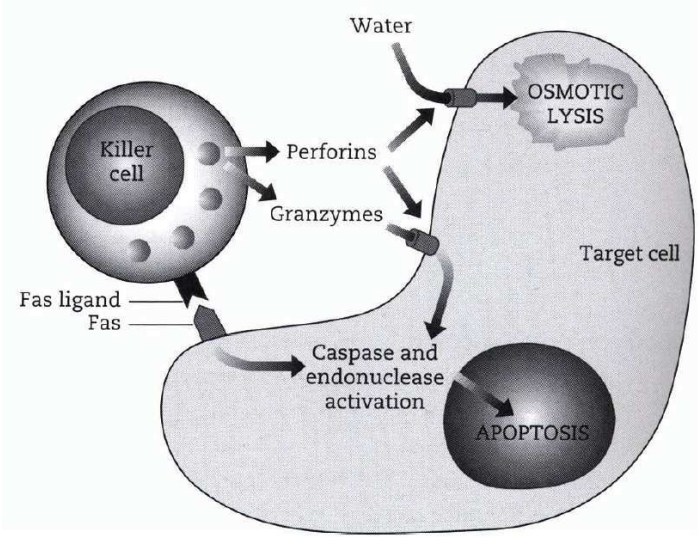
- **Specific component**: antibodies
  - Neutralization: eg. viruses, toxins
  - Opsonization: binds pathogens for recognition by other immune cells (eg. phagocytes)
  - Promotion of complement-mediated lysis



2. Cell-Mediated Immunity

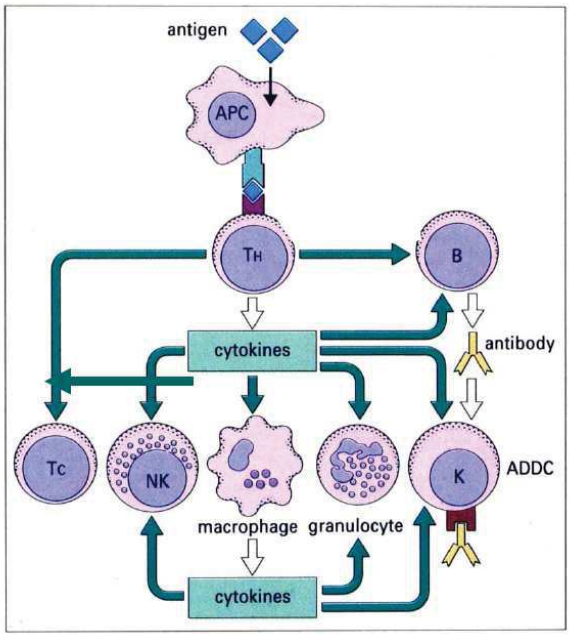
- **T-independent, non-specific components**: (early phase)
  - Phagocytes
  - Macrophages (MQ)
  - Natural killer cells (NK)
- **T-dependent, specific components**:
  - Cytotoxic T cells (T_c): kill infected cells directly
  - Helper T cells (T_H): help other cells (T_C, MQ, NK) to kill

a. Cell-mediated Cytotoxicity

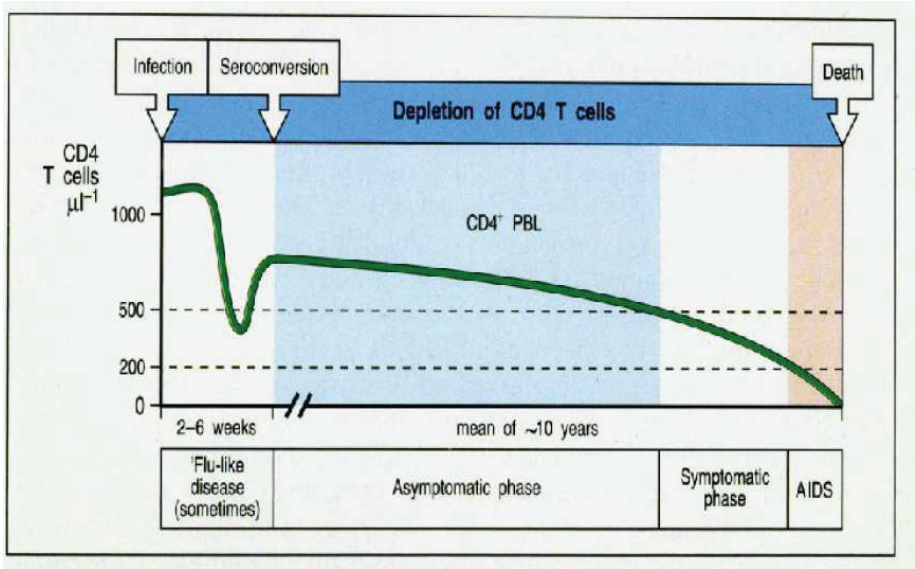


- Cytotoxic T cells and natural killer cells secrete **cytotoxic factors** to kill infected cells:
  - **Perforins** punch a hole in cell membrane → osmotic lysis
  - **Granzymes** (a serine protease) pass through perforin holes and cleaves caspase in cytosol → endonuclease activated → apoptosis
  - Expresses **Fas ligand** on cell membrane → binds with **Fas** receptor on infected cell membrane → caspase apoptotic pathway activation

b. Role of Helper T Cells



- **Helper T cells (T_H, $CD4^+$)** are responsible for secreting cytokines to mediate immune responses
  - TH1 secretes **IL-2**, **IFN-γ**
  - TH2 secretes **IL-4**, **IL-10**

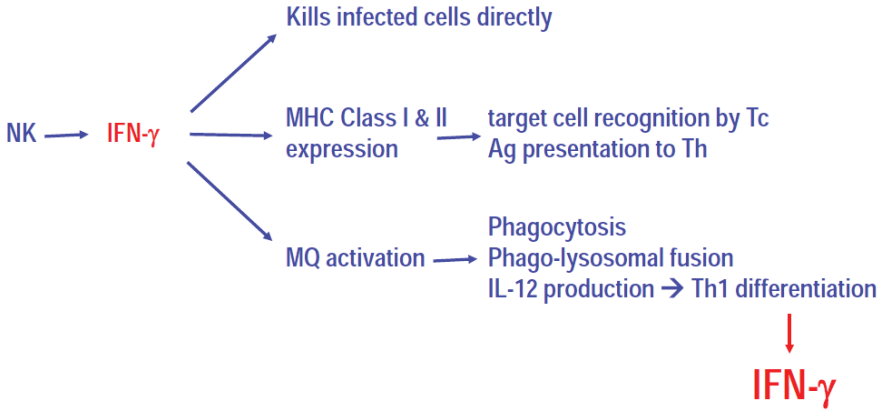


- HIV infection targets $CD4^+$ cells → immune dysfunction

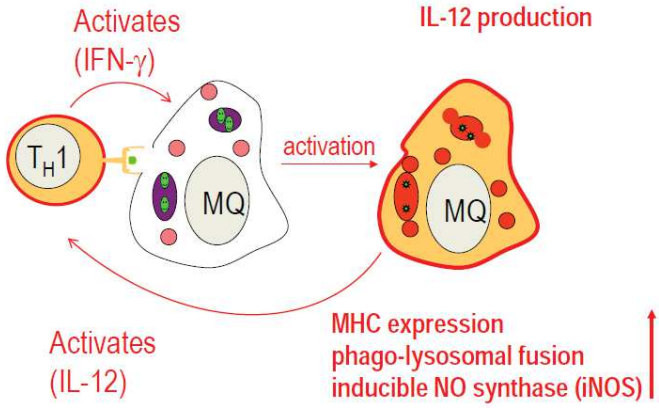
c. Interferons

- **Interferons**: cytokines that play a role in interaction of inflammatory cells
- Binds with **interferon receptors** on cell membranes to induce anti-viral activities (for type I IFN) or mediate other immune responses (for type II IFN)

Type	Cell source	Functional activities
I (α/β)	Virus-infected cells	- Induce anti-viral activities of cells (major effect) - Activates **large granular leukocytes (LGL)** or **NK cells** - Enhance MHC class I
II (γ)	NK cells, T cells	- Induce anti-viral activities of cells (to a small degree only) - Activate macrophages - Stimulate IL-12 and NO production - Enhance MHC classes I and II - Kill infected cell directly

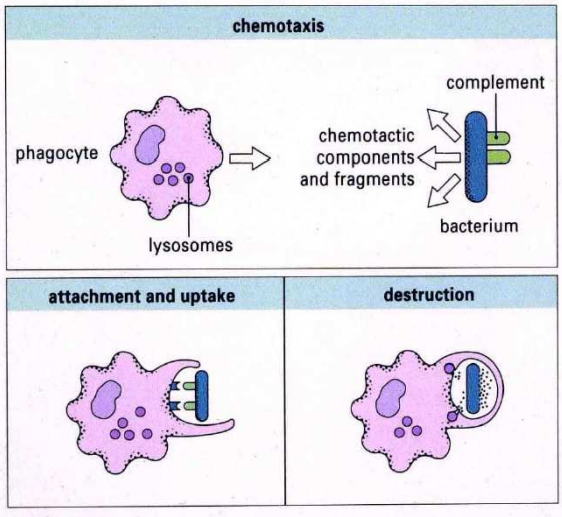


i. T-dependent Macrophage Activation



- Th-1 secretes IFN-γ to activate MQ to increase its potency in killing microbes
- In turn, activated MQ secretes IL-12 to promote differentiation of Th1

d. Phagocyte-mediated Killing



- Mechanism for phagocyte-mediated killing:
  - **Reactive oxygen intermediates (ROIs)**: eg. superoxide anion
  - **Reactive nitrogen intermediates (RNIs)**: eg. nitric oxide
  - Other mediators eg. lysozyme, prostaglandins
  - **Cytokines**: eg. IL-1, IL-6, TNF, IFN-γ

D. Immunodeficiency and Immune Disorders

1. Immunodeficiency

- Primary immunodeficiency
  - Intrinsic cause
  - Most are genetic linked
  - Example: **severe combined immunodeficiency (SCID)** characterized by defective T and B cells
- Secondary immunodeficiency:
  - Extrinsic (i.e. acquired) cause:
    - → Infections: eg. HIV infects T_H cells
    - → Drugs
    - → Irradiation
    - → Malnutrition
- Clinical implication:
  - **Recurrent (pyogenic) infection** can be caused by encapsulated bacteria infection when there are
    - → Defects in Ig
    - → Defects in complements
    - → Defects in phagocytosis
  - **Opportunistic infections**:
    - → Caused by defective cell-mediated immunity
    - → Caused by **opportunistic pathogens** eg. some viruses and yeasts
- **Opportunistic pathogen**: a microorganism that causes infectious disease only in individuals with compromised host defense mechanisms

2. Immune Response against Tumours

- Human immune system will effect response against tumours
- Evidence:
  - Tumour prevalence higher in both neonatal and elderly stages
  - Immune cell infiltration in tumours
  - Spontaneous regression of tumours
  - Post-mortem finding often reveal more tumours than clinically diagnosed
  - **Graft versus leukaemia (GVL) responses**: injection of external T cells causes tumour regression
- Immunodeficiency may cause cancer (eg. **Kaposi's sarcoma**, **Burkitt's lymphoma** etc.)
- Tumours escape from immune surveillance due to:
  - Lack of molecules important in immunity
  - Suppression on immunity by tumour-derived factors

3. Hypersensivity

- **Hypersensitivity**: state of heightened reactivity to innocuous antigens (**allergens**)
- May lead to **anaphylaxis** and other adverse reactions

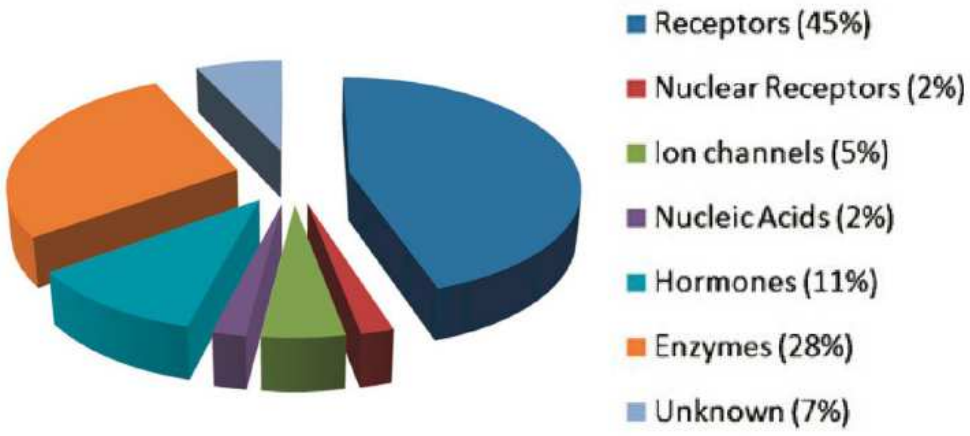
4. Autoimmunity

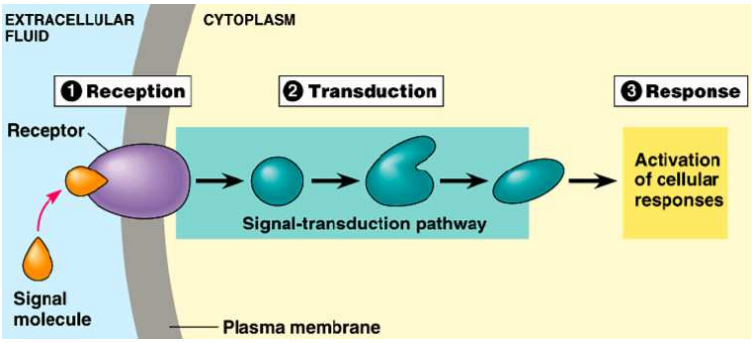
- **Autoimmunity**: an adaptive immune response directed against self-antigens
- **Immunological tolerance**: a state of unresponsiveness to a particular antigen
  - Antigen specific
  - Mechanism:
    - → **Central tolerance** by **thymic education**: elimination of those responding to self-antigens
    - → **Peripheral tolerance** by **failed-safe mechanisms**: overlapping control mechanisms in activating T cells
- Loss of self-tolerance → **autoimmunity**

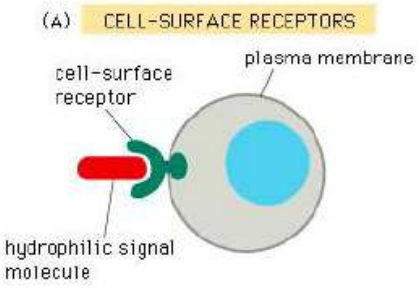
L88 Drug-receptor Interactions

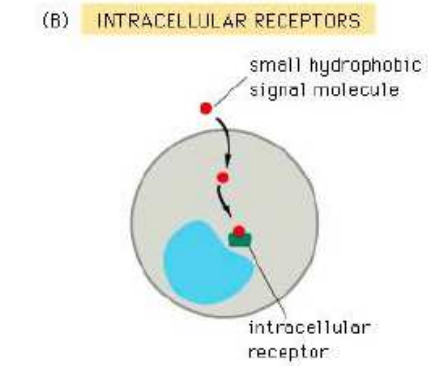
A. Principle of Drug Action

- **Pharmacology**: a study of the effect of chemicals on biological systems
- Effects of most drugs result from interaction with macromolecular components (eg. receptors) of organism
  - These interactions alter function of the pertinent component and thereby initiate biochemical and physiological changes that are characteristic of the response to the drug
  - Drugs merely modify or manipulate existing physiological response
- **Drug targets**: specific molecular components of a biologic system with which drugs interact to produce changes in the function of the system
  - Examples: receptors, transporters, ion channels, DNA, nuclear receptors, enzymes

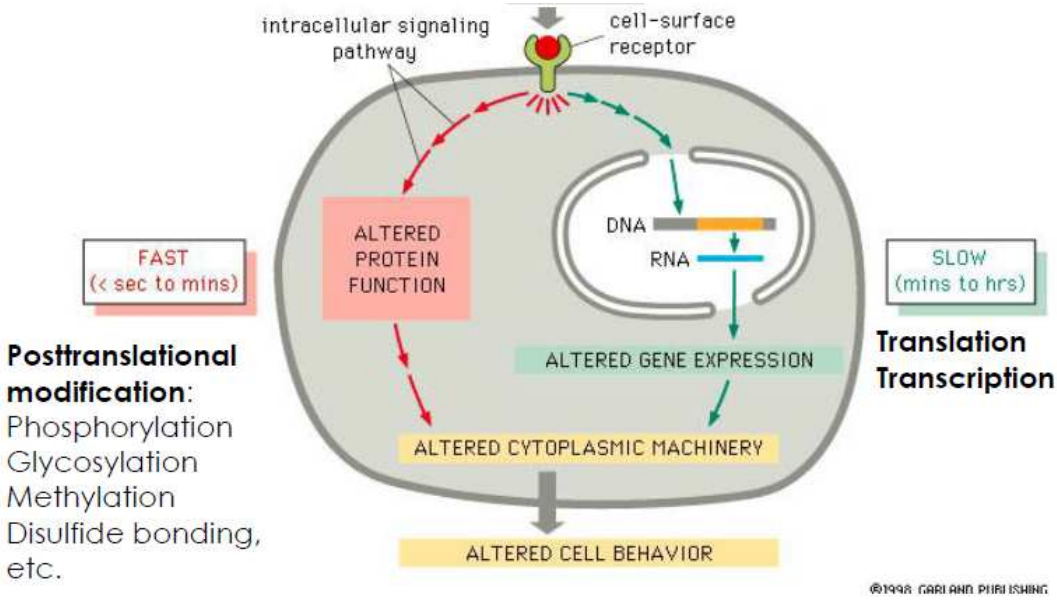








- **Ligand**: the chemical signal that ligates to the specialized cellular macromolecule
- **Receptor**: the cellular macromolecule that receives the ligand
  - May either be in the cell membrane or intracellular
- Interaction of the ligand with receptors → binding triggers cellular response

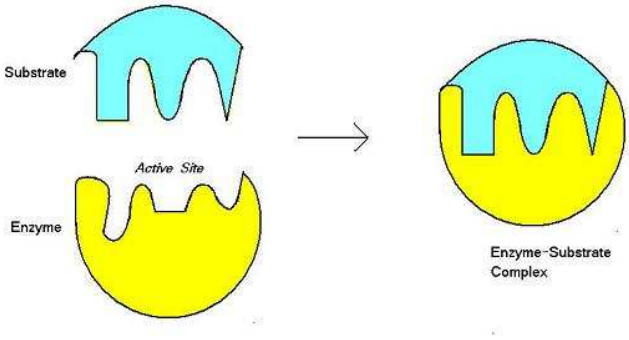


- Speed of drug response (on a cellular level):
  - Drugs that act on **posttranslational modification** pathways (eg. phosphorylation, glycosylation, methylation, disulphide bonding) tends to be <u>fast</u>
  - Drugs that act on **translation** and **transcription** tends to be <u>slow</u>

1. Drug Receptors

- **Drug receptors**: specific target molecules with which drugs interact to modulate cell function
- Differ based on:
  - Cellular location
  - Specific for different chemical messengers
  - Time scale of effects in cells
- Each cell has a variety of receptors making it responsive to different chemical messengers
- Functions:
  - Recognition of specific ligand molecule
  - Transudction of signal into response
- Domains:
  - **Ligand-binding domain**
  - **Effectors domain**: undergo functional conformational change
- Functions determined by the interaction of lipophilic or hydrophilic domain of peptide chain with drug molecule
  - Non-polar part buried in membrane, polar part on cell surface
  - Hydrophilic drug cannot pass through membrane → only binds to hydrophilic peptide domain
  - Binding of polar drug in ligand induces conformational changes (alter distribution of charges and transmission to coupling domain followed by effector domain)
- Two different types of drug action on receptors:
  - Drug may act on **physiological receptors** and mediates response of hormones, transmitters, **autacoid** (local hormone-like molecule) and others (eg. cholinergic, histaminic, adrenergic etc.)
  - Drug may act on **true drug receptors** (i.e. body cannot synthesize the ligand) eg. benzodiazepine receptors

2. Ligand-receptor Binding



Induced-fit Model. - The enzyme active site forms a complementary shape to the substrate after binding.

- Receptors contain a binding site that is recognized by ligand
- Binding of ligand involves <u>intermolecular bonds</u>
- Binding results in an **induced fit** of receptor protein
- Change in receptor shape results in signal transduction → chemical signal received inside the cell
- Two types of binding:
  - Irreversible covalent bonding:
    - → Long, persistent drug effect
    - → Few drug examples
  - Reversible non-covalent bonding:
    - → Usual type of interaction
    - → Eg, ionic bonds (electrostatic), hydrogen bonds, hydrophobic interactions, van der Waals interactions

a. Drug Selectivity

- **Drug selectivity**: drug binding to one type of receptor in preference to another
  - A drug with <u>high</u> degree of selectivity is likely to show a <u>greater difference</u> between dose required for different biological action
- **Receptor selectivity**: extent to which it can recognize and respond to only one ligand or a group of related ligands
  - 3D organization of different sites for reversible binding interactions corresponds to 3D structure of endogenous ligand
  - For **stereoisomers**, only one type has the necessary complementarity of receptor complex (eg. L-norepinephrine)
    - → Some drugs are a mixture of stereoisomerism → different isomers may show very different binding characteristics and biological properties
    - → A drug that is an equal mixture of levo and dextro isomers (i.e. **racemate**) could be a mixture of 5-% active compound plus 50% inactive (eg. levodopa)

b. Receptor Binding

- **Receptor occupancy** (extent of drug binding to receptor) proportional to drug concentration
- Intensity and duration of intracellular changes dependent on continuing presence of ligand
- Binding of ligand alters receptor conformation → intracellular changes

i. Receptor Binding Curves

c. Drug Affinity

- **Dissociation constant (K_d)**: free ligand concentration at which 50% of receptors are occupied
- Affinity of drug for a receptor is expressed as reciprocal of K_d
  - High affinity drugs have low K_d
  - Low affinity drugs have high K_d
- Note: each ligand has its own specific affinity to a particular receptor

d. Agonists and Antagonists

- **Agonist**: an agent that can bind to a receptor and elicit a biologic response
- **Antagonist**: an agent that decreases or blocks actions of another drug or endogenous ligand

3. Drug Tolerance

- **Drug tolerance**: decrease in response with repeated doses
- An increase in dose is required to maintain the same response
- May result from:
  - A decrease in concentration of drug at receptor
  - A decrease in drug response produced by receptor to the same concentration of drug
  - Decrease in number of receptors (eg. due to **receptor regulation**) so that the percentage occupancy to produce the same effect is elevated

a. Receptor Regulation

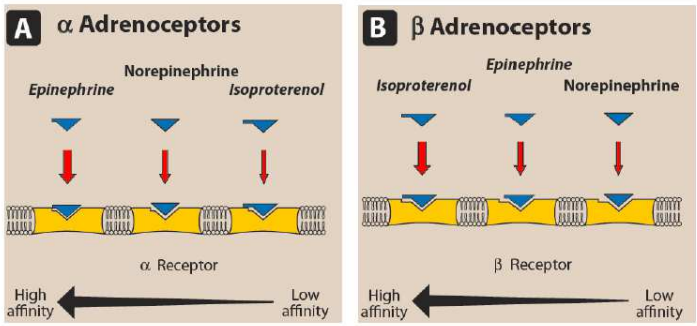
- Number of receptors within the cell membrane may be altered as a consequence of continuous or repeated exposure to its ligand, with either an increase (**upregulation**) or a decrease (**downregulation**) in receptor numbers
- Tolerance to effects of some drugs (eg. opioids) may arise from downregulation of opioid receptor numbers → a need for increased doses to produce the same analgesic activity

b. Drug Dependence

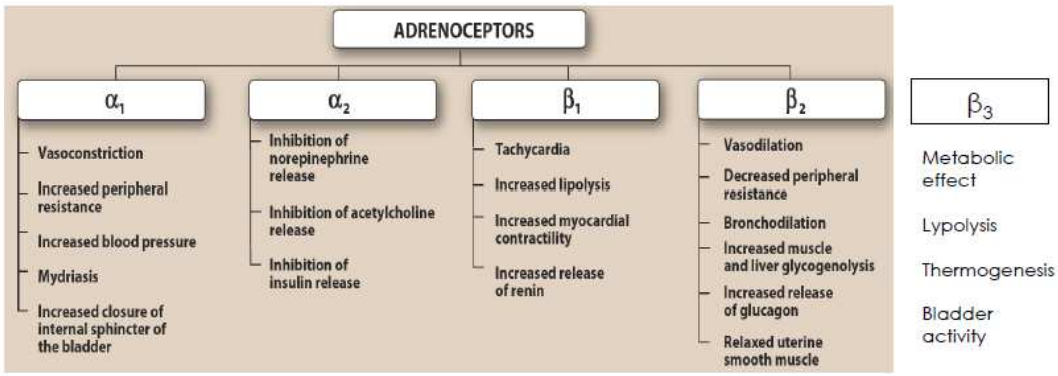
- **Drug dependence**: a person needs a drug to function normally
- An altered or adaptive physiologic state produced in an individual by the repeated administration of a drug (eg. some CNS acting drugs)
- Manifests itself as intense physiologic disturbance called **withdrawal** or **abstinence syndrome** when the drug (eg opiod) is abrupty discontinued or when its actions are diminished by the administration of a specific antagonist (eg. naloxone)
- Often these withdrawal symptoms are opposite to physiological effects of the drug itself

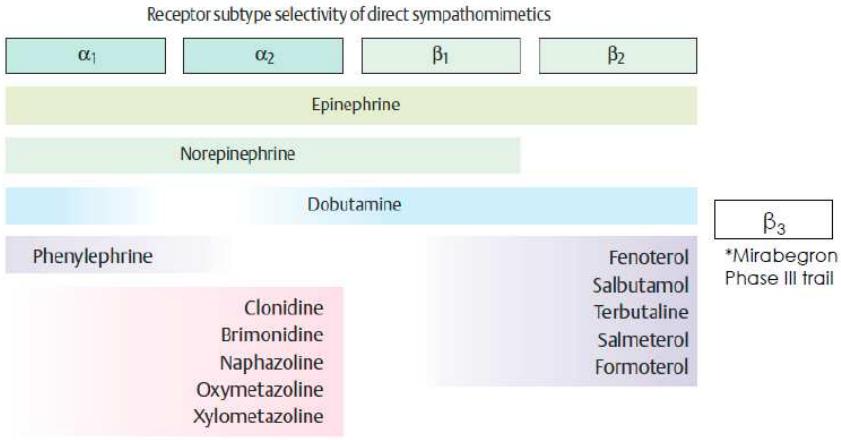
B. Receptor Types and Subtypes

- Receptors tend to occur in different families (receptor **types**)
  - Distinct families show different characteristics



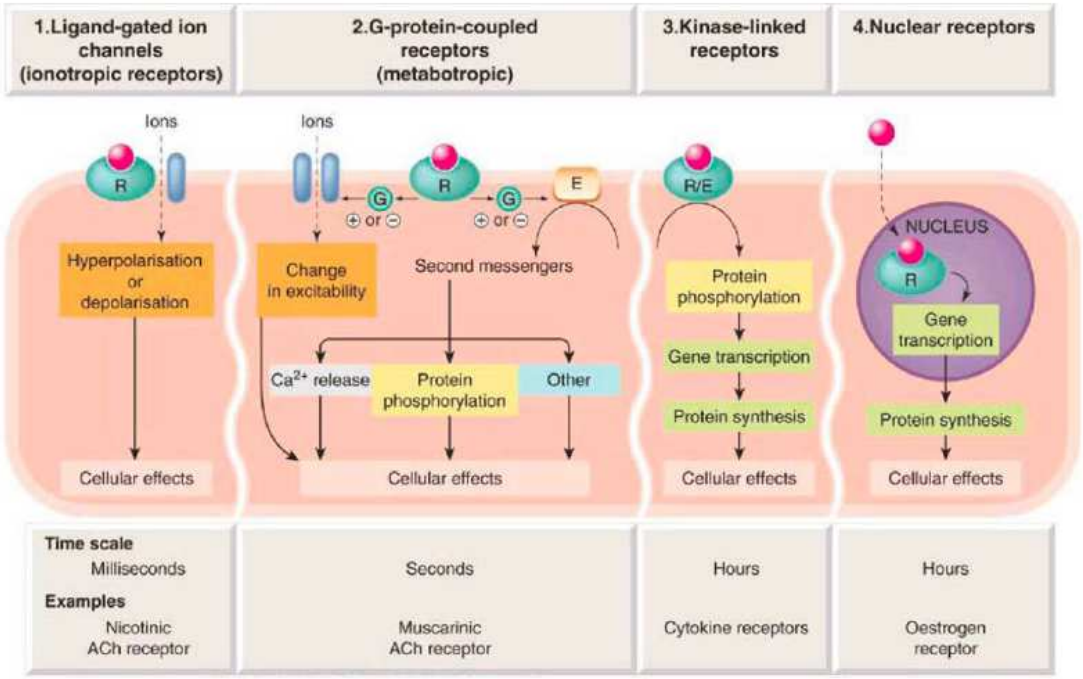
- Within any one family of receptors there are different family members (receptor **subtypes**), each of which specifically recognizes or binds the same ligand
  - Individual members of the same family share some common traits inherent in the family
  - Also possible for some subtypes to produce distinct different biological effects





- Example: **adrenoreceptors**
  - α_1, α_2, β_1, β_2 all bind to adrenaline
  - Occur to a different extent in different tissues
  - Produce different intracellular changes when stimulated or blocked

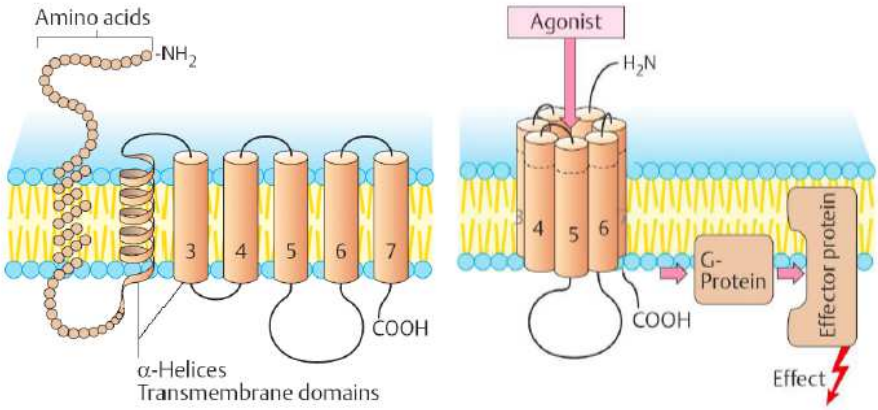
1. Major Superfamilies of Receptors



a. Ligand-gated ion channel

- Receptor is a transmembrane ion channel
- Binding of ligand controls passage of ions across membranes
- Example: **nicotinic acetylcholine receptor**, **benzodiazepine receptor**

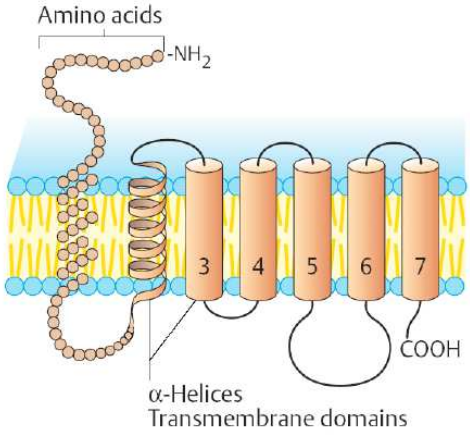
b. G-protein Coupled Receptors



Adrenergic receptors (α and β): Salbuterol, Propanolol, Dobutamine, etc.
Glucagon receptor

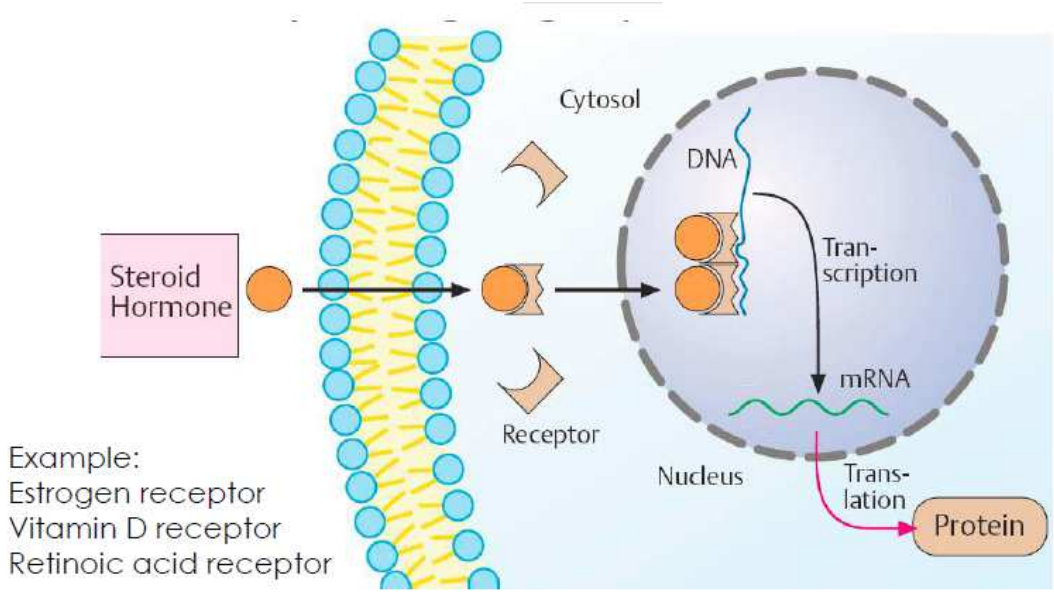
- Also called**7-transmembrane receptors** (with 7 transmembrane α helices)
- Named because of their interaction with guanine nucleotides (GTP/GDP);
- Receptor linked to an enzyme via a G-protein
- Example: **adrenergic receptors**, **glucagon receptors**

c. Kinase-linked Receptors



- Transmembrane receptors with a cytosolic enzymatic component
- Binding activates an intracellular kinase site
- Examples: **insulin receptors**, **toll-like receptors** (recognize microbes breaching physical barrier and activates immune response), **epidermal growth factor receptor**

d. Nuclear Receptors



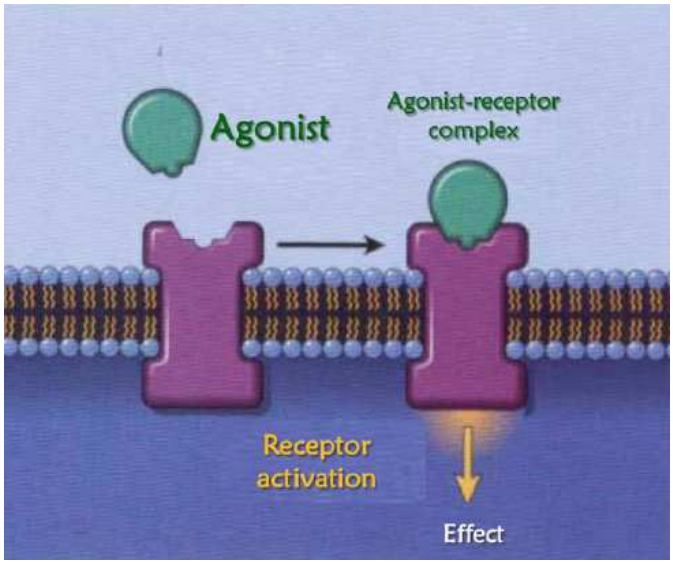
- Intracellular receptors responding to ligands by modification of gene transcription
- Membrane solubility allows lipid-soluble drugs to cross cell membrane and bind to nuclear receptors
- Examples: **oestrogen receptors**, **vitamin D receptor**, **retinoic acid receptor** (activation of transcription machinery)

L89 Dose-response Relationships

A. Pharmacodynamics

- **Pharmacodynamics**: relationship between drug concentration at site of action and resulting effect (incl. time course and intensity of therapeutic and adverse effect)
- Study of biochemical and physiological effects of drugs and mechanism of action:
  - Drug-receptor interactions
  - Dose-response phenomena
  - Mechanisms of therapeutic toxic actions

B. Receptor Occupancy Theory



- Premise: drug response is proportional to number of receptors occupied at equilibrium
- Assumptions:
  - All interactions are result of a reversible drug-receptor interaction (**occupancy**)
  - All responses are result of **mass action**
  - All responses are measured at equilibrium
  - 1:1 drug-receptor stoichiometry
  - Concentration of bound drug (eg. to plasma proteins) is negligible compared to total drug concentration
  - 100% occupancy = maximum effect

By **law of mass action**, for an equilibrium $\alpha[A] + \beta[B] + \cdots \rightleftharpoons \sigma[S] + \tau[T]$,

$$K = \frac{k_+}{k_-} = \frac{[S]^\sigma [T]^\tau \ldots}{[A]^\alpha [B]^\beta \ldots}$$

Therefore, for the equilibrium $[A] + [R] \rightleftharpoons [AR]$,

$$k_1[A][R] = k_2[AR]$$

$$k_d = \frac{k_2}{k_1} = \frac{[A][R]}{[AR]} \ldots\ldots (1)$$

where k_d is the **dissociation constant**

Let R_{total} be total number of receptors, therefore $R_{total} = [R] + [AR]$

And $[R] = R_{total} - [AR]$……(2)

Substitute (2) into (1), we get,

$$k_d = \frac{[A][R]}{[AR]}$$

$$= \frac{[A](R_{total} - [AR])}{[AR]}$$

$$= \frac{[A]R_{total}}{[AR]} - [A]$$

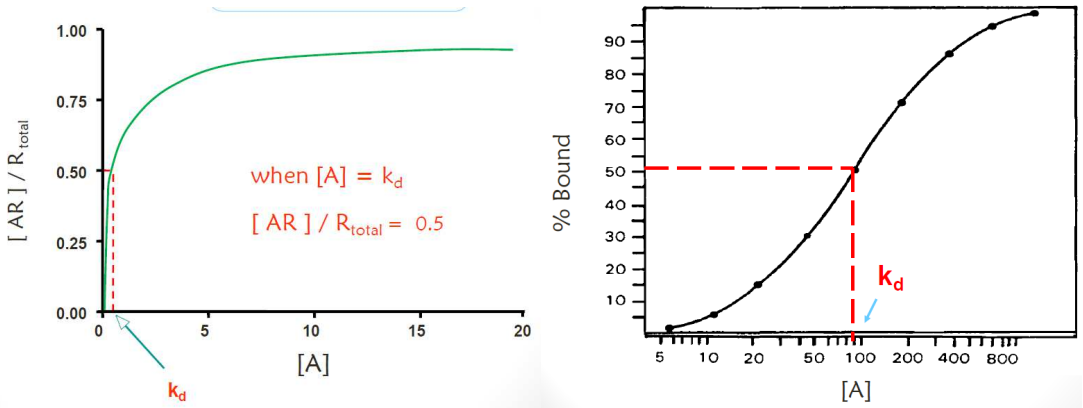
$$k_d + [A] = \frac{[A]R_{total}}{[AR]}$$

$$\frac{[AR]}{R_{total}} = \frac{[A]}{k_d + [A]}$$

Note that LHS is equal to **receptor occupancy**, we can also write

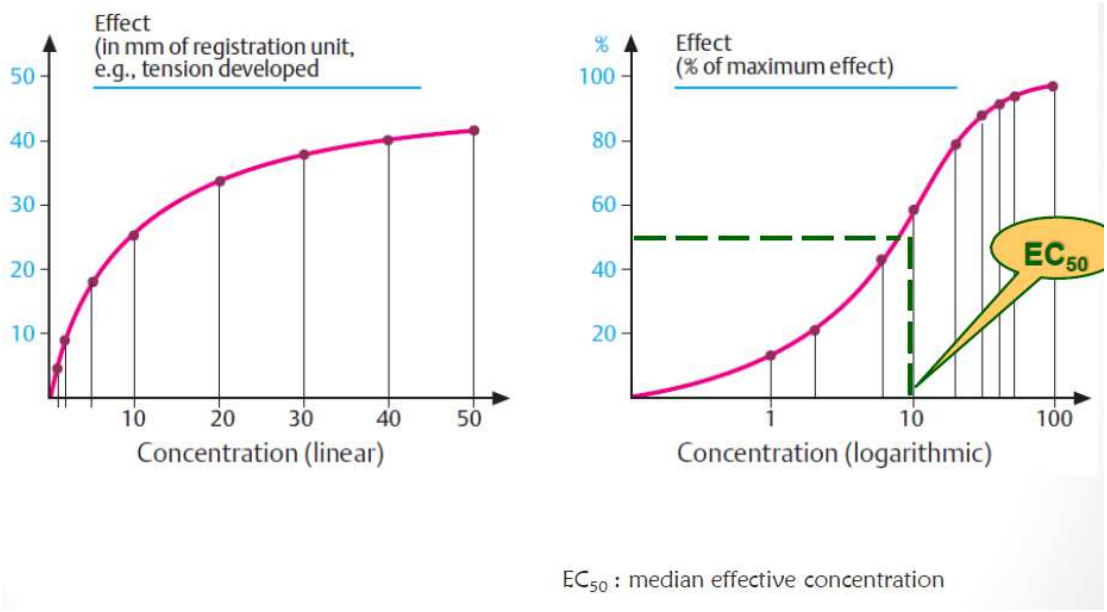
$$\frac{E}{E_{max}} = \frac{[A]}{k_d + [A]}$$

Also note that k_d is the drug concentration at which <u>50%</u> of all receptors are bound.



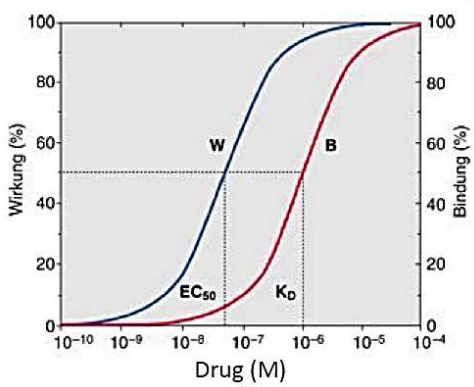
C. Dose-Response Relationship

1. Concentration-effect Relationship



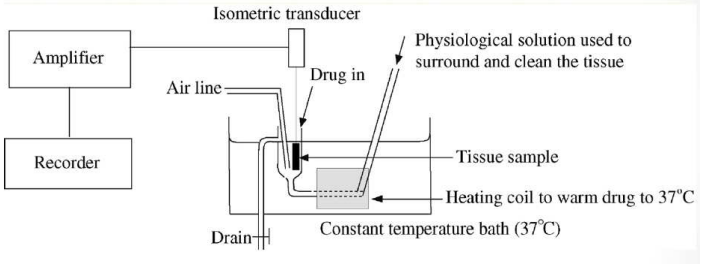
- Magnitude of drug effect is generally proportional to receptor occupancy (subject to modification by **efficacy**)
- **Median effective concentration (EC_{50})**: molar concentration of an agonist that produce 50% of <u>maximal possible effect</u> of that agonist

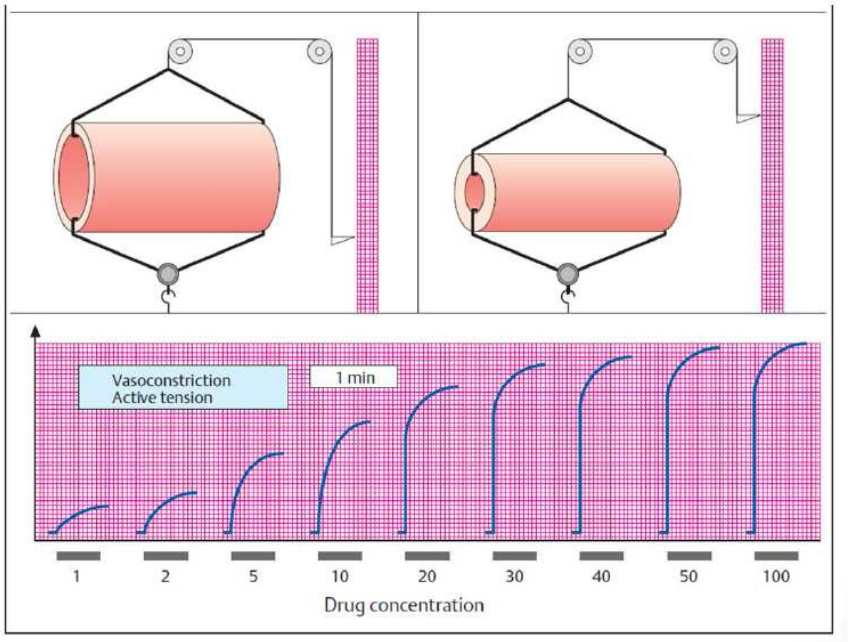
a. Receptor Reserve



- Some agonists are so efficacious that they do not need to occupy the entire receptor pool to produce a full response
- Fraction of receptors not needed called **receptor reserve** or **spare receptors**
- Receptor reserve for a given agonist can vary among tissues
- Note difference between k_d and EC_{50}:
  - k_d: concentration at which half of all receptors are occupied
  - EC_{50}: concentration at which half of maximum response is produced

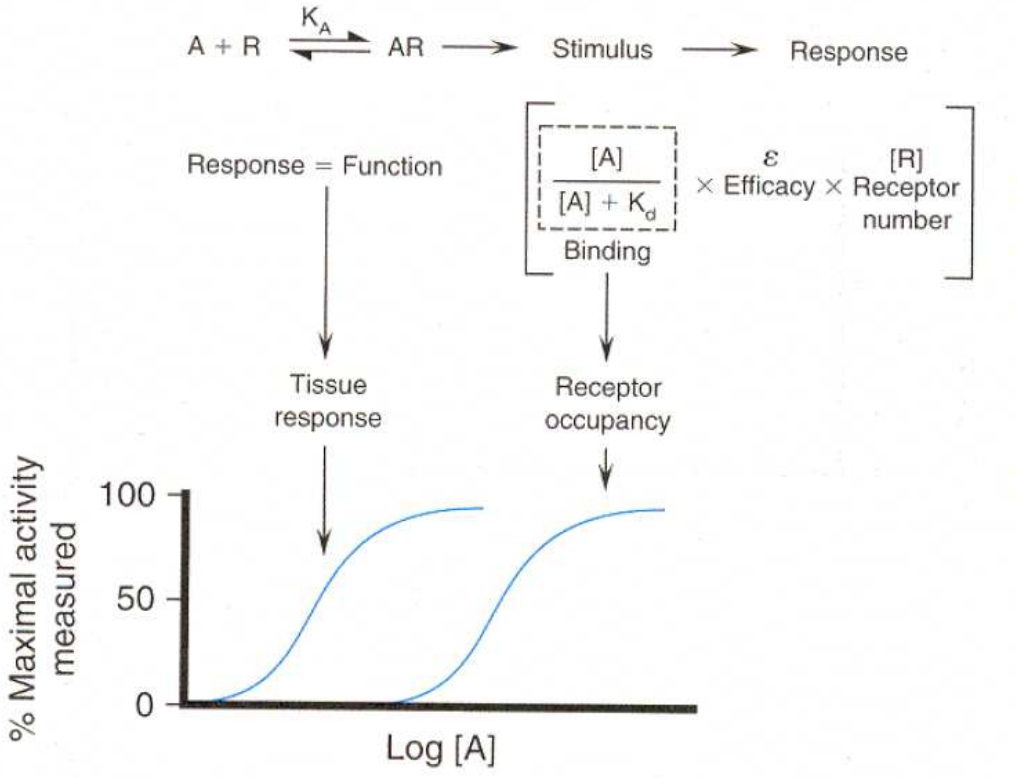
b. Quantification of Drug Effects





- Drug effect can be quantified by a **bioassay system**
- **Isometric transducer** measures minute changes in tension in place of drug response

2. Drug Response



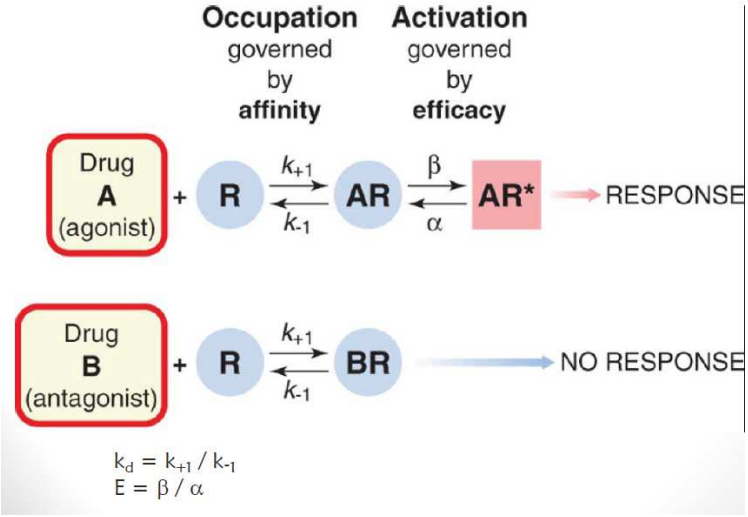
- Drug response can be expressed as a <u>function of the magnitude of stimulus</u>

$$\text{Response} = f\left(\frac{[A]}{k_d + [A]}\right) \cdot \varepsilon \cdot R_{total}$$

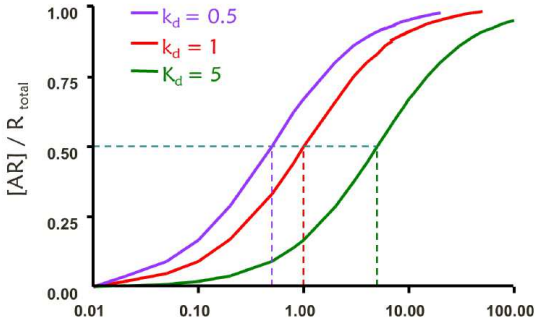
f: **transducer function**

ε: intrinsic efficacy

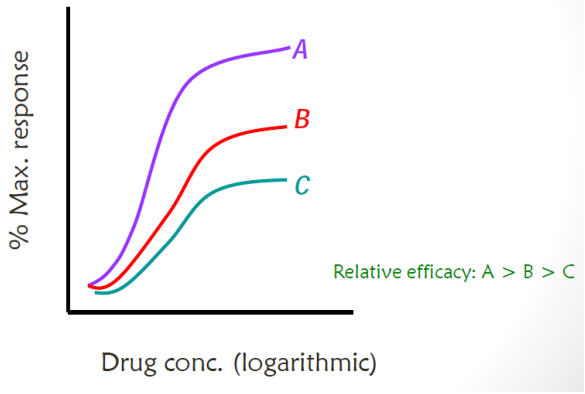
3. Drug Affinity, Efficacy and Potency



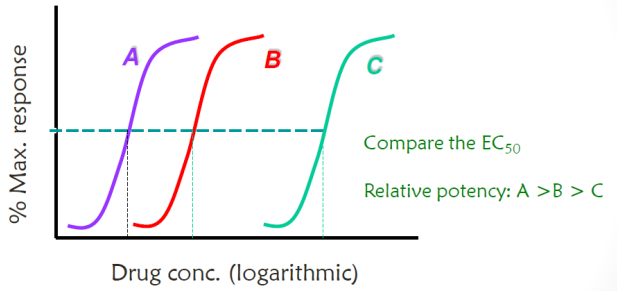
- **Affinity**: attraction of drug for receptor
  - Can be seen as the reciprocal of k_d
  - Best drugs have high affinity for target receptor (for beneficial effects) but little or no affinity for other receptors (for unwanted effects)
  - Applies to both **agonists** and **antagonists**



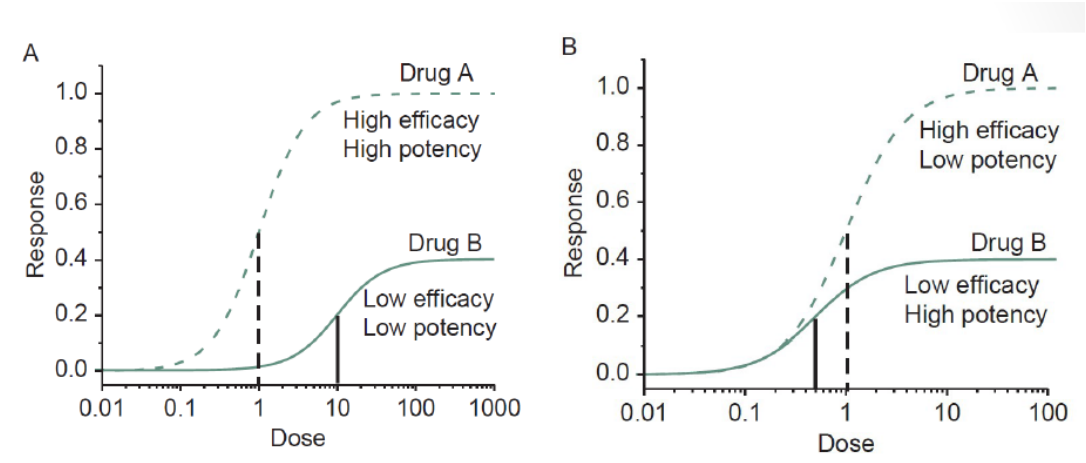
- **Efficacy**: intrinsic activity or effect
  - Can be seen as a <u>proportionality constant</u> between **receptor occupancy** (modified by transducer function) and **drug effect**
  - Low affinity: many receptors bound but no effect
  - Efficacy = 1: effect equivalent to endogenous compound
  - Efficacy = 0: antagonist



- **Potency**: an expression of activity of the drug in terms of <u>concentration</u> or amount needed to produce a <u>defined effect</u> (either % max effect or absolute effect)
  - Can be seen as the reciprocal of EC_{50}
  - Affected by both **affinity** and **potency**

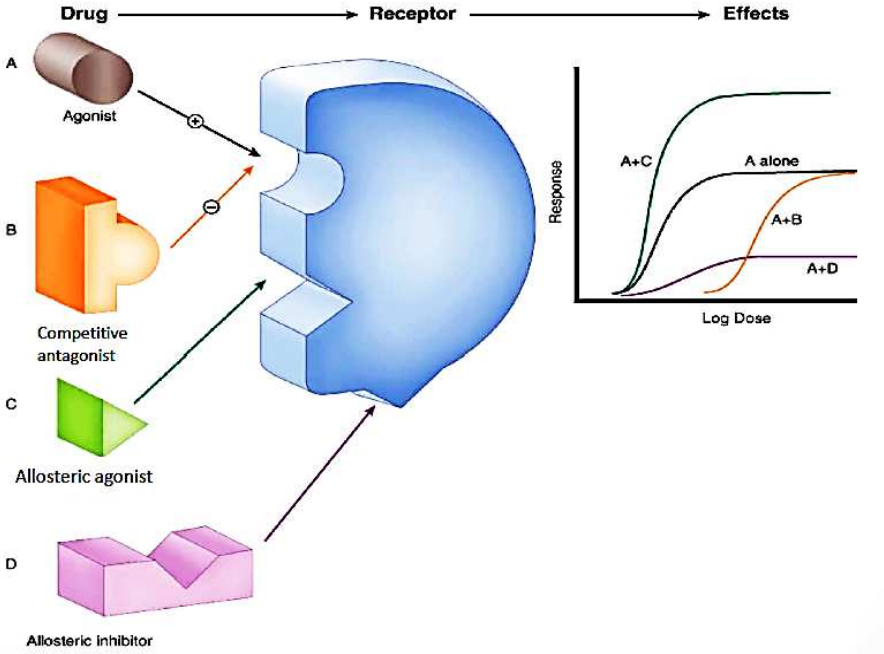


*Note the difference between potency and efficacy:

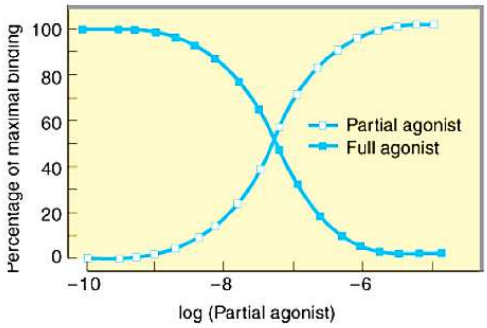


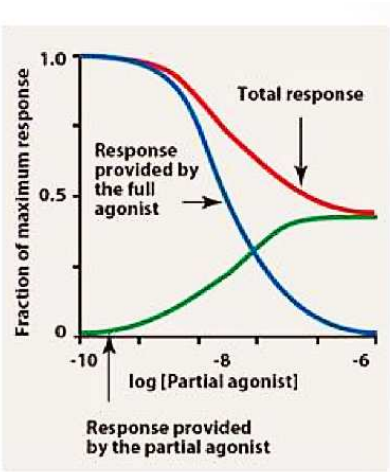
These panels illustrate the concepts of efficacy and potency in comparing drugs with similar effects. Efficacy is the maximum response. Potency is the inverse of ED_{50}. A drug with low potency but high efficacy may be more effective than an equal dose of a drug with higher potency but lower efficacy.

4. Agonists and Antagonists



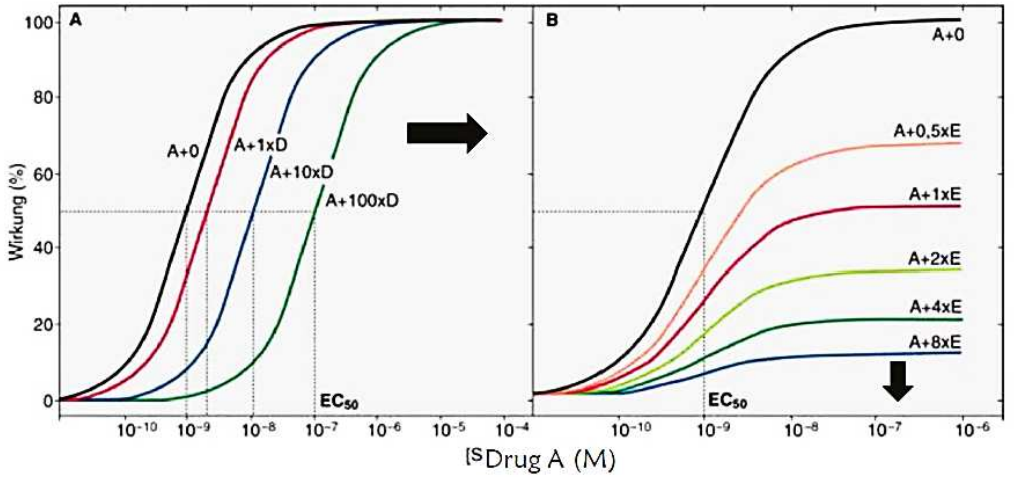
- **Agonist**: an agent that activates a receptor to produce an effect that is similar to that of physiological signal molecule
  - EC_{50} can be much lower than k_d in full agonists ($\because$ receptor reserve)
  - Example: muscarine, nicotine





Partial agonists can act as competitive antagonists by interfering with full agonist binding

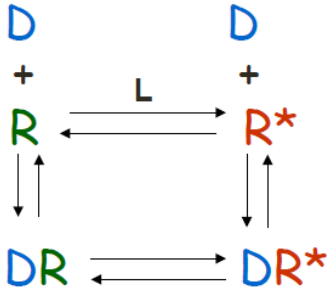
- **Partial agonist**: an agent that activates a receptor to produce submaximal effect but antagonize the action of a full agonist
  - EC_{50} approximates k_d in partial agonists
  - Example: pentazocine



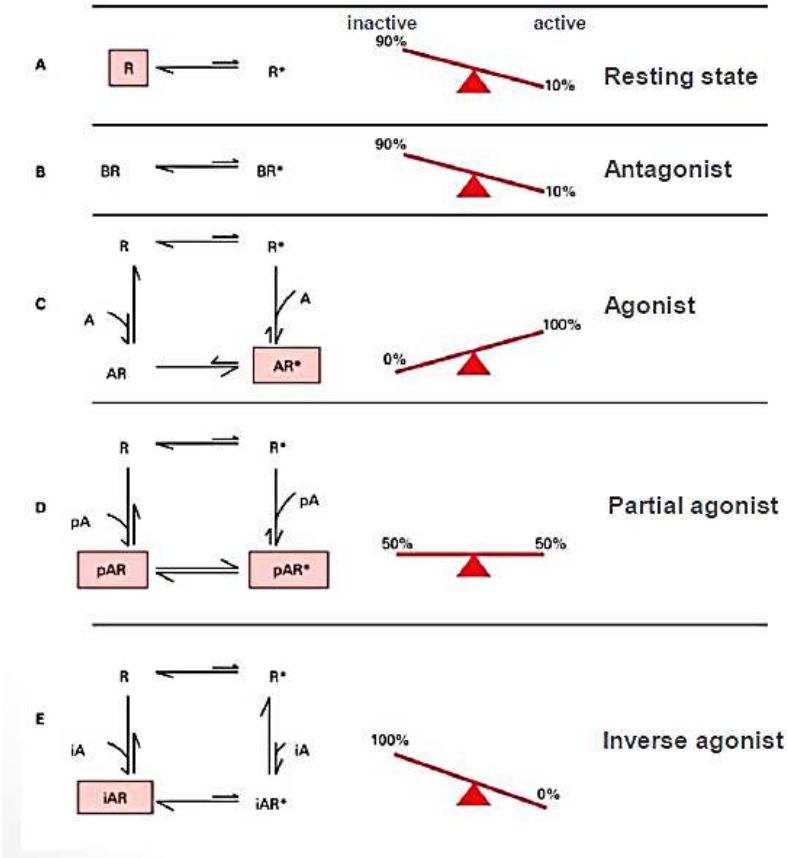
A: Drug D, a competitive antagonist parallel shifts the concentration-response relationship for Drug A. The shift depends on the concentration of Drug D.
B: Drug E, a non-competitive antagonist, reduces the maximum effect of Drug A.

- **Antagonist**: an agent that prevent action of an agonist on a receptor or subsequent response, but dose does not have an effect on its own
  - **Competitive antagonism**: compete with agonist for the same active site but have no effect on its own
  - **Non-competitive antagonism**: binds to another site at receptor to diminish effect of agonist on the receptor
  - Example: atropine
- **Inverse agonist**: an agent that activates a receptor to produce an effect in an opposite direction to that of the agonist
  - Example: DMCM

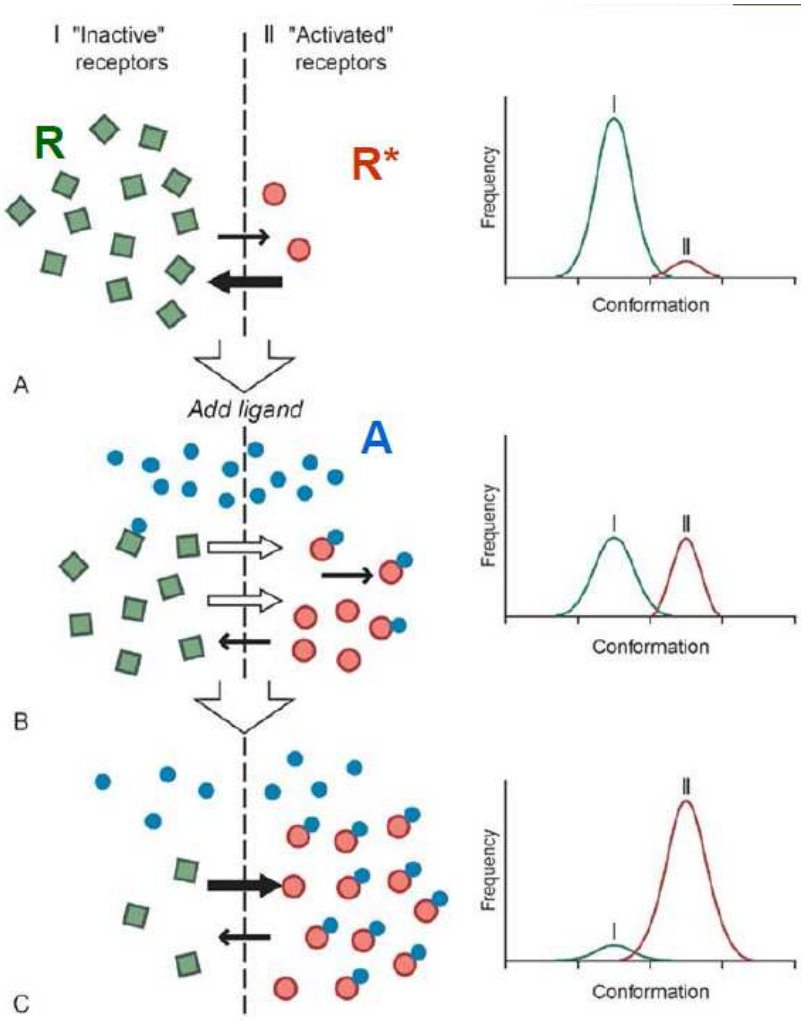
5. Two-state Model of Receptor Activation



D: drug
L: allosteric constant
R: inactive receptor
R*: active receptor



- Receptors can exist in two states: inactive conformation (R) and active conformation (R*)
- Receptors exist in an equilibrium: $R \rightleftharpoons R^*$
- Relative quantities of R and R* controlled by an **allosteric constant (L)**
- **Agonist** is a ligand with <u>preferential affinity for R* over R</u>



Compare

$$A + R \rightleftharpoons AR$$
$$A + R^* \rightleftharpoons AR^*$$

We have $k_{[R^*]} > k_{[R]}$,

i.e. $\frac{[AR^*]}{[R^*]} > \frac{[AR]}{[R]}$

Rewrite as $\frac{[AR^*]}{[AR]} > \frac{[R^*]}{[R]}$,

Consider

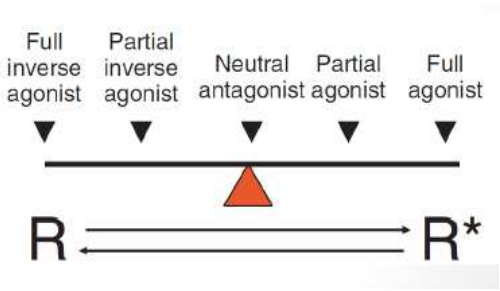
$$R \rightleftharpoons R^*$$
$$AR \rightleftharpoons AR^*$$

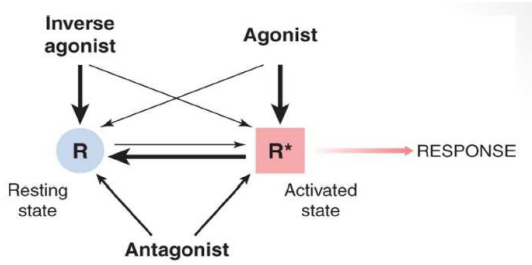
We also have

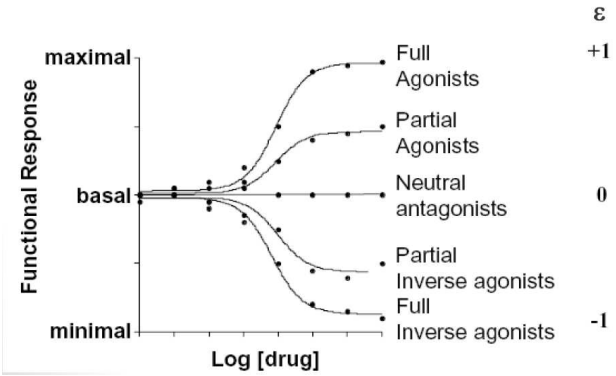
$$k_{[AR]} > k_{[R]}$$

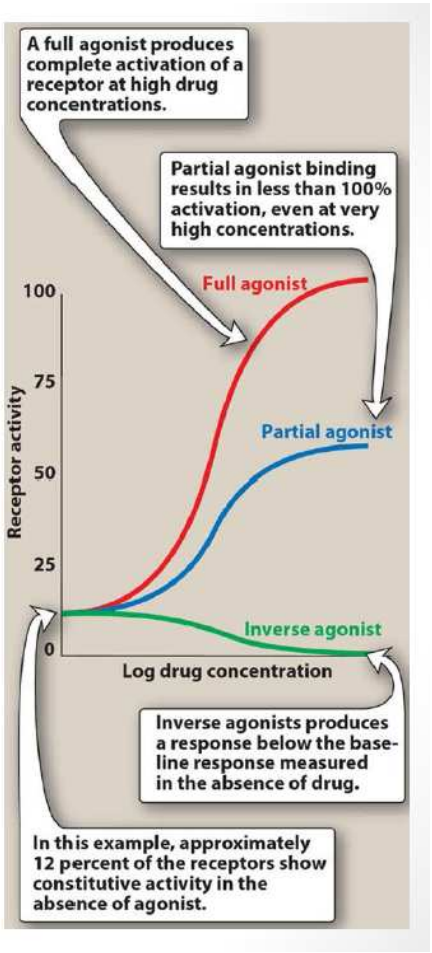
Thus, agonist binding shifts R-R* eqm to the right.

Note that when selectivity ($\propto \frac{k_{[R^*]}}{k_{[R]}}$) ↑ , efficacy ($\propto \frac{k_{[AR]}}{k_{[R]}}$) also ↑.









For **partial agonists**, the extent to which the R-R* eqm is affected is NOT as high as full agonists. Thus, they create a submaximal effect and have lower efficacy ($0 < \varepsilon < 1$).

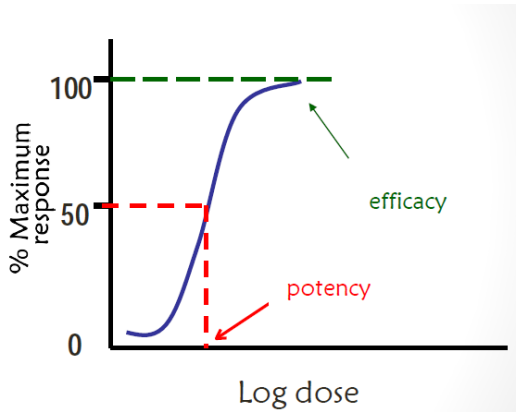
For **neutral antagonists**, they bind to the receptor but do NOT affect R-R* eqm. They bind to the receptor but have nil effect. They have <u>zero</u> efficacy.

For **partial inverse agonists** and **full inverse agonists**, they shift R-R* eqm to the left. They create an opposite effect to that of agonists and reduces basal constitutive activity. They have <u>negative</u> efficacy.

D. Dose-response Relationship

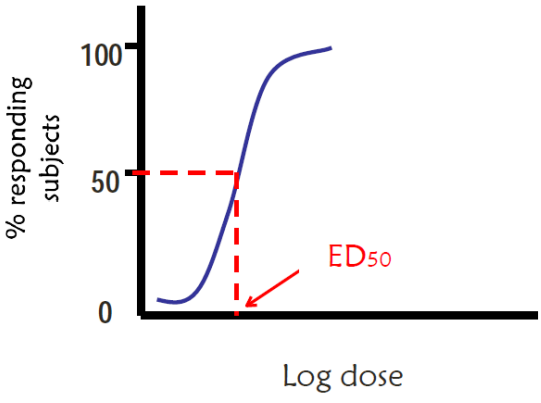
1. Graded Dose-response Relationship

- **Graded dose-response relationship**: drug response is considered <u>continuous</u> and <u>gradual</u>
- Strength assessed by:
  - **Drug potency**: amount of drug necessary to produce an effect of a given magnitude
  - **Drug efficacy**: ability of a drug to illicit a physiological response when interacting with a receptor
  - Drug with greater <u>efficacy</u> is therapeutically more beneficial than one that is more potent

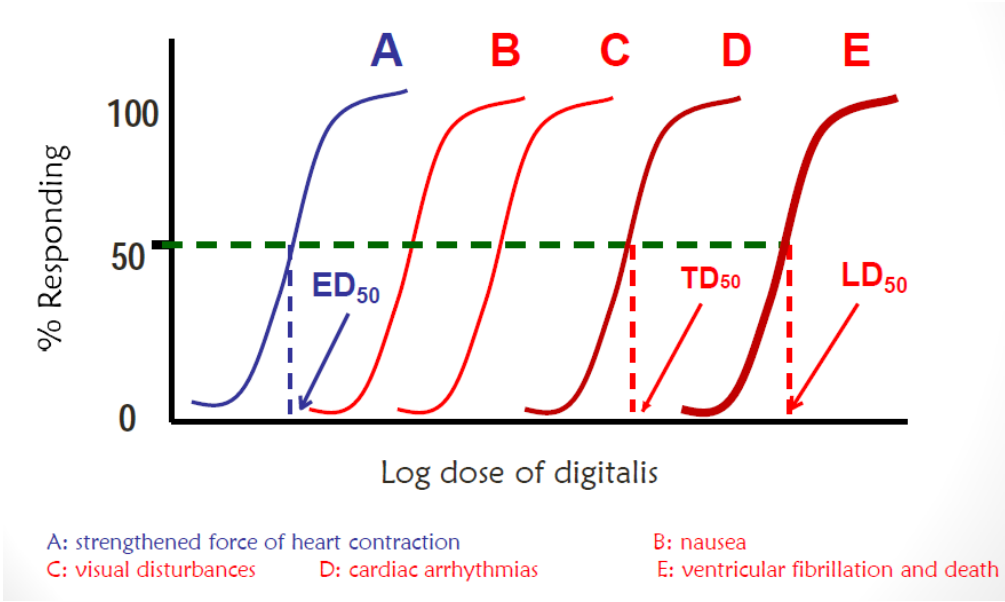


2. Quantal Dose-response Relationship

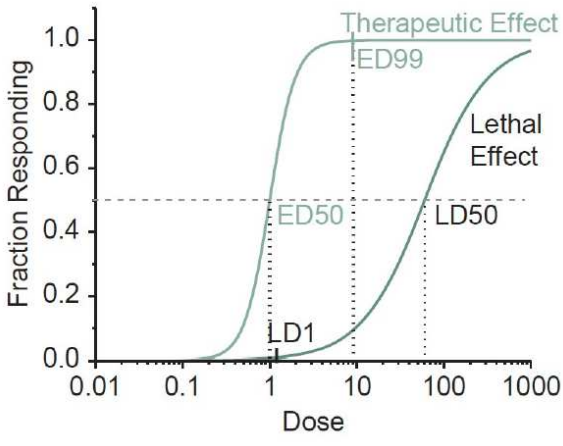
- **Quantal dose-response relationship**: drug response is considered an <u>all-or-none</u> response (either occurs or not)
- Useful for determining doses to which most of the population responds
- **Median effective dose (ED_{50})**: dose that produces therapeutic effect in <u>50%</u> of population



3. Therapeutic and Toxic Effect

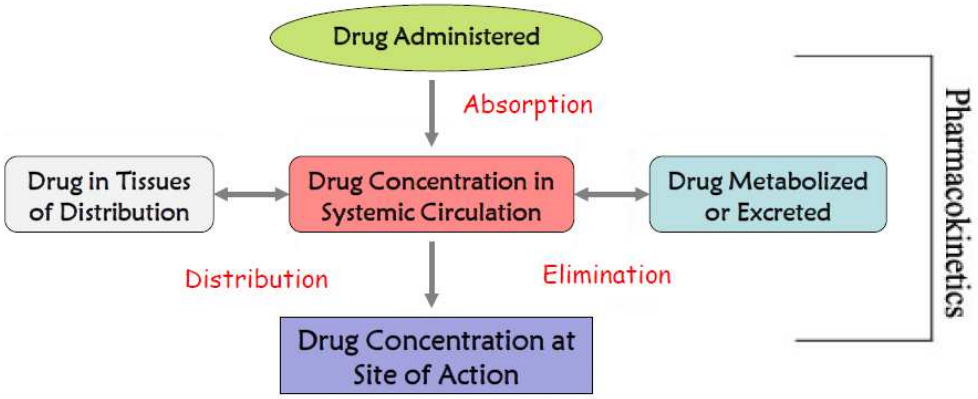


- Consider the <u>percentage of population</u> that shows a specified response to increasing doses of a drug,
  - **Median effective dose (ED_{50})**: dose that causes specified pharmacologic effect in 50% of the population
  - **Median toxic dose (TD_{50})**: dose that causes the specified adverse side effect in 50% of the population
  - **Median lethal dose (LD_{50})**: dose that causes lethality in 50% of population (in experimental animal studies)
- **Therapeutic index**: ratio of dose that produces toxicity to the dose that produces a clinically desired or effective response in a population of individuals
  - Therapeutic Index $= \frac{TD_{50}}{ED_{50}} \left(= \frac{LD_{50}}{ED_{50}} \text{ for animal studies} \right)$
  - A measure of drug safety
  - A drug with low T.I. has a narrow safety margin
  - Examples:
    - → Large T.I.: penicillin
    - → Low T.I.: warfarin
- **Certain safety factor**: $\frac{LD_{01}}{ED_{99}}$
  - In the figure, although T.I. = 60 is high, the certain safety factor is too low (0.1)

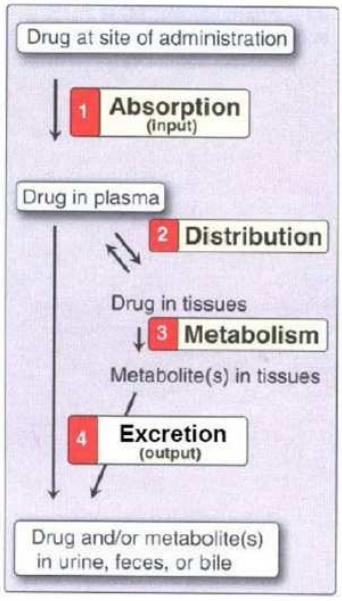


L90 Drug Absorption and Distribution

A. Pharmacokinetics

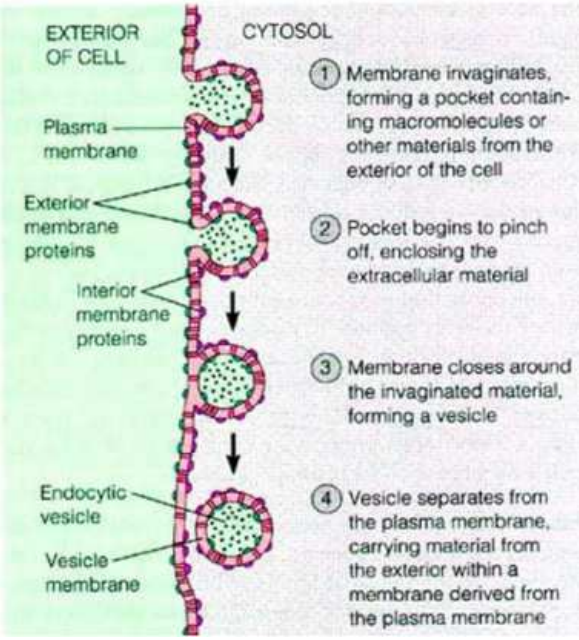


- **Pharmacokinetics**: action of the body on the drug
- Explores the factors that determine relationship between drug dosage and time-varying concentration at site of action
- Important in determining route of administration, dose, onset of action, peak action time, duration of action and frequency of dosing
- Includes:
  - **Absorption** into the body
  - **Distribution** throughout the body
  - **Elimination** from the body by:
    - → **Metabolism** by metabolizing enzymes
    - → **Excretion** from the body



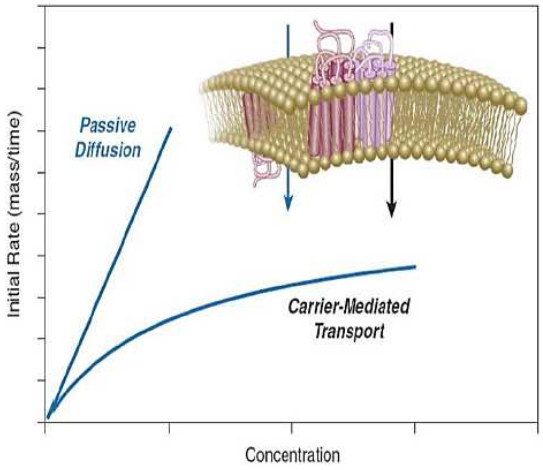
B. Passage of Drugs across Cell Membrane

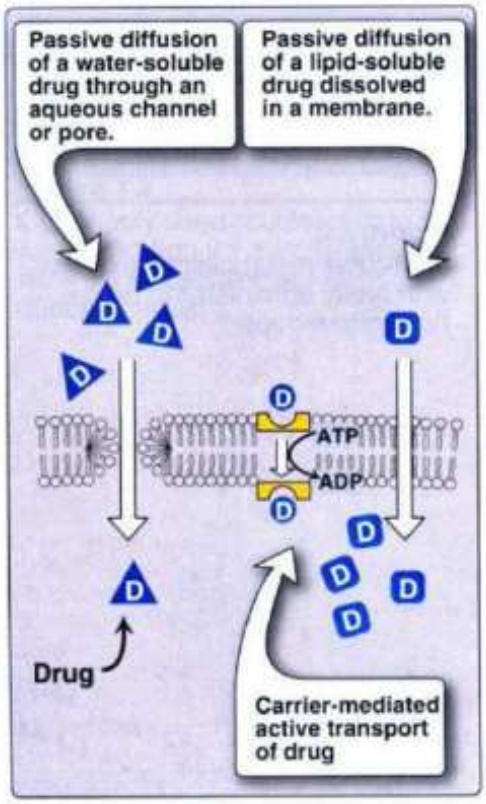
- **Carrier-mediated transport**: movement of drug molecules mediated by transport proteins
  - Drug molecules may be transported by mechanisms that carry similar endogenous substances (eg. amino acid carriers)
  - Not governed by Fick's law
  - Capacity limited: reaches a maxima when all carrier proteins are saturated



- **Endocytosis** and **pinocytosis**:
  - Occurs through binding to specialized components on cell membranes with subsequent internalization
  - Permits very large or very lipid insoluble chemicals to enter cells
  - Smaller polar substances (eg. vitamin B_{12}, Fe) transported by **pinocytosis**

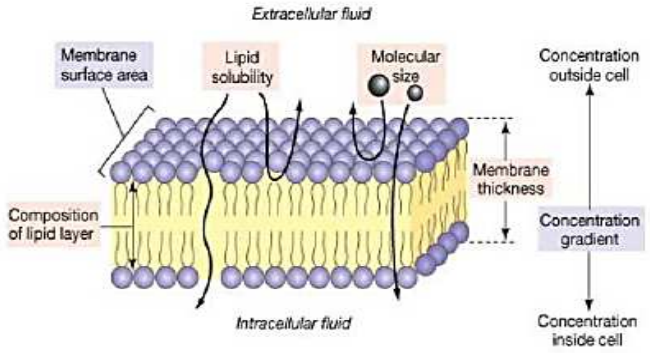
1. Passive Diffusion





- **Passive diffusion**: movement of drug driven by a concentration gradient
  - **Aqueous diffusion**: movement of drug molecules through watery extracellular and intracellular spaces
  - **Lipid diffusion**: movement of drug molecules through the lipid membrane
  - Governed by **Fick's law of diffusion**
  - Not saturable

a. Fick's Law of Diffusion



- Rate of passage of substance through lipid membranes is proportional to concentration gradient:

$$\frac{dQ}{dt} = \frac{DAK_p}{h}(C_1 - C_2)$$

where

$\frac{dQ}{dt}$: rate of diffusion

D: **diffusion coefficient** (barrier-specific)

A: surface area of membrane

K_p: **partition coefficient** (particle-specific, ratio of drug concentration in two immiscible phases (a non-polar phase (eg. oil) and a polar phase (eg. water)))

h: membrane thickness

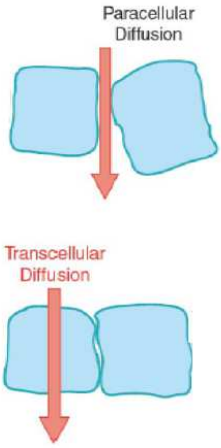
C: concentration

Let $P = \frac{DAK_p}{h}$ be the **permeability coefficient**,

$$\frac{dQ}{dt} = P(C_1 - C_2)$$

- At $t = 0$, $C_2 = 0 \rightarrow \frac{dQ}{dt} = PC_1$
- As $C_2 \rightarrow C_1$, the gradient gradually drops to zero $\rightarrow$ no net flow

b. Factors Affecting Drug Permeability



- **Lipophilicity** of drug particle as expressed as **partition coefficient (K_p)**
- Molecular size:
  - ↑ molecular weight (MW) ↑ size
  - MW < 200kDa: **paracellular diffusion**
  - MW > 250kDa: **transcellular diffusion**
  - MW > 500kDa: decreases in permeability
- H-bonding capacity: number and strength (polarity)

i. Effect of Drug Ionization on Drug Permeability

- Many drugs are weak acids or bases
- pH of medium determines fraction of ionized and non-ionized molecules
- Non-ionized form penetrates cell membrane much easier than ionized form

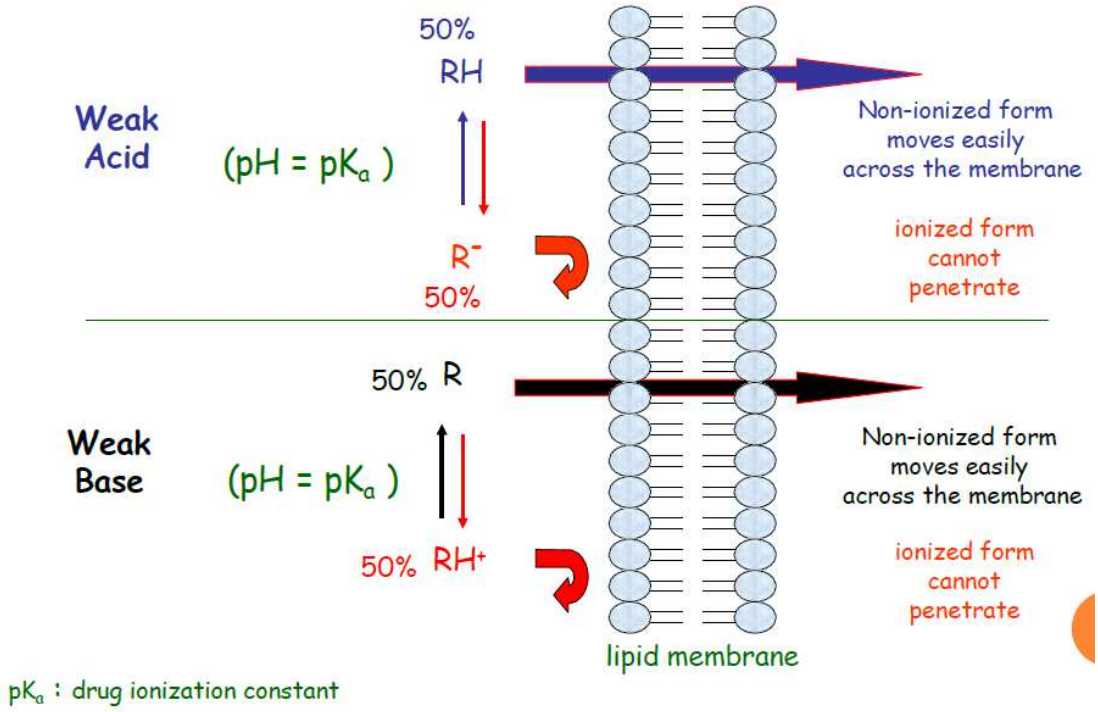
For weak acids, $RH \rightleftharpoons R^- + H^+$

For weak bases, $R + H^+ \rightleftharpoons RH^+$

Note that pH = - log [H^+], $pK_a = -\log\frac{[R^-][H^+]}{[RH]} = pH - \log\frac{[R^-]}{[RH]}$

$$pH = pK_a + \log\frac{[R^-]}{[RH]}\left(= pK_a + \log\frac{[R]}{[RH^+]}\right)$$

This is called the **Henderson-Hasselbalch equation**.



When pH = pK_a,

WLOG consider weak acids,

$$pH = pK_a + \log\frac{[R^-]}{[RH]}$$

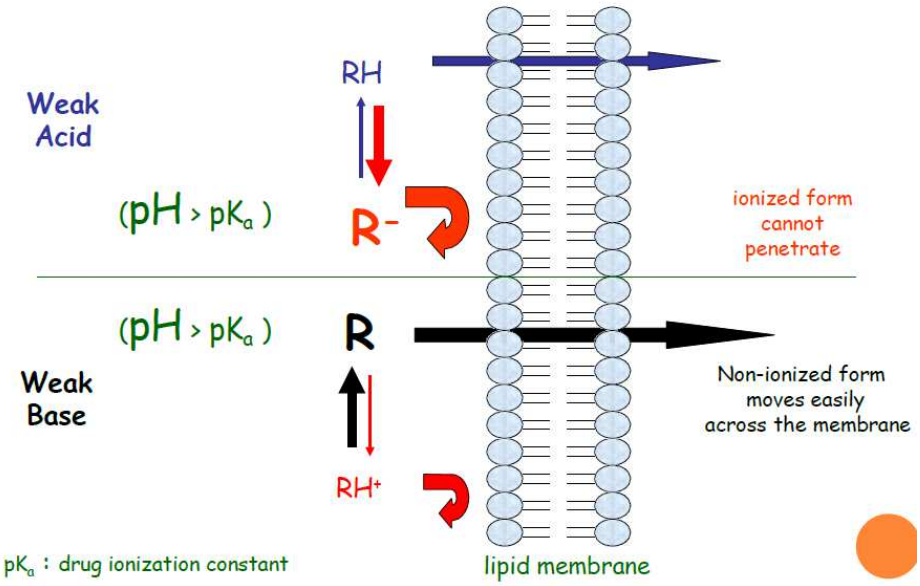
$$\log\frac{[R^-]}{[RH]} = 0$$

$$[R^-] = [RH]$$

Half of all drug molecules are in protonated form.

For weak acids, only the protonated form (50%) can be absorbed.

For weak bases, only the non-protonated form (50%) can be absorbed.



For pH > pK_a,

WLOG consider weak acids,

$$pH = pK_a + \log\frac{[R^-]}{[RH]}$$

$$\log\frac{[R^-]}{[RH]} > 0$$

$$[R^-] > [RH]$$

Most of the drug molecules are non-protonated.

For weak acids, most of the drug molecules are charged and <u>cannot</u> be absorbed.

For weak bases, most of the drug molecules are uncharged and <u>can</u> be absorbed.

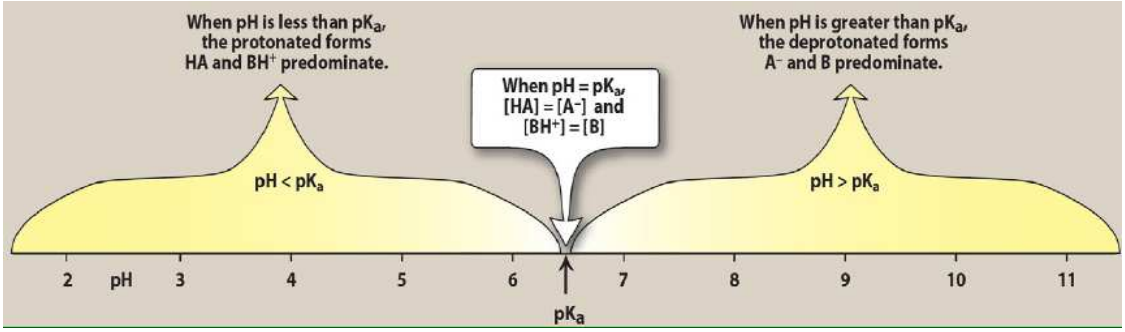
Similarly, for pH < pK_a,

Most of the drug molecules are protonated.

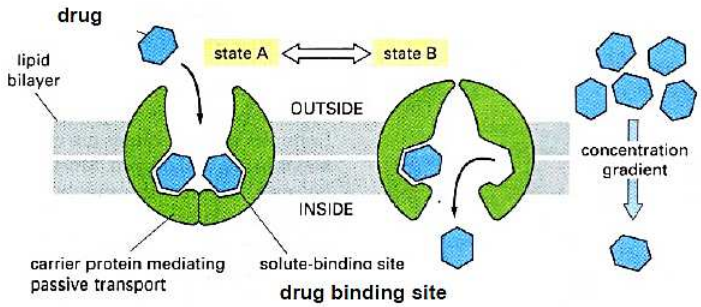
For weak acids, most of the drug molecules are uncharged and <u>can</u> be absorbed.

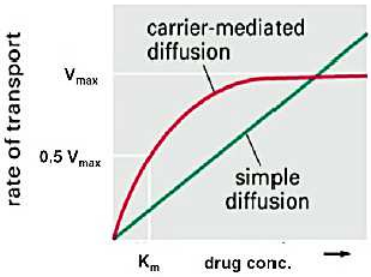
For weak bases, most of the drug molecules are charged and <u>cannot</u> be absorbed.

Thus,



2. Carrier-mediated Drug Transport



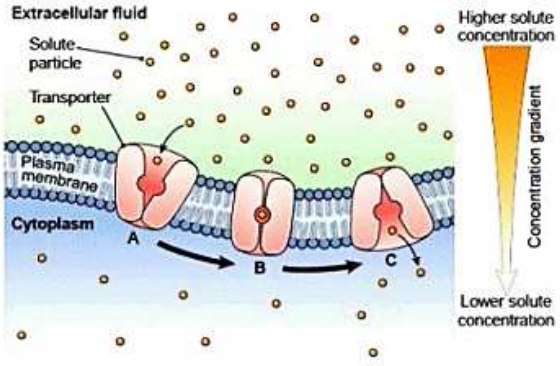


$$\frac{dD}{dt} = \frac{[D] \times V_{max}}{[D] + K_m}$$

- Carrier proteins bind one or more drug molecules on one side of the membrane and then undergo a conformational change that transfer the drug molecules to the other side of the membrane
- Governed by **saturation kinetics** (i.e. $\frac{d[D]}{dt} = \frac{[D]V_{max}}{[D]+K_m}$)

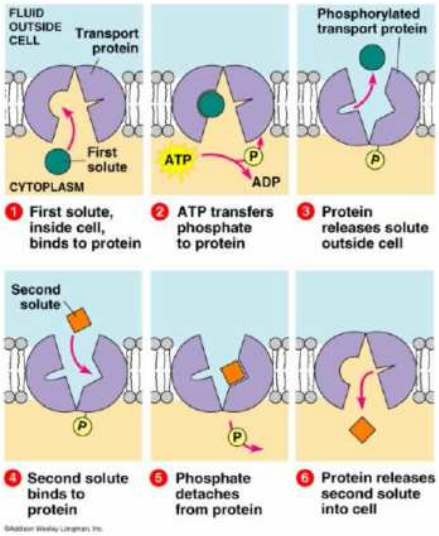
a. Facilitated Diffusion

- Passive process (does not require energy)
- Downhill transport (down concentration gradient)
- Follows saturation kinetics
- High chemical specificity
- Example: vitamin B_{12}

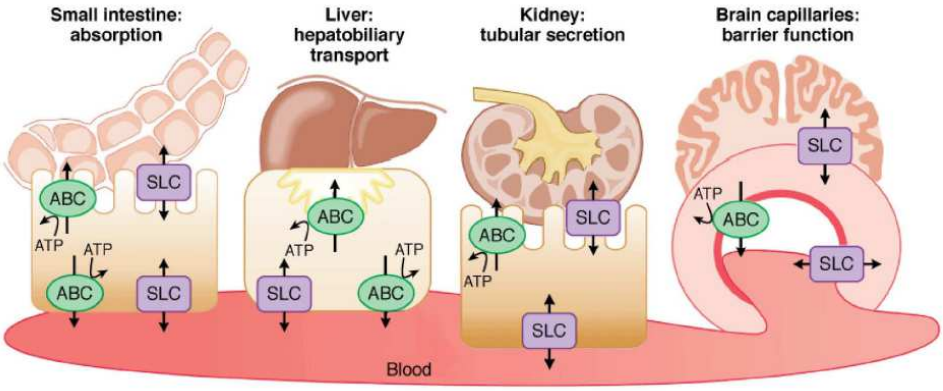


b. Active Transport

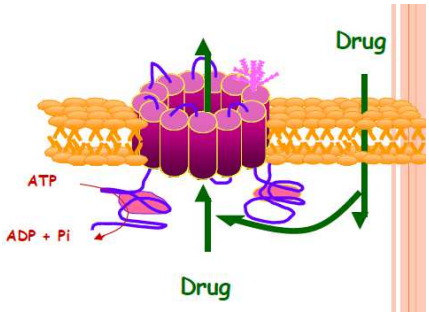
- Requires energy (eg. ATP)
- Against concentration gradient
- Follows saturation kinetics
- High chemical specificity
- Examples: levodopa, fluorouracil, penicillin



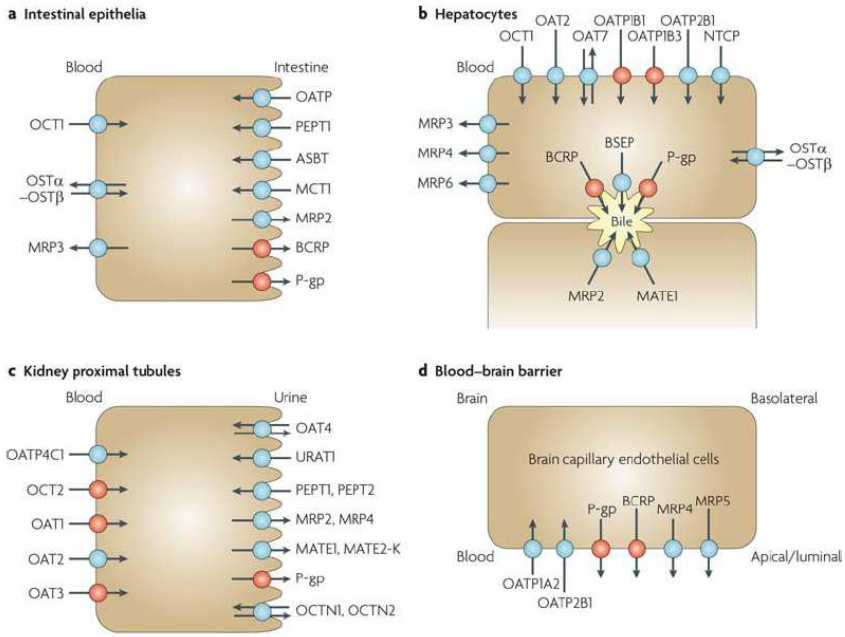
c. Drug Transporters



- Two major superfamilies:
  - **ATP-binding cassette transporter (ABC)**
    - → Energy-dependent efflux transporter
    - → 7 families, including: **P-glycoprotein** (MDR1 (ABCB1), MDR3 (ABCB4)), **multi-drug resistance protein** (MRP2 (ABCC2)) and **breast cancer resistance protein** (BCRP (ABCG2))
  - **Soluble carrier transporter (SLC)**
    - → Facilitated bilateral transporter
    - → 43 families

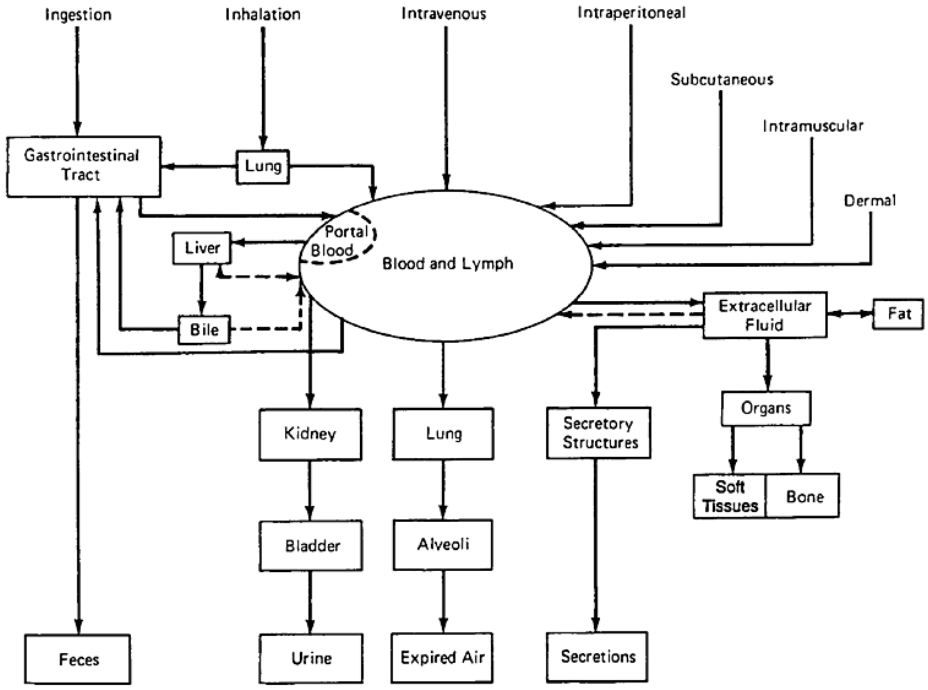


- Organ-specific expression of drug transporters:



Transporter	Substrates
Amino acid transporters	Baclofen, L-dopa, gabapentin, methyldopa
Peptide transporters (hPEPT1, HPT1)	β-lactam antibiotics, ACE inhibitors, cephalexin, cyclosporin
Nucleoside transporters (CNT1, CNT2)	Zidovudine, zalcitabine, dipyridamole
Organ anion transporters (OATP1, OATP3, OATP8)	Ceftriaxone, benzoic acid, methotrexate, pravastatin
Organic cation transporters (OCT1, OCT2)	Thiamine, desipramine, quinidine, midazolam, verapamil
Bile acid transporters (IBAT/ISBT)	Chlorambucil, thyroxine

C. Drug Disposition

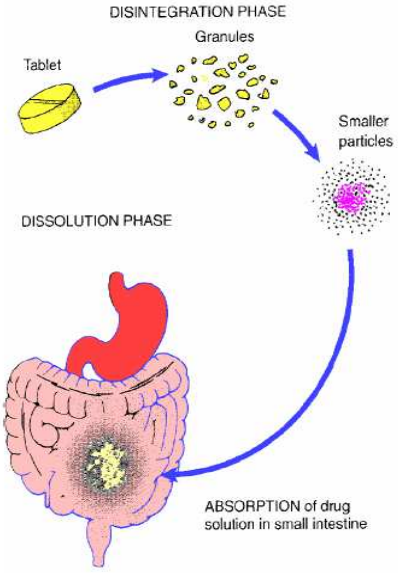


- Rate at which a drug reaches its site of action depends on:
  - **Absorption**: passage of drug from its site of administration into the blood
  - **Distribution**: delivery of the drug to the tissues

1. Drug Absorption

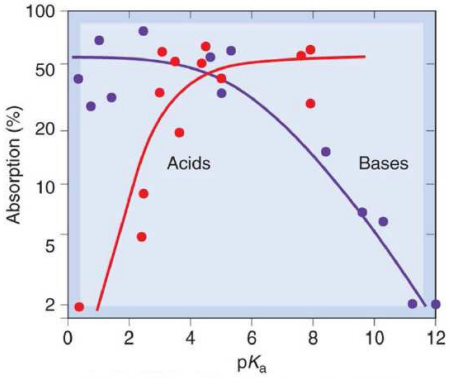
- Factors modifying absorption:
  - Drug solubility
  - Drug dissolution
  - Drug concentration
  - pH (for ionizable drugs)
  - Circulation to site of absorption
  - Absorbing surface
  - Route of administration

a. Peroral Administration (P.O. route)

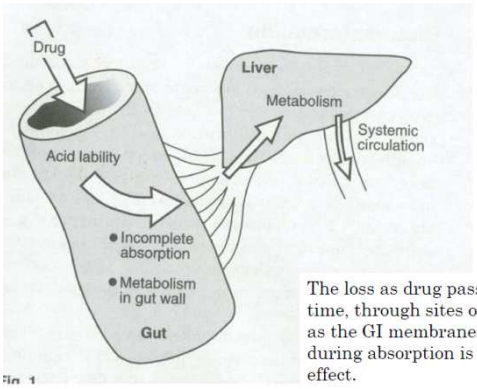


- Involves swallowing of the drug
- Also known as 'oral administration'
- Most convenient and economic method of systemic drug delivery
- Dosage forms: eg. tablets, capsules, syrups, etc.
- Drug release depends on **formulation**, eg.
  - Particle size
  - Surface area
  - **Excipients**: inert substances added to drug
- Process of absorption:
  - Disintegration (as a solid)
  - Dissolution (into solution)
  - Absorption into systemic circulation
- Drug has to be not too lipophilic (prevents dissolution) nor too hydrophilic (prevents absorption)
- **Bioavailability**: fraction of administered dose of a drug that is actually available in systemic circulation for distribution to target organ
  - Usually estimated via fraction of dose entering bloodstream (∵ measurability)

- Physiological considerations of GI system:
  - Surface area:
    - → Small intestines: 200 m^2
    - → Stomach: 1 m^2
  - Permeability:
    - → Intestinal membrane > stomach
  - Blood flow:
    - → Small intestine: 1000 mL/min (through intestinal capillaries)
    - → Stomach: 150 mL/min
  - pH:
    - → Stomach: pH 1.5-2 rising to pH 5-6 after a meal
    - → Duodenum: pH 5-7
    - → Jejunum and ileum: pH 6-7
  - GI transit:
    - → Stomach: about 0-3 hour (variable)
    - → Small intestine: ~3-5 hours
    - → Large intestine: 18 hours
    - → Rate of gastric emptying is a controlling step for rapid absorption

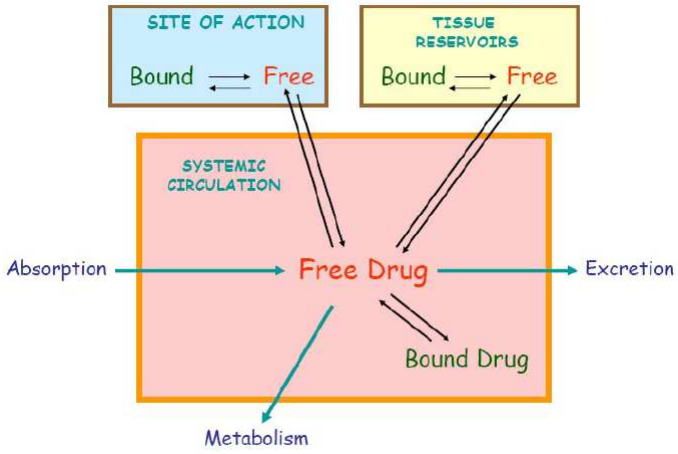


- Drug absorption in GI:
  - Takes place through whole GI tract
  - Predominantly absorbed in upper small intestine, some in stomach and colon
  - Non-polar lipophilic drugs absorbed rapidly by passive diffusion
  - Polar, hydrophilic drugs slower and may be incomplete
  - Drugs get absorbed into portal vein (except in oral cavity and rectum) → liver for **first pass metabolism**
  - **Presystemic metabolism** also occur in small intestine
- **Bioavailability** is incomplete because:
  - **First pass effect**: some drugs are lost through sites of elimination during absorption
  - Uptake and efflux transporter in small intestine play a significant role in determining oral drug bioavailability

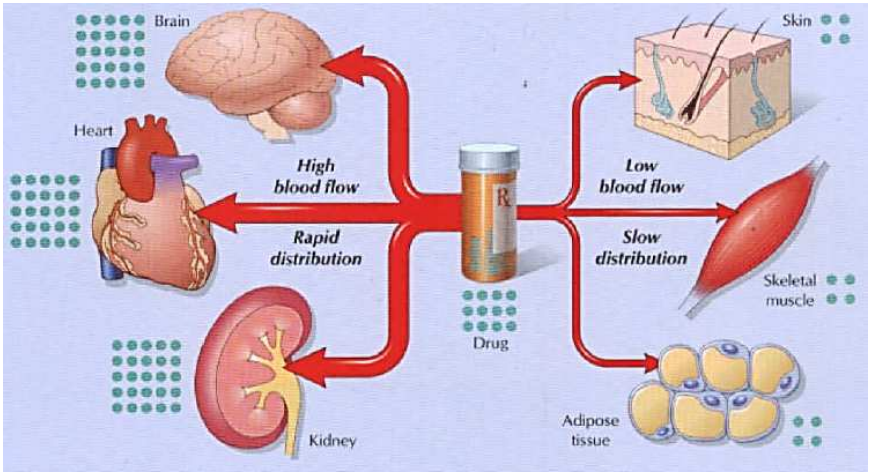


The loss as drug passes, for the first time, through sites of elimination, such as the GI membranes and the liver, during absorption is termed as first-pass effect.

2. Drug Distribution



- **Drug distribution**: transfer of drugs from blood to extravascular fluids
- Drugs in plasma (~5% body weight) exists in a distribution equilibrium with drug in blood cells (~3%), in other bodily fluids (~15%) and in tissues (~40%)
- Changes of plasma drug concentration indicative of changes in drug level in other tissues including sites of pharmacologic effect
- Factors affecting <u>rate</u> of drug distribution:

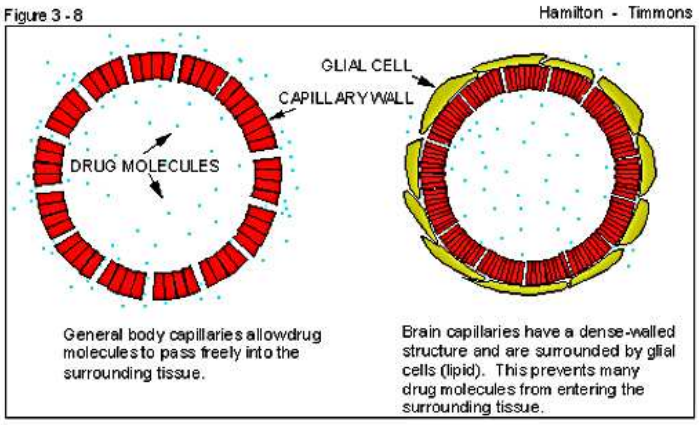


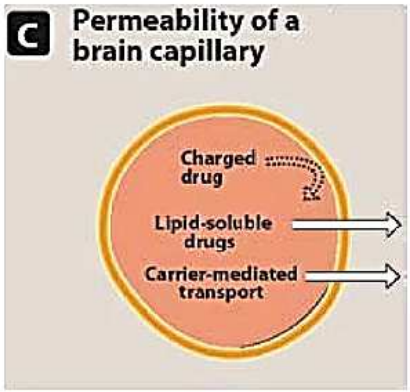
  - □ Blood perfusion into tissues: rate of delivery to tissues determined by amount of blood flow to tissue

Well-perfused	**Poorly perfused**
Kidney (360 mL/min/100g)	Muscle (4 mL/min/100g)
Liver (95 mL/min/100g)	Skin (3 mL/min/100g)
Heart (70 mL/min/100g)	Fat (1 mL/min/100g)
Brain (50 mL/min/100g)	

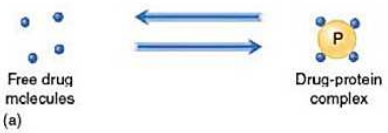
  - □ Drug permeability across cell membrane
    - → Passive diffusion
    - → Carrier-mediated transport

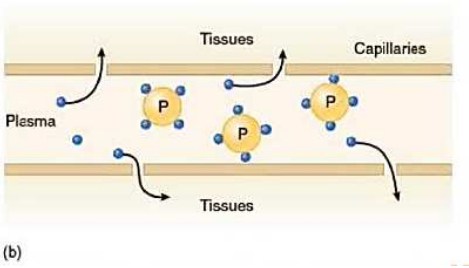
- Factors affecting <u>extent</u> of distribution:
  - Organ size, eg. large amounts of drugs are distributed to skeletal muscles
  - Capillary permeability
    - → Most free drugs can pass between endothelial cells
    - → **Blood-brain barrier (BBB)** mostly impermeable to water-soluble drugs





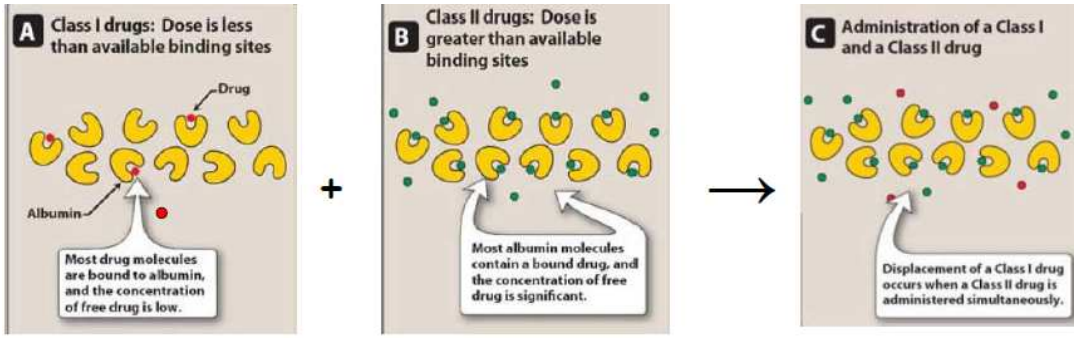
  - Drug lipophilicity
    - → Affect ability for drug to cross lipid membrane barriers
    - → Very highly lipid soluble drugs subsequently slowly redistribute into body fat
  - Protein/tissue binding capacity
    - → Drugs may bind to plasma proteins or other proteins
    - → Protein-bound drugs cannot leave capillaries to exert systemic effects
    - → Example: warfarin is strongly bound to plasma albumin
    - → Example: chloroquine is strongly bound to tissue protein





a. Plasma Proteins Binding to Drugs

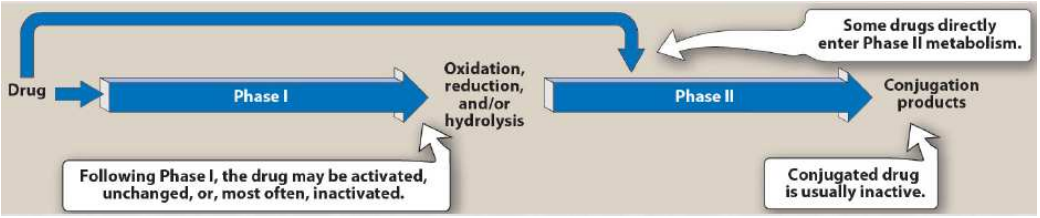
- Drugs can bind reversibly to two primary plasma proteins:
  - Albumin (conc. = 4.6 g/100mL)
  - α_1-acid glycoprotein (conc. = 0.04-0.1g/100mL)
- Many acidic drugs bind principally to albumin
- Basic drugs frequently bind to other plasma proteins (eg. α_1-acid glycoprotein (AGP) and lipoproteins)
- Drugs bound to plasma proteins
  - Cannot reach target site and other body tissues
  - Not filtered by glomerulus nor biotransformed by metabolizing enzymes
  - Pharmacologically inactive and act as a <u>drug reservoir</u>
  - Binding sites can be saturable
  - Can be displaced by other drugs
- **Drug displacement**: use of one drug to displace another from binding with plasma proteins
  - Drugs with high affinity for albumin divided into two classes:
    - → Class I drugs: clinical dose < binding capacity (eg. warfarin)
    - → Class II drugs: clinical dose >> binding capacity (eg. aspirin)
  - Class I drugs can be displaced by class II drugs → rapid ↑ in its free form in plasma

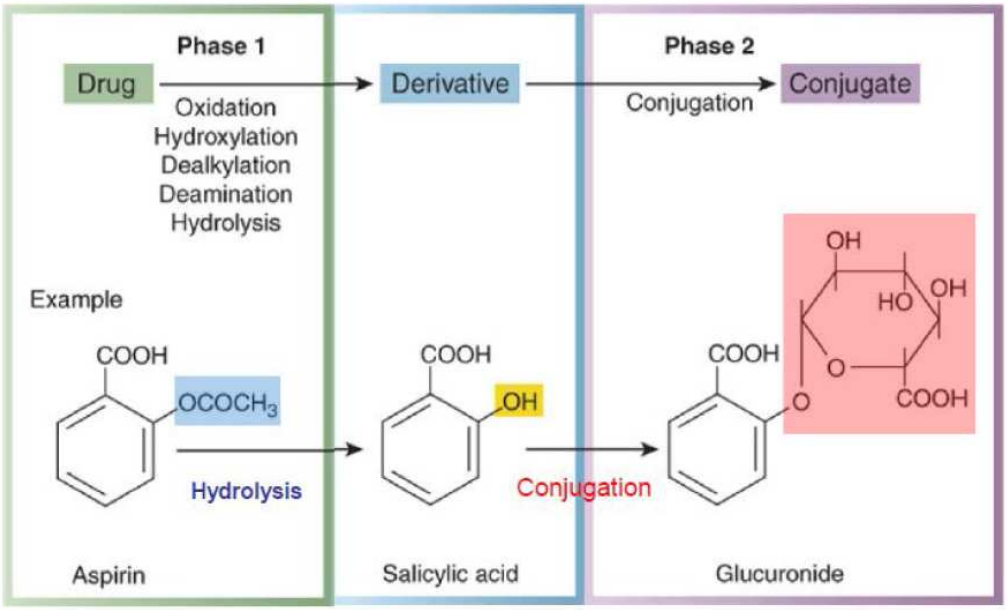


L91 Drug Metabolism and Excretion

A. Drug Metabolism

- **Drug metabolism**: biotransformation of drugs
  - Catalyzed mainly by enzymes in liver
  - Can also occur in blood or other tissues (eg. lungs, kidney, gut wall)
- Metabolites usually less active than parent compound
- Metabolites usually more polar and readily excreted





- Two phases:
  - **Phase I reactions**: oxidation, reduction and/or hydrolysis
    → Eg. dealkylation, hydroxylation, oxidation, deamination
  - **Phase II reactions**: conjugation reactions
    → Eg. glucuronidation, sulphation, acetylation, GSH-conjugation

1. Phases of Drug Metabolism

a. Phase I Reactions

i. Oxidative Reactions

- **N- and O- dealkylation**:
  - $RNHCH_3 + [O] \rightarrow RNHCH_2OH \rightarrow RNH_2 + HCHO$
  - $ROCH_3 + [O] \rightarrow ROCH_2OH \rightarrow ROH + HCHO$
  - Example: caffeine, codeine
- **Aliphatic and aromatic hydroxylation**:
  - $RCH_2CH_3 + [O] \rightarrow RCHOHCH_3$
  - Example: barbiturates
- **N-oxidation**:
  - $R_3{\equiv}N + [O] \rightarrow R_3{=}N^+OH \rightarrow R_3{=}N{\rightarrow}O$
  - Example: nicotine
- **Sulphoxide formation**:
  - $R\text{-}S\text{-}R' + [O] \rightarrow R\text{-}S^+OH\text{-}R' \rightarrow R\text{-}S(=O)\text{-}R'$
  - Example: cimetidine
- **Deamination of amines**:
  - $R_2{=}CHNH_2 + [O] \rightarrow R_2{=}C(OH)NH_2 \rightarrow R_2{=}C{=}O$
  - Example: amphetamine

ii. Hydrolysis

- **Ester hydrolysis**:
  - $RCOOR' \rightarrow RCOOH + R'OH$
  - Example: aspirin
- **Amide hydrolysis**:
  - $RCONR' \rightarrow RCOOH + R'NH_2$
  - Example: lignocaine

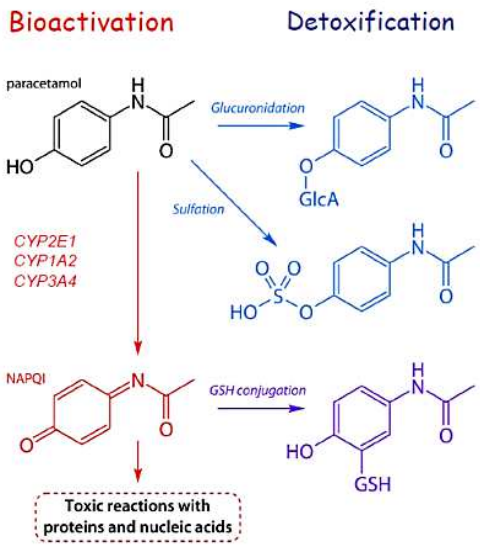
iii. Reduction

- **Azo reduction**:
  - $R\text{-}N{=}N\text{-}R' \rightarrow RNH_2 + R'NH_2$
  - Example: azo dyes
- **Nitro reduction**:
  - $RNO_2 \rightarrow RNH_2$
  - Example: chloramphenicol

b. Phase II Reactions

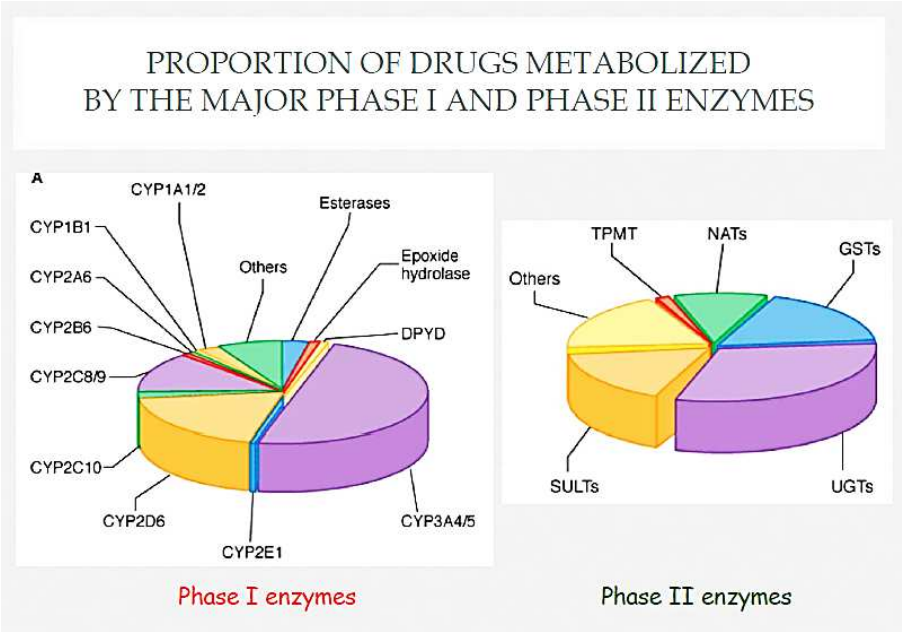
- **Glucuronidation**:
  - R + UDP-glucuronic acid → R-glucuronic acid + UDP
  - Example: paracetamol
- **Acetylation**:
  - $RNH_2 + CH_3\ CO{\sim}SCoA \rightarrow RNHCOCH_3 + HSCoA$
  - Example: isoniazid
- **Sulphation**:
  - $ROH + PAPS \rightarrow ROSO_3^- + PAP$
  - **PAPS**: 3'-phosphoadenosine 5'phosphosulphate
  - Example: paracetamol
- **O-, S- and N- methylation**:
  - R-XH + S-adenosyl methionine → $R\text{-}X\text{-}CH_3$ + S-adenosyl cysteine
    - → X: O, S or N
    - → Note that the difference between methionine and cysteine is an extra methyl group attached to the S atom (of the side chain)
- **Glutathione conjugation**:
  - R + GSH → GSR
  - Example: ethacrynic acid
- **Glycine conjugation**:
  - $RCOOH + NH_2CH_2COOH \rightarrow RCONH_2CH_2COOH$
  - Example: salicylic acid

2. Drug Bioactivation

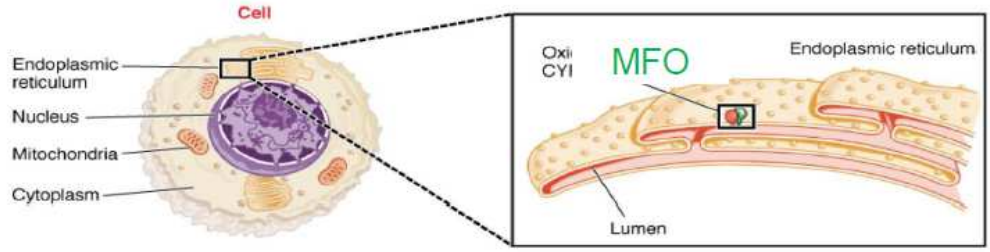


- Some drugs are not active in taken form
- **Drug bioactivation** can occur by:
  - Conversion of an inactive **prodrug** to a pharmacologically active metabolite
    → Example: cyclophosphamide (anticancer drug)
  - Conversion of a non-toxic drug to a toxic metabolite
    → Example: paracetamol overdose may cause liver damage

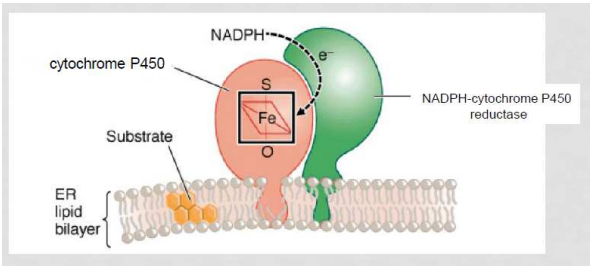
3. Major Enzymes for Drug Metabolism

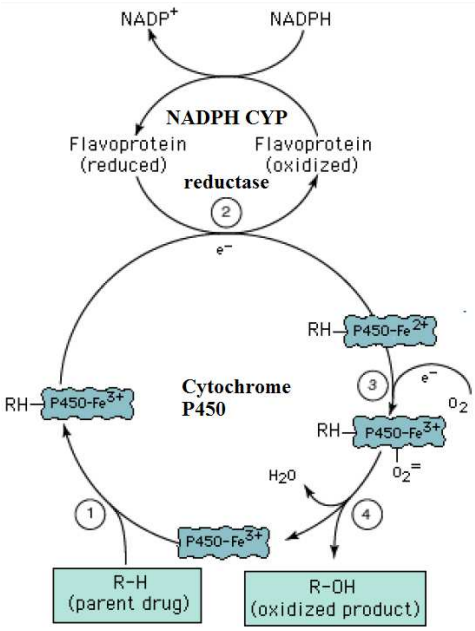


a. Mixed Function Oxidase System (MFO)

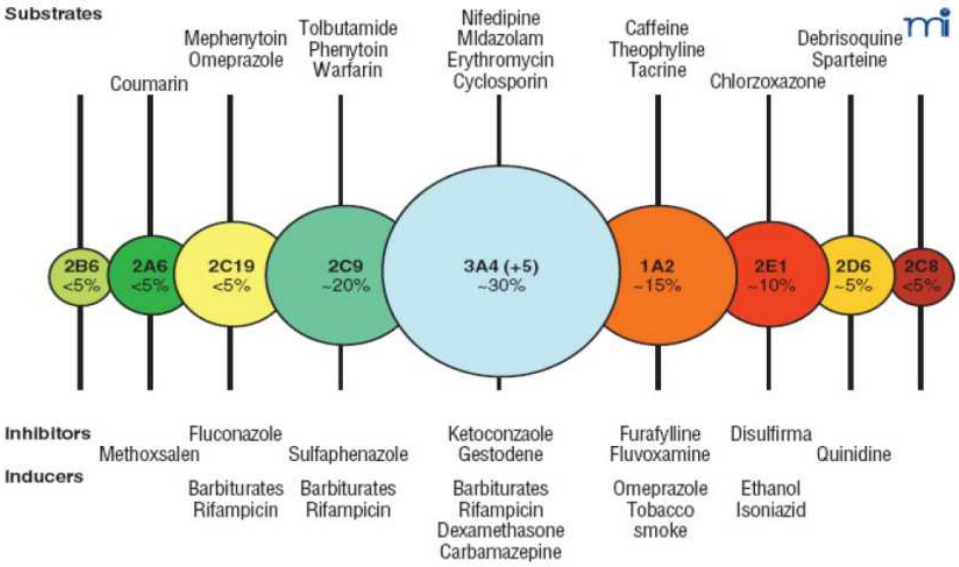


- **Mixed function oxidase system (MFO)**: an electron transport system located in the lipophilic membranes of endoplasmic reticulum of liver and other tissues
  - Also called **monooxygenase**
- Function: catalyze drug oxidations and reductions
- Typical reaction:
  $NADPH + H^+ + O_2 + RH\ (drug) \rightarrow ROH + H_2O + NADP^+$

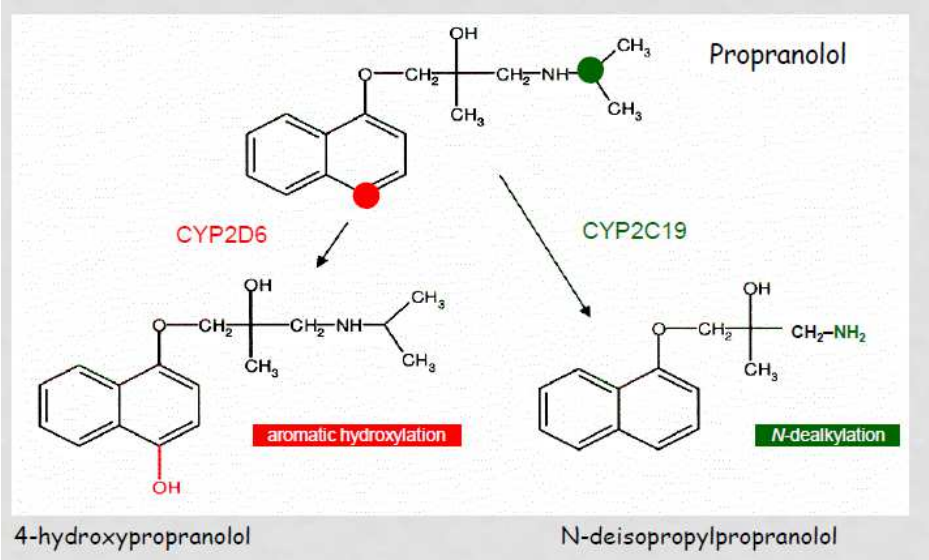




- System comprises of:
  - NADPH-cytochrome P450 reductase
  - Cytochrome P450 (CYP) (exists in isoforms with varied substrate specificity)
- Contains **heme** group for oxygen and drug substrate binding
- Human liver P450 enzymes:
  - Major isoforms: CYPs 1A2, 2C9, 2C19, 2D6, 2E1 and 3A4/5
  - CYP3A4 responsible for metabolizing >50% of clinically prescribed drugs metabolized in liver
    → Grapefruit juice inhibits CYP3A4 → interferes with drug metabolism



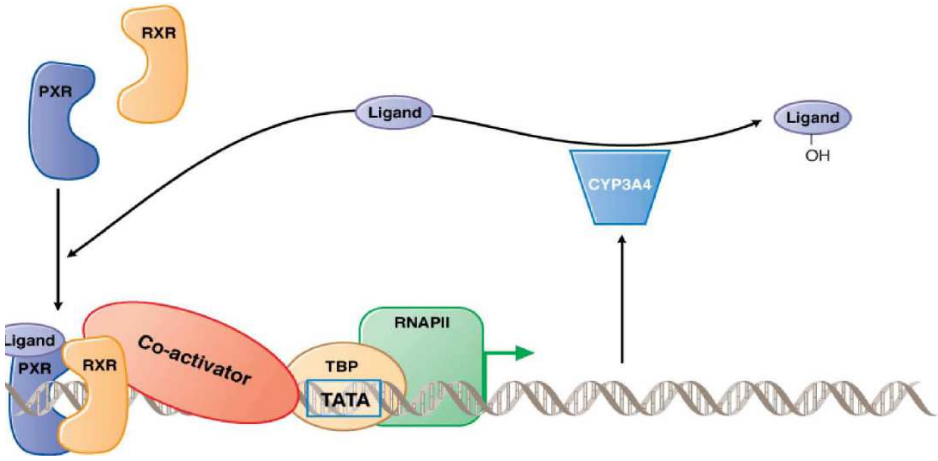
- Example: oxidative metabolism of propranolol



b. Modification of Enzymes for Drug Metabolism

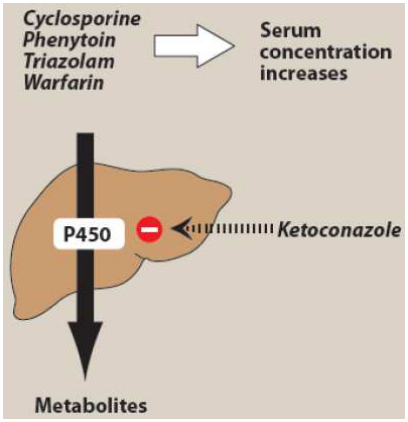
i. Induction of Enzymes for Drug Metabolism

- Repeated or chronic exposure of **inducers** of **enzymes for drug metabolism** can cause:
  - ↑ content of drug metabolizing enzymes (eg. cytochrome P450)
  - ↑ rate of drug metabolism activities (i.e. shorter duration of action)
- Dosing rates need to be altered
- Examples of enzyme inducers:
  - Coal tar (CYP1A2)
  - Phenobarbitone (CYP2C9)
  - Alcohol (CYP2E1)
  - Corticosteroids (CYP3A4)



- Nuclear receptors that induce drug metabolism:
  - A drug can bind to a nuclear receptor such as **pregnane X receptor (PXR)** when enter cell
  - PXR then forms complex with **retinoid X receptor (RXR)**
  - PXR-RXR complex binds to DNA upstream of target genes and recruits **coactivator**
  - Coactivator binds to **TATA box-binding protein (TBP)** to activate transcription by **RNA polymerase II (RNAP II)**
  - CYP3A4 is a PXR target gene

ii. Inhibition of Enzymes for Drug Metabolism



- Competition by two or more drugs for the same system for metabolism may occur
  - Eg. ketoconazole, diltiazem, erythromycin are potent CYP3A4 inhibitors
- Enzyme inhibition may lead to slower rates of metabolism → ↑ plasma levels with time → prolonged pharmacological drug effect and enhanced drug-induced toxicities

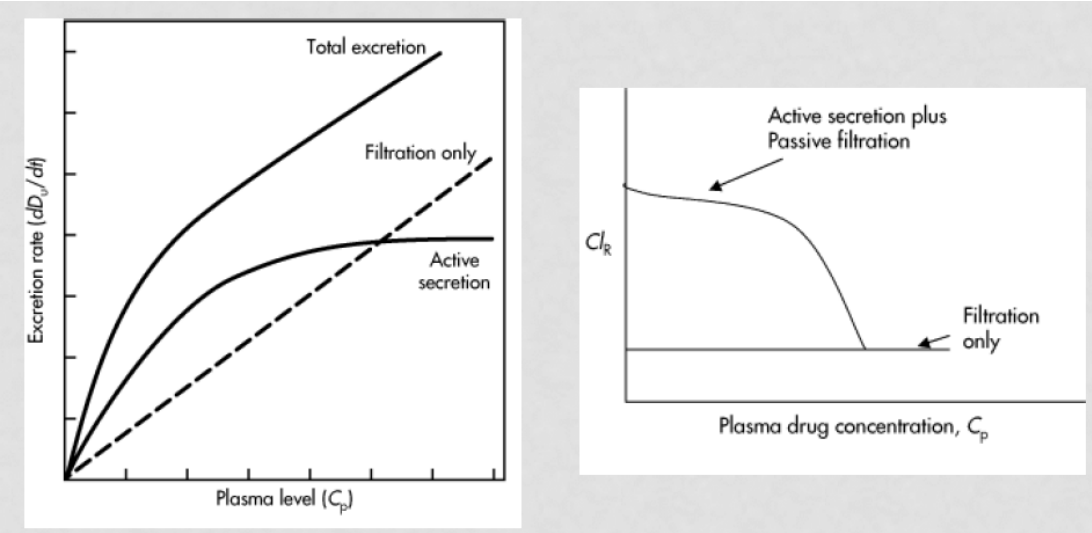
4. Factors Affecting Drug Metabolism

- Age: metabolic rate may be different in children
- Gender: hormone-related effect on drug metabolism
- Genetics
- Food and diet
- Environment
- Hormonal and disease states
- Drug interactions

B. Drug Excretion

- **Drug excretion**: the mechanism by which a certain drug is removed from the body
- Paths of drug excretion:
  - Renal excretion
    - → **Glomerular filtration**
    - → **Tubular secretion**
    - → **Tubular reabsorption**
  - Biliary excretion
  - Other excretion

1. Renal Excretion

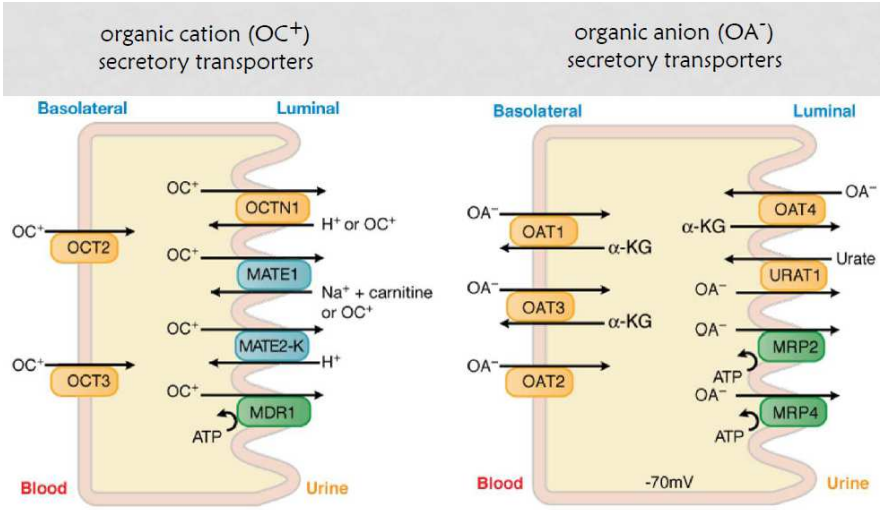


- Main route of elimination for many drugs
- Applicable for drugs that are non-volatile, water soluble, low molecular weight
- Drug excreted via kidneys by any combination of **glomerular filtration**, **tubular secretion** and/or **tubular reabsorption**
- **Renal clearance (CL_R)**: rate at which a drug is cleared from blood via kidneys
  - $CL_R = \frac{\text{filtration rate} + \text{secretion rate} - \text{reabsorption rate}}{C_p}$

a. Glomerular Filtration

- **Glomerular filtration**: a unidirectional process that occurs for most small drug molecules, but not protein-bound drugs
- Driving force: hydrostatic pressure within glomerular capillaries
- Directly related to free drug concentration in plasma

b. Tubular Secretion

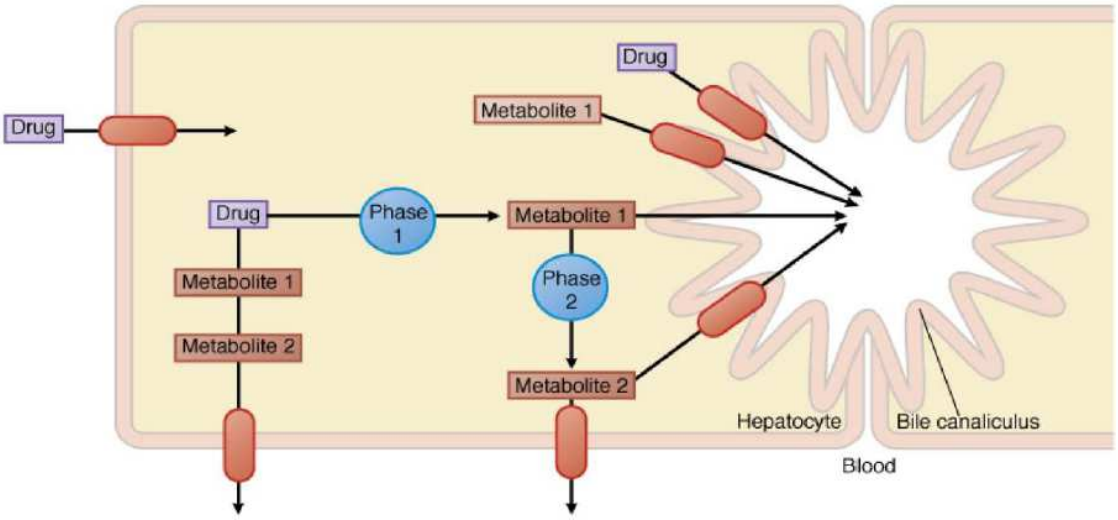


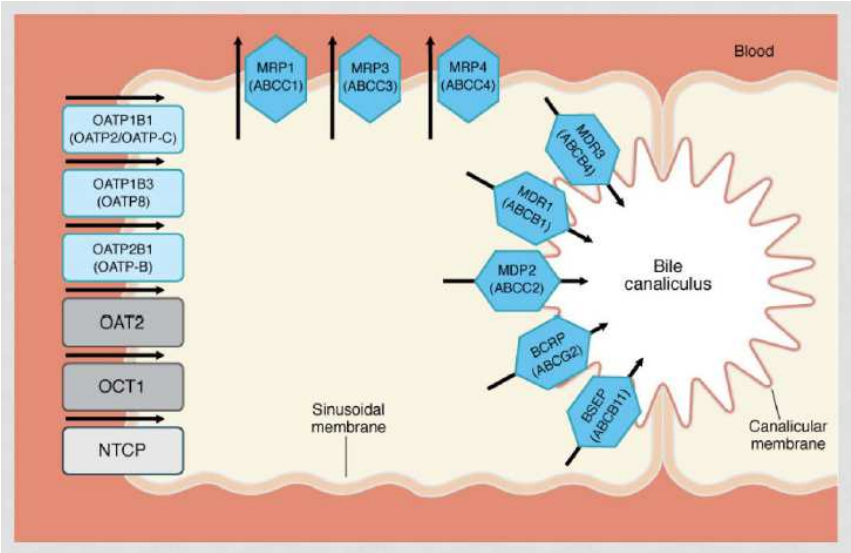
- Two active transport processes:
  - Weak acids: anions actively transported from plasma to tubular lumen
  - Weak bases: cations actively transported
- Secretion rate dependent on renal plasma flow
- Protein-bound drugs can be secreted (given that there is a specific carrier)

c. Tubular Reabsorption

- Occurs after the drug is filtered through glomerulus
- Process can be active or passive
- Reabsorption of acid/weak base drugs influenced by urine pH and pK_a of drug
  - Drug excretion can be facilitated by drugs changing urine pH
- Unionized drug easily reabsorbed from renal tubule back into body
- Rate of urine flow influences amount of filtered drug reabsorbed

2. Biliary Excretion of Drugs

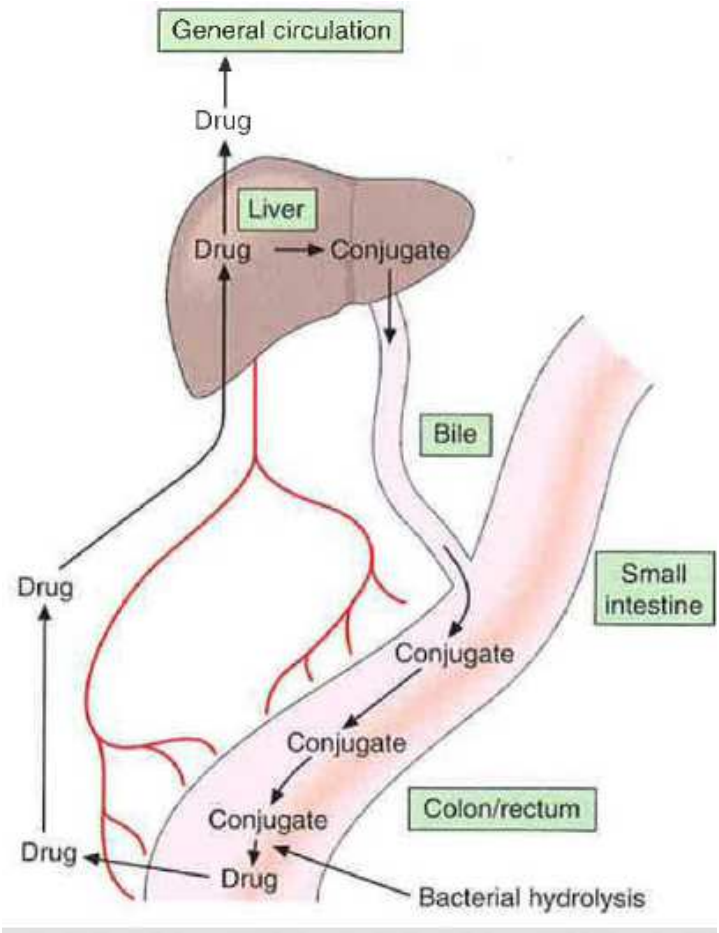




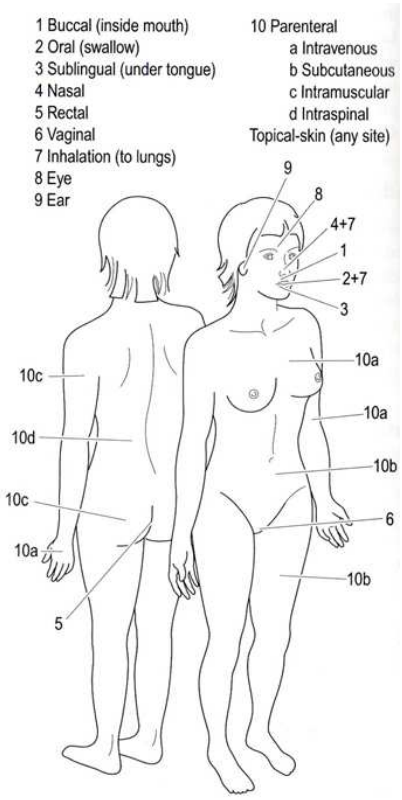
- Some drugs and their metabolites are actively transported by liver into bile
- Molecular weight 300-500 appears optimal for biliary excretion (esp. glucuronide conjugate)
- Membrane transporter work in concert with phase 1 and phase 2 drug metabolizing enzymes in hepatocyte to mediate uptake and efflux of drugs and metabolites

a. Enterohepatic Circulation of Drugs

- Bile conjugates are released into gut and hydrolyzed by β-glucuronidase enzymes in gut microflora to regenerate parent drugs
- Lipophilic parent drugs reabsorbed back to systemic circulation
- May delay elimination and prolong effect of drug

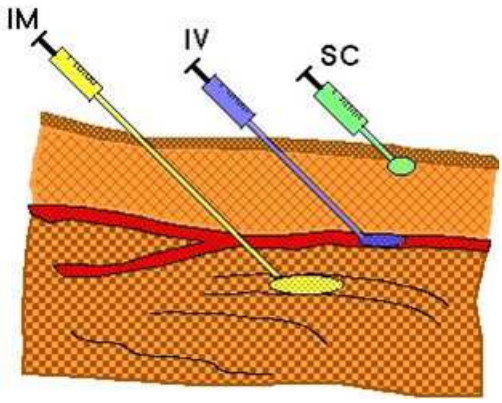


L93 Drug Administration and Dosage Forms



A. Injections

- **Parenteral route**: desired effect is systemic but is administered outside GI (para-enteral)
- Administration routes:
  - **Intravenous route (IV)**: drugs injected directly into systemic circulation via a vein
  - **Subcutaneous route (SC)**: drugs injected into subcutaneous layer of skin
  - **Intramuscular route (IM)**: drugs injected into muscle layers



- Dosage forms for injection
  - Solution
  - Suspension (solid in liquid)
  - Emulsion (oil in water/water in oil)
    - → Eg. total parenteral nutrition: IV injection of all nutrients needed for patients that have faulty GI
  - Powder-reconstitution (for unstable drugs that may be hydrolyzed by water)

- Injection dosage forms must be sterile

1. Intravenous Route

- **IV injection**: a small volume of fluid given intravenously and rapidly at one time
- **IV infusion**: a large volume of fluid given intravenously at a slow rate
  - Importance: ensure that drug enters general circulation at a constant rate
  - Fluid moves through catheter by gravity
  - Rate affected by physical characteristics (eg. viscosity) of fluid, fluid pressure, height of infusion above the patient
- Advantages:
  - Quick response
  - 100% bioavailability
  - Some drugs have no specific transporter for GI absorption (eg. cisplatin)
  - Can be delivered to unconscious patients
- Disadvantages:
  - Trained personnel required to give IV injections (∵ may be difficult to find a suitable vein)
  - Cannot be used to administer **water in oil emulsion** (i.e. oil >> water) or suspension (∵ may block blood vessels)
- Procedures:
  - Fluid commonly administered into superficial vein on back of hand or in internal flexure of elbow
  - Subclavian vein preferred if a large volume is administered (i.e. **central venous catheter**)
    - → ∵ Slow blood flow in arm veins → drug accumulate in arms → inflammation
    - → Risk: bleeding and nerve damage
    - → **Peripheral inserted central catheter (PICC)**: ultrasound-guided catheter inserted at arm into subclavian vein

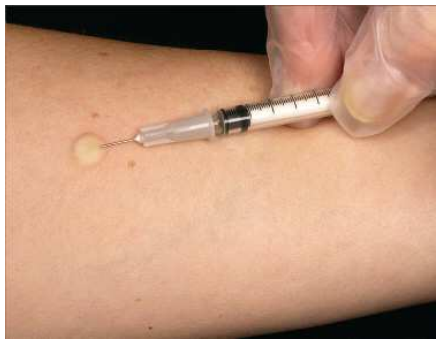
2. Subcutaneous Route

- **Subcutaneous injection**: aqueous solutions, solutions in oil and suspensions injected into loose connective and adipose tissues immediately under skin
- Absorption slower than IV injection
- Delay in systemic effect due to time required to pass through epithelial cells and basement membrane of capillaries
- Advantages:
  - Can be given by patients (eg. insulin)
  - Absorption is slow but usually complete
- Disadvantages:
  - Can be painful (∵ many nerve endings in subcutaneous layer)
  - May cause **lipohypertrophy** (lump of excess fat, possibly due to drug effects eg. insulin stimulates proliferation of fat cells) or **lipoatrophy** (loss of fat in an area, possibly due to immune response)
  - Typical volume of injection does not exceed 1mL
- Common injection sites include abdomen, upper back, upper arms and lateral upper hips
- Factors affecting drug distribution:
  - Site of injection → amount of subcutaneous fat
  - Body temperature → blood flow
  - Age (↑ with subcutaneous fat)
  - Degree of massaging of injection site

3. Intramuscular Injection

- **Intramuscular injection**: aqueous solutions, solutions in oil and suspensions administered directly into body of relaxed muscle
- Advantages:
  - Larger volume than subcutaneous injection (maximum: 2mL)
  - Faster onset of action than subcutaneous injection (∵ less fat at site of injection)
- Disadvantages:
  - Trained personnel required for injections
  - Site of injection influence absorption
- Common injection sites: **gluteal muscle** in buttock, **deltoid muscle** in shoulder, **vastus lateralis** of thigh
  - Adults: **gluteal muscles** often used ∵ larger volumes can be tolerated
  - Infants and small children: **vastus lateralis** used ∵ usually more developed than other muscle groups
  - **Deltoid muscle** used often for rapid absorption (∵ less fat)

4. Intradermal Injection



- **Intradermal injection**: a volume of about 0.1mL injected into skin between epidermis and dermis
- Slow absorption
- More often used for diagnostic test for allergy or immunity

5. Intra-arterial Injection

- **Intra-arterial injection**: injection directly into an artery
- Advantages:
  - Rapid dispersal ∵ fast blood flow
  - Can be used to target a specific organ or tissue served by the target artery
    - → Eg. liver cancer drug directly injected into hepatic artery when abdomen is opened up in surgery
- Disadvantages:
  - Risk of bleeding
  - Manipulative difficulties

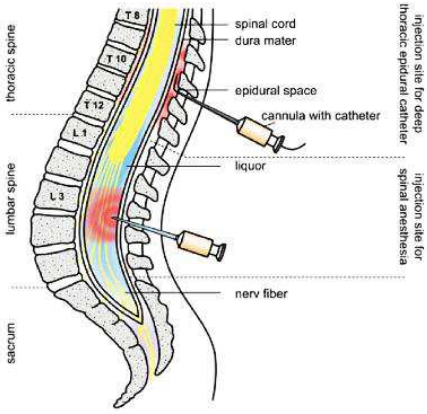
6. Intracardiac Injections

- **Intracardiac injection**: aqueous solutions administered directly into a ventricle or cardiac muscle
- Used in emergency
- Local effect desired only

7. Intraspinal Injections

- **Intraspinal injections**: aqueous solutions injected into particular areas of the spinal column
- Volume less than 20mL
- Types:
  - **Subarachnoid injection**: injection into space between **arachnoid meter** (middle membrane) and **pia meter** (inner membrane)
    - → Advantage: quick action (∵ close proximity)
    - → Disadvantage: dangerous (∵ risk of damaging spinal nerve)
  - **Epidural injection**: injection into space around **dura meter** (outer membrane

Figure 1 - Method of CSTEA.



8. Intra-articular Injections

- **Intra-articular injections**: injection s administered into synovial fluid in a joint cavity
- Can be solution or suspension
- Often used for local administration of anti-inflammatory agents

B. Implants

- **Implant**: a solid dosage form inserted under skin by a small surgical incision
- Most commonly used for hormone replacement therapy or as contraceptive
- Release of drug from implants slow → long-term therapy
- Implant must be sterile

C. Oral Route

- Desired effect can be:
  - Local: eg. antacids and laxatives
  - Systemic: drugs absorbed from GI then delivered to sites of action
- Advantages:
  - Simple: patient can administer drug to himself
  - Safe
  - Convenient: portable, no pain, easy to take
  - Cheap: no need to sterilize, automated machines can produce tablets in large quantities
- Disadvantages:
  - Onset of action relatively slow (several hours) compared to parenteral route
  - Absorption from GI may be irregular
  - Some drugs destroyed by enzymes in GI tract (eg. antibiotics)
  - First pass effect: liver metabolism → loss of effect → need a higher dose
  - Drug solubility may be altered by presence of other substances in GI tract
    → Eg. Calcium in antacids can chelate tetracycline → ↓ absorption
  - Food and GI motility can affect drug absorption
    → Eg. penicillins absorption is slower with food
  - Not suitable for unconscious or vomiting patients
  - Not suitable for immediate pre- or post-operative use (∵ anaesthetics induce vomiting)

- Dosage forms available:
  - Solution
  - Suspension
  - Emulsion
  - Tablets
  - Capsules
    - → Hard gelatin cell capsule for holding powder
    - → Soft gelatin cell capsule for holding oil
  - Granules/powders
- Advantage of liquid oral dosage forms:
  - Much easier to swallow (useful for children, elderly and patients with difficulty in swallowing)
  - Medicament readily absorbed from GI tract (∵ no need for dissolution)
- Advantages of solid oral dosage forms
  - Less bulky and convenient to carry around
  - More microbiologically and chemically stable (∵ without water)
  - Dosage more accurate
  - Drugs with unpleasant taste can be masked by coatings
  - **Enteric coated tablets** and **sustained-release tablets** available
- **Enteric coated tablets**: tablets lined by an acid-stable coating which breaks down in basic conditions (i.e. only dissolve at high pH)
  - Used for drugs that can be degraded by gastric juice or irritate gastric mucosa
  - Note: avoid indigestion remedies ∵ affect pH → premature breakdown of coating
- **Sustained-release tablets**: tablets surrounded by a polymer that retard release of drugs
  - Should be swallowed whole, but not chewed
  - Can be used to combine multiple doses into one single dose

D. Buccal Route

- **Buccal route**: drug tablet placed under tongue (i.e. in buccal cavity) for absorption via the mucous membrane
  - Presence of saliva facilitates dissolution of drugs
  - High vascular nature of tongue and buccal cavity facilitate drug absorption
- Desired effect can be:
  - Local: eg. lozenge for sore throat
  - Systemic: eg. nitroglycerine for angina ($\because$ first pass effect $\rightarrow$ cannot use oral route)
- Dosage forms:
  - Buccal tablets
  - Lozenge (hard)
  - Pastilles (soft)
- Advantages:
  - Rapid absorption: good blood supply to area of absorption
  - No first pass: liver is bypassed $\rightarrow$ no loss of drug
  - Drug stability: pH in mouth relatively neutral (cf stomach)
  - Can be administered to unconscious and vomiting patients
- Disadvantages:
  - Holding dose in mouth is inconvenient
    $\rightarrow$ Any part of dose swallowed $\rightarrow$ the same as oral dose $\rightarrow$ subject to first pass metabolism
  - Only small doses can be accommodated

E. Topical Route

- **Topical route**: administration of drug directly into body surface
  - Same term can also be used to describe all routes of administration for local effect (i.e. include local injections etc.)
- Commonly used for local effects
- Dosage forms:
  - Lotion: suspension
  - Liniment: alcoholic solution
  - Ointment: oily semisolid without powdery solid
  - Paste: oily semisolid with powdery solid
  - Cream: emulsion-type semisolid
  - Gel: aqueous semisolid
  - Paint
  - Collodian
  - Powder
  - Irrigation solution

Lotion

Liniment

Paint


Ointment


Cream

Collodian


Gel


Powder

- **Transdermal patch**: medicated adhesive patch placed on skin
  - Drug can pass through skin and produce a systemic effect
  - Deliver a <u>controlled</u> dose of drug over a specific time period
  - Advantages:
    - → Avoids first pass effect
    - → No drug deactivation by digestive juices
  - Disadvantages:
    - → Skin permeability is usually inadequate → mainly for potent drugs

F. Rectal Route

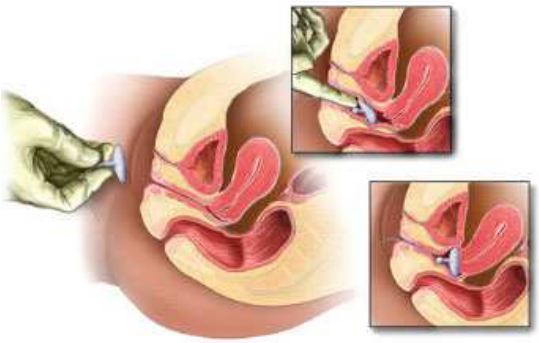
Suppository

Enema

- **Rectal route**: chosen preparation inserted into rectum
- Desired effect:
  - Local eg. drugs for haemorrhoids and constipation
  - Systemic eg. aspirin (for fever), diazepam (for epilepsy)
- Dosage forms available:
  - Suppository (solid)
  - Enema (fluid)
- Advantages:
  - Can be used when oral route is unsuitable (eg. severe vomiting, unconscious patients, uncooperative patients (eg. children, elderly or mentally disturbed) and patients with **dysphagia** (difficulty in swallowing))
  - Bypasses liver: most veins draining rectum lead directly to general circulation → ↓ first-pass effect
- Disadvantages:
  - Absorption can be irregular and unpredictable → variable effect
  - Less convenient than oral route

G. Vaginal Route

- Used for local effect only (eg. vaginal infection)
  - Blood supply to vagina not enough to produce systemic effect
- Dosage forms available: pessaries, tablets, capsules, solution, sprays and creams



Pessary

H. Inhalation Route

- **Inhalation route**: administration of drugs by inhalation through the nose or mouth
- Desired effects:
  - Local eg. bronchodilators (for asthma) and drugs for nasal congestion
  - Systemic eg. general anaesthesia
- Dosage forms available:
  - Inhalation: one or two drops put to hot water and the steam is inhaled
  - Aerosol
  - Powder

Inhalation

aerosol

Powder

- Advantages:
  - Local effect: drug dose required to produce desired effect much smaller than that for oral route → ↓ side effects
  - Systemic effect: extremely rapid absorption (∵ high blood flow and large surface area)
- Disadvantages:
  - Special techniques required
  - Solids and liquids excluded if larger than 20 micron (→ impact in mouth and throat)
- Use of aerosol:

1) Remove cap and shake canister thoroughly;
2) Hold canister upright and breathe out fully;
3) Place mouthpiece between lips;
4) Fire inhaler and inhale slowly and deeply <u>simultaneously</u>;
5) Hold breath for 10 seconds (or as long as comfortable);
6) Wait at least 1 min before taking 2nd dose.

I. Nasal Route

- Traditionally used for local effect (eg. decongestants for common cold, local steroids for allergic rhinitis)
- More recently been used for systemic action (eg. desmopressin for **diabetes insipidus** (disorder characterized by lack of ADH))
  - Good vascularity supply promote drug absorption
  - Avoids first pass metabolism
- Dosage forms available:
  - Nasal spray
  - Nasal drop

Nasal spray

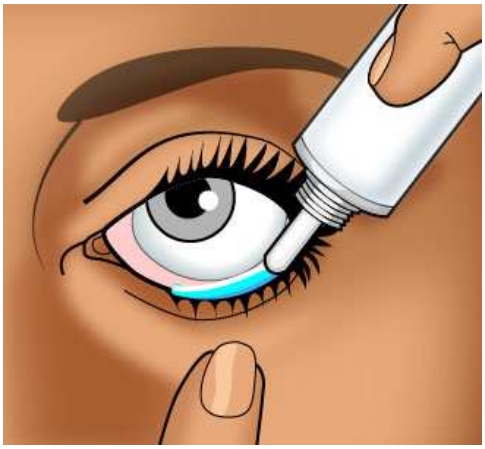
Nasal drop

J. Eye Products

- For local effect only (eg. eye infection or allergy)
- Dosage forms available:
  - **Eye drops**
  - **Eye ointment**
- All eye products should be sterile and discarded after 4 weeks of opening
- Instillation of eye drops:

1) Wash hands thoroughly;
2) While tilting head back, pull down lower lid of eye with index finger to form a pocket;
3) Hold the dropper (tip down) with the other hand as close to the eye as possible without touching it;
4) While looking up, gently squeeze the dropper so that a single drop falls into the pocket made by the lower eyelid;
5) Close the eye for 2-3 mins and tip the head down as though looking at the floor. Try not to blink or squeeze the eyelids;
6) Place a finger on tear duct and apply gentle pressure;
7) Wipe any excess liquid from face with a tissue;
8) Repeat steps 2-7 if more than one drop needs to be administered.

- ► Instillation of eye ointment:



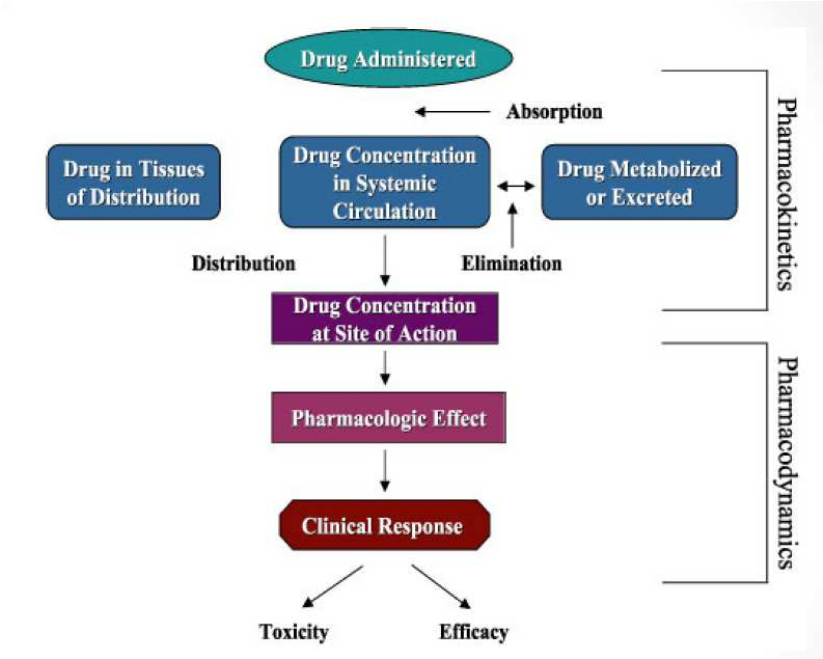
1) Wash hands;
2) Tip back head;
3) Gently pull down on cheek to create eyelid pouch;
4) Position tube over eye;
5) Look up;
6) Squeeze tube adding 1/4 to 1/2 inch of ointment without tube touching eye;
7) Slowly close eye gently for 1-2 mins.
   - □ Vision may be temporarily blurry
   - □ If also using eyedrops, use drops 10 mins before ointment (greasy semi-solid ointment may prevent absorption of eye drop.)

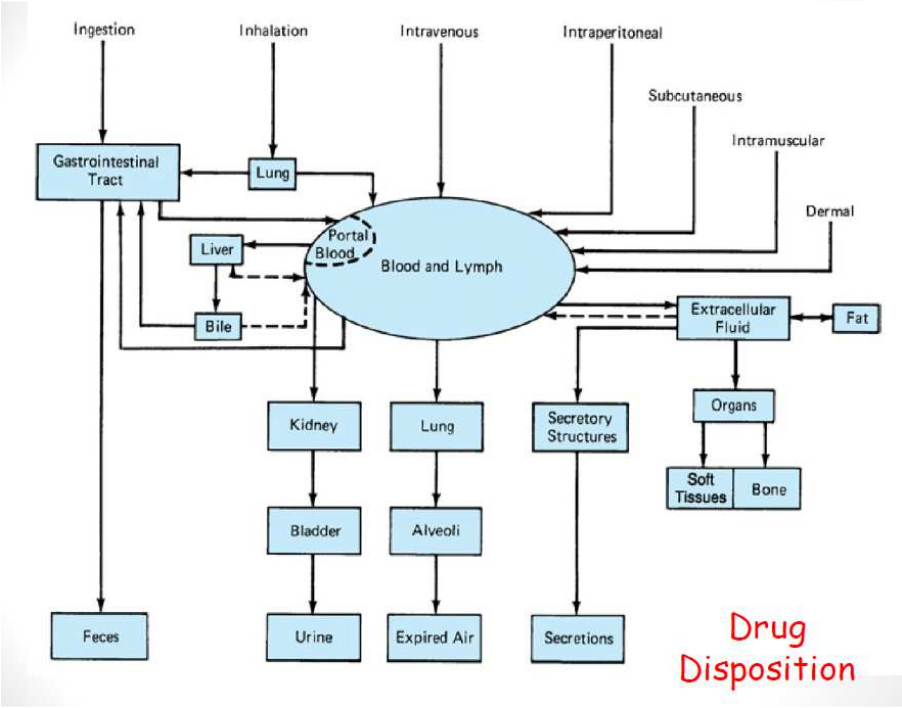
K. Ear Drops

- ► For local effect only (ear infections, earwax)
- ► Instillation of ear drops:

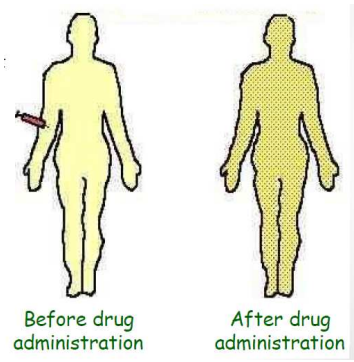
1) Either lie on your side or tilt your head over so that the ear, which needs the drops, is facing upwards;
2) For adults, the earlobe should be held up and back;
3) For children, hold the earlobe down and back, away from your neck;
4) Squeeze the correct number of drops into the ear. Keep your head tilted up for several minutes so that the drops can spread into the ear;
5) Straighten your head and wipe away any extra liquid with a clean tissue.

L93 Practical Pharmacokinetics

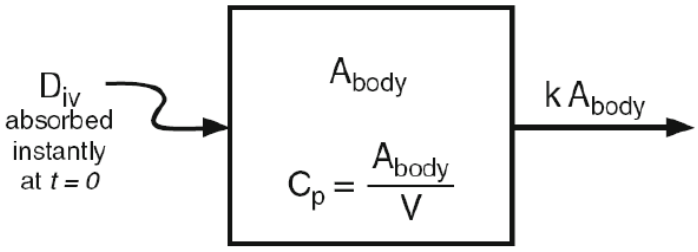




A. One Compartment IV Bolus Pharmacokinetic Model



- Runs with the assumption that the drug is evenly distributed throughout the body into a single compartment
- Only appropriate for drugs which rapidly and readily distribute between plasma and other body tissues
- Drug elimination follows **first order kinetics**



$$\frac{dA_{body}}{dt} = -kA_{body}$$

When $t = 0, A_{body} = D_{iv}$

Therefore, we have

$$A_{body} = D_{iv}e^{-kt}$$

and

$$C_p = \frac{A_{body}}{V} = \frac{D_{iv}}{V}e^{-kt} = C_0e^{-kt}$$

Where

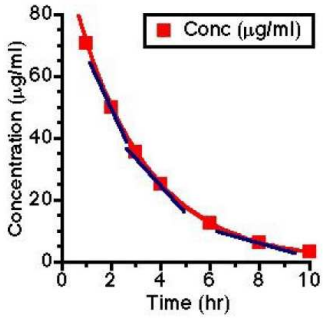
V: volume of distribution

A_{body}: amount of drug in body

D_{iv}: size of intravenous dose

C_p: drug concentration in plasma

C_0: initial drug concentration



- **Volume of distribution (V)**: apparent volume that the drug is distributed into
  - Not a physiological volume
- **Elimination half-life ($t_{1/2}$)**: time taken to reduce drug concentration in blood by 50%
  - Affects frequency by which a drug is given to patient

Given

$$C_p = C_0 e^{-kt}$$

Let

$$C_p = \frac{1}{2} C_0$$

when $t = t_{\frac{1}{2}}$.

We have

$$\frac{1}{2} C_0 = C_0 e^{-kt_{\frac{1}{2}}}$$

$$e^{-kt_{\frac{1}{2}}} = \frac{1}{2}$$

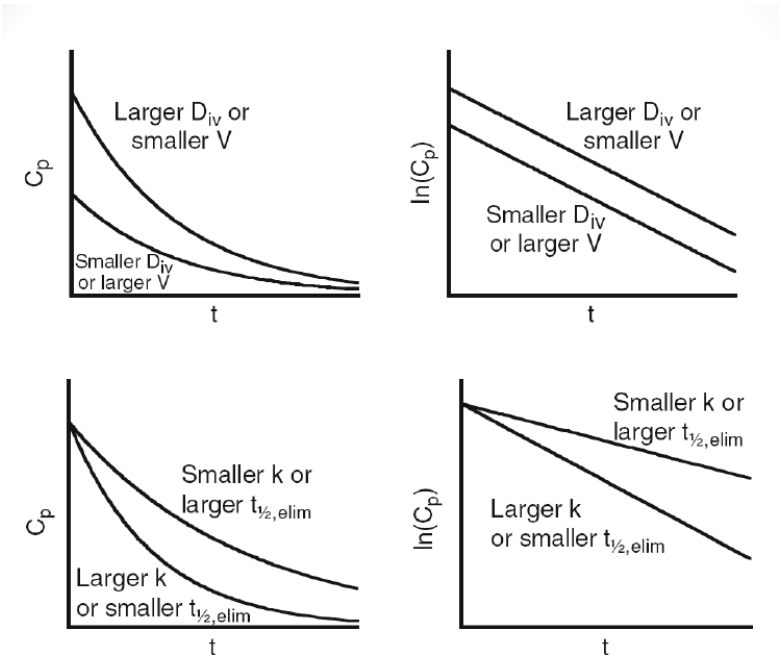
$$-kt_{\frac{1}{2}} = -\ln 2$$

$$t_{\frac{1}{2}} = \frac{\ln 2}{k} \approx \frac{0.693}{k}$$

- Variation of C_p-t pattern with D_{iv}, V, k and $t_{1/2}$:

$$C_p = \frac{D_{iv}}{V} e^{-kt} = C_0 e^{-\frac{t \ln 2}{t_{\frac{1}{2}}}}$$

$$\ln C_p = (\ln D_{iv} - \ln V) - kt = \ln C_0 - \frac{t \ln 2}{t_{\frac{1}{2}}}$$



- **Drug clearance (CL)**: measure of efficiency with which a drug is removed from the body

Drug clearance is defined as:

$$CL = Vk$$

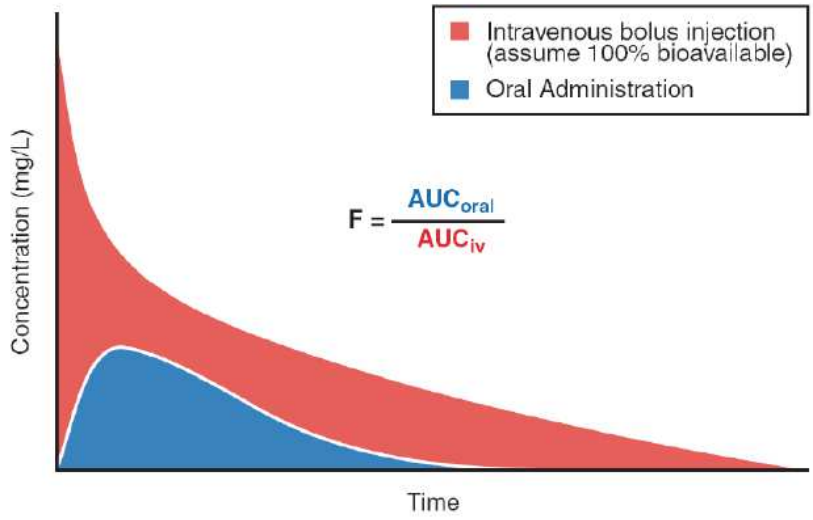
where

$$k = k_{met} + k_{excr}$$

Note that

$$\frac{dA_{body}}{dt} = -kA = -\frac{CL}{V}(A) = -CL \cdot C_p$$

- **Bioavailability (F)**: extent of absorption of a given drug, expressed as fraction of administered dose
  - Varies with dosage forms
  - IV by definition has F = 100%



Area under curve (AUC): area under C_p-t curve, useful for calculating bioavailability of different drug products

For IV dose,

$$AUC = \int C_p dt$$

$$= \int C_0 e^{-kt} dt$$

$$= \left(-\frac{1}{k}\right) C_0 e^{-kt} + C$$

$$= -\frac{D_{iv}}{Vk} e^{-kt} + C$$

$$= -\frac{D_{iv}}{CL} e^{-kt} + C$$

Consider when $t = 0$,

$$-\frac{D_{iv}}{CL} e^{-k(0)} + C = 0$$

$$C = \frac{D_{iv}}{CL}$$

Thus,

$$AUC = -\frac{D_{iv}}{CL} e^{-kt} + \frac{D_{iv}}{CL}$$

$$= \frac{D_{iv}}{CL}(1 - e^{-kt})$$

For AUC_{iv}, we are actually considering AUC when $t \rightarrow \infty$.

$$AUC_{iv} = \lim_{t \to \infty} \frac{D_{iv}}{CL}(1 - e^{-kt})$$

$$= \frac{D_{iv}}{CL}$$

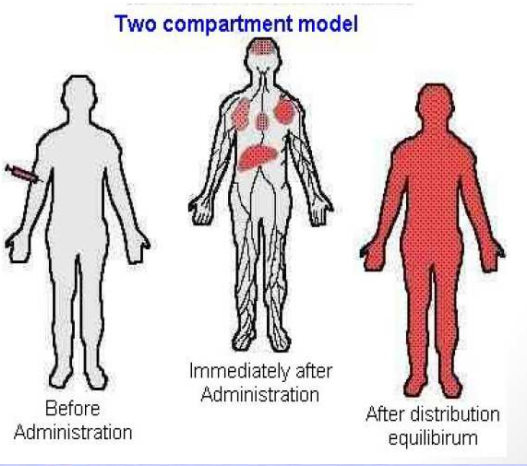
Therefore,

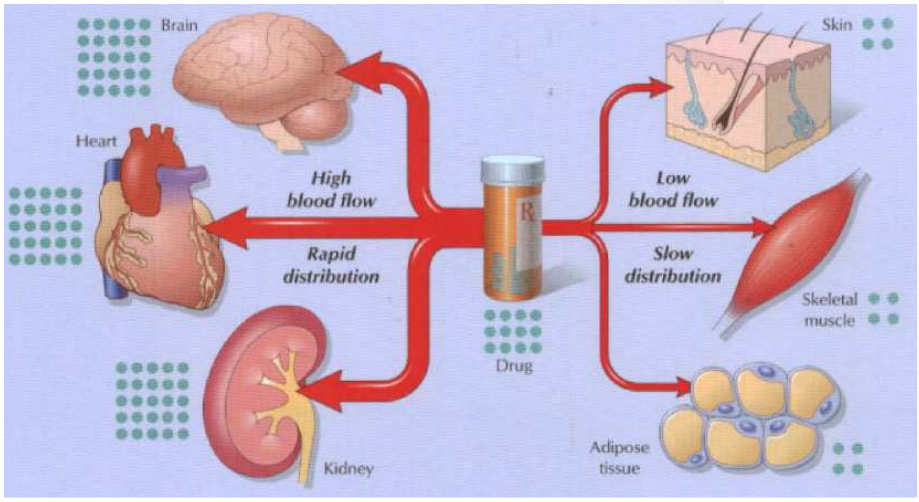
$$F = \frac{AUC_{oral}}{AUC_{iv}}$$

$$= \frac{AUC_{oral}}{\frac{D}{CL}}$$

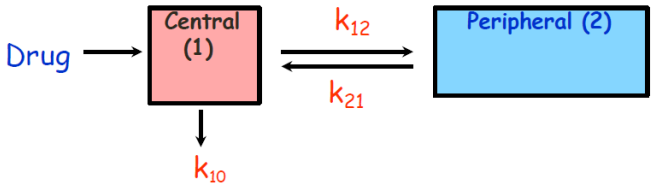
$$= \frac{AUC \cdot CL}{D}$$

B. Two-compartment Intravenous Bolus Pharmacokinetic Model

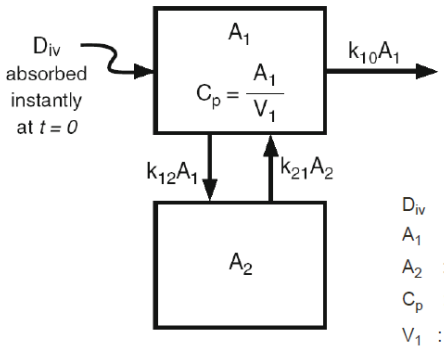




- Applicable for drugs exhibiting a slow equilibration with peripheral tissues



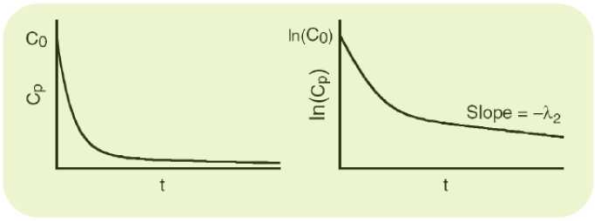
- Drug appears to distribute between two compartments
- Drug <u>not</u> instantaneously equilibrated in various tissues
- **Central compartment**: rapidly perfused tissues
- **Peripheral compartment**: slowly perfused tissues



D_{iv} : intravenous dose
A_1 : amount of drug remaining in compartment 1
A_2 : amount of drug remaining in compartment 2
C_p : plasma drug concentration
V_1 : volume of drug distribution in compartment 1
k_{12} : rate constant for distribution from 1→2
k_{21} : rate constant for distribution from 2→1
k_{10} : micro elimination rate constant

For small *t*, $A_1 \gg A_2$.
Thus, $k_{12}A_1 \gg k_{21}A_2$ i.e. $k_{21}A_2$ is negligible and $\frac{dA_1}{dt} \approx (k_{10} + k_{12})A_1$

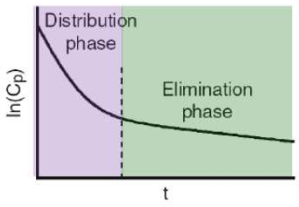
This is called the **distribution phase**.



When *t* increases, A_2 increases while A_1 decreases.
The situation gradually approach the equilibrium when $A_1 \approx A_2$
This is called the **elimination phase**.

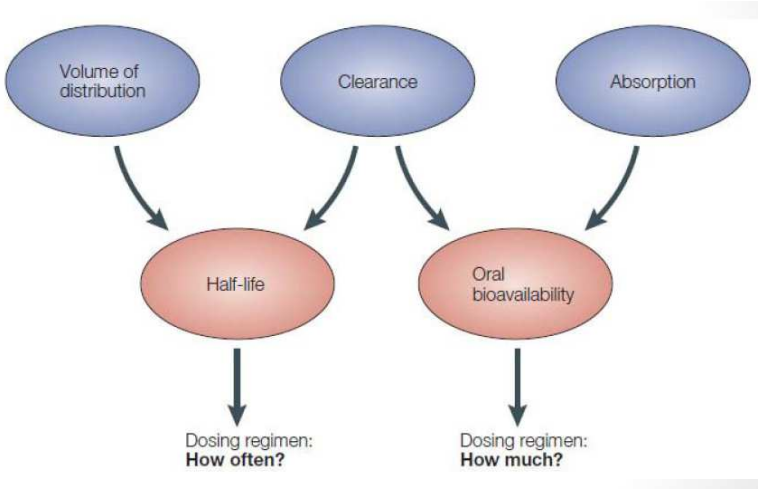
$$t_{1/2,dist} = \frac{\ln(2)}{\lambda_1} \approx \frac{0.693}{\lambda_1}$$

$$t_{1/2,elim} = \frac{\ln(2)}{\lambda_2} \approx \frac{0.693}{\lambda_2}$$



Each phase has its own unique half-life, depending on distribution and drug elimination characteristics.

C. Designing the Dose Regimen



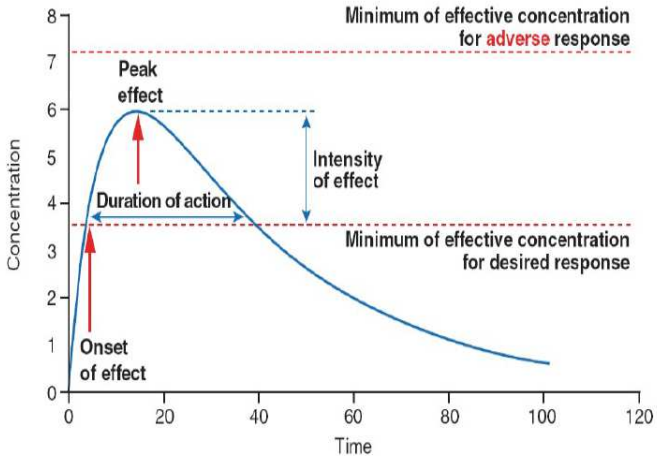
$$t_{\frac{1}{2}} = \frac{\ln 2}{k} = \frac{\ln 2}{\frac{CL}{V}} = \frac{V \ln 2}{CL}$$

$$F = \frac{AUD \cdot CL}{D}$$

- Dose regimen and dose size is affected by three factors:
  - Volume of distribution: body size of individual
  - Clearance: renal function
  - Absorption: GI function

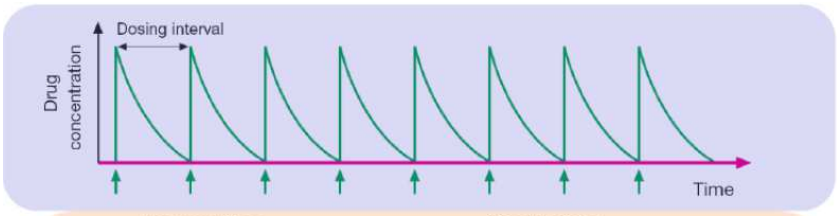
1. Single Dose Regimen

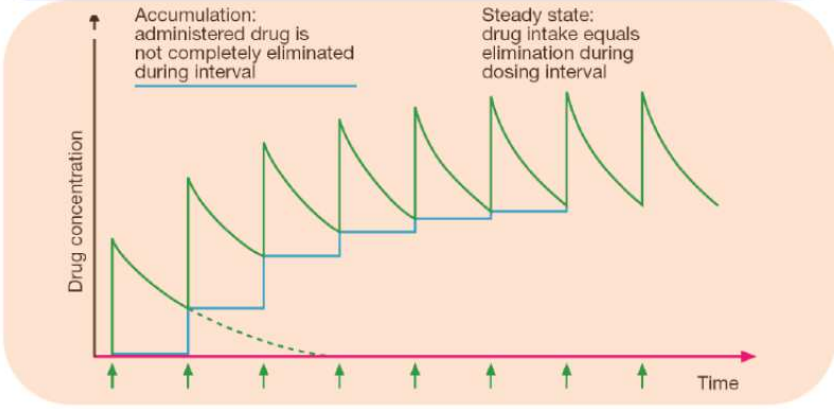
Time course of action of a single oral dose

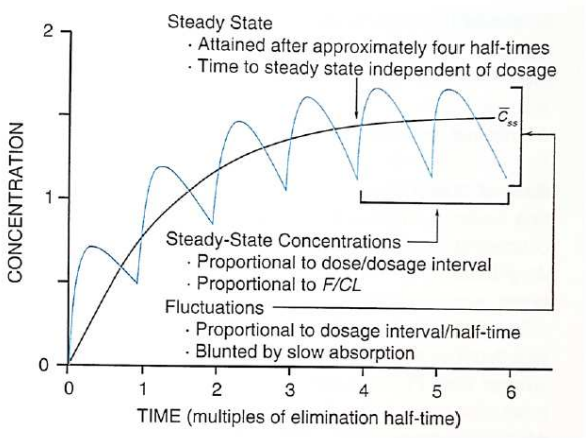


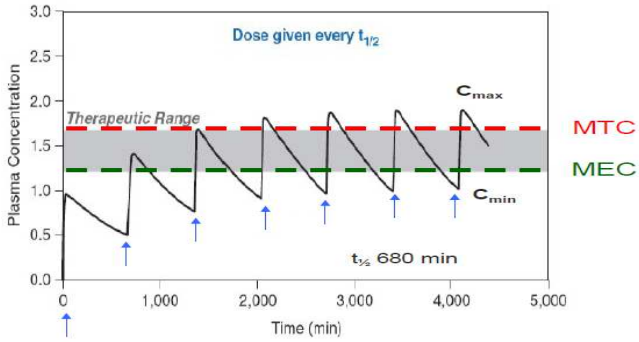
- **Time of onset**: time taken for the drug to produce a response
- **Time to peak effect**: time taken for the drug to reach its highest blood concentration
- **Duration of action**: time during which the drug produces a response

2. Multiple Doses Regimen









C_{ss} = steady state conc.

C_{max} = maximum C_{ss} (peak)

C_{min} = minimum C_{ss} (trough)

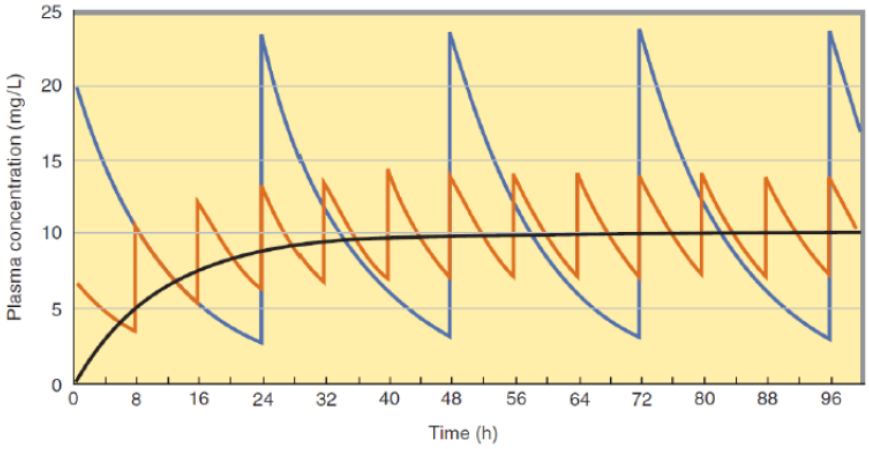
MEC = minimum effective concentration

MTC = minimum toxic concentration

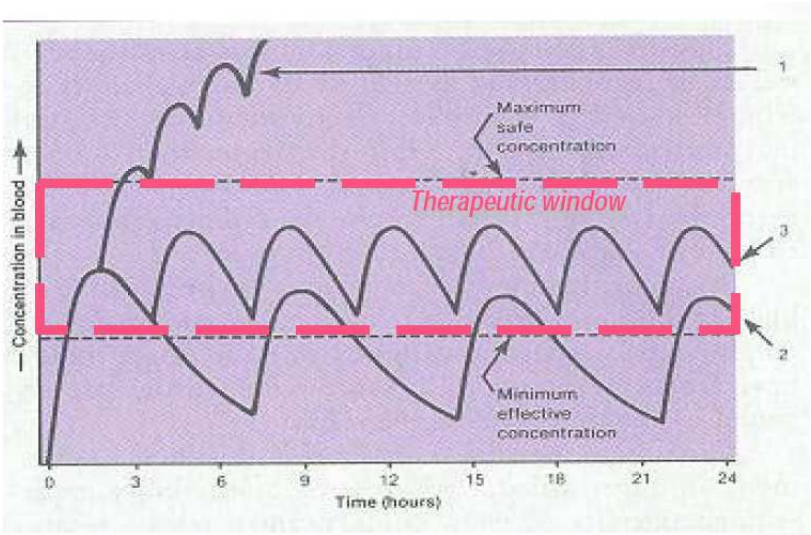
therapeutic range = MTC – MEC

- In multiple dosing, drug accumulation occurs when repeated doses are given before drug is completely eliminated
- **Plateau principle**: repeated drug administration at fixed dosage intervals will produce a <u>plateau</u> concentration of drug in blood (i.e. **steady state**)
  - Repeated drug administration at **dose interval (τ)** will give a **steady state** with plasma concentration fluctuating between a maximum (C_{max}) and minimum (C_{min})
- **Steady state**: state at which rate of drug adminsitration = rate of elimination
  - Usually 4-5 half-lives are required t reach steady state (unless a loading dose is given)
- **Therapeutic range**: range between **minimum effective concentration (MEC)** and **minimum toxic concentration (MTC)**

- Effect of dosing frequency on C_p-t pattern:

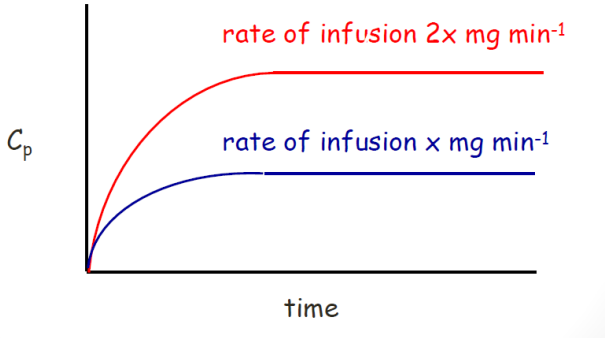


- Effect of dosage interval on C_p-t pattern:

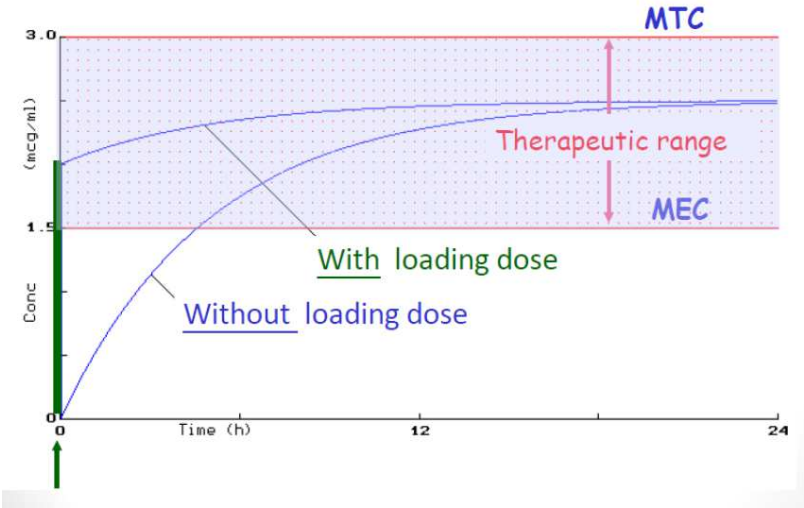


curve 1 -- dosage interval too short; curve 2 -- too long; curve 3 -- ideal

- When continuous IV infusion is used, the resulting C_p-t graph is a smooth curve approximating the steady state concentration:



a. Combined Infusion and Bolus Administration



- A **loading dose** is given first by rapid IV injection followed by the slower **maintenance infusion**
- **Loading dose**: a large dose given to achieve therapeutic concentration rapidly
- **Maintenance dose**: a dose given to maintain drug concentration at steady state

At t = 0, steady state concentration is reached immediately due to the loading dose. Thus,

$$\text{Loading dose} = C_{ss} \cdot V$$

At equilibrium position,

$$\frac{dA}{dt} = \text{rate of administration} = \frac{\text{maintenance dose}}{\tau}$$

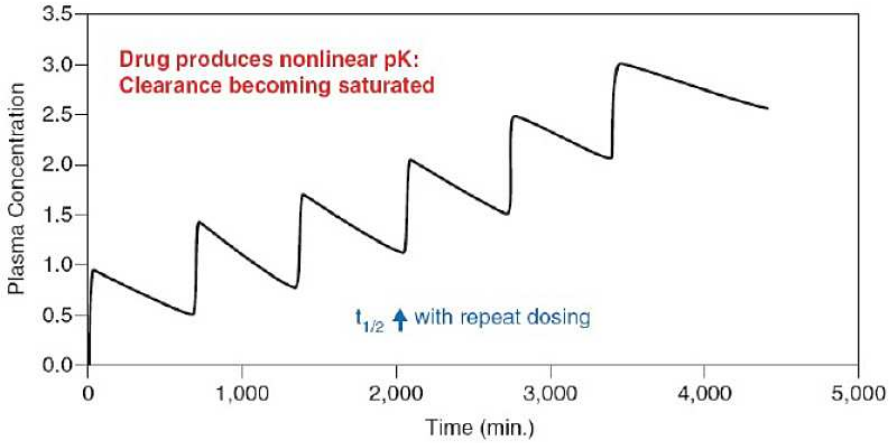
Thus,

$$\begin{aligned} \text{maintenance dose} &= \frac{dA}{dt}(\tau) \\ &= kA\tau \\ &= \frac{CL}{V}(A\tau) \\ &= CL \cdot C_{ss} \cdot \tau \end{aligned}$$

If drug is given by other routes, dosage is adjusted by dividing F (bioavailability).

- Advantage: can achieve a therapeutic concentration more quickly

b. Saturation Kinetics



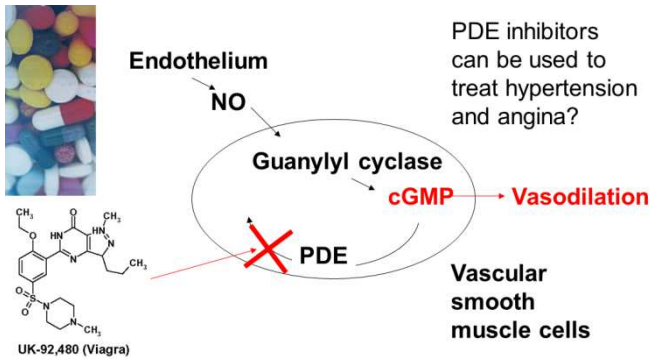
- Occasionally some drugs follow **zeroth order elimination kinetics** (i.e. elimination rate = constant) at high doses
- Examples: alcohol, phenytoin
- Caused by a limitation on capacity of drug metabolism or drug transport
- Great importance in multiple dose therapy ∵ drug accumulation may result in toxic response

L94 Development and Regulation of Drugs

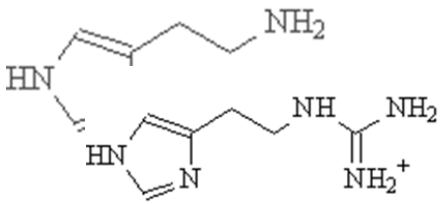
A. Development of Drugs

1. Discovery of Drugs

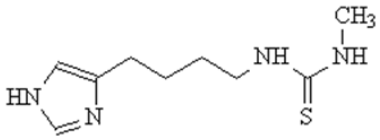
- Discovery based on history:
  - **Cocaine**: mood stimulant
  - **Morphine**: painkiller
  - **Quinine**: anti-malarial agent
- Discovery by chance observation:
  - **Penicillin**: antibiotic
    - → Discovered in mould by Alexander Fleming
    - → Found to have antibacterial activity
    - → Used to treat severe infection
    - → Structure is found later → open door to synthesis
  - **Cisplatin**: anti-cancer drug
    - → Discovered by Barnett Rosenberg
    - → Pt electrodes + NH_4Cl electrolyte to study effect of E-field on *E. coli*
    - → $Pt(NH_3)_2Cl_2$ inhibits cell division
- Discovery by random screening:
  - **Taxol**: anti-cancer drug
    - → Discovered by random screening of natural products
    - → Found in pacific yew
- Discovery by clinical observation of side effects
  - **Viagra**: drug used to treat erectile dysfunction
    - → A **phosphodiesterase (PDE)** inhibitor
    - → Originally a candidate drug for treating hypertension and angina, but no obvious effect
    - → Always produces a side effect → penile erection
    - → Reason: specific in inhibiting PDE-5 found mainly in penile vascular smooth muscles (PDE-1 is the most common isoform in vascular smooth muscles)



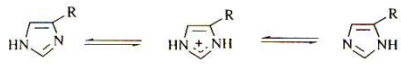
- Discovery based on physiological substances
  - **H2 receptor blocker**: drug for reducing stomach acid secretion
    - → **Histamine** can induce allergic response and gastric acid secretion
    - → Traditional antihistamines are only **H1 receptor** antagonists → only reduce allergic response
    - → Gastric acid secretion related to **H2 receptor**
    - → **Guanylhistamine** has a charged side chain and is only a partial agonist
    - → **Burimamide** is an effective H2 blocker but not orally active due to existence of charged transition state → not absorbable
    - → **Metiamide** with CH_3 and S electron withdrawal groups added to stabilize structure but will cause **granulocytopenia** (low granulocyte count)
    - → **Cimetidine** with =S group (source of granulocytopenia) replaced by NC=N group → successful drug

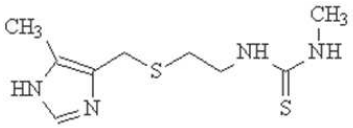


Guanylhistamine

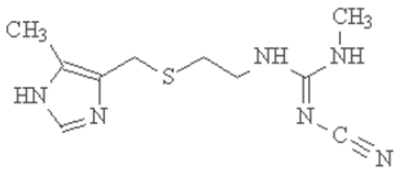


Burimamide





Metiamide



Cimetidine

2. Course of Drug Development

a. Early Development Phase

- **Pharmacokinetic studies**:
  - Numerous routes of administration: oral, IV, IM, IP (intraperitoneal), SC,…
  - Absorption
  - Distribution
  - Metabolism
  - Excretion
- **Toxicology studies**:
  - Acute toxicology
  - Chronic toxicology
  - Reproductive toxicology
  - Carcinogenicity

b. Clinical Development Phase

i. Phase I Trial

- Objective: verify safety and tolerability of the candidate drug in human
- Length: typically 6-9 months
- Subject: 20-100 healthy volunteers
- Documentation of how the drug acts on the boy: how it is absorbed, distributed, metabolized and excreted

ii. Phase II Trial

- Objective: determine effectiveness of drugs in human
- Length: 6 months – 3 years
- Subject: up to several hundred patients suffering from the disease
- Establish minimum and maximum effective dose
- Randomized: randomly divided into groups (investigational drug, placebo or standard treatment)

iii. Phase III Trial

- Objective: expanded testing of effectiveness and safety of an investigational drug
- Length: 1-4 years
- Subject: several hundred to thousands of volunteer patients suffering from the condition

iv. Phase IV (Post-Marketing Studies)

- Collect information from patients after drug has been marketed
- Objectives:
  - Expand testing of a drug to broader patient populations and compare long-term effectiveness
  - Focus on previously unknown side effects or related risk factors

B. Drug Regulation

1. Registration of Pharmaceutical Products

- No person shall sell, offer for sale or distribute any pharmaceutical product unless the product is registered with the **Pharmacy and Poisons Board**
- Factors relevant to determination of application for registration:
  - Safety
  - Efficacy
  - Quality

2. Regulation on Sale of Pharmaceutical Products

a. Laws Regulating Sale of Pharmaceutical Products

- **Pharmacy and Poisons Ordinance, Cap. 138** (藥劑業及毒藥條例, 第一三八章)
  - Pharmacy and Poisons Regulations (藥劑業及毒藥規例)
  - Poisons List Regulations (毒藥表規例)
- **Antibiotics Ordinance, Cap. 137** (抗生素條例)
  - Antibiotics Regulations (抗生素規例)
- **Dangerous Drugs Ordinance, Cap. 134** (危險藥物條例)
  - Dangerous Drugs Regulations (危險藥物規例)

b. Listed and Authorized Sellers of Poisons

- **Listed sellers of poisons (列載毒藥銷售商)**:
  - Also called 藥行
  - No pharmacist
  - Cannot use the logo of Rx
  - Cannot dispense
    - → Drug must be in a closed and original container
    - → No broken-bulk is allowed
- **Authorized sellers of poisons (獲授權毒藥銷售商)**:
  - Also called pharmacy, dispensary, drug –store or 藥房
  - Pharmacist preset
  - Can use the logo of Rx
  - Can dispense drugs according to prescription

c. Classification of Pharmaceutical Products

i. Non-poisons (NP)

- Drugs not found in poison list
- Used for treatment of minor ailments/conditions and have a history of being safe and effective
- Examples: paracetamol, vitamins, loperamide (anti-diarrhoea drug), laxatives, dequalinium, antacids, contraceptives
- No restriction for sale
- Can be sold as over-the-counter drug in any retail premises including
  - Supermarkets
  - Hospitals
  - Clinics
  - Listed sellers of poisons
  - Authorized sellers of poisons
    - → Can be sold without supervision of pharmacist
    - → No prescription required

ii. Part II Poisons

- Drugs included in the part II of the **Poisons List** in the **Poisons List Regulation**
- Example: antihistamines (eg. chlorpheniramine)
- Sold in:
  - Hospitals
  - Clinics
  - Listed sellers of poisons
  - Authorized sellers of poisons:
    - → Can be sold without supervision of pharmacist
    - → No prescription required

iii. Part I Poisons

- Drugs include in part I of the **Poisons List** in the **Poisons List Regulation**
- Examples: bronchodilators, antifungal agents, glyceryl trinitrate (anti-anginal drug)
- Most of them are used in emergency → sale cannot be too highly regulated
- Sold in:
  - Hospitals
  - Clinics
  - Authorized sellers of poisons
    - → Must be sold by a pharmacist or under supervision of a pharmacist
    - → No prescription required

iv. Part I Schedule 1 Poisons

- Drugs included in the part I of the Poisons List and the First Schedule in the **Pharmacy and Poisons Regulation**
- Example: dextromethorphan (cough medicine)
- Sold in:
  - □ Hospitals
  - □ Clinics
  - □ Authorized sellers of poisons
    - → Must be sold by pharmacist or under supervision of a pharmacist
    - → Prescription required
    - → No prescription → record in **poison book** (fill in date of sale, name of patient, ID number, address, name of drug, quantity and purpose; signed by pharmacist and patient)

FORM OF ENTRY TO BE MADE IN THE BOOK TO BE KEPT BY SELLERS OF POISONS IN ACCORDANCE WITH SECTION 22(3)

Date of Sale	Name and quantity of poison supplied	Purchaser's			Purpose for which stated to be required	Date of certificate (if any)	Name and address of person giving certificate (if any)	Signature of purchaser (or reference number of Signed order in case of wholesale)	Signature of registered pharmacist
		Name and number of identity card	Address	Business, trade or occupation					

(L N. 366 of 1995; L N. 63 of 1997)

v. Part 1 Schedule 3 Poisons

- Drugs included in part I of the **Poisons List** and Third Schedule in the **Pharmacy and Poisons Regulation**
- Mainly include drugs which would lead to death in cases of overdose
- Examples: antihypertensives, steroids, certain sedatives (eg. zolpidem, zoplicone), anticancer drugs, antidepressant, antipsychotropic drugs
- Sold in:
  - Hospitals
  - Clinics
  - Authorized seller of poison
    - → Must be sold by pharmacist or under supervision of pharmacist
    - → Prescription required

(1) Prescription Requirements

- Prescription should:
  - Be in writing and signed by person giving it with his usual signature and be dated by him
  - Specify the address of the person giving it
  - Specify name and address of person for whose treatment it is give
  - Indicate drug name, dosage, frequency of use, duration of treatment (or total amount of drugs to be supplied if used only when required)
  - Have either of the following phrases written:
    - → "For Dental Treatment only" (for dentists)
    - → "For Animal Treatment only" (for veterinary surgeon)

vi. Antibiotics

- Drugs included in the **Antibiotics Regulations**
- Same regulations as **Part 1 Schedule 3 Poisons**
- Example: amoxicillin
- Sold in:
  - Hospitals
  - Clinics
  - Authorized sellers of poisons:
    - → Must be sold by pharmacist or under supervision of pharmacist
    - → Prescription required

vii. Dangerous Drugs (DD)

- Drugs specified in Part I of the First Schedule in **Dangerous Drugs Ordinance, Cap. 134**
- Examples:
  - Opioid drugs, eg. morphine, methadone, codeine (>0.5%)
  - Benzodiazepines (sleeping pill), eg. diazepam, chlordiazepoxide, tiazolam, nitrazepam
  - Amphetamines, eg. dexamphetamine, phentermine
  - Hallucinogens, eg. LSD, ketamine, MDMA
- Sold in:
  - Hospitals
  - Clinics
  - Authorized sellers of poisons
    - → Must be sold by pharmacist or under supervision of pharmacist
    - → Stored in locked receptacle
    - → Prescription required (must be in ink, indelible and contain ID card number of patient)
- Recoded in **Dangerous Drug Register**

(1) Dangerous Drug Register

- Head of register:
  - Specify name and strength of DD
  - Separate pages are used for entries made with reference to each of the dangerous drugs and different strengths of preparations
- Written in ink or otherwise indelible
- Enter in chronological sequence, with respect to every quantity of a dangerous drug obtained or supplied
- Make an entry when transaction takes place or on the day next following the said day (within 48 hours)
- No cancellation, obliteration or alteration
  - Correction of an entry should be made only by way of a marginal note or footnote

Form of Register

Morphine Sulphate 30mg Tablet 30's

Date of receipt/supply	Name and address of person* or firm from whom received/to whom supplied	Patient's identity card number #	Amount		Invoice No.	Balance
			received	supplied		
3.5.2004	ABC firm 382 Kowloon Bay, Kowloon		100		ABC12345	100
6.5.2004	Pshk CHAN 388 Shek kip Mei, Kowloon	A123456(2)		30		70
8.5.2004	Society LAU 99 Pak Tin Estate, Kowloon	G883456(9)		**50***		**20***

* Cross reference of the person to whom supplied may be made in which case only the reference number of the person's treatment

For a patient who is not resident in Hong Kong, the reference number of any proof of identity, other than an identity card, specified

Row 3 'Amount supplied' should read as 30 and 'Balance' should read as 40

3. Regulation on Advertising Pharmaceutical Products

- Governed by **Undesired Medical Advertisements Ordinance (UMAO)**
- Advertising is allowed for:
  - Disease and conditions in Column 2 of Schedule 1 of UMAO
  - Examples: common cold, hay fever, blocked sinuses, headache, indigestion, diarrhoea and travel sickness
- Advertising is prohibited for:
  - Medicine, surgical appliances or treatments for prevention or treatment of disease and conditions in Column 1 of Schedule 1 of UMAO
  - Examples: benign or malignant tumours, STDs and heart disease
  - Exemption: publication of a technical character intended for circulation mainly to registered medical practitioners, pharmacists, professional staff of hospitals and registered Chinese medicine practitioners

L96, 97 Autonomic Pharmacology

A. Autonomic Nervous System

- **Autonomic nervous system (ANS)** is a branch of PNS that is responsible for regulation of visceral (internal) functions, eg. cardiac output, gut motility and blood vessel tone
- Not under conscious control
- Some similarity between transmitters and receptors of ANS and CNS → pharmacology requires selective application or properties to drug to target each system
  - Eg. local application
  - Eg. drugs that do not pass BBB → no CNS effect
  - Eg. lipophilic drugs → target the brain
- ANS and CNS work together to produce neurological control of body functions

1. Transmission of ANS Signals

- **Preganglionic synapse**:
  - Transmitter: **acetylcholine (ACh)**
  - Receptor: **cholinergic nicotinic receptor** (can be activated by nicotine)
  - Activation will produce a non-selective effect (both SN and PN) → little clinical use
- **Postganglionic synapse**:
  - Transmitter:
    - → Sympathetic: **norepinephrine (NE)**
    - → Parasympathetic: **acetylcholine (ACh)**
  - Receptor:
    - → Sympathetic: **adrenergic receptor** (α or β)
    - → Parasympathetic: **cholinergic muscarinic receptor**

a. Cholinergic Receptors

i. Muscarinic receptors:

- Subtypes: M_1, M_2, M_3, M_4, M_5
- Most drugs are non-selective to muscarinic subtypes
- ANS targets: heart, smooth muscles and glands
- Also present in CNS

ii. Nicotinic receptors:

- Subtypes:
  - N_N: nerves (CNS and preganglionic synapses)
  - N_M: muscles (skeletal muscle motor end-plates)
- Drugs that target N_N has no selectivity for SN or PN
- Nicotine (in cigarettes) activate N_N (in CNS and ANS) and has some effects on N_M → little clinical application (except nicotine for smoking cessation)
- Blockade of N_M → muscle paralysis (eg. use in surgical operation)

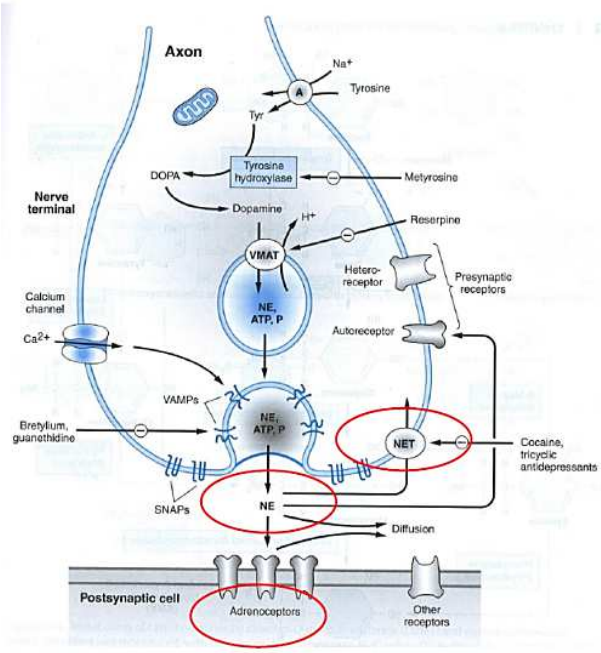
b. Adrenergic Receptors

i. α Adrenergic Receptors

- Subtypes: α_{1A}, α_{1B}, α_{1D}, α_{2A}, α_{2B}, α_{2C}
- Targets: blood vessels, airway, glands and CNS
- Little clinical use in selective activation or blocking subtypes

ii. β Adrenergic Receptors

- Subtypes: β_1, β_2, β_3 (found in fat cells, more relevant in endocrinology)
- Targets: heart and smooth muscles



2. Effects of ANS Activation

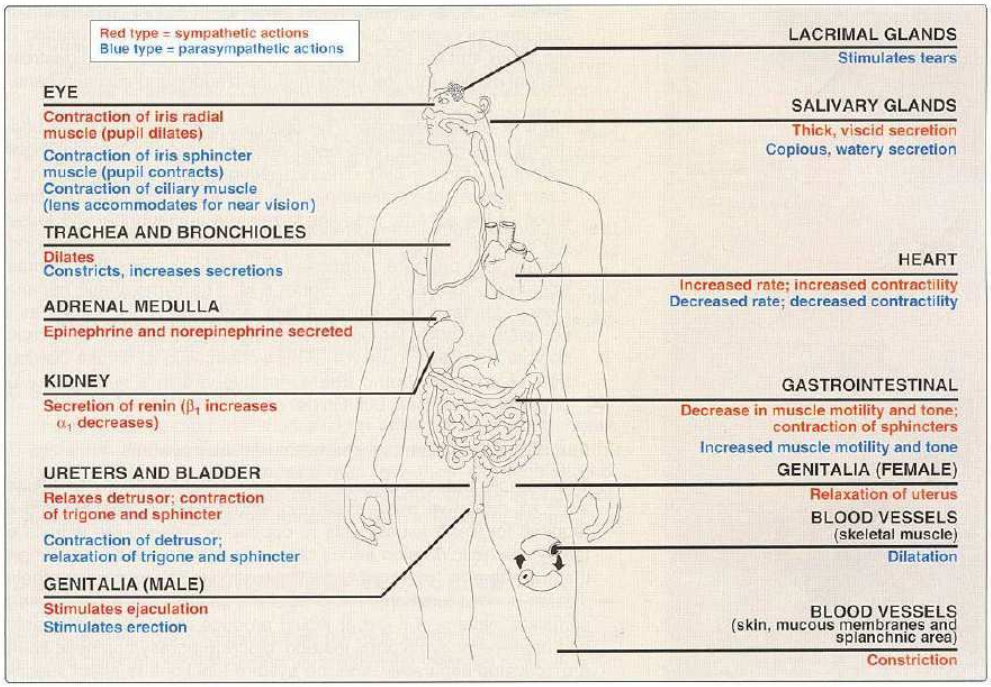


Figure 3.3
Action of sympathetic and parasympathetic nervous systems on effector organs.

- Effects of sympathetic activation:

- Effect of muscarinic activation: **SLUGBAM**
  - Salivation/Secretions/Sweating
  - Lacrimation
  - Urination
  - GI upset
  - Bradycardia/Bronchoconstriction/Bowel movement
  - Abdominal cramp/Anorexia
  - Miosis

B. Pharmacology of Autonomic Nervous System
1. Pharmacology of Parasympathetic Nervous System

- **Parasynpathomimetic**: describe drugs that mimic the effects of parasympathetic nervous system activation (depend on innervation)
- **Cholinomimetics**: drugs that mimic action of acetylcholine (depends on presence of receptor)
- Modes of action:
  - Direct: by activation of receptor (via an agonist)
  - Indirect: by decreasing breakdown of acetylcholine (via inhibition of enzymes cholinesterase or acetylcholinesterase) → ↑ duration of action of acetylcholine
- Selectivity of action achieved by:
  - Selective activation of muscarinic nicotinic receptors (less selective for subtypes)
  - Route of administration

a. Direct Acting Agents

- **Direct acting agents** bind to receptors and activate them → effects
- Action dependent on presence of receptors but not on presence of nerve terminals

i. Acetylcholine

- Breakdown quickly: duration of action only 5-30s → little clinical use
- Non-selective activation of all nicotinic (N) and muscarinic (M) receptors
- Used when very short-term effect is required (eg. diagnostic use)

ii. Muscarine

- Naturally-occurring compound in some mushrooms (eg. *Amanita muscaria*)
- Selective for muscarinic receptors
- No clinical application
- Certain mushroom poisoning due to presence of muscarine
- Use of antimuscarinic agonists (eg. atropine) for treatment

iii. Carbachol

- Not an ester → not degraded by cholinesterase
- Duration of action: 30min – 2h
- Non-selective activation for both nicotinic and muscarinic receptors
- Clinical effect: relax bladder sphincter → treatment of urinary retention
  - Not usually used in clinical setting ∵ side effects
  - Usually put catheter into bladder instead
- Side effects: nausea, vomiting and blurred vision (CNS effects)

iv. Bethanechol

- Selective agonist for muscarinic receptors
- Not degraded by cholinesterase
- Poor lipid solubility → cannot cross BBB → little CNS effects
- Not commonly used

v. Pilocarpine

- Selective agonist for muscarinic receptors
- Not degraded by cholinesterase
- Good lipid solubility
- More commonly used than bethanechol
- Clinical use:
  - Contract ciliary muscle in eye (local application)
  - Improve drainage and reduce pressure in **glaucoma**
    → Early treatment (not first line anymore)
- Side effects: constrict pupil and blur vision

b. Indirect Acting Agents

- No effect on receptor
- Inhibits cholinesterase → decrease breakdown of acetylcholine → increased acetylcholine level → increased effect
- Action dependent on intact nerve terminal

i. Edrophonium

- Cholinesterase inhibitor
- Competitive inhibition
- Short acting, 5-15 mins
- Charged molecule → poor lipid solubility
- Use for diagnostic testing (improvement of muscle weakness)

ii. Neostigmine

- Cholinesterase inhibitor
- Competitive inhibition
- Orally active
- Duration of action: 30min – 2h
- Charged molecule → poor lipid solubility → no CNS effect
- Used to treat muscle weakness
- Side effects: salivation, sweating, gastric secretion and diarrhea

iii. Pyridostigmine

- Similar mechanism and effect to neostigmine
- Little CNS effect
- Orally active
- Duration of action: 3-6h

iv. Physostigmine

- Cholinesterase inhibitor
- Duration of action: 30 min – 2h
- Lipid-soluble → has CNS effect
- Contract ciliary muscle in eue (local application) → improve drainage and reduce pressure in glaucoma
- Side effects: constrict pupil and blur vision
- Not normally used ∵ CNS effect

v. Organophosphate Cholinesterase Inhibitors

- Lipid soluble cholinesterase inhibitors
- Phosphorylated active site of enzyme → irreversible inhibition of cholinesterase
- Different metabolism between mammals and insects → use as insecticides
- Normally have low toxicity for humans
- **Nerve gas**: extremely potent and volatile cholinesterase inhibitors that are sensitive to humans

(1) Parathion

- Organophosphate
- Irreversible cholinesterase inhibitor
- High lipid solubility
- Duration of action: 7-30 days
- Common ingredient in insecticides

(2) Insecticide and Organophosphate Poisoning

- Symptoms:
  - Excessive salivation and sweating
  - Bronchial constriction → difficulty in breathing
  - Vomiting and diarrhea
- Caused by neuromuscular blockade
- Treatment: atropine (to block muscarinic receptors)

c. Cholinergic Receptor Blockers

i. Antimuscarinic Cholinergic Blockers

(1) Atropine

- Isolated from natural sources (*Atropa belladonna*)
- Muscarinic blocker for all subtypes
- No effect on nicotinic receptor
- Well-absorbed
- Half-life: ~2h
- Less effective for endogenous release of Ach but more against exogenous muscarinic agonists
- Some distribution to CNS: quaternary structure → less lipid soluble → less CNS effect

(a) Effects and Side-effects of Atropine

- Dilate pupil: used for eye examination (local application)
- ↓ salivary and bronchial secretion: used as premedication for surgery (not as common now)
- ↓ gastric and intestinal motility: treatment of diarrhea
- ↓ cholinergic poisoning (eg. organophosphate poisoning)
- ↑ heart rate (due to blockage of PN function)
- Side effects: dry mouth, urinary retention, constipation, tachycardia
- Certain antihistamines, antipsychotics and antidepressants also have antimuscarinic properties → share some side effects eg. dry mouth etc

(2) Ipratropium

- Synthetic analog of atropine
- Charged molecule → less absorption and CNS effect
- Apply by inhalation
- Used to dilate bronchial muscle in treatment of asthma
- Useful for COPD

(3) Scopolamine (Hyoscine)

- Similar action to atropine
- More central than peripheral effects
- Effective for motion sickness (CNS effect)

(4) Oxybutynin and Tolterodine

- Orally active muscarinic antagonists
- Some selectivity for M_3 receptors
- M_2 and M_3 receptor subtypes involved in some smooth muscle contraction
- Blocks M_3 receptors → block contraction (i.e. relax smooth muscle) → relieve bladder spasm
- Used for treatment of urinary urgency
- Little CNS effect

ii. Antinicotinic Receptor Blocker

- Antagonists at N_N receptors (ganglion blockers) → block both SN and PN
  - Used in the past for treatment of hypertension
  - Rarely used nowadays
- Antagonists at N_M receptors used in anaesthesia as muscle relaxants
  - Examples: tubocarine, pancuronium

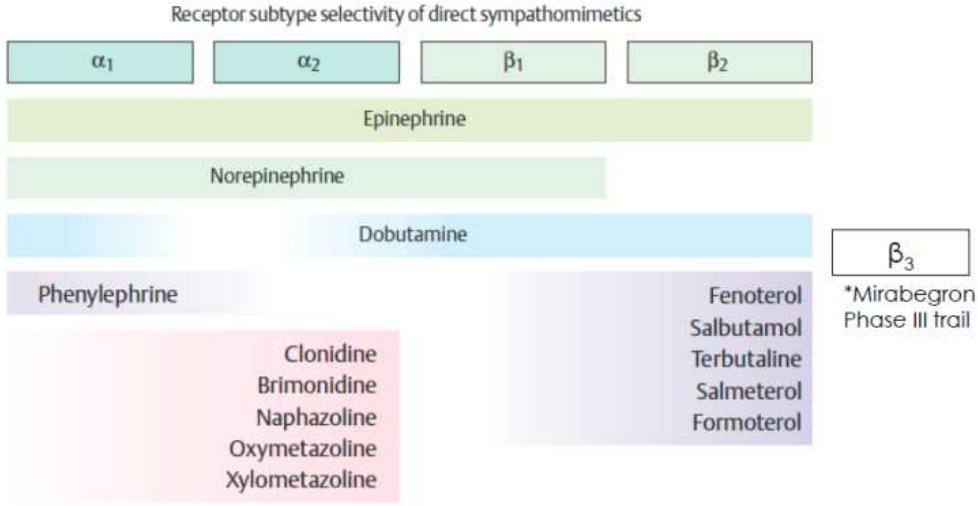
2. Pharmacology of Sympathetic Nervous System

- **Sympathomimetics**: mimic action of sympathetic nervous system activation
  - Mode of action:
    - → Direct: activation of the receptor (agonist)
    - → Indirect: increase release of transmitter, decrease breakdown or removal
- **Sympatholytics**: reduce the action of sympathetic nervous system action
  - Mode of action:
    - → Block receptor activation (receptor antagonist or blocker)
- Factors affecting drug effect on adrenergic system:
  - Receptor selectivity
  - Structure: **catecholamine** (class of chemicals that include noradrenaline and adrenaline) structure → metabolism and breakdown by enzymes → short duration of action (in minutes)
  - Lipophilicity → tissue distribution → presence/absence of CNS effect
  - Reflex mechanism (control system): changes in BP → changes in heart rate
    - → Eg. ↑ BP → activates baroreceptor reflex → ↑ PN effect on heart → ↓ heart rate

- Effects of different receptor subtypes:
  - Alpha = constrict → α-receptors usually bring about constriction responses
  - Beta = dilate → β-receptors usually bring about dilatation responses
  - Hearts beats as one → β_1 receptors are responsible for increasing rate and force of heartbeat
  - Most α effects are due to α_1 activation
  - α_2 activation in CNS → negative feedback mechanism → ↓ NE secretion in presynaptic neurone → less NE effects (eg BP)

Organ	Response	Receptor type
Heart	↑ rate & force	β_1
Blood vessel		
skin	constriction	α
skeletal	constriction	α
	dilation	β_2
Bronchial muscle	relaxation	β_2
Intestine		
motility	decreased	α, β_2
sphincters	contraction	α
Urinary tract		
bladder	relaxation	β_2
sphincters	contraction	α

a. Sympathomimetic Agents



i. Adrenaline (Epinephrine)

- Non-selective agonist (for both α and β)
- Potent cardiac stimulant
- ↑ in BP and cardiac output
- β_1 effect → ↑ cardiac output (positive **inotropic** and **chronotropic** actions)
  - **Inotropic**: ↑ force of heart (muscle) contraction (ino-: fibre)
  - **Chronotropic**: ↑ heart rate (chrono-: time)
- β_2 effect → relax blood vessels → ↓ BP
- α effect → constrict blood vessels → ↑ BP
- Opposing β_2 and α effects → little or no change in BP

ii. Noradrenaline (Norepinephrine)

- Non-selective agonist (for both α and β)
- Similar effect as adrenaline on β_1 receptor
- Little effect on β_2 receptor
- Similar effect as adrenaline on α receptor → ↑ constriction → ↑ systolic and diastolic BP
- ↑ BP → activation of vagal reflex → ↓ heart rate → counteract β_1 effect on heart rate
- Net result: ↑ systolic and diastolic BP with small or no increase in heart rate

iii. Phenylephrine and Methoxamine

- Agonist: mostly α effect with little or no β effect
- Activates α receptor → constrict blood vessels → ↑ BP
- Used as a decongestant (∵ vasoconstriction)
- Other commonly used topical decongestants: **xylometazoline** and **oxymetzoline** (large doses have central effect on α_2 receptors → ↓ BP and sedation)

iv. Clonidine

- Centrally-acting α_2 agonist
- Little effect on β receptor
- α_2 effect on CNS → NE release inhibited → ↓ BP
  - Cf. α_1 activation in ANS → ↑ BP
- Side effects: sedation
- Limited use of centrally acting α_2 agonists ∴ side effects

v. Dexmedetomidine

- Centrally acting α_2 agonist
- Used as sedative agent or to enhance anaesthetic effect

vi. Isoproterenol (Isoprenaline)

- Potent β agonist (for both β_1 and β_2)
- Little effect on α receptor
- β_1 effect on heart → positive inotropic and chronotropic effects → large increase in cardiac output
- β_2 effect on blood vessels → relax blood vessels → ↓ BP
- Net results: ↑ cardiac output, ↑ systolic pressure and ↓ diastolic pressure

vii. Dobutamine

- Relatively selective for β_1
- β_1 effect on heart → positive inotropic and chronotropic effects → large increase in cardiac output
- No β_2 effect on blood vessels → little effect on BP
- Some α effect → small ↑ BP
- Mostly used to ↑ cardiac output and maintain or raise BP (in treatment of shock and heart failure)

viii. Terbutaline and Salbutamol (Albuterol)

- β_2 selective
- Mostly administered by inhalation
- Useful for treatment of asthma (airway disease and acute bronchospasm)
- Terbutaline can be used to delay or prevent premature labour ∵ activation of β_2 receptors cause relaxation of uterine smooth muscle

ix. Amphetamine

- Strong CNS stimulant
- Crosses BBB easily
- Produces mood elevation, increase awareness and suppress appetite
- Subject to abuse
- Amphetamine and analogs used for treatment of **attention-deficit hyperactivity disorder (ADHD)** (CNS effect)
    - **Attention-deficit hyperactivity disorder (ADHD)**: disorder in children with short attention span and hyperactive physical behaviour

x. Cocaine

- Local anaesthetic with sympathomimetic action
- Inhibition of transmitter reuptake at noradrenergic synapses
- Strong central effect → amphetamine-like effect
- Short acting but more intense effect than amphetamine
- Can be absorbed by inhalation (snorted or smoked) or by injection
- Popular drug of abuse

b. α-blockers

i. Phentolamine and Tolazoline

- Competitive antagonist for α receptors
- Non-selective for α_1 and α_2 receptors
- Effect: ↓ BP
- Use: treatment of hypertension and reduction of BP

ii. Phenoxybenzamine

- Non-competitive antagonist for α receptors
- Non-selective for α_1 and α_2 receptors
- Use: ↓BP in **pheochromocytoma**

iii. Selective α_1 Receptor Blockers

- Selective to α_1 receptor than α_2 receptor
- Peripheral effect
- **Prozosin** and **terazosin**: block α_1 receptors → inhibit contraction of blood vessels → ↓ BP
- **Doxazosin**: longer acting, more suitable for treatment of hypertension
- **Tamsulosin**: more selective for prostate smooth muscles → used in **benign prostatic hypertrophy (BPH)**

c. β-blockers

- Clinically useful β-blocking agents are competitive antagonists for β receptors
- Prototype of this group: **propranolol**
- Major use: <u>reduce cardiac workload</u>
- Very little difference among β blockers in terms therapeutic outcome
- Difference in duration of action and potency
- Can be selective for $\beta_1$or β_2 receptors
  - No clinical use for β_2 selective antagonists

i. Selective β_1 Receptor Blockers

- β_1 selective blockers: low doses block β_1 in heart but not β_2 receptors in respiratory tract → less likely to block β_2 effect to precipitate an asthmatic attack
- Retain ability to respond to β_2 agonists to relief bronchospasm in asthmatic patients
- Blood levels highly variable → always danger of blocking β_2 receptors
- Examples: **metoprolol** and **atenolol**

3. Clinical Application of ANS Drugs

- Considerations when applying ANS drugs to target ANS:
  - Cholinergic and adrenergic receptors found outside ANS also → side effects
  - Lipophilic → cross BBB → CNS effects
  - Local/target application → localized/specific effects
- Examples of ways of targeting effect:
  - Use of selective muscarinic agonist → sphincter muscle of iris contracts → ↓ pupil size
  - Route of administration: use of eye drops → specific effect on eye only
  - Different metabolism paths: local vs systemic

a. Eye Examination

- Require light → constriction of pupil
- Need to dilate pupil (**mydriasis**)
- Local application to block muscarinic receptors on iris sphincter
- Duration of effect for different muscarinic blockers:
  - Atropine: 7-10 days
  - Homatropine: 3-7 days
  - Cyclopentolate: 1 day
  - Tropicamide: 1/4 day
- Shorter duration preferred
- Alternative: activate receptor of dilator muscle of iris instead

b. Sjögren's Syndrome

- **Sjögren's syndrome**: an autoimmune disorder in which immune cells attack and destroy exocrine glands that produce tears and saliva
- 9/10 of patients with Sjögren's syndrome are women
- Average age of onset is late 40s
- Treatment: pilocarpine or bethanechol → activation of muscarinic receptor → stimulate saliva secretion → reduce symptoms

c. Pheochromocytoma

- A tumour of adrenal medulla
- Production of a large amount of adrenaline and noradrenaline
- Symptoms: high BP
- Treatment: surgical removal of tumour
- Pre-surgery management: control of BP by α-blockers
- α-blockers → ↓ smooth muscle contraction → ↓ BP
- Phentolamine: competitive blocker, less effective
- Phenoxybenzamine: non-competitive blocker, more effective
- β-blocker may also be needed to control heart rate

d. Myasthenia Gravis

- **Myasthenia gravis**: a neuromuscular autoimmune disorder in which circulating antibodies block ACh receptors at post-synaptic neuromuscular junctions, causing muscle weakness and fatigue
- Treatment: cholinesterase inhibitors
- Diagnosis: edrophonium (short acting)
- Treatment: neostigmine and pyridostigmine
- Can also treat with immunosuppressants and in some cases thymectomy

e. Muscle Paralysis

- Muscle relaxants may cause reversible and temporary blockade of neuromuscular transmissions (used in general anaesthesia)
- Irreversible blockade of nerve transmission or release of transmitters → highly toxic
  - Eg. Toxins from microorganisms, insects, fish and snakes
- **Botulinium toxin (Botox)** → blocks release of ACh in cholinergic nerves
  - Localized application
  - Treatment of ocular muscle spasms
  - Cosmetic treatment of wrinkles

f. Clinical Application of Interference with Removal of Transmitters

- Cholinesterase inhibitors → ANS and CNS effects
  - ANS: enhance PN action
  - CNS: enhance effect of ACh in CNS → treatment of Alzheimer's disease
- Breakdown of transmitters of adrenergic nerves mediated by **monoamine oxidase (MAO)** and **catechol-O-methyltransferase (COMT)**
  - ANS: little clinical effect
  - CNS: important clinical applications (eg. MAO inhibition for treatment of depression)

i. Alzheimer's Disease

- Pathophysiology: neuronal degeneration → ↓ production and secretion of ACh → impairment of cognitive functions
- Cholinesterase inhibitors → ↑ action of ACh → improvement of neuronal function
- Require good distribution to brain
- **Tacrine**: first compound available, but limited by liver toxicity
- Current drugs: donepezil, galantamine and rivastigmine

-The End-