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PPT Bank

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Index

Immunosuppressive Therapy

- Allograft transplantation always requires some degree of immunosuppression if the transplant is to survive.
- Most immunosuppressive treatments are nonspecific, resulting in generalized suppression of responses to all antigens, not just those of the allograft.

  This places the recipient at increased risk of infection and cancer. In fact, infection is the most common cause of transplant-related death.
- Patients on long-term immunosuppressive therapy are also at increased risk of hypertension and metabolic bone disease.

Generalized Immunosuppressive Therapy

- 6-mercaptopurine 1st used in animal models
- its chemical analogues for eg. azathioprine, in combination with corticosteroids, dramatically showed increased survival of allografts in humans.
- Azathioprine (Imuran) is a potent mitotic inhibitor often given just before and after transplantation to diminish both B- and T-cell proliferation.
- Other mitotic inhibitors that are sometimes used in conjunction with immunosuppressive agents are cyclophosphamide and methotrexate.

- Cyclophosphamide is an alkylating agent that inserts into the DNA helix and becomes cross-linked, leading to disruption of the DNA chain.
- It is especially effective against rapidly dividing cells and is therefore sometimes given at the time of grafting to block T-cell proliferation.
- Methotrexate acts as a folic-acid antagonist to block purine biosynthesis.
- Side effects of these drugs-
- Because mitotic inhibitors act on all rapidly dividing cells, they cause significant side effects, especially affecting the gut and liver, in addition to their target, bone marrow-derived cells.

- Fungal metabolites
- cyclosporin A (CsA), FK506 (tacrolimus), and rapamycin (also known as sirolimus)

  Function-

  blocking the activation and proliferation of resting T cells. Some of these also prevent transcription of several genes encoding important T-cell activation molecules, such as IL-2 and the high-affinity IL-2 receptor (IL-2R).

  CsA is effective immunosuppressive drug but toxic to kidney.

  FK506 and rapamycin are 10 to 100 times more potent immunosuppressants than CsA and therefore can be administered at lower doses and with fewer side effects.

Specific Immunosuppressive Therapy

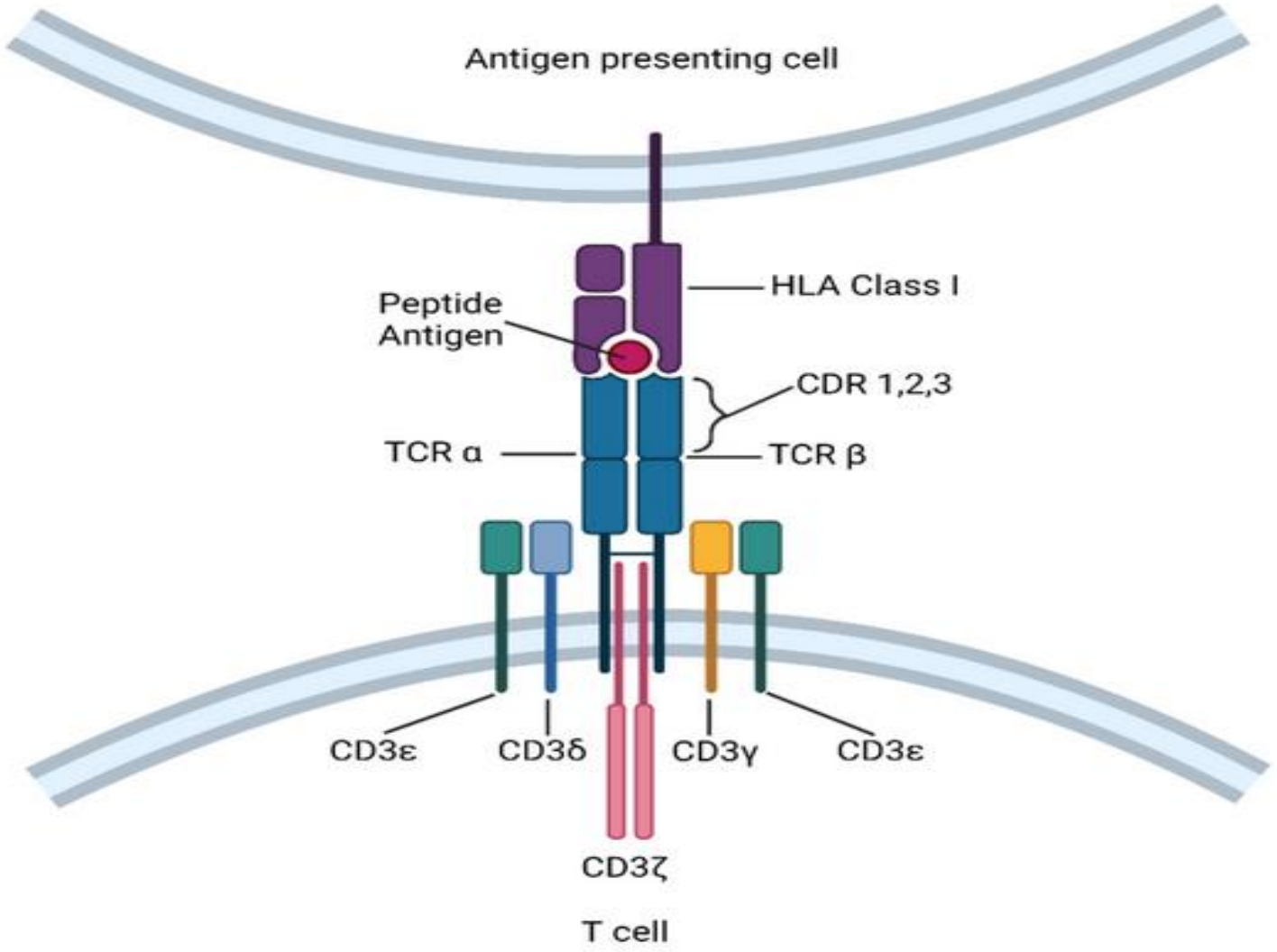
- The ideal immunosuppressant would be antigen-specific, inhibiting the immune response to the alloantigens present in the graft while preserving the recipient's ability to respond to other foreign antigens.

Monoclonal antibody therapy

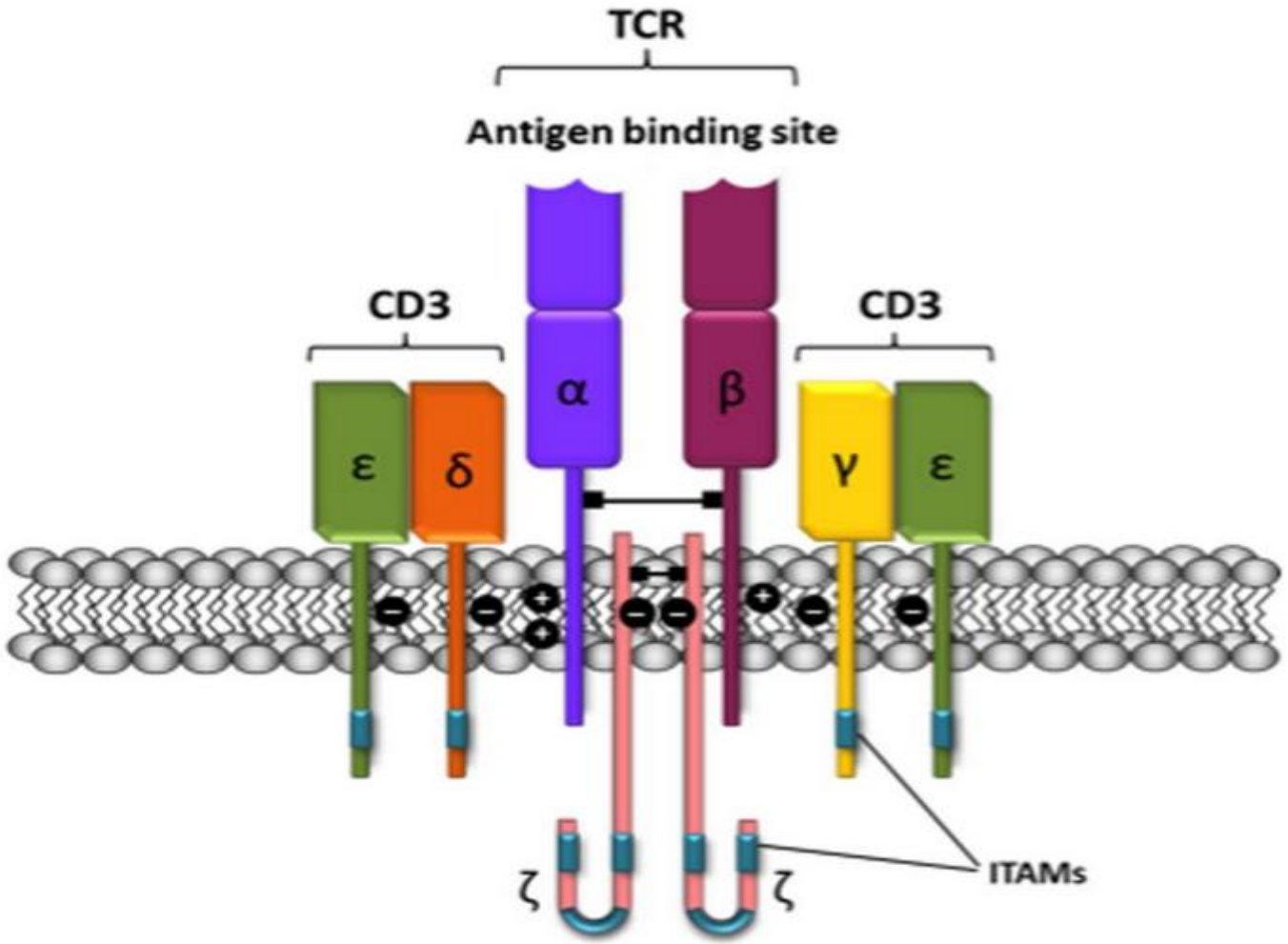
a) OKT3

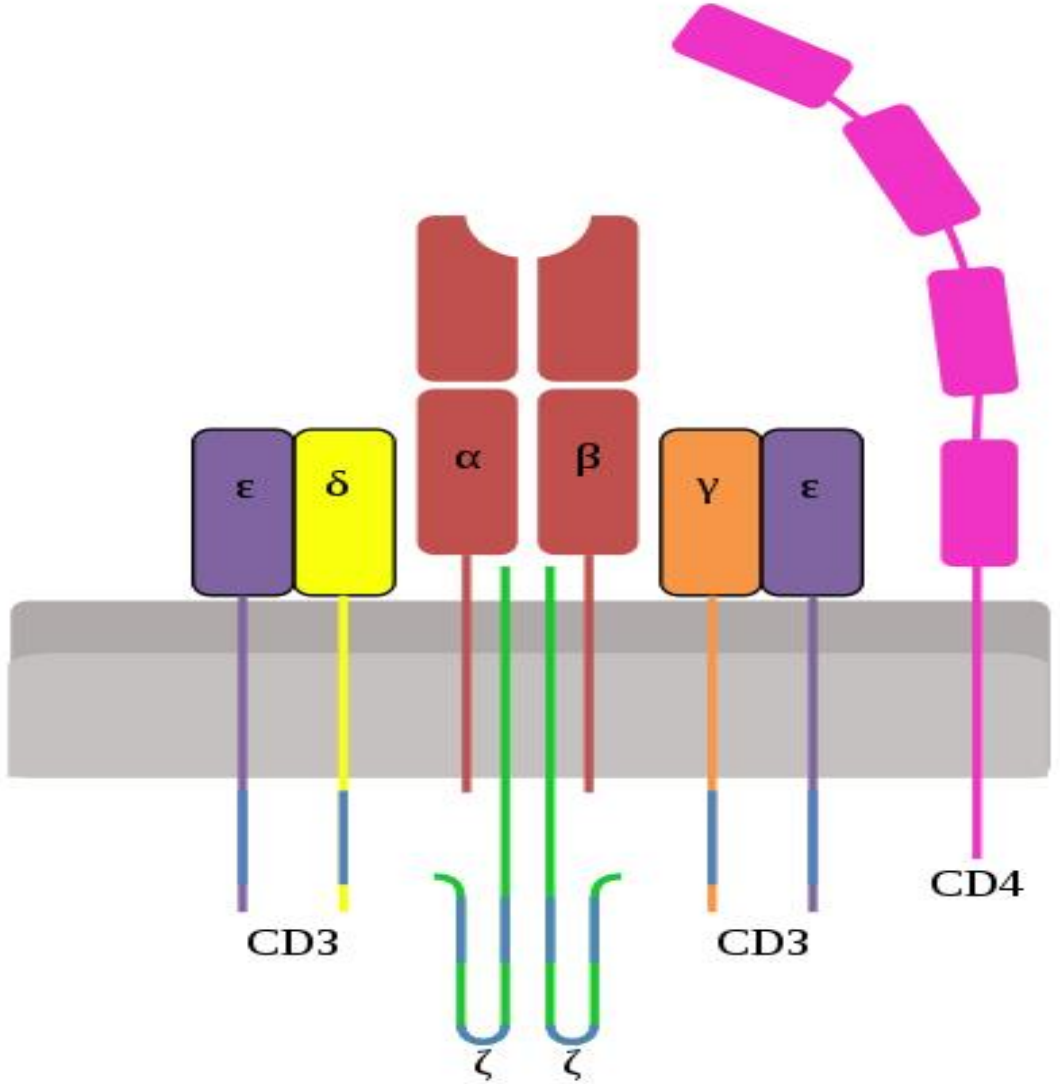
A mAb to the CD3 molecule of the TCR, called OKT3, and rapidly depletes mature T cells from the circulation. This depletion appears to be caused by binding of antibody-coated T cells to Fc receptors on phagocytic cells, which then phagocytose and clear the T cells from the circulation.

Another strategy is, a cytotoxic agent such as diphtheria toxin is coupled with the mAb. Antibody-bound cells then internalize the toxin and die.


Antigen presenting cell
HLA Class I
Peptide
Antigen
CDR 1,2,3
TCR α
TCR β
CD3ε
CD3δ
CD3γ
CD3ε
CD3ζ
T cell

TCR-CD3 complex majorly recognizes the Ag-MHC molecules




ε
δ
α
β
γ
ε
CD3
CD3
CD4
ζ
ζ

b) Rituximab

A mAb against CD20 (Rituximab) is used to deplete mature B cells and is aimed at suppressing AMR responses. CD20 is predominantly expressed on the surface of pre-B and mature B-lymphocytes, allowing rituximab to target and promote lysis in this specific type of cells

c) Because cytokines appear to play an important role in allograft rejection, these compounds can also be specifically targeted. Animal studies have explored the use of mAbs specific for the cytokines implicated in transplant rejection, particularly TNF-alpha, IFN-gamma, and IL-2.

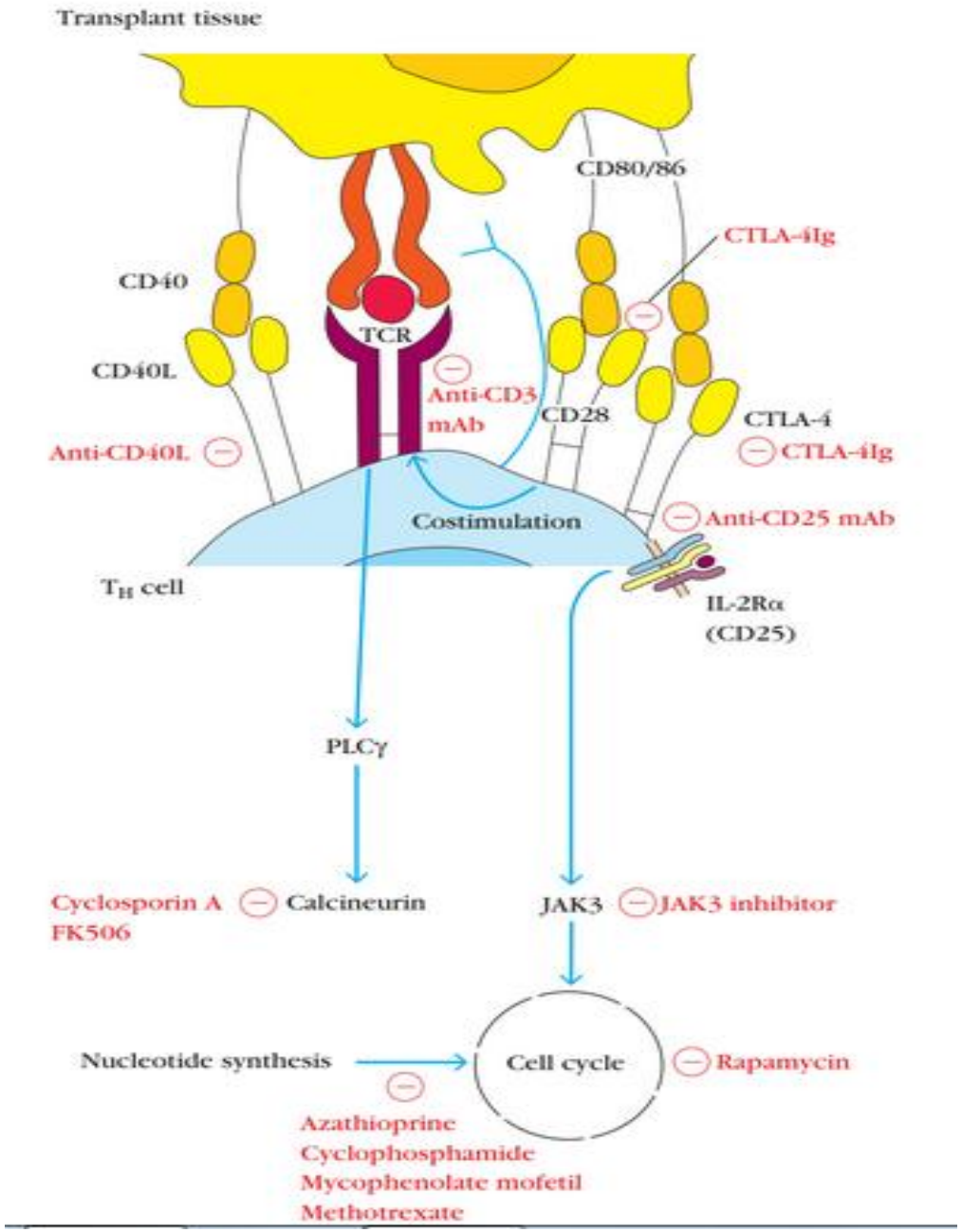
d) Belatacept

TH-cell activation requires a costimulatory signal in addition to the signal mediated by the TCR. The interaction between CD80 (B7-1)/86 (B7-2)on the membrane of APCs and the CD28 or CTLA-4 molecule on T cells provides one such signal. Without this costimulatory signal, antigen-activated T cells become anergic.

CD28 is expressed on both resting and activated T cells, while CTLA-4 is expressed only on activated T cells and binds CD80/86 with a 20-fold-higher affinity.

Belatacept, sold under the brand name Nulojix, is a fusion protein composed of the Fc fragment of a human IgG1 immunoglobulin linked to the extracellular domain of CTLA-4. Belatacept, was shown to induce anergy in T cells directed against the grafted tissue and has been approved by the FDA for prevention of organ rejection in adult kidney transplant patients.

Sites of action for various agents used in clinical transplantation



Transplantation Immunology

- What is transplantation?

  a surgical procedure in which an organ/s, tissue or a group of cells are removed from one person (the donor) and surgically transplanted into another person (the recipient), or moved from one site to another site in the same person.
- What is graft?

  a section of tissue or a complete organ that is removed from its original natural site and transferred to a new position in the same or in a separate individual.

Donor? , Recipient?

- Donor – living or dead
- Living – can donate one of the two kidneys, One of two lobes of their liver/part of liver, A lung or part of a lung, part of the pancreas, or part of the intestines
- People of all ages should consider themselves potential donors.
- Dead – Kidneys (2), Liver, Lungs (2), Heart, Pancreas, Intestines, Hands and Face, Eyes
- When a person dies, they are evaluated for donor suitability based on their medical history and age.
- Organs that cannot be donated by live donor- heart, brain
- Recipient –

  Who receives graft

- **How do I become an organ donor?**
- Individuals who wish to be organ donors should complete the following steps:
- You might join a donor registry. A registry is more than just an expression of interest in becoming a donor. It's a way to legally give consent for the anatomical gift of organs, tissue and eyes.
- You can also join the registry at any time
- For more information, go to www.lifebanc.org and click on donor registry. Donor registry information for any state might be obtained from www.donatelife.net.
- Sign and carry an organ donor card. This card can be downloaded at: www.organdonor.gov.
- Let your family members and loved ones know you'd like to be a donor.
- You might also want to tell your family healthcare provider, lawyer that you'd like to be a donor.

What One Donor Can Do

MEASURING THE IMPACT OF ORGAN & TISSUE DONATION

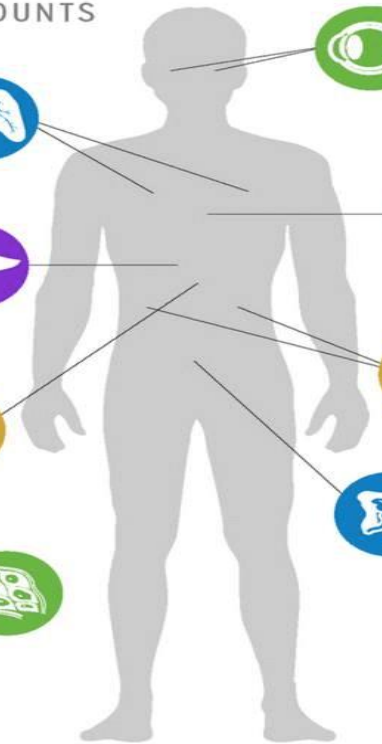
1 DONOR

=

8 life-saving **organs**

tissues & corneas that can improve **75 LIVES**

EVERY DONATION COUNTS



Lungs

3-year survival rate of **68%**

Liver

70% last 5 years or more

Pancreas

improve lives for an average of **10+ YEARS**

Tissues

1 IN 20 Americans will benefit from tissue transplants

Corneas

can help improve sight for **20 YEARS**

Heart

5-year survival rate of **70% OR MORE**

Kidneys

improve lives for an average **12-15 YEARS**

Intestines

NEARLY 3000 LIVES improved in the U.S. to date

DONATE LIFE®

REGISTER TO MAKE A DIFFERENCE TODAY **RegisterMe.org**

- Transplants can be for:
- organs – heart, kidney, liver, lung, pancreas, stomach and intestine.
- tissue – part of eye- cornea, bone, tendon (a strong, thin part inside body that joins a muscle to a bone), skin, pancreas islets, heart valves, nerves and veins.
- cells – bone marrow and stem cells.

- The first human kidney transplant, attempted in 1935 by a Russian surgeon
- It failed because a mismatch of blood types between donor and recipient caused almost immediate rejection of the kidney. It was hyperacute rejection.

- Finally, in 1954 a team in Boston headed by Joseph Murray performed the first successful human kidney transplant between identical twins
- 3 years later another transplant between non identical individual was performed.

- Today, the transfer of various organs and tissues between individuals is performed with ever-increasing frequency and rates of success, at least for their short-term survival.

- Immunosuppressive agents can delay or prevent rejection of transplanted organs, but they have side effects.

- New treatments that promise longer transplant survival and more specific tolerance to the graft without suppressing other immune function are under development.

- The following terms denote different types of transplants:

1) Autograft

is self tissue transferred from one body site to another in the same individual.

Examples include transferring healthy skin to a burned area in burn patients and using healthy blood vessels to replace blocked coronary arteries.

2) Isograft is tissue transferred between genetically identical individuals. This occurs in inbred strains of mice or identical human twins, when the donor and recipient are syngeneic (genetically similar).

3) Allograft is tissue transferred between genetically different members of the same species.

In mice this means transferring tissue from one strain to another, and in humans this occurs in transplants in which the donor and recipient are not genetically identical (the majority of cases).

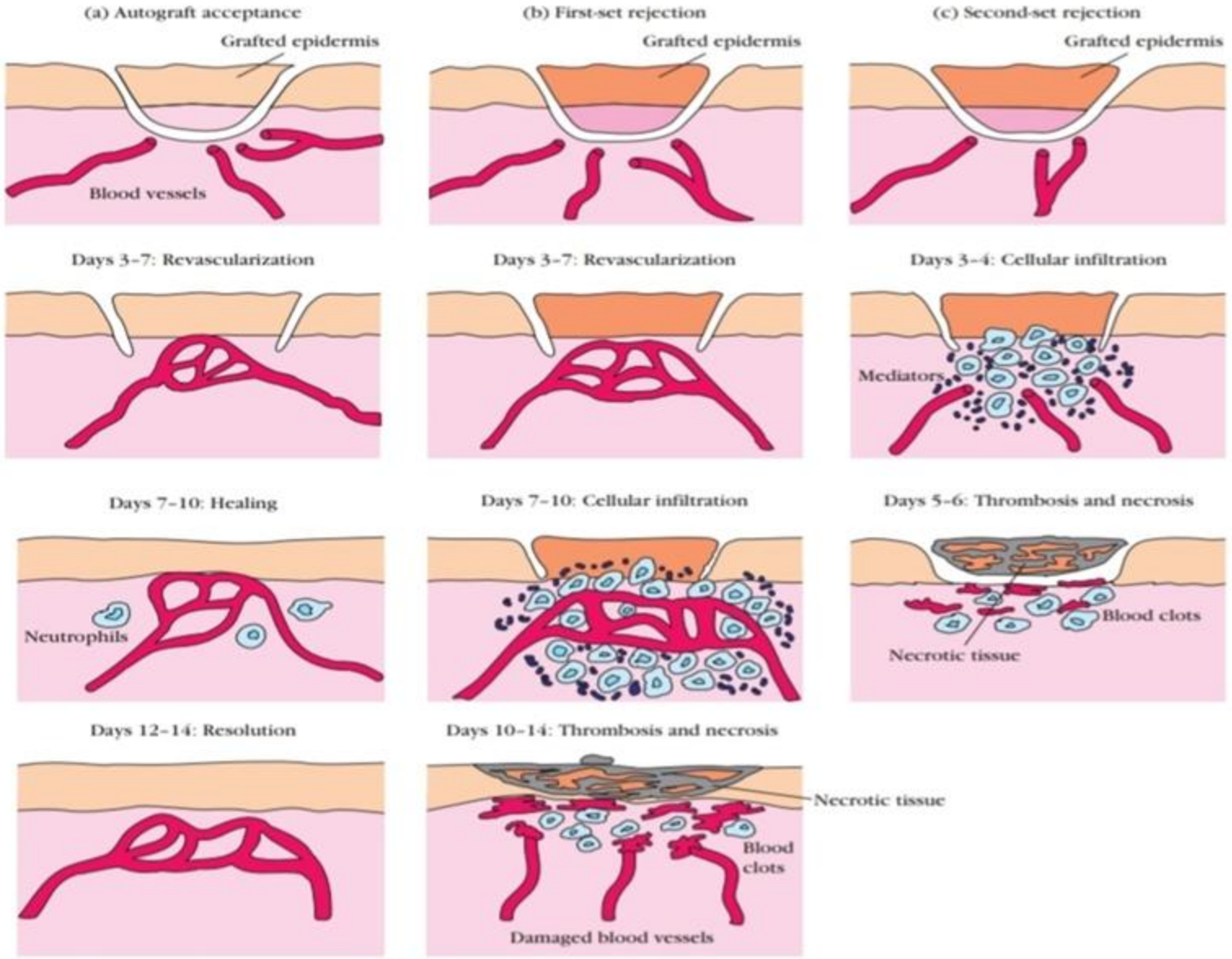
4) Xenograft is tissue transferred between different species
e.g., the graft of a baboon (Asian or African monkey) heart into a human).

Because of significant shortages of donated organs, raising animals for the specific purpose of serving as organ donors for humans is under serious consideration.

Graft	Procedure	Complications
Autograft	From self to self	No rejection concerns
Isograft	From identical twin to twin	Little concern of rejection
Allograft	From relative or nonrelative to individual	Rejection possible
Xenograft	From animal to human	Rejection possible

- Graft Rejection Occurs Based on Immunologic Principles

- The degree and type of immune response to a transplant varies with the type and source of the grafted tissue.



Schematic diagrams of the process of graft acceptance and rejection. (a) Acceptance of an autograft is completed within 12 to 14 days. (b) First-set rejection of an allograft begins 7 to 10 days after grafting, with full rejection occurring by 10 to 14 days. (c) Second-set rejection of an allograft begins within 3 to 4 days, with full rejection by 5 to 6 days. The cellular infiltrate that invades an allograft (b, c) contains lymphocytes, phagocytes, and other inflammatory cells.

- Autografts and isografts are usually accepted, owing to the genetic identity between donor and recipient.

- Because an allograft is genetically dissimilar to the host and therefore expresses unique antigens, it is often recognized as foreign by the immune system and is therefore rejected.

- Obviously, xenografts exhibit the greatest genetic and antigenic dissimilarity, hence show a vigorous graft rejection response.

- Cells in Graft Rejection
- Both CD4 and CD8-T cells
- Dendritic cells
- Tissues that share sufficient antigenic similarity, allowing transfer without immunologic rejection, are said to be histocompatible.

  This is the case when the transfer occurs between identical twins.

- Tissues that display significant antigenic differences are histoincompatible and typically induce an immune response that leads to tissue rejection.

- Role of Blood Group and MHC Antigens in Graft Tolerance
- The most intense graft rejection reactions are due to differences between donor and recipient in ABO blood-group and MHC antigens.

1) ABO Blood gr antigens

- The blood-group antigens are expressed on RBCs, epithelial cells, and endothelial cells, requiring the donor and recipient to first be screened for ABO compatibility.
- If the recipient carries antibodies to any of these antigens, the transplanted tissue will induce rapid antibody-mediated lysis of the incompatible donor cells.
- For this reason, most transplants are conducted between individuals with a matching ABO blood group.

	Blood gr A	Blood gr B	Blood gr AB	Blood gr O
RBC type	A	B	AB	O
Ab’s	B	A	-	A and B
Ag	A	B	A and B	-

2) MHC compatibility

- The MHC variation in the human population is high
- Testing for this is called cross-matching, and is the most important level of compatibility testing that occurs prior to solid organ transfer; a positive cross-match means that the recipient has antibodies against HLA proteins carried by the donor.

- The most common method used today is the Luminex assay, which employs fluorochrome-labeled microbeads impregnated with specific HLA proteins

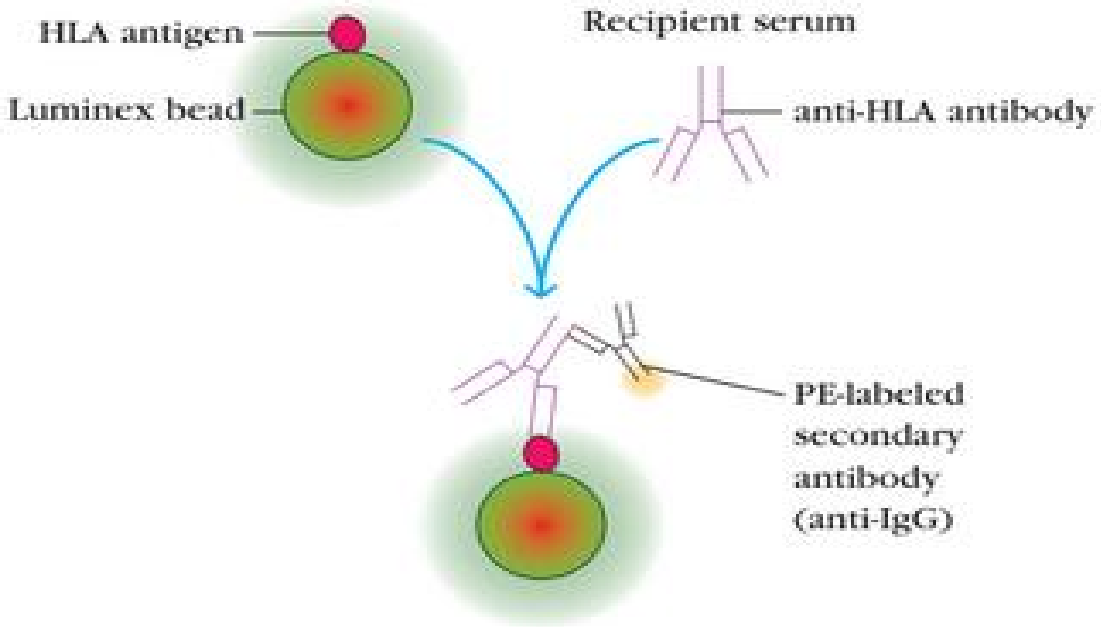


FIGURE 16-13 The Luminex cross-matching assay. Microbeads impregnated with fluorochromes of different intensity each carry a different HLA protein. Recipient serum is incubated with these beads, and any antibody binding is detected using *phycoerythrin* (PE)-labeled secondary anti-human immunoglobulin. Laser excitation and detection are used to determine the fluorochrome intensity of bound beads, and therefore the associated HLA molecule(s) with which the serum reacts. *[B. D. Tait, 2009, Luminex tech-*

	Transplant Rejection		
	Type	Onset	Mechanism & Vessel Histology
"Host vs. Graft"	Hyperacute	Immediate	**Preformed antibodies** directed against the donor tissue. Caused by accidental ABO **blood type incompatibility** which is very rare. Presents while still in surgery with **thrombosis and occlusion** of graft vessels
	Acute	Weeks to months	T-Cell mediated immune response directed against the foreign MHC. Inflammation and **leukocyte infiltration of graft vessels** results. Most common type.
	Chronic	Months to years	T-Cell mediated process resulting from the foreign MHC "looking like" a self MHC carrying an antigen. Results in **intimal thickening and fibrosis of graft vessels** as well as graft atrophy

Hyperacute rejection – Ab mediated rejection

- Hyperacute rejection reactions typically occur within the first 24 hours after transplantation
- Careful cross matching can avoid most cases of hyperacute rejection.
- Hyperacute reactions are caused by preexisting host serum antibodies specific for antigens of the graft and have the greatest impact in highly vascularized grafts (such as kidney and heart).
- Preexisting recipient antibodies bind to HLA antigens on the endothelial cells of the graft . These antigen-antibody complexes activate the complement system and result in an intense accumulation of neutrophils. It results into inflammatory reaction which causes endothelial damage and obstructing blood clots within the capillaries, preventing vascularization of the graft

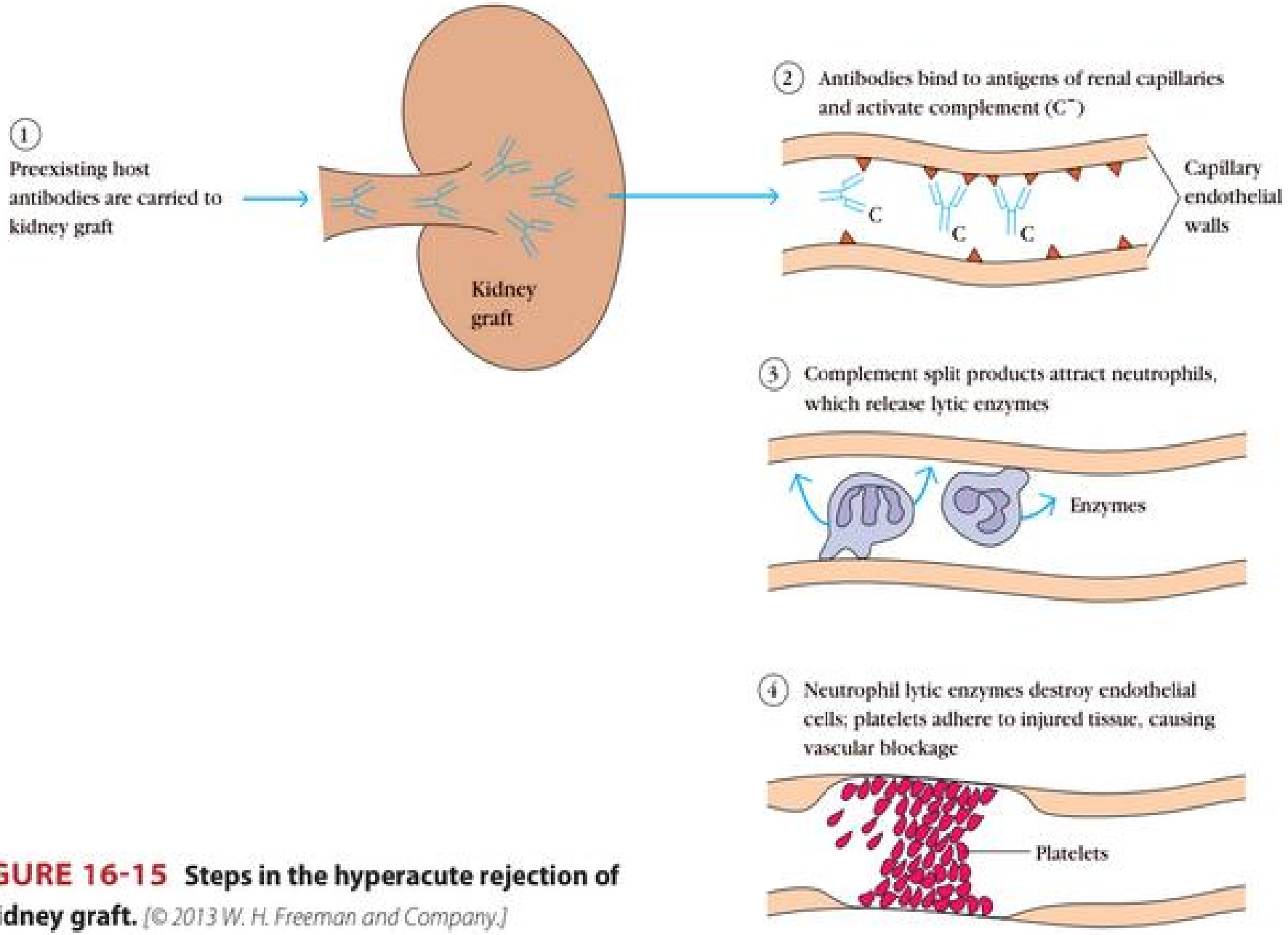


FIGURE 16-15 Steps in the hyperacute rejection of a kidney graft. *[© 2013 W. H. Freeman and Company.]*

Acute rejection – T cell mediated rejection

Acute rejection reactions usually begin in the first few weeks after transplantation

Cell-mediated allograft rejection manifests as an acute rejection of the graft beginning about 7 to 10 days after transplantation.

Histopathologic examination reveals a massive infiltration of macrophages and lymphocytes at the site of tissue destruction, suggestive of TH-cell activation and proliferation.

Chronic rejection

Chronic rejection reactions can occur from months to years after transplantation.

The mechanisms include both humoral (Ab)and cell-mediated (T cell) responses by the recipient.

Histoplasmosis

Systemic Mycosis

Primarily an intracellular infection of the reticuloendothelial system

Mostly asymptomatic/ relatively mild, self limiting pulmonary infection

Epidemiology- Distribution- worldwide, most common in endemic areas of North, Central, and South America, Africa and Asia. Histoplasmosis isn't contagious, so it can't be spread from person to person. If you've had histoplasmosis, you can get it again. However, if you do get it again, the illness will likely be milder the second time.

Causative agent

Dimorphic fungus- *Histoplasma capsulatum*

Two varieties -

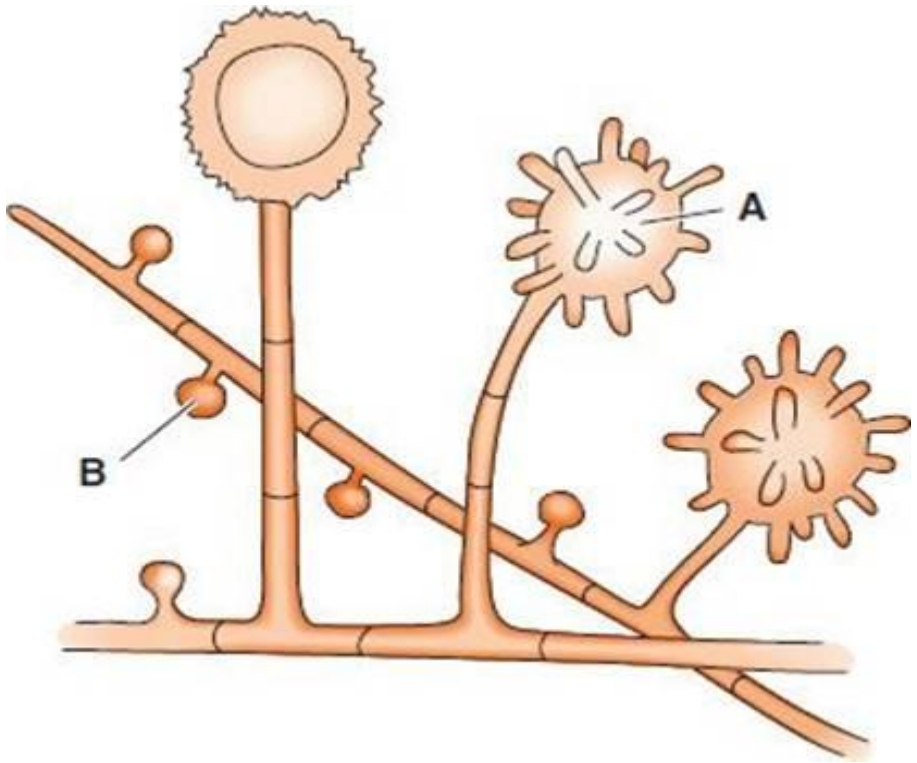
Histoplasma capsulatum var *capsulatum* – in most endemic areas

Histoplasma capsulatum var *duboisii* - in Africa

- Habitat- soil enriched with the dropping of birds, chickens, or bats. It's particularly common in chicken and pigeon coops, old barns, caves, and parks.
- In soil- grows well as septate mycelium
- In culture media- as septate mycelium
- In animal tissue- as yeast
- Temp- 25-30◦C
- In mycelial phase produce 2 types of asexual spores

(1) tuberculate macroconidia, with typical thick walls and finger like projections that are important in laboratory identification; and (2) microconidia, which are smaller, thin, smooth-walled spores that, if inhaled, transmit the infection.

Asexual spores of *Histoplasma capsulatum*. A: Tuberculate macroconidia. B: Microconidia.

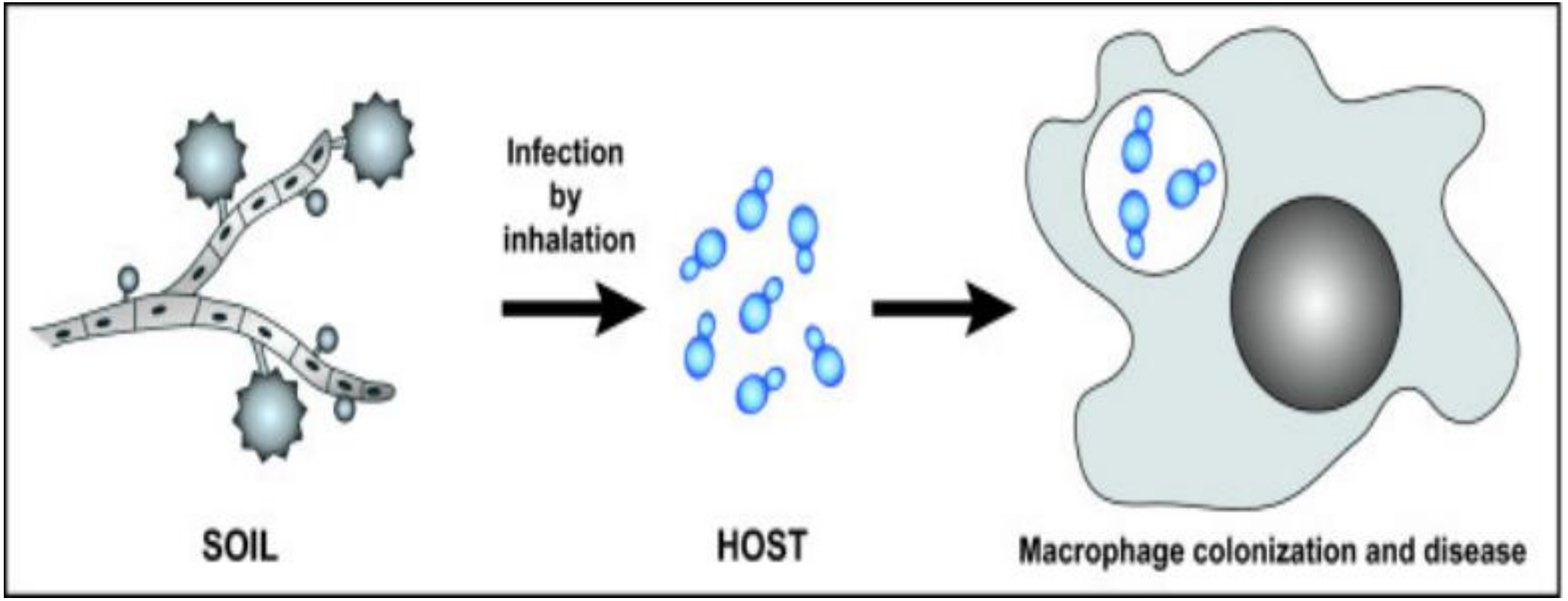


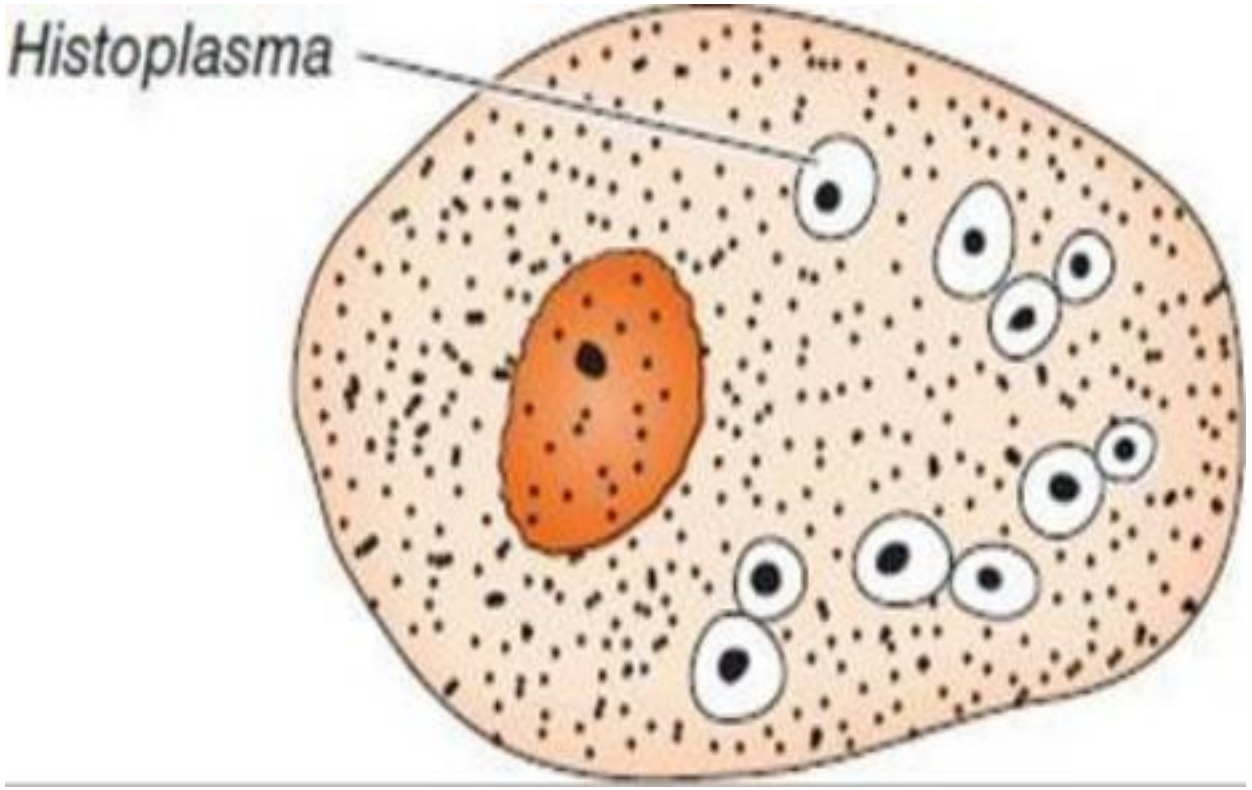
Pathogenesis

- Portal of entry- by inhalation of microconidia or hyphal fragments. Spores float into the air when dirt or other material is disturbed.

- Travel in respiratory tract and finally reach at alveolar macrophages

- In macrophages, microconidia transforms into budding yeast cells, yeasts behave as facultative intracellular organisms (i.e. they can survive and proliferate inside phagocytic cells).


Infection
by
inhalation
SOIL
HOST
Macrophage colonization and disease


Histoplasma

- The chances of developing histoplasmosis symptoms increase with the number of spores you inhale. People more likely to be exposed include:

- Farmers
- Pest control workers
- Poultry keepers
- Construction workers
- Gardeners
- Cave explorers
- Demolition workers

- In persons with normal immunity (immunocompetent), most infections are asymptomatic, form granulomas which undergo fibrosis and calcification

- Primary infection leads to development of immunity

- In patients with impaired immunity, histoplasmosis may spread to other organs of reticuloendothelial system. Eg. Bone marrow, liver, spleen

Examples-

- HIV or AIDS
- Cancer chemotherapy
- Corticosteroid drugs, such as prednisone
- Tumor necrosis factor inhibitors, often used to control rheumatoid arthritis
- Medications that prevent rejection of organ transplants

Clinical syndrome

- Types are

1. Acute pulmonary histoplasmosis
   self limited illness characterized by fever and non productive cough

2. Chronic pulmonary disease
   mostly found in adults with formation of cavities in the lung- due to primary lesion or reactivation of apparently healed old lesion. Signs of chronic histoplasmosis include weight loss and a bloody cough.

3. Disseminated disease – most severe type

dissemination is rare, occasionally spread to reticuloendothelial system/ any organ of the body – in old persons, infants with underlying disease or immunosuppression. It can affect nearly any part of your body, including your mouth, liver, central nervous system, skin. If untreated, disseminated histoplasmosis is usually fatal.

4. Ocular histoplasmosis

is seen in endemic areas

a hypersensitivity reaction and not true retinal infection.

Symptoms

- The mildest forms of histoplasmosis cause no signs or symptoms. But severe infections can be life-threatening. When signs and symptoms occur, they usually appear 3 to 17 days after exposure and can include:
- Fever
- Chills
- Headache
- Muscle aches
- Dry cough
- Chest pain
- Tiredness

Lab diagnosis

- Specimen-

  Sputum, blood, bone marrow, scrapings from lesions

  Biopsy material from pulmonary disease or from sites of lesion

1. Microscopy- smear (sputum, blood, bone marrow, scrapings from lesions) stained by Wright or Giemsa stains and observed microscopically

   H. capsulatum appears as small oval yeast cells (2-3 x 3-4 micrometer) packed within macrophages or monocytes.

   Blood and bone marrow smears may also be positive in persons suffering from AIDS

2. Cultural examination-

   Sabouraud's dextrose agar/ brain heart infusion agar with cycloheximide or chloramphenicol – 25◦C – 2- 6 weeks – white cottony mycelial growth

   At 37◦C – small, oval yeast forms appear that produce whitish tan colonies.

3. Histoplasmin skin test - The histoplasma (An allergen (antigen) of mycelial phase of *Histoplasma capsulatum* is used to check if you have been exposed to a fungus *Histoplasma capsulatum*

How to perform? –

- Clean an area of skin, usually the forearm. An allergen is injected just below the cleaned skin surface.
- An allergen is a substance that causes an allergic reaction.
- The injection site is checked at 24 hours and at 48 hours for signs of a reaction. Occasionally, the reaction may not appear until the fourth day.
- No reaction (inflammation) at the site of the test is normal.
- A reaction (inflammation) means you have been exposed to *Histoplasma capsulatum (past/present infection)*. It does not mean you have an active infection and also does not differentiate active and past infection.
- This test is rarely used today. It has been replaced by a variety of blood and urine tests.

3. Serological test –

CFT, Precipitation, Latex agglutination tests are useful

Antigen used – Histoplasmin or killed whole yeast cells

In immunocompromised patients with disseminated disease, tests for *Histoplasma* antigen in the urine are especially useful because antibody tests may be negative.

4. Histopathology test –

Histopathology is the study of the signs of the disease using the microscopic examination of a biopsy or surgical specimen that is processed and fixed onto glass slides. To visualize different components of the tissue under a microscope, the sections are dyed with one or more stains. Gomori methenamine silver (GMS) and periodic-acid Schiff (PAS) are the two most commonly used broad-spectrum fungal stains in routine histopathology practice. These stains help to distinguish fungi based on morphologic characteristics such as size, type of budding, presence of hyphae, and branching. *Histoplasma capsulatum* can be seen in yeast form within mononuclear cells after staining with above stains.

Treatment

- For some people, the symptoms of histoplasmosis will go away without treatment.
- However, antifungal medication is needed to treat severe histoplasmosis in the lungs, chronic histoplasmosis, and infections that have spread from the lungs to other parts of the body (disseminated histoplasmosis).
- Itraconazole is one type of antifungal medication that’s commonly used to treat histoplasmosis.
- Depending on the severity of the infection and the person’s immune status, the course of treatment can range from 3 months to 1 year.

Prevention and control

- It can be difficult to avoid breathing in Histoplasma in areas where it's common in the environment.
- In areas where Histoplasma is known to live, people who have weakened immune systems (for example, by HIV/AIDS, an organ transplant) should avoid doing activities that are known to be associated with getting histoplasmosis, including

➢ Disturbing material (for example, digging in soil or chopping wood) where there are bird or bat droppings

➢ Cleaning chicken coops

➢ Exploring caves

➢ Cleaning, remodelling, or tearing down old buildings

- Large amounts of bird or bat droppings should be cleaned up by professional companies that specialize in the removal of hazardous waste.

Special microbial metabolites and their applications in health care– Lovastatin, Daunorubicin

LOVASTATIN

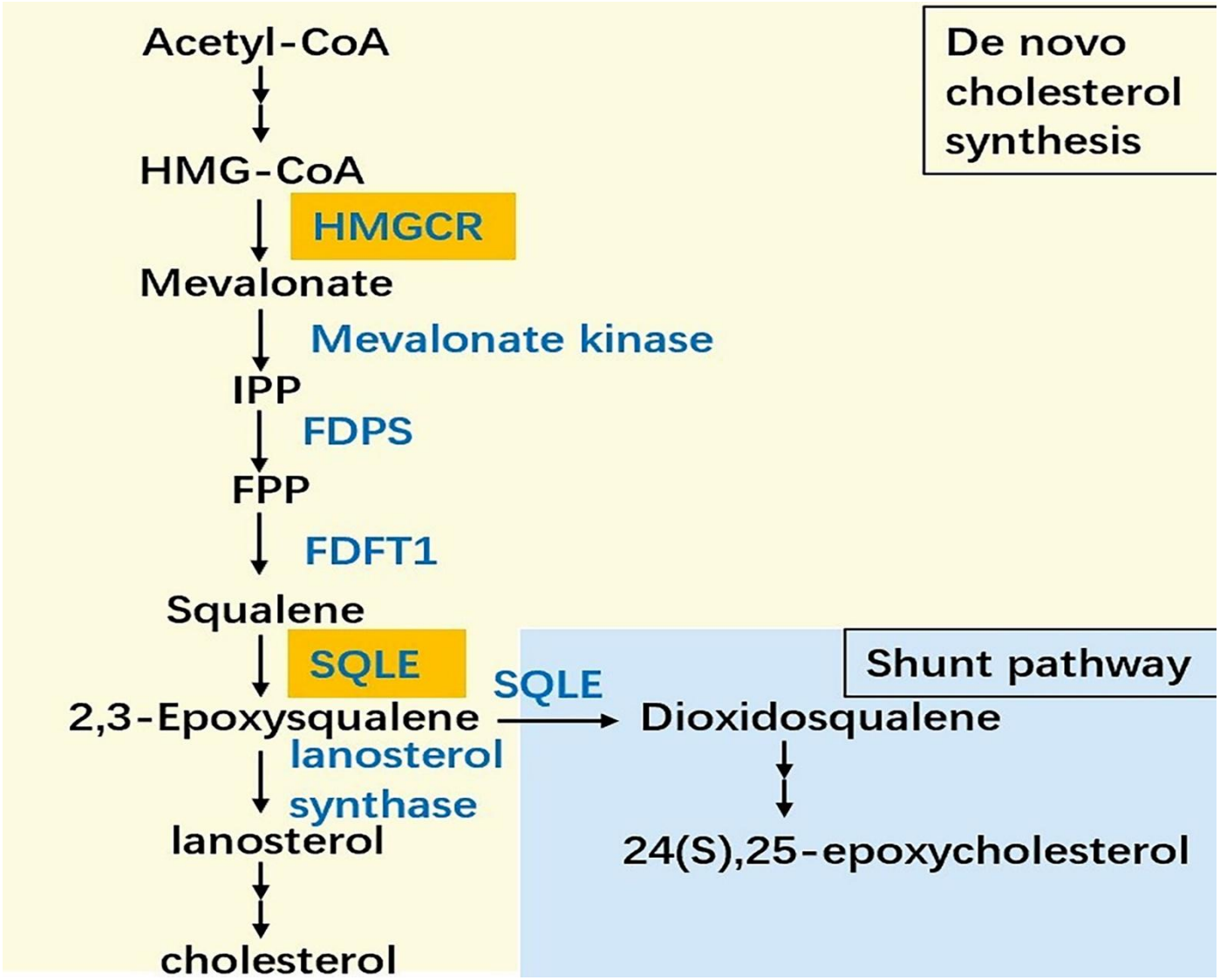
- Commercially lovastatin is produced by a variety of filamentous fungi including Penicillium species, Monascus ruber and Aspergillus terreus as a secondary metabolite.

- Production of lovastatin by fermentation decreases the production cost compared to costs of chemical synthesis.

- Lovastatin is an effective competitive inhibitor of the enzyme hydroxymethylglutaryl coenzyme A (HMG-CoA) reductase (mevalonate: NADP1 oxidoreductase) that catalyzes the reduction of HMG-CoA (3-hydroxy-3-methyglutaryl coenzyme A) to mevalonate during synthesis of cholesterol

- Act as substrate analogue of substrate (HMG-CoA) of HMG-CoA reductase

- HMG-CoA reductase is an important enzyme that can regulate the synthesis of cholesterol in cells


De novo cholesterol synthesis
Acetyl-CoA
HMG-CoA
HMGCR
Mevalonate
Mevalonate kinase
IPP
FDPS
FPP
FDFT1
Squalene
SQLE
2,3-Epoxysqualene
lanosterol synthase
lanosterol
cholesterol
SQLE
Shunt pathway
Dioxidosqualene
24(S),25-epoxycholesterol

- Cholesterol is an important sterol (a type of lipid) that is biosynthesised in all animal cells.
- It also forms an essential component of the animal cell membrane.
- It acts as a precursor for the synthesis of bile acid, steroid hormones and vitamin D.
- Cholesterol is synthesised in hepatic cells of the liver in large amounts. The synthesis takes place in the cytosol and endoplasmic reticulum of the hepatocytes.
- Cholesterol is totally absent in prokaryotes, one exception being *Mycoplasma* which requires cholesterol to grow.

- Cholesterol can be found in our body from two different sources: it is either synthesised de novo in our cells or is taken in the form of dietary cholesterol from different food sources. The de novo synthesis of cholesterol in our cells is enough for our bodily requirements, and there is no need to take cholesterol in dietary supplements.

- Lovastatin, is a lipid-lowering drug and fungal metabolite derived synthetically from a fermentation product of Aspergillus terreus.
- lovastatin belongs to the statin class of medications
- used to lower the risk of cardiovascular disease and manage abnormal lipid levels by inhibiting the endogenous production of cholesterol in the liver

- Minimal side effects or long term effects has resulted in this class becoming one of the most widely prescribed medications
- Take this medication by mouth as directed by your doctor, usually once daily with your evening meal. Some patients may be directed to take this medication twice daily.
- Dosage is based on your medical condition, response to treatment, age, and other medications you may be taking

BIOMEDICAL APPLICATIONS OF LOVASTATIN

- Lovastatin is used together with diet, weight-loss, and exercise to reduce the risk of heart attack and stroke and to decrease the chance that heart surgery will be needed in people who have heart disease or who are at risk of developing heart disease. Lovastatin is also used to decrease the amount of cholesterol (a fat-like substance) and other fatty substances in the blood.
- It works by slowing the production of cholesterol in the body to decrease the amount of cholesterol that may build up on the walls of the arteries and block blood flow to the heart, brain, and other parts of the body.
- Accumulation of cholesterol and fats along the walls of your arteries (a process known as atherosclerosis) decreases blood flow and, therefore, the oxygen supply to your heart, brain, and other parts of your body. Lowering your blood level of cholesterol and fats with lovastatin may help prevent heart disease, angina (chest pain), strokes, and heart attacks.

Daunorubicin

- The antibiotic daunorubicin is prepared by
- 1. aerobically cultivating Streptomyces bifurcus, strain DS 23,219 (NRRL 3539), or a daunorubicin-producing mutant thereof
- 2. **Streptomyces peucetius subsp. caesius**
- Daunorubicin is in a class of medications called **anthracyclines**. It works by slowing or stopping the growth of cancer cells in your body.

- **Daunorubicin**, also known as **daunomycin**, is a chemotherapy medication used to treat cancer. Specifically it is used for acute myeloid leukemia (AML) (cancer of white blood cells), acute lymphoblastic leukemia (ALL) (cancer of white blood cells), chronic myelogenous leukemia (CML) (cancer of white blood cells), and Kaposi's sarcoma (skin cancer)
- It is administered by injection into a vein

- It slows or stops the growth of cancer cells in the body. Treatment is usually performed together with other chemotherapy drugs (such as cytarabine), and its administration depends on the type of tumor and the degree of response.
- In addition to its major use in treating AML, daunorubicin is also used to treat neuroblastoma (cancer of nerve tissue). Daunorubicin has been used with other chemotherapy agents to treat the blastic phase of chronic myelogenous leukemia.
- Daunorubicin is also used as the starting material for semi-synthetic manufacturing of doxorubicin, epirubicin and idarubicin.

- It acts by intercalating between the DNA base pairs, causing the DNA double helix to uncoil and inhibit the topoisomerase II enzyme, resulting in single and double-strand breaks, thus inhibiting DNA and RNA synthesis.

Streptococcus mutans

- facultative anaerobic,
- gram-positive coccus
- mesophilic and grow at temperatures between 18-40 degrees Celsius
- commonly found in the human oral cavity
- significant contributor to tooth decay

Role in disease

- **Tooth decay**
- Metabolizing sucrose to lactic acid
- The acidic environment created in the mouth by this process is what causes the highly mineralized tooth enamel to be vulnerable to decay.
- *S. mutans* uses the enzyme glucansucrase to convert sucrose into a sticky, extracellular, dextran-based polysaccharide that allows them to adhere, forming plaque.

- Early colonizers of the tooth surface are mainly *Neisseria* spp. and streptococci, including *S. mutans*.
- They must withstand the oral cleansing forces (e.g. saliva and the tongue movements) and adhere sufficiently to the dental hard tissues.
- The growth and metabolism of these pioneer species changes local environmental conditions (e.g., Eh, pH, and substrate availability), thereby enabling more fastidious organisms to further colonize after them, forming dental plaque.

- *S. mutans* produces dextran via the enzyme dextransucrase (a hexosyltransferase) using sucrose as a substrate in the following reaction:
- n sucrose $\rightarrow$ $(\text{glucose})_n$ + n fructose sucrose is the only sugar that bacteria can use to form this sticky polysaccharide
- However, other sugars—glucose, fructose, lactose—can also be digested by *S. Mutans* but they produce lactic acid as an end product.
- The combination of plaque and acid leads to dental decay.

- Infectious Dose
- Colony Forming Units CFU *S. mutans* /ml saliva
- class 0-1 <100,000
- class 2 100,000 - 1,000,000
- class 3 >1,000,000

- class 0-3 is referring to how many *Streptococcus mutans* reside in the mouth with class 0-1 acting as best case with good oral hygiene, while class 3 acts as the worst case.

Cadiovascular disease

- *Streptococcus mutans* has been associated with bacteraemia and infective endocarditis (IE)
- IE is divided into acute and subacute forms, and the bacterium is isolated in subacute cases
- The common symptoms are: fever, chills, sweats, anorexia, weight loss, and malaise

Symptoms

- chalky white spot on the surface of the tooth
- it can turn brown and will eventually result in a cavity
- once the *Streptococcus mutans* forms the cavity, the tooth structure is lost and cannot be regenerated
- The affected area of the tooth changes color and becomes sensitive.
- When the decay passes through the enamel, the dentin tubules allow passages to the nerves making the tooth exposed, which results in pain that temporarily worsens with exposure to heat, cold, sweet food or drinks.
- In addition, bad breath and foul tastes, and gum disease are other symptoms.

Diagnosis

- Clinical sample – Saliva
- Mitis- Salivarius agar and tryptone, yeast extract, cystine (TYC) agar in which antibiotic Bacitracin is added is used for isolation of *S. mutans*
- X-Rays of the mouth to examine the teeth as well as the gums.
- Diagnostic kit

Prophylaxis

- Practice of good oral hygiene including daily brushing, flossing and the use of appropriate mouthwash can significantly reduce the number of oral bacteria, including *S. mutans* and inhibit their proliferation.
- *S. mutans* often live in dental plaque, hence mechanical removal of plaque is an effective way of getting rid of them.
- Brushing twice daily can help decrease the caries risk.
- Reduce sugar intake

Specific prophylaxis

- *S. mutans* secretes Glucosyltransferase on its cell wall, which allows the bacteria to produce polysaccharides from sucrose. These sticky polysaccharides are responsible for the bacteria's ability to aggregate with one another and adhere to tooth enamel, i.e. to form biofilms.
- Use of Anti Cell-Associated Glucosyltransferase (Anti-CA-gtf) Immunoglobulin Y disrupts *S. mutans*' ability to adhere to the teeth enamel, thus preventing it from reproducing. Studies have shown that Anti-CA-gtf IgY is able to effectively and specifically suppress *S. mutans* in the oral cavity.

Treatment

- Treatment for tooth decay depends on how severe the decay is.

1) Fluoride ions - detrimental to bacterial cell metabolism.

Fluoride directly inhibits-

- Adherence of bacteria

- glycolytic enzymes and H+ATPases. Fluoride ions also lower the pH of the cytoplasm. This means there will be less acid produced during the bacterial glycolysis

2) Various other natural remedies have been suggested or studied to a degree, including tea tree oil, macelignan (found in nutmeg), curcuminoids (the main components of turmeric), and eugenol (found in bay leaves, cinnamon leaves and cloves).

3) To fix cavities caused by the bacterial strain *Streptococcus mutans* a dentist will need to adequately perform either a composite filling or an amalgam filling

Composite filling-

made from a mixture of plastic and fine glass particles.

Advantages-

- filling closely matches the color of the tooth
- composite fillings are chemically bonded to the tooth they provide better support
- Disadvantages-
- wear out sooner- last for 5 years
- increased chair time because the process to apply the filling is longer
- Additional visits are required,
- Expensive

- Amalgam fillings

made from a mixture of silver, tin, zinc, copper and mercury.

Aadvantages

- Durability- more durable than strength and expense. composite fillings, and do not need to be replaced for approximately 10-15 years.
- Strong since they can withstand the chewing forces
- less expensive than composite fillings.

Disadvantage

- cause: discoloration, cracks and fractures, allergic reactions, and destroys more tooth structure.
- These fillings also have poor aesthetics (attractive) since the silver fillings are not the color of one's natural tooth.

- In case of extreme tooth decay a root canal or crown may be used.
- The dentist will choose the root canal procedure when the decayed tooth is deep and has reached the pulp of the tooth, which is the center of the tooth where the nerves are
- A crown should also be installed if the tooth decay has traveled deep under the gum line.
- In most extreme case – extraction of the tooth.

- A root canal treatment is a dental procedure to remove inflamed or infected pulp on the inside of the tooth which is then carefully cleaned and disinfected, then filled and sealed.
- Root canal treatment is designed to eliminate bacteria from the infected root canal, prevent reinfection of the tooth and save the natural tooth.
- A dental crown is a tooth-shaped cap that restores a decayed, broken, weak or worn-down tooth. Dentists also use crowns to cover dental implants and root canal-treated teeth.
- Made from a variety of materials, including metal, resin and porcelain, crowns last between five and 15 years with proper care

Congenital Thymic aplasia

DiGeorge syndrome is also known as congenital thymic aplasia.

- Thymic hypoplasia is a condition in which the thymus is underdeveloped or involuted.
- Thymic aplasia is congenital absence of the thymus.

  Cause of thymic aplasia-

It occurs due to the deletion of chromosome number 22

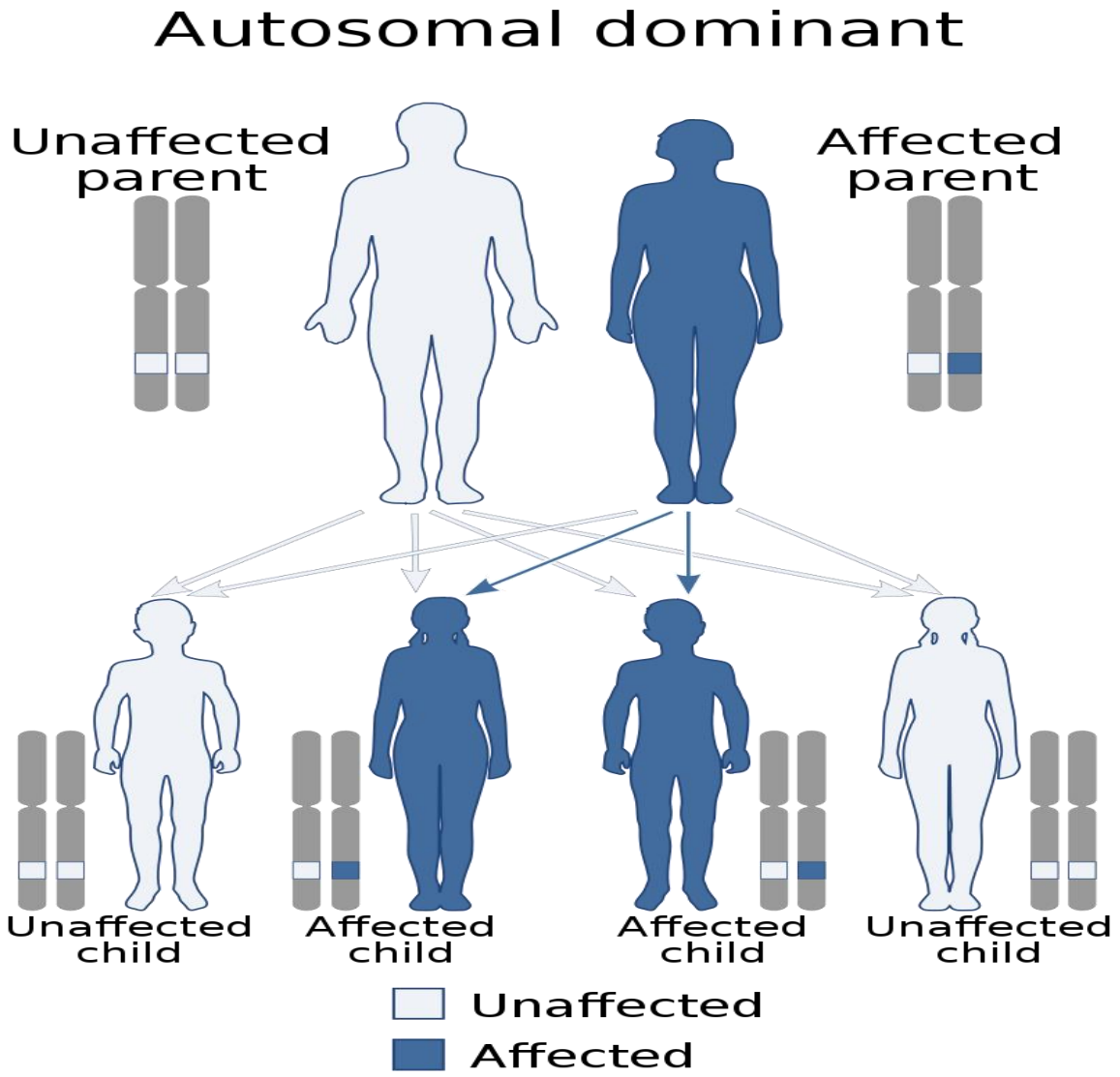
Complete lack of thymus gland occurs when it becomes more severe.

A missing thymus is a sign of DiGeorge syndrome.

- The thymus gland is a very unique organ that is at its largest in children and shrinks away as the body grows older.
- It is about 2.5 to 5 cms wide, 4 to 6 cms long and 1 cm thick at birth. At its largest instance, (viz. during puberty) the thymus weighs just under 30 grams.
- After puberty, the thymus slowly starts to shrink and by the age of 75, it is nothing more than fatty tissue.
- One of the most important functions of the thymus is to stimulate the production of very specialized cells called T-cells (also called T-lymphocytes).
- Being born without a thymus makes you more susceptible to infections.

- A missing thymus is a sign of DiGeorge syndrome.
- DiGeorge syndrome can impact multiple body systems.
- DiGeorge syndrome is a genetic condition caused by a missing piece of chromosome 22.
- Another name for DiGeorge syndrome is 22q11.2 deletion syndrome (Each chromosome holds thousands of genes. The term “22q11.2” gives the specific location on the chromosome where genes are missing - deletion of part of the long arm (q) of chromosome 22, region 1, band 1, sub-band 2 (22q11.2)).
- Approximately 80-90% of patients have a deletion of 3 Mb and 8% have a deletion of 1.5Mb. The number of genes affected by the deletion has been cited as approximately 30 to 50
- It can affect the function of your immune system and heart, and it can delay development during childhood.

- The disorder has an autosomal dominant inheritance pattern.



Prevalance

- DiGeorge syndrome can affect anyone since 90% of cases occur as a result of a random deletion on chromosome 22.
- This happens when the egg and sperm meet in the early stages of fetal development.
- The rate of occurrence is unpredictable.
- DiGeorge syndrome isn’t common.
- About 1 in 4,000 people in the U.S. receive a 22q11.2 deletion syndrome diagnosis annually.
- Some studies suggest that this estimate could be more common since the condition is underdiagnosed.

Symptoms

- Can range from mild to severe
- Vary from person to person.
- In some people asymptomatic
- Can affect several parts of your body.
- The classic features are problems with the heart, and decreased calcium levels.

1. Heart problem

Babies born with abnormalities in heart structure

Enough oxygen is not transported to entire due to abnormalities in heart's structure.

2. Affects function of brain

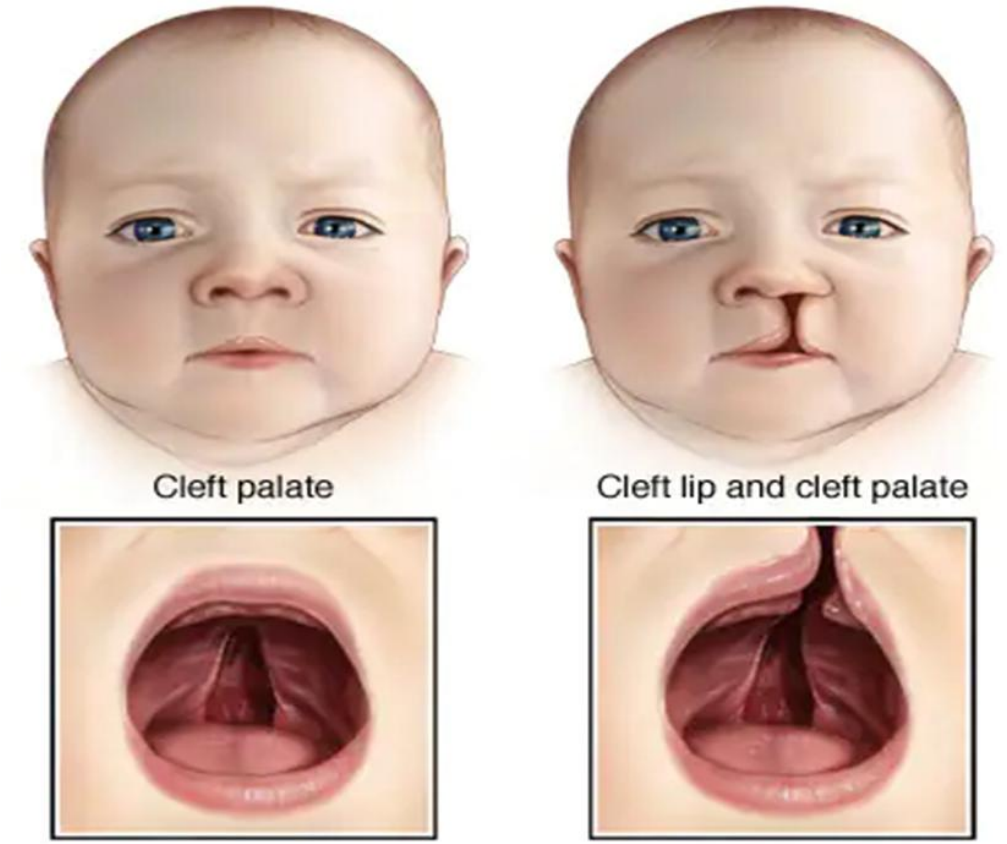
Anxiety (nervousness, panic and fear as well as sweating and a rapid heartbeat), Schizophrenia, Seizures

3. Immune system disorder

High risk of getting an infection

- A deletion of part of chromosome 22 can cause physical symptoms that include:
- Hearing loss
- Spine (the bones of the back) curvature (scoliosis).
- Vision loss
- Breathing difficulties.
- Lower-than-normal levels of calcium in your blood (hypocalcemia).
- Kidney structure and function abnormalities
- Endocrine system (hormone) function abnormalities.
- Feeding difficulties during infancy.

- **Facial features**
- DiGeorge syndrome can also cause unique facial features that include:
- Hooded eyelids (excess skin folds down from the eye brow line).
- Flat cheeks.
- A prominent or bulbous nose.
- An underdeveloped chin.
- Ears with attached lobes.
- Cleft lip and palate.



Diagnosis

- DiGeorge syndrome can be detected prenatally based upon prenatal ultrasound results and amniocentesis.
- (Amniocentesis is a prenatal test that can diagnose genetic disorders and other health issues in a foetus. A small amount of amniotic fluid is removed from inside uterus, and it is tested for specific conditions).
- Or soon after child is born tests are done if infant is born with DiGeorge syndrome or blood tests that show low calcium levels.

- Tests-
- Genetic testing: a small sample of blood from new born is tested to detect genetic abnormalities.
- Imaging tests: X-ray and CT scan tests provide images of the inside of child’s body. The images help identify growth abnormalities in child’s heart and other organs.
- Physical exam: Examination of child’s face, ears and eyes for characteristics of the condition

Treatment

- Antibiotic medications to treat infections.
- Calcium supplements to treat low calcium levels.
- Ear tubes or hearing aids to improve hearing.
- Hormone replacement therapy to treat endocrine (a gland which secretes hormones) abnormalities.
- Surgery to repair internal organ symptoms or a cleft palate.
- Medicine to reduce seizures (uncontrolled body movement because of abnormal activity of brain) or neurological conditions.
- Special education programs to address learning challenges in school.

Prophylaxis – Cant prevent as it is genetic condition

X-linked agammaglobinaemia

- X-linked agammaglobulinemia — also called XLA — is a rare genetic disorder discovered in 1952 that affects the body's ability to fight infection.

- X-linked agammaglobulinemia is caused by a genetic mutation.

- XLA is caused by a mutation on the X chromosome (Xq21.3-q22) of a single gene identified in 1993 which produces an enzyme known as Bruton's tyrosine kinase, or Btk.

- A mutation occurs at the Bruton's tyrosine kinase (Btk) gene that leads to a severe block in B cell development (at the pre-B cell to immature B cell stage) and a reduced immunoglobulin production in the serum

- It is the first known immune deficiency, and is classified with other inherited (genetic) defects of the immune system, known as primary immunodeficiency disorders.

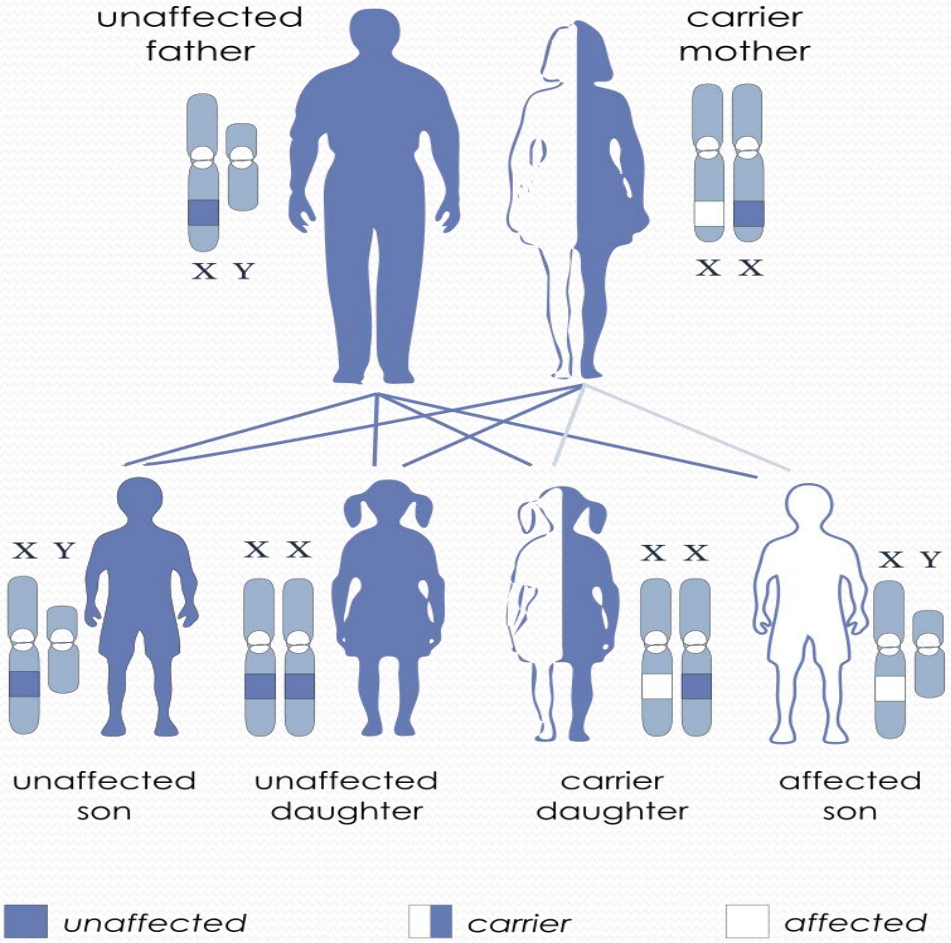
- People with the condition can't produce antibodies that fight infection. About 40% of people with the condition have a family member who has it.

- People with XLA might get infections of the inner ear, sinuses, respiratory tract, bloodstream and internal organs.

- Patients with untreated XLA are prone to develop serious and even fatal infections

- XLA affects males almost exclusively, although females can be genetic carriers of the condition.
- The disorder is inherited in an X-linked recessive fashion (as the gene linked to it is on the X chromosome) and is almost entirely limited to the sons of asymptomatic female carriers
- In people with XLA, the white blood cell formation process does not generate mature B cells, which manifests as a complete or near-complete lack of proteins called gamma globulins, including antibodies, in their bloodstream.
- Most people with XLA are diagnosed in infancy or early childhood, after they've had repeated infections.
- Some people aren't diagnosed until adulthood.

X-linked recessive inheritance



- **Image- X-linked inheritance pattern with carrier mother**
- Women can pass down X-linked recessive disorders such as X-linked agammaglobulinemia. A woman who is a carrier of an X-linked recessive disorder has a 25% chance of having an unaffected son, a 25% chance of having an affected son, a 25% chance of having an unaffected daughter and a 25% chance of having a daughter who also is a carrier.

- Babies with XLA generally appear healthy for the first few months (6-8) because they're protected by the antibodies they got from their mothers before birth.

- When these antibodies clear from their systems, the babies begin to develop often severe, recurrent bacterial infections — such as of the ears, lungs, sinuses (small, air-filled cavities behind your cheekbones and forehead) and, skin — that can be life-threating.

Complications

- People with XLA can live relatively normal lives and should be encouraged to participate in regular activities for their ages. However, recurrent infections related to XLA will likely require careful attention and aggressive treatment. They can cause organ damage and be life-threatening.
- Possible complications include:
- Chronic lung disease
- Increased risk of certain cancers
- Infectious arthritis
- Increased risk of central nervous system infections from live vaccines

Diagnosis

- XLA diagnosis usually begins due to a history of recurrent infections, mostly in the respiratory tract, through childhood. This is due to humoral immunodeficiency.
- The diagnosis is probable when blood tests show the complete lack of circulating B cells (determined by the B cell marker CD19 and/or CD20), as well as low levels of all antibody classes, including IgG, IgA, IgM, IgE and IgD
- Western Blot test to determine whether the Btk protein is being expressed

Treatment

- There's no cure for XLA. The goal of treatment is to boost the immune system, preventing infections and aggressively treating infections that occur

Medications to treat XLA include:

1. Gammaglobulin- intravenous infusion of immunoglobulin (IVIg, human IgG antibodies) every week, for life. IVIg does not cure XLA but increases the patient's lifespan and quality of life,

2. Antibiotics- Antibiotics are another common supplementary treatment. Local antibiotic treatment (drops, lotions) are preferred over systemic treatment (pills) for long-term treatment, if possible.

3. Gene therapy- in future cure from XLA could be possible by gene therapy.

Precautions to avoid infections

- follow-up visits every six to 12 months to screen for complications of XLA
- not get live vaccines, such as live polio, measles-mumps-rubella or chickenpox vaccines.

As live vaccines carry live organisms – development of disease occurs as no synthesis of antibodies in body.

Selective IgA deficiency

- is a genetic immunodeficiency, a type of hypogammaglobulinemia.
- Selective IgA deficiency is an immune system condition in which you lack or don't have enough immunoglobulin A (IgA), a protein that fights infection (antibody).
- Selective IgA deficiency is an IgA level < 7 mg/dL (< 70 mg/L, < 0.4375 micromol/liter) with normal IgG and IgM levels.
- It is the most common primary immunodeficiency

IgA

- IgA is present in the blood, most of the IgA in the body is in the secretions of the mucosal surfaces, including tears, saliva, colostrum, genital, respiratory and gastrointestinal secretions.

- The IgA antibodies in the secretions play a major role in protecting us from infections in these areas.

Signs and symptoms

- Many patients are asymptomatic, but some develop recurrent infections and autoimmune disorders.
- Some people who have IgA deficiency experience pneumonia, ear infections, sinus infections, allergies, asthma and diarrhea
- Autoimmune diseases, in which your immune system attacks particular organs or tissues in your own body, can be found with selective IgA deficiency. Common autoimmune conditions found with IgA deficiency include rheumatoid arthritis, lupus, celiac disease or inflammatory bowel disease.

Cause

- Selective IgA deficiency is inherited and has been associated with differences in chromosomes 18, 14 and 6.
- Selective IgA deficiency is often inherited

Diagnosis

- Diagnosis is by measuring serum immunoglobulins

  When suspected, the diagnosis can be confirmed by laboratory measurement of IgA level in the blood. SIgAD is an IgA level < 7 mg/dL with normal IgG and IgM levels (reference range 70–400 mg/dL for adults; children somewhat less)

- Most such persons remain healthy throughout their lives and are never diagnosed.

Treatment

- Most people with selective IgA deficiency don't need treatment unless they have frequent infections.
- In some cases, treatment may include a long course of antibiotics to help prevent an infection from returning.

Selective IgM deficiency (Isolated primary immunoglobulin M deficiency)

- Selective IgM deficiency (SIgMD) is a rare immune disorder in which a person has no immunoglobulin M (IgM) antibodies, or too little IgM (less than 30 mg/dl in adults, less than 20 mg/dl in children), with normal levels of IgG and IgA antibodies.
- IgM is the first antibody the immune system makes to fight a new infection. Therefore, when a person does not have enough IgM, the body may have difficulty fighting infections.

- SIgMD can occur in infants, children, or adults.
- The disorder may occur as a primary disorder (on its own) or more commonly, as a secondary disorder (associated with another underlying disease or condition).

Symptoms

- May start to appear at any time in life.
- Symptoms of SIgMD may include repeated viral, bacterial, or fungal infections, such as ear infections, bronchitis, sinusitis, and pneumonia.
- Infections may be life-threatening. Repeated infections are common in infants with SIgMD.
- In some people, diarrhea or a skin rash is the first symptom. Others do not have symptoms specific to SIgMD

Diagnosis

- The diagnosis is made by blood tests showing low or absent IgM and normal levels of other antibodies

Cause

- The cause of SIgMD is still unclear.
- This condition is caused by a change in the genetic material (DNA).
- It may occur in some people with chromosome disorders such as 22q11.2 deletion syndrome.

Ataxia-telangiectasia (AT or A–T)

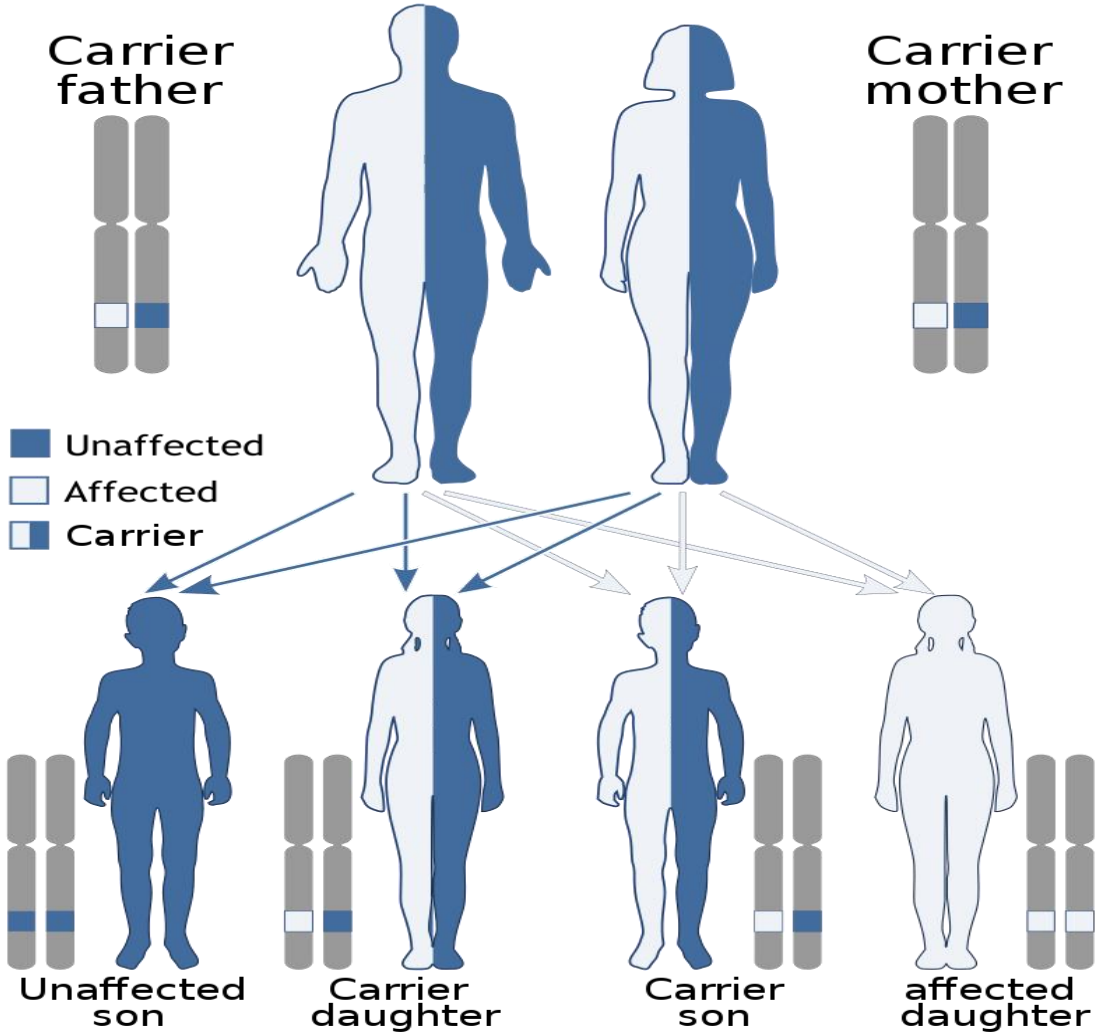
ataxia–telangiectasia syndrome or Louis–Bar syndrome

- a rare, neurodegenerative, autosomal recessive disease causing severe disability.
- A–T is caused by a defect in the ATM gene, named after this disease, which is involved in the recognition and repair of damaged DNA
- The prevalence of A–T is estimated to be as high as 1 in 40,000 to as low as 1 in 300,000 people
- A–T affects many parts of the body
- It impairs certain areas of the brain including the cerebellum, causing difficulty with movement and coordination.
- It weakens the immune system, causing a predisposition to infection.
- It prevents repair of broken DNA, increasing the risk of cancer.

Autosomal recessive

- A genetic condition can occur when the child inherits one copy of a mutated (changed) gene from each parent. The parents of a child with an autosomal recessive condition usually do not have the condition.
- (An autosome is any chromosome that is not a sex chromosome. In humans -22)

Autosomal recessive



Symptoms

- difficulty with control of movement
- difficulty with coordination of head and eye movement
- Problems with infections, especially of the ears, sinuses and lungs
- Increased incidence of cancer (primarily, but not exclusively, lymphomas and leukemias)
- Delayed onset or incomplete pubertal development, and very early menopause
- Slowed rate of growth (weight and/or height)
- slurred, slow, or distorted speech sounds
- Diabetes in adolescence or later
- Premature changes in hair and skin

Diagnosis

- physical examination of symptoms
- blood tests to verify the genetic mutation

Tests-

Genetic testing- blood tests to verify the genetic mutation

Elevated and slowly increasing alpha-fetoprotein levels in serum after 2 years of age

Magnetic resonance imaging (MRI)- An MRI will take images of the brain to look for weakened neurons or cerebellar cells

Treatment

- There is no cure for A-T. Treatment helps alleviate symptoms and prolong the life and comfort of each person diagnosed with the condition. Treatment is unique for each person diagnosed with the condition and could include:

- Avoiding overexposure to sunlight to control dilated blood vessels
- Chemotherapy treatment for cancer.
- Physical therapy to strengthen muscles.
- Receiving immunoglobulin therapy to address a weakened immune system.
- Taking antibiotics to treat infections.
- Taking diazepam to control slurred speech and involuntary muscle movements.

Collection and transport of clinical specimens (clinical samples from throat, alimentary tract, genito-urinary tract, conjuctiva, ear, blood), preliminary processing of specimens

Clinical sample

- Portion or quantity of human material that is tested, examined/studied to determine the presence or absence of particular microorganisms
- Sample to be collected depends on type of infection

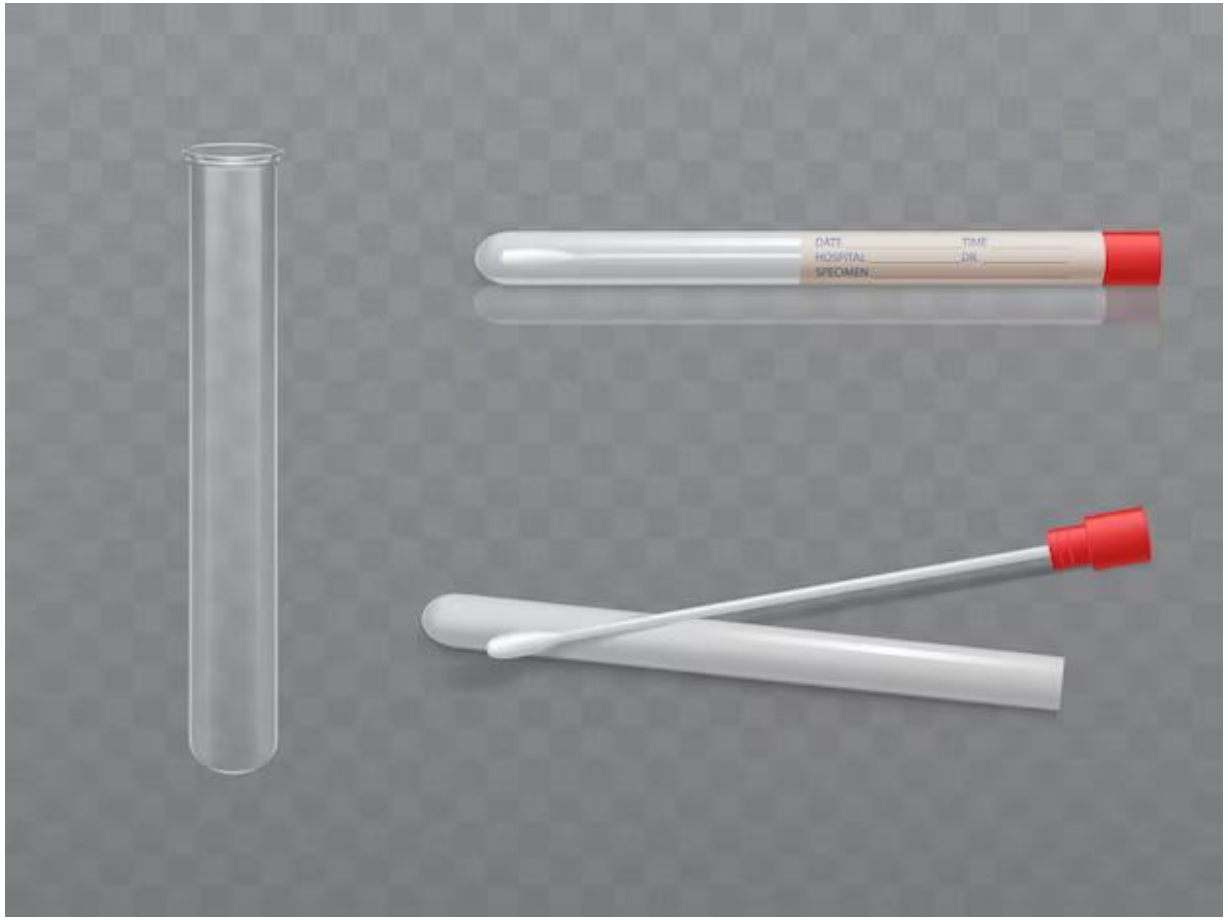
Infection	Clinical sample
Skin / wound infections	Skin scrapings, swabs, biopsies
URT	Throat swab, nasal swabs
LRT	Sputum
GIT	Stool, vomitus, endoscopic biopsies
UT	Urine
GUT	Vaginal swabs, cervical swabs, urethral discharge
CNS	CSF
Blood	Blood

Precautions during sample collection

- Asepsis during collection – to avoid contamination by skin flora or environment
- Container must be sterile and adequate
- For anaerobic cultures – sample is collected in anaerobic container
- Transport sample without delay or stored at appropriate temp
- For culture of viruses –use viral transport media to maintain viability of viruses, to avoid overgrowth of contamination flora.

Clinical samples from throat

- A nose-throat swab specimen is used to detect pathogenic microorganisms in the nose and throat.
- Throat swab specimens are generally collected to diagnose group A streptococcal pharyngitis, *Neisseria gonnorheae* infection or to detect viral infections such as enteroviruses, HSV, or CMV.
- a sterile swab is a plastic or wire shaft and a rayon, Dacron, or calcium alginate tip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SPECIMEN

Procedure

1. Wash hands thoroughly
2. Put on gloves, surgical mask and protective eye-wear
3. Open the package containing swab and aseptically remove swab from package
4. Use a wooden tongue depressor to hold the tongue in place
5. Without touching the sides of the mouth, collect the throat culture by rubbing the sterile swab tip
6. Gently move the swab without touching the teeth, gums or tongue.
7. Insert swab into sterile liquid transport system vial
8. Label the collection tube with patient name, date of birth, source and date of collection.
9. Transport specimen.
10. Specimens that cannot be transported or processed immediately should be refrigerated (2-8°C)

Swab and transport medium


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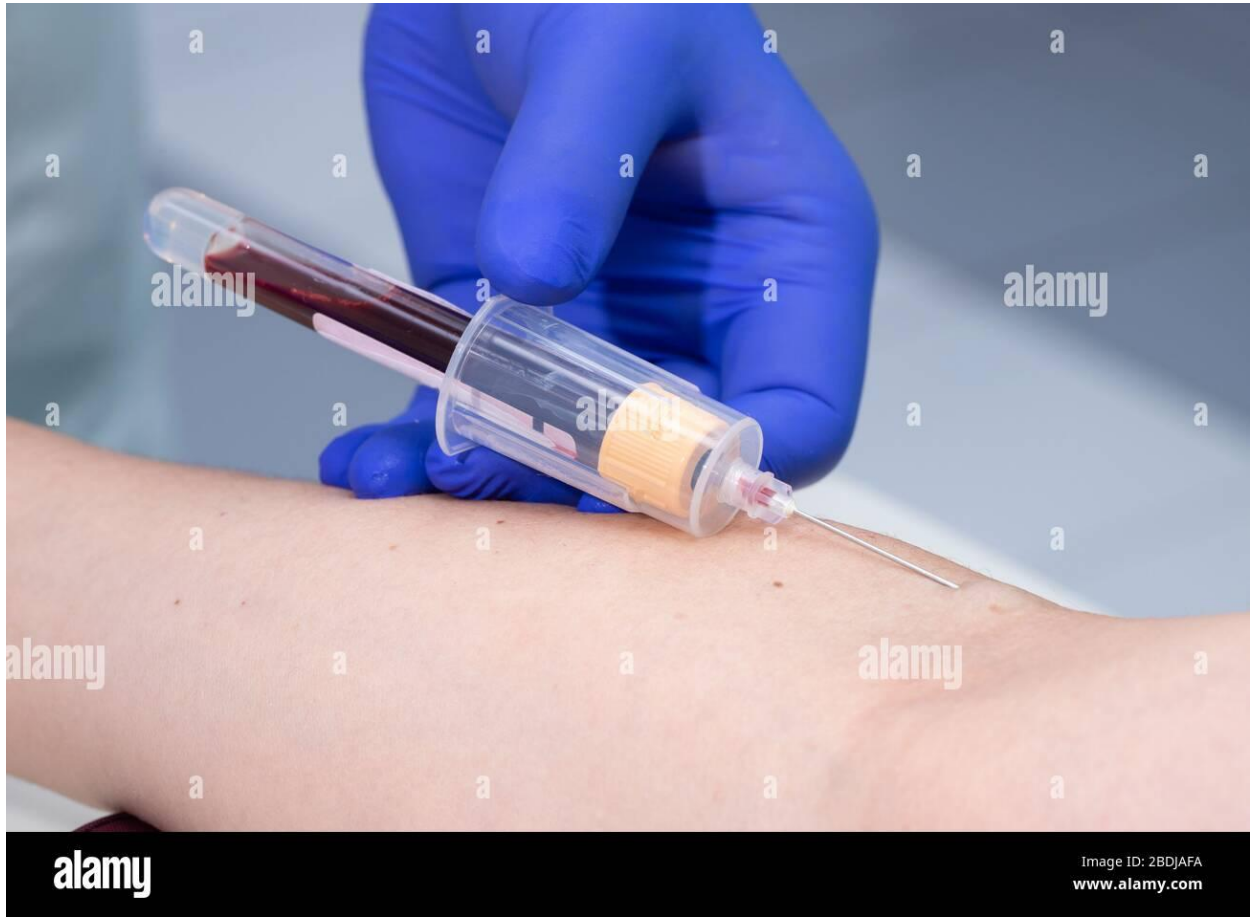
Blood as clinical sample

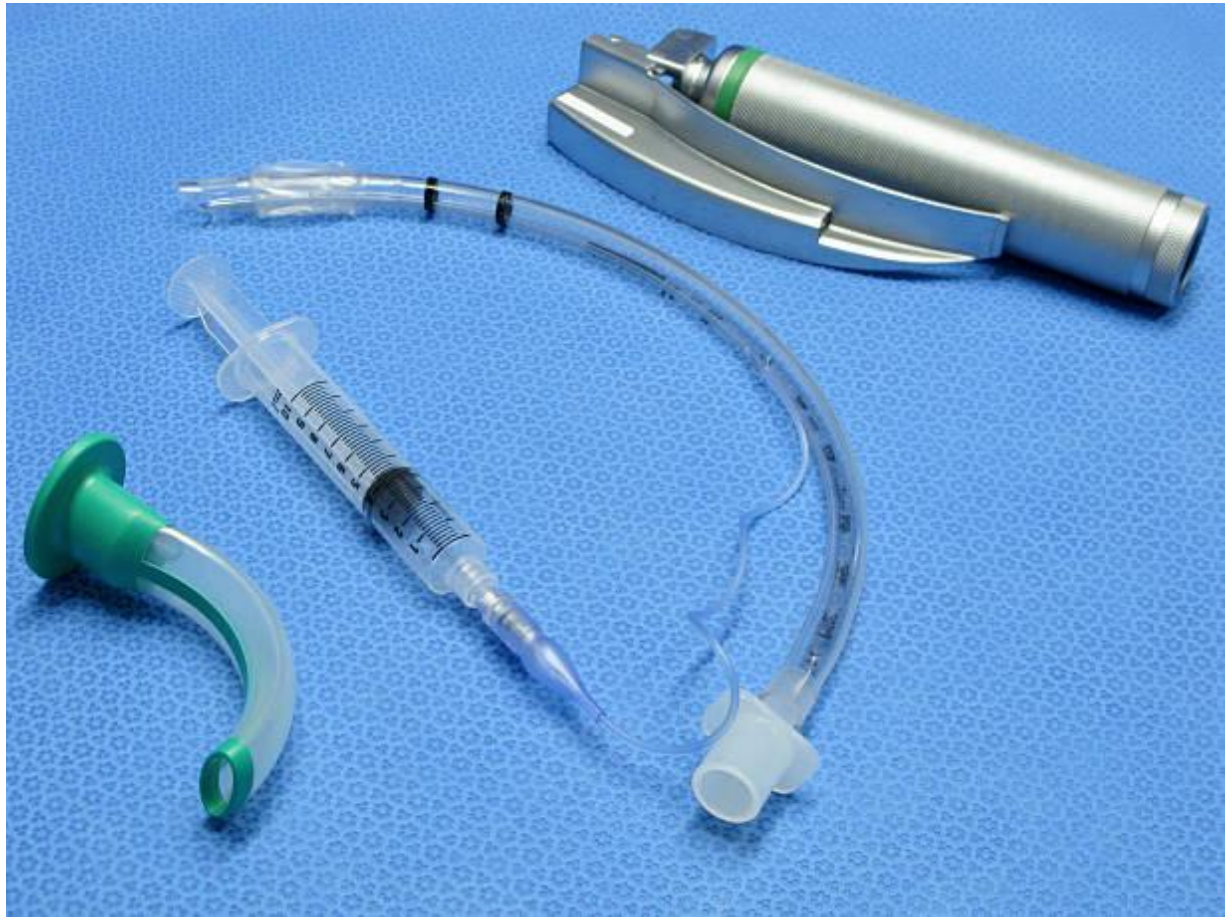
- Method used is Needle aspiration (The removal of fluid, cells, or tissue with a thin needle)
- To prevent blood from clotting various anticoagulants eg heparin, sodium citrate are included within specimen bottle or tube
- Eg Diagnosis of typhoid

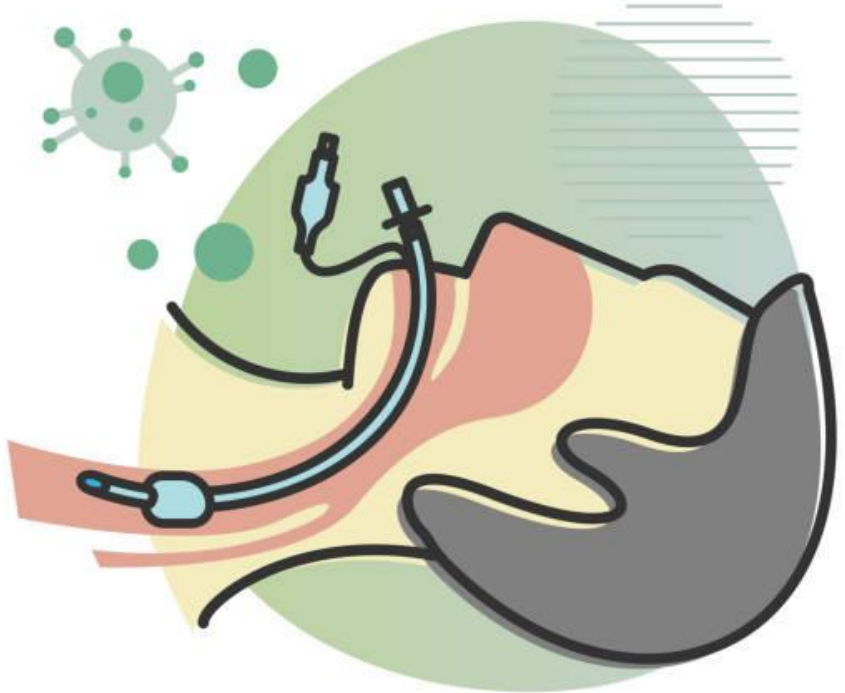
Blood collection needle



Clinical sample from alimentary tract (GIT)

- Method used is Intubation – is the insertion of a tube into a body canal /hollow organ
- Eg. From stomach
- A long sterile tube is attached to a syringe and the tube is either swallowed by the patient or passed through nose into the patients stomach. Specimen is then withdrawn into sterile syringe.





Clinical sample from genito-urinary tract

- Organs of the genitourinary tract include the kidneys, bladder, fallopian tubes, and penis.
- Clinical sample – urine
- Catheter is used
- It is tubular instrument used which inserted into body cavity to withdraw sample.
- For eg in newborns who can not give a voluntary urinary specimen.
- Methods used

1) Clean-catch method-
   most common, time of collection- early morning – as urine contains more microorganisms of both urethra and bladder
2) Clean-catch midstream method
   Here midstream urine is collected. It contains organisms of bladder mainly

Sample from conjunctiva

- Conjunctival and corneal swabs and smears are the usual specimens collected to diagnose acute fungal, bacterial or viral (kerato) conjunctivitis.
- specimens should be collected before starting any antimicrobial therapy.
- Use aseptic technique.
- Gently evert the lower eyelid to expose the conjunctival membrane.
- Rub the swab gently over the conjunctival membrane avoiding the cornea.
- Immediately after collection the swab should be returned to its plastic sheath or placed into the transport medium.
- Label the sample properly and place into a specimen bag.
- Transport to the laboratory as soon as possible.
- If transport is delayed, refrigeration is preferable to storage at ambient temperature.

Sample from ear

- Ear swabs are collected to diagnose acute fungal, bacterial or viral (kerato) ear infection.
- specimens should be collected before starting any antimicrobial therapy.
- Use aseptic technique.
- Place swab in the canal. Rotate gently.
- Immediately after collection the swab should be returned to its plastic sheath or placed into the transport medium.
- Label the sample properly and place into a specimen bag.
- Transport to the laboratory as soon as possible.
- If transport is delayed, refrigeration is preferable to storage at ambient temperature.

Handling of sample

- Immediately after collection, the specimen must be properly labeled and handled.
- The proper information should be written / imprinted on culture request form as well as on specimen container.
- It is the duty of the person collecting the sample.

The information should include-

- Patient name
- Age, sex, and address of patient
- Hospital name
- Registration no.
- Current antimicrobial therapy
- Name of attending physician
- Admission date
- Type of specimen

Transport of specimen

- Speed in transporting the specimen to the laboratory after collection is of prime importance
- Transportation is done -

  through a medium that preserves microorganisms and helps to maintain ratio of interested microorganism to non interested organisms
- Sometimes transport medium should be supplemented with antimicrobials that support survival of interested microbe and inhibit normal flora of specimen. e.g. 50,000 U of penicillin, 10mg of streptomycin or 0.2 mg chloramphenicol can be added /ml specimen to ensure recovery of fungi.
- Special treatment – for anaerobes- 1) specimen is aspirated with a needle or syringe and transported within 10 minutes to lab. 2) transport specimen in anaerobic transport vial containing transport medium with indicator which will show anaerobic conditions in vial e.g. resazurin (a color change from pink to white indicates anaerobic conditions)

Processing of specimen

- After collection of sample, it should be processed as early as possible to ensure that the organisms do not die before transferred to the culture media
- Microscopy

Microbial Cyst

- A microbial cyst is a resting or dormant stage of a microorganism, usually a bacterium or a protist, that helps the organism to survive in unfavourable environmental conditions.
- Unfavourable environmental conditions such as lack of nutrients or oxygen, extreme temperatures, lack of moisture and presence of toxic chemicals, which are not conducive for the growth of the microbe trigger the formation of a cyst.
- The main functions of cysts are to protect against adverse changes in the environment such as nutrient deficiency, desiccation, adverse pH, and low levels of oxygen
- **Encystment,** the formation of the cyst, also helps the microbe to disperse easily, from one host to another or to a more favourable environment. When the encysted microbe reaches an environment favourable to its growth and survival, the cyst wall breaks down by a process known as **excystation**.

- The production of cysts, an integral part of the life cycle of many free-living protozoa, allows these organisms to survive adverse environmental conditions.
- Eg. *Toxoplasma gondii*, *Giardia lamblia*, *Entamoeba histolytica*, *Cryptosporidium parvum*, and *Balantidium coli*
- The encysted form leaves the host in search of a new host.

- Protists, especially protozoan parasites, are often exposed to very harsh conditions at various stages in their life cycle.
- For example, *Entamoeba histolytica*, a common intestinal parasite that causes dysentery, has to endure the highly acidic environment of the stomach before it reaches the intestine and various unpredictable conditions like desiccation and lack of nutrients while it is outside the host. An encysted form is well suited to survive such extreme conditions.
- In addition to survival, the chemical composition of certain protozoan cyst walls may play a role in their dispersal. The sialyl groups present in the cyst wall of *Entamoeba histolytica* confer a net negative charge to the cyst which prevents its attachment to the intestinal wall thus causing its elimination in the faeces.

- Other protozoan intestinal parasites like *Giardia lamblia* and *Cryptosporidium* also produce cysts as part of their life cycle.
- Due to the hard outer shell of the cyst, *Cryptosporidium* and *Giardia* are resistant to common disinfectants used by water treatment facilities such as chlorine
- While the cyst component itself is not pathogenic, the formation of a cyst is what gives *Giardia* its primary tool of survival and its ability to spread from host to host. Ingestion of contaminated water, foods, or fecal matter gives rise to the most commonly diagnosed intestinal disease, Giardiasis

Mitochondria

- Popularly known as the “Powerhouse of the cell
- A double membrane-bound organelle found in plant and animal cell
- They play a major role in breaking down nutrients and generating energy-rich molecules for the cell.
- Many of the biochemical reactions involved in cellular respiration take place within the mitochondria.

Structure

- The mitochondrion is a double-membraned, rod-shaped structure found in both plant and animal cell.
- Its size ranges from 0.5 to 1.0 micrometre in diameter.
- The structure comprises an outer membrane, an inner membrane, and a gel-like material called the matrix.
- The outer membrane and the inner membrane are made of proteins and phospholipid layers separated by the intermembrane space.
- The outer membrane covers the surface of the mitochondrion and has a large number of special proteins known as porins.

Cristae

- The inner membrane of mitochondria is rather complex in structure. It has many folds that form a layered structure called cristae, and this helps in increasing the surface area inside the organelle.
- The cristae and the proteins of the inner membrane aid in the production of ATP molecules.
- The inner mitochondrial membrane is strictly permeable only to oxygen and ATP molecules.
- A number of chemical reactions take place within the inner membrane of mitochondria.

- Mitochondrial Matrix

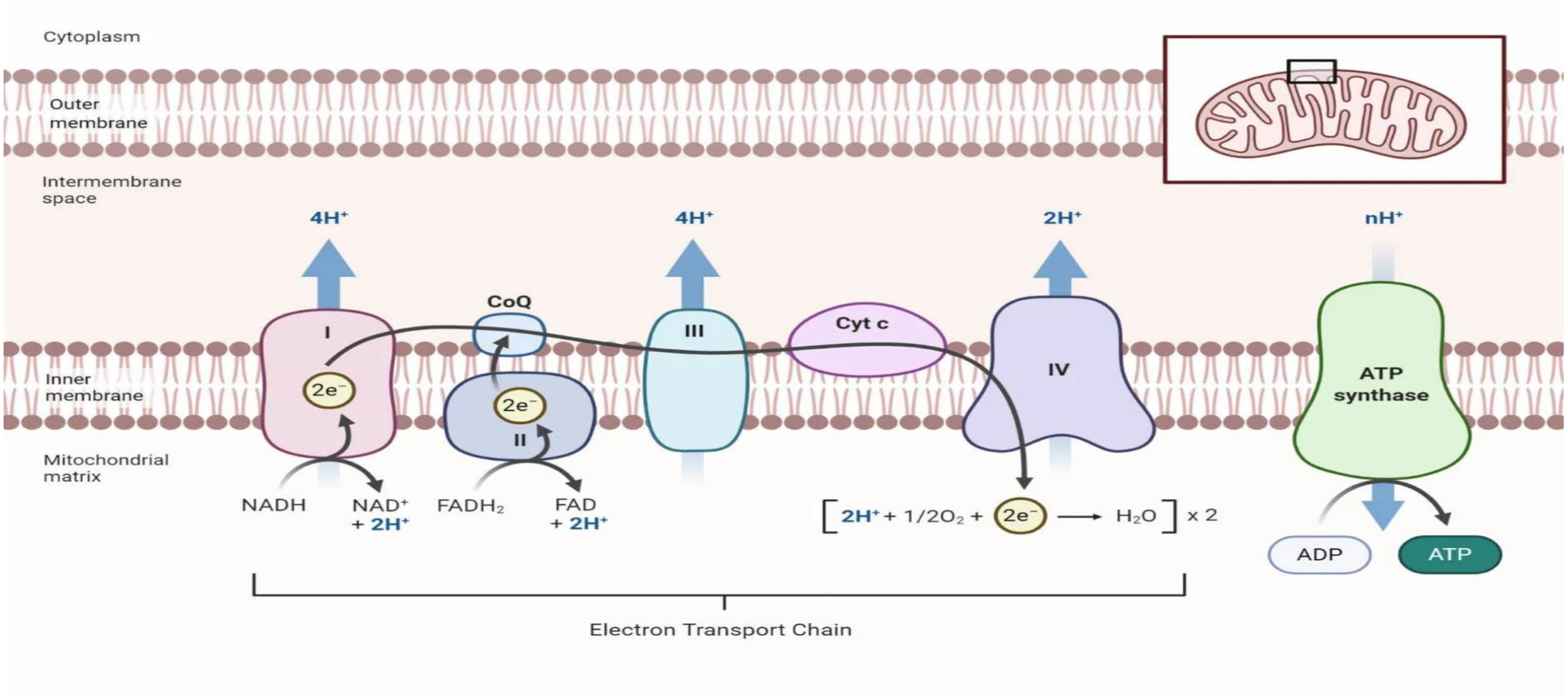
- The mitochondrial matrix is a viscous fluid that contains a mixture of enzymes and proteins. It also comprises ribosomes, inorganic ions, mitochondrial DNA, nucleotide cofactors, and organic molecules.
- The enzymes present in the matrix play an important role in the synthesis of ATP molecules.

Functions of Mitochondria

- The most important function of mitochondria is to produce energy through the process of oxidative phosphorylation. It is also involved in the following process:

- Regulates the metabolic activity of the cell
- Promotes the growth of new cells and cell multiplication
- Helps in detoxifying ammonia in the liver cells
- Plays an important role in apoptosis or programmed cell death
- Responsible for building certain parts of the blood and various hormones like testosterone and oestrogen
- Helps in maintaining an adequate concentration of calcium ions within the compartments of the cell
- It is also involved in various cellular activities like cellular differentiation, cell signalling, cell senescence, controlling the cell cycle and also in cell growth.

Mitochondrial ETC



Pasteur effect, Crabtree effect and Autotrophy

Pasteur effect

- The effect was described by Louis Pasteur in 1857 in experiments showing that aeration of yeasted broth causes cell growth to increase while the fermentation rate decreases, based on lowered ethanol production.

- Yeast fungi, being facultative anaerobes, can either produce energy through ethanol fermentation or aerobic respiration.

- When the O_2 concentration is low, the two pyruvate molecules formed through glycolysis are each fermented into ethanol and carbon dioxide.

- Typically, yeast is a facultative anaerobe that is able to produce energy using two major metabolic pathways.

- When the oxygen concentration is low, they give ethanol and carbon dioxide from pyruvate in glycolysis. Here, the efficiency of energy produced is very low.

- At high oxygen concentration, pyruvate converts into acetyl Co-A and the energy efficiency become high.

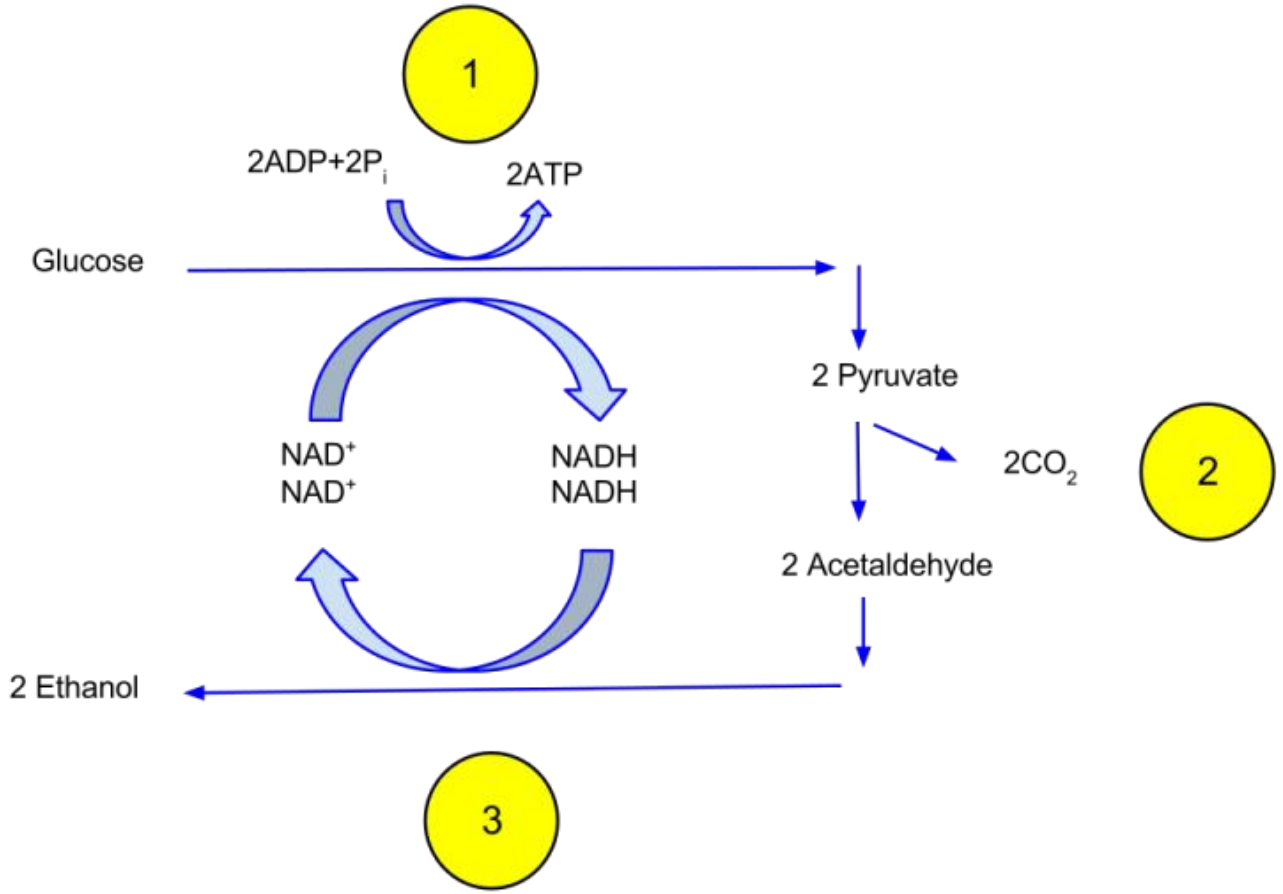
- The Pasteur effect occurs only if glucose concentration is low and under the limited concentrations of nitrogen and other nutrients.

Crabtree effect

- Crabtree effect is the phenomenon in which yeast produces ethanol in aerobic conditions at high external glucose concentrations.
- This concept was first introduced by the English biochemist Herbert Grace Crabtree.
- The usual process aerobically occurring in yeast is the production of biomass via the tricarboxylic acid cycle.

- Increasing the concentrations of glucose can cause an acceleration of the glycolysis process and produce appreciable amounts of ATP through substrate-level phosphorylation.

- Moreover, this effect causes the reduction of the need for oxidative phosphorylation that occurs through the TCA cycle (through the electron transport chain), decreasing the oxygen consumption.


1
2ADP+2P$_i$
2ATP
Glucose
2 Pyruvate
2CO$_2$
2
NAD$^+$
NAD$^+$
NADH
NADH
2 Acetaldehyde
2 Ethanol
3

Autotrophy - Concept, factors for, types of autotrophs, mechanisms

- Autotrophs are organisms that are capable of producing their own food by using various inorganic components like water, sunlight, air, and other chemical substances.

- Autotrophs are the source of all the organic compounds found on the planet that are utilized by organisms that cannot prepare their own food.

- The term “autotrophy” is formed by the combination of two terms, “auto” meaning self, and “trophy” meaning nutrition. The literal meaning of this term is self-nutrition.

- These are also called producers in ecology as these produce organic compounds from inorganic compounds

Types of Autotrophs

- According to how they obtain their energy to produce food, types of autotrophs include photoautotrophs, and chemoautotrophs.

Photoautotrophs

- Photoautotrophs are autotrophs that utilize solar energy and carbon dioxide to prepare their food by the process of photosynthesis.
- The photoautotrophs have a photosynthetic reaction centre consisting of chlorophyll. The chlorophyll pigment is responsible for the transduction of radiation in cells.

- Green plants are producers of the terrestrial and aquatic ecosystems, which are consumed by heterotrophs known as consumers.

- Photoautotrophs include green plants and algae that contain chlorophyll. The chlorophyll is involved in capturing energy from sunlight which is utilized to assemble carbon dioxide into glucose.

- The energy assembled by green plants is transferred to other animals through the food chain, which forms the basis of the ecosystem.

- Besides green plants, there are numerous photosynthetic bacteria that contain other photosynthetic pigments like rhodopsin, carotenoids, etc.

- Photoautotrophs are also important in the carbon cycle as these utilize carbon dioxide released by heterotrophs during respiration.

- Photoautotrophs can be further divided into two groups based on the number of reaction centres in the photosynthetic apparatus and the ability to utilize water;

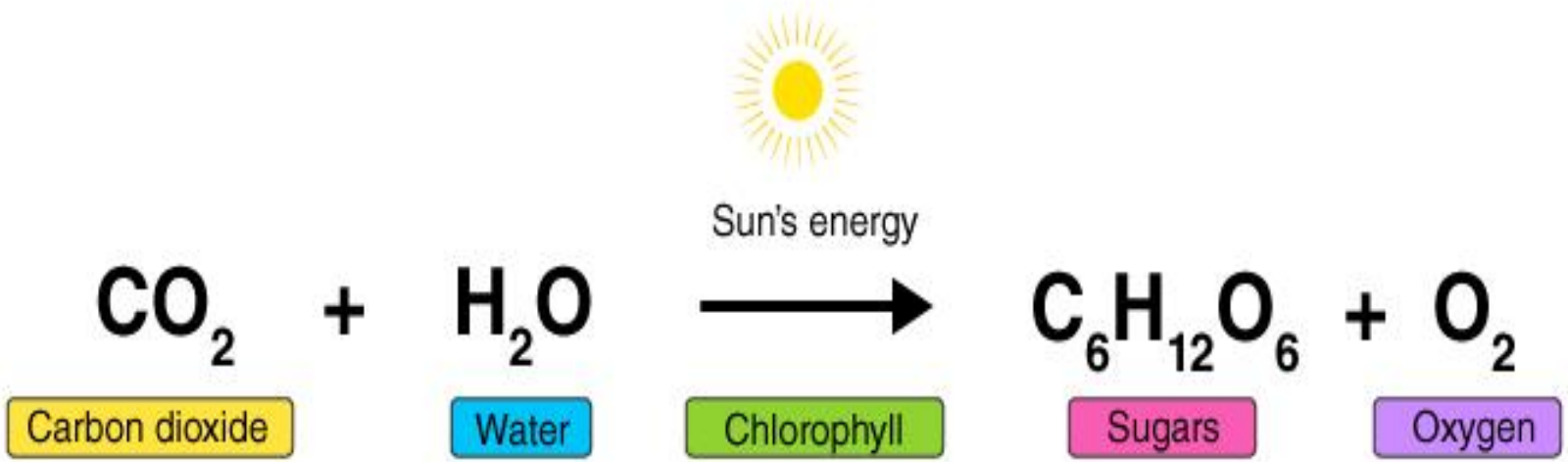
  oxygenic photoautotrophs and anoxygenic photoautotrophs.

- Oxygenic photoautotrophs

  - utilize water as a source of reducing power in order to generate oxygen.

  - use both systems for harvesting energy from light, Photosystem I and Photosystem II

- The anoxygenic phototrophs, or anoxygenic photosynthesizing bacteria
  - don't use water, so they don't give off molecular oxygen as a waste product.
  - depend on environmental reducing power in order to assimilate CO2
  - eg. green sulfur bacteria
  - For example, some microbes use hydrogen sulfide, and others use arsenic.
  - use only one system for harvesting energy from light, Photosystem I.


Sun's energy
CO_2 + H_2O $C_6H_{12}O_6$ + O_2
Carbon dioxide
Water
Chlorophyll
Sugars
Oxygen

Chemoautotrophs

- Chemoautotrophs are autotrophs that utilize energy obtained from a chemical reaction involving oxidation in order to prepare their food.
- These differ from photoautotrophs in that they do not depend on sunlight for energy.
- The source of carbon, in the case of chemoautotrophs, is an oxidized form of carbon like carbon dioxide.
- The most well-known group of chemoautotrophs includes the chemolithoautotrophic organisms that are found in rocks and utilize inorganic sources like ferrous ion, hydrogen, and hydrogen sulphide.

- These autotrophs are found in extreme habitats like deep-sea vents, acidic environments, and deep tranches.

- All chemoautotrophs are microorganisms belonging to the Archaea and Bacteria domains.

- The energy used by these microorganisms is obtained from chemical reactions occurring in the environment. The energy is then transformed into cellular energy by the microorganisms.

The essential conditions for autotrophic nutrition are:

- Source of energy – sunlight / chemical reaction
- Supply of carbon dioxide
- Source of reducing power

Photo-phosphorylation in bacteria-

Photosynthetic and non-photosynthetic ETC

Cyclic and non-cyclic photophosphorylation

- In the process of photosynthesis, the phosphorylation of ADP to form ATP using the energy of sunlight is called photophosphorylation

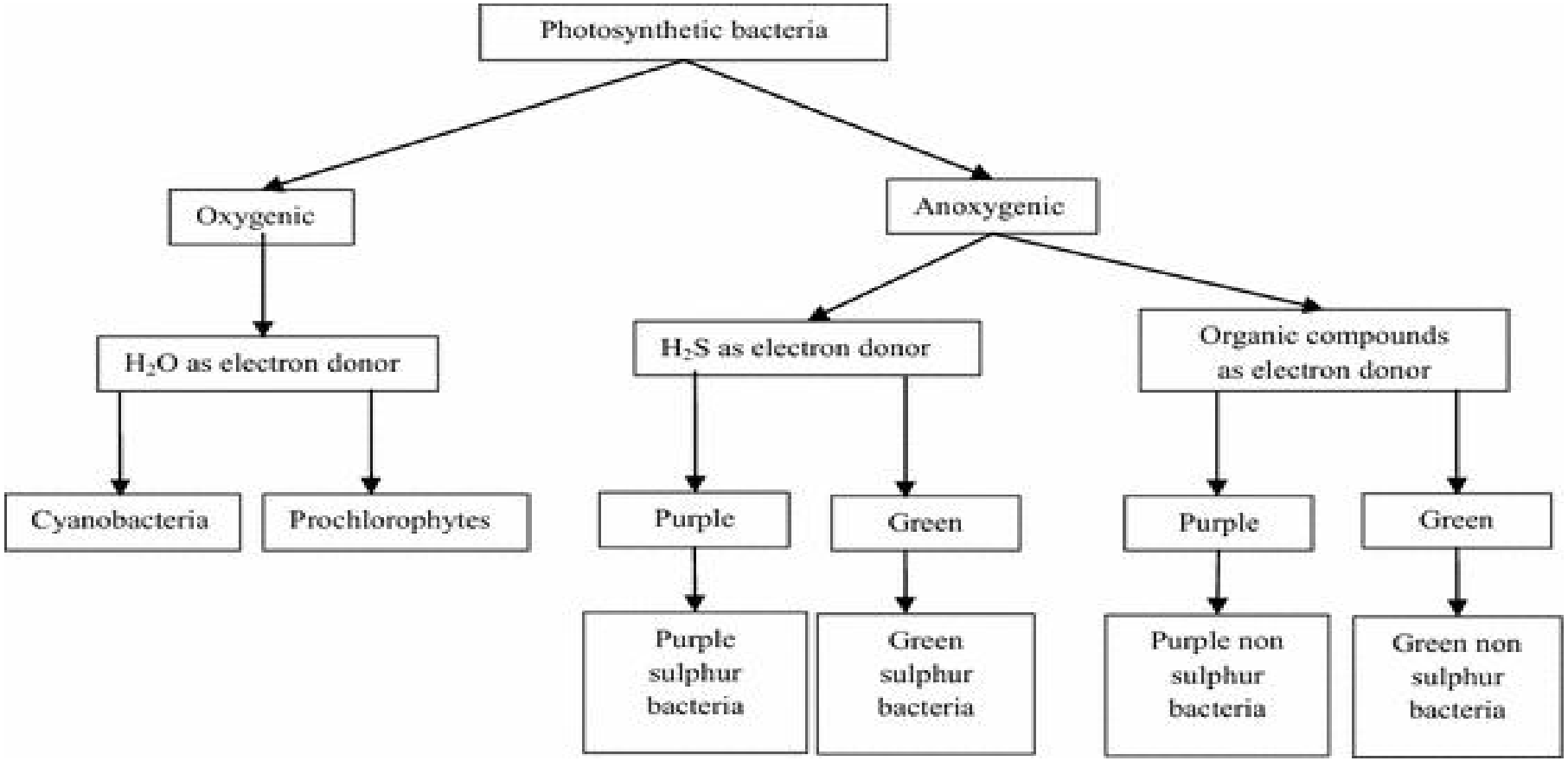
- In photophosphorylation, light energy is used to pump protons across a biological membrane, mediated by flow of electrons through an electron transport chain. This stores energy in a proton gradient. As the protons flow back through an enzyme called ATP synthase, ATP is generated from ADP and inorganic phosphate. ATP is essential in the Calvin cycle to assist in the synthesis of carbohydrates from carbon dioxide and NADPH.

- Like plants, some bacteria also have the ability to perform photosynthesis, and therefore, they are called photosynthetic bacteria.

- With the help of light energy they can also extract electrons from molecules other than water.

- Photosynthetic bacteria possess photosynthetic apparatus and are also capable of harvesting light energy. They can be divided into two groups, oxygenic and anoxygenic.

Classification of photosynthetic bacteria based on photosynthetic characteristics



- Photosynthesis occurs by 2 ways

Light reaction- Preparation of chemical energy

Dark reaction- Production of carbohydrates using chemical energy

Light reaction – Photosynthetic apparatus- consists of 3 essential components

Visible rays – wavelength 200-1200 nm – useful for synthesis of ATP

A) Light harvesting antenna- contains components which are capable of absorbing light energy

B) Photochemical reaction center- This light energy get transferred into chemical energy

C) The photosynthetic ETC- Transport of electrons and energy is liberated

A) LIGHT HARVESTING SYSTEMS IN BACTERIA

- Photosynthetic bacteria contain a well organized and developed system for harvesting sunlight and converting it into biochemical energy.

- In this process, photons of sunlight are absorbed by specialized pigment-protein complexes, known as light harvesting complexes (LHC) which are similar to those of plants. The energy of photons is used to excite the reaction centre which initiates the electron flow through a series of redox carriers eventually culminating into phosphorylation of ADP to produce ATP.

- Types of pigments-

  1) Carotenoids- yellow orange pigment, capable of absorbing wavelength in between 450-550 nm

  2) Phycobiliproteins- capable of absorbing wavelength in between 560-650 nm

  2 types- a) phycocynins- found in BGA

  b) phycoerytrins- found in eukaryotes, red algae

  3) Chlorophylls- are heme proteins, capable of absorbing light of 2 different regions- violet region- 400nm and near infra red region- 600-1100nm

  Various types- Chla, Chlb, Chlc, Chld.

B) Photochemical reaction center

- Contains chlorophyll molecules only, which are different from chlorophyll - capturing light.

C) Photosynthetic ETC

- is located in the thylakoid membrane in cyanobacteria and in the plasma membrane in green and purple bacteria
- contains – Ubiquinone's

  Iron Sulphur proteins (Fe-S)

  Ferredoxin

  Cytochromes- b, c, f

As electrons flow through the chain, a proton motive force is generated which is used to synthesize ATP by

2 ways in which electron transport occurs

A) Cyclic photophosphorylation

B) Noncyclic photophosphorylation

Cyclic photophosphorylation

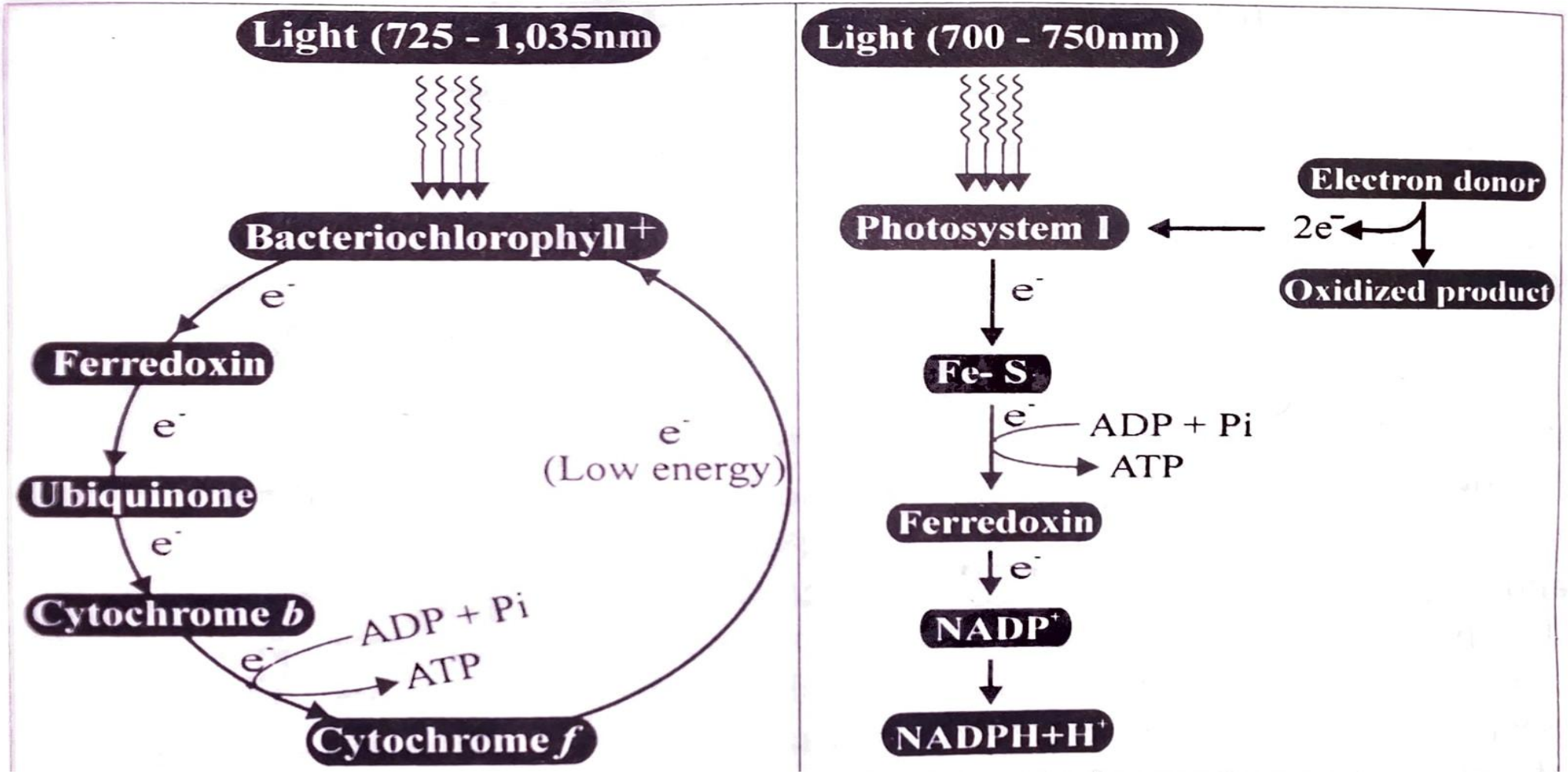
- Occurs in anoxygenic photosynthesis
- Eg. Bacterial photosynthesis
- Cyclic photophosphorylation occurs in both aerobic and anaerobic conditions.
- Cyclic Photophosphorylation is a process of photophosphorylation in which an electron expelled from reaction centre chlorophyll by light energy are passed through photosynthetic ETC and finally returned back chlorophyll. ATP is formed when electrons pass Ferredoxin to PQ and from PQ to the Cytochrome system.
- There is no generation of reducing power in the form of NADH or NADPH

Noncyclic photophosphorylation

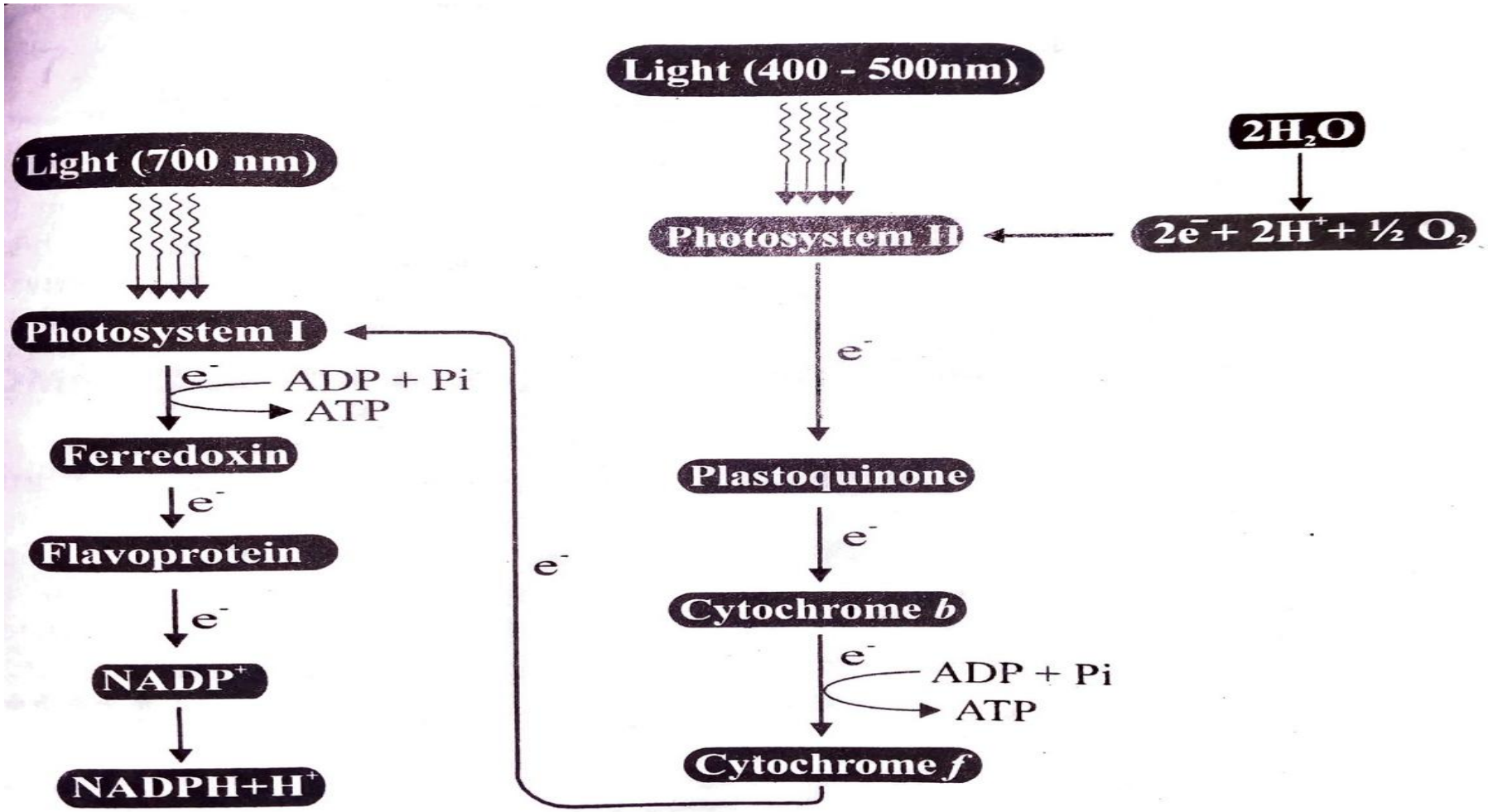
- Occurs in oxygenic photosynthesis
- electrons expelled from reaction centre chlorophyll by light energy are finally used for reduction of NADP to NADPH. So the flow of electrons become noncyclic, reaction centre chlorophyll does not receive electron back. The oxidised chlorophyll must be reduced by a chemical reductant.
- Green Sulphur bacteria contain only one type of reaction center (PS I)
- The electron ejected by light from PSI is given first to iron Sulphur proteins, then to ferredoxin and finally to NADP.
- The oxidized reaction center chlorophyll is reduced by electrons derived from reduced sulfur compounds

Cyclic photophosphorylation

Non cyclic photophosphorylation in green bacteria



Non cyclic photophosphorylation in green bacteria



- Photosystem I and photosystem II are the two multi-protein complexes that contain the pigments necessary to harvest photons and use light energy to catalyse the primary photosynthetic endergonic reactions producing high energy compounds.

- The first stage of the light reaction occurs in PS II (in higher plants, algae, and cyanobacteria) whereas the final stage of the light reaction occurs in PS I.

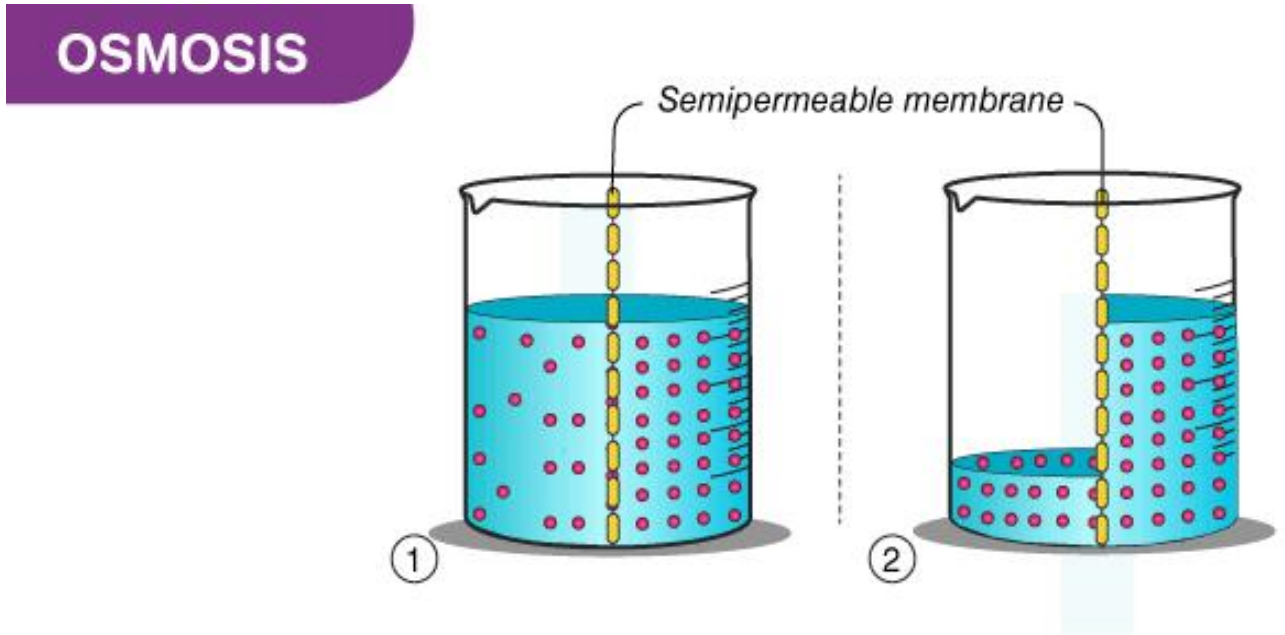
Osmosis and Transport across membrane

Osmosis- Effect of osmotic stress on microorganisms, plasmolysis and plasmoptysis, Microbial response to osmotic stress

Permeation- Primary active transport, secondary active transport, co transport
Transport of ions across the membrane V-type, F-type and P-type ATPases

Osmosis

Osmosis is the spontaneous net **movement** or diffusion **of solvent** molecules through a selectively-permeable membrane **from** a region of high water potential (region of **lower solute concentration**) **to** a region of low water potential (region of **higher solute concentration**), in the direction that tends to equalize the solute concentrations on the two sides.



- It is a type of passive transport and is directed towards the direction that tends to equalize the solute concentration across a semi-permeable membrane.
- Osmosis is the movement of a solvent across a semipermeable membrane toward a higher concentration of solute.
- A common process taking place in most of the biological membrane in the organisms.
- In a biological system, the solvent mostly is water; however, osmosis can also take place in other liquids and even gases.
- As it is a means of passive transport, it doesn't require any energy.
- **Low solute** → **High solute**

Solvent

Osmotic Solutions

- There are three different types of solutions
- Isotonic Solution, Hypertonic Solution, Hypotonic Solution

- An isotonic solution is one that has the same concentration of solutes both inside and outside the cell.

- A hypertonic solution is one that as a higher solute concentration outside the cell than inside.

- A hypotonic solution is one that has a higher solute concentration inside the cell than outside.

- **Isotonic solution**

- When an extracellular solution has the same concentration of solute as that inside the cell, the solution is termed an isotonic solution.

- When a cell is placed in an isotonic solution, no movement of water occurs across the cell membrane.

- In this case, the size of the cell is not influenced as no movement of water takes place.

- **Hypotonic solution**

- If an extracellular solution has less concentration of solute than that inside the cell, the solution is termed a hypotonic solution.

- When a cell is placed in a hypotonic solution, the movement of water occurs into the cell resulting in endosmosis.

- The cell in such condition will swell up and might even burst.

Plasmoptysis

- Plasmoptysis is when a cell bursts because it has taken in too much water as a result of being place in a hypotonic environment.
- Occurs to red blood cells when placed in water. Plasmolysis is cell shrinkage due to water loss as a result of being placed in a hypertonic environment.

- **Hypertonic solution**

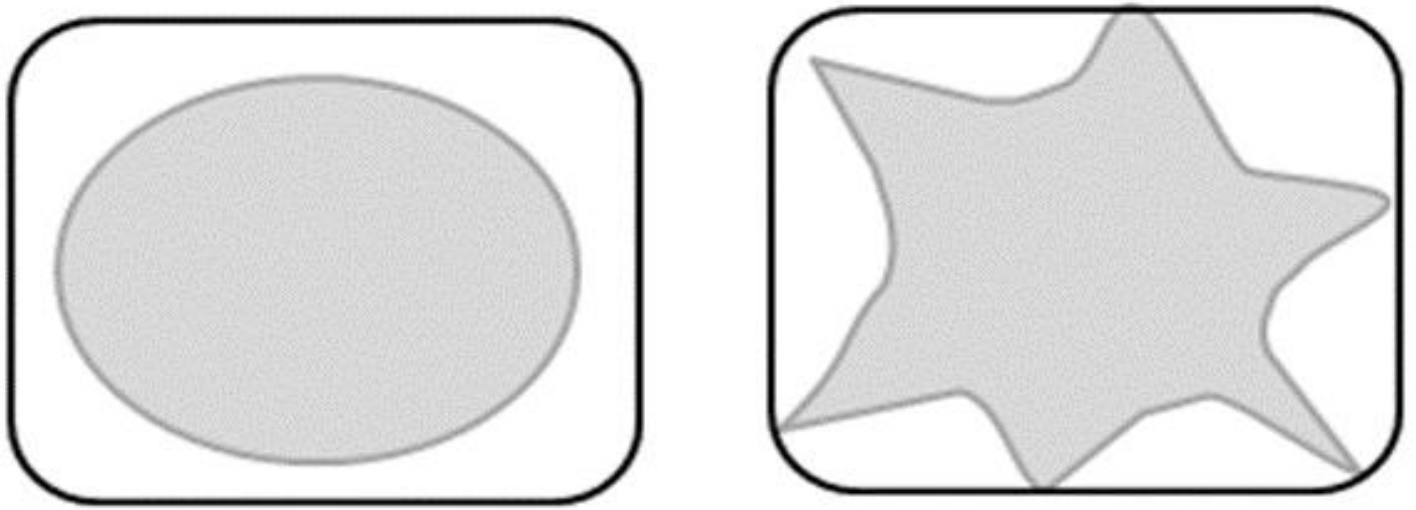
- If an extracellular solution has more concentration of solute than that inside the cell, the solution is termed a hypertonic solution.

- When a cell is placed in a hypertonic solution, the movement of water occurs out of the cell resulting in exosmosis.

- The cell shrinks down, losing the ability to divide, and even function.

Plasmolysis

- Plasmolysis is the shrinking of protoplasm away from the cell wall of a plant or bacterium.
- The protoplasmic shrinking is often due to water loss via exosmosis, thereby resulting in gaps between the cell wall and the plasma membrane.
- There are two types of plasmolysis: concave plasmolysis and convex plasmolysis.
- In concave plasmolysis, the contraction of the protoplasm and the plasma membrane resulted in concave pockets. There are still points of attachment between the cell wall and the protoplasm. Thus, the condition can still be reversed with a hypotonic solution.
- Convex plasmolysis is a type of plasmolysis that is irreversible. In this case, the plasmolyzed cell is a spherical protoplast that completely detaches from the cell wall.



- Two major types of plasmolysis: convex (left) and concave (right).

Permeation

- The cellular membrane of biological organisms is semi-permeable in nature, meaning they allow only a fraction of ions, molecules, and water across a membrane.
- The permeability of these molecules varies based on the structural pattern of lipid bilayer among different types of cells and organisms. The lipid bilayer, in accordance with macromolecules such as proteins, carbohydrates, and lipids, facilitates the transport of molecules.
- The ability of any membrane to control the transportation of molecules across a membrane is considered as one of the essential life processes in an organism..

- The membranes apply two modes of transport for molecular movement, this includes:

- **Passive Transport** – This transport mechanism involves **the movement of substances** across the membrane down (along) the concentration gradient **from high to low** without employing any energy expenditure.

Active Transport

- Active transport is a kind of cellular transport in which substances like amino acids, glucose and ions are transported across cell membranes to a region that already has a high concentration (against concentration gradient) of such substances.
- As a result, active transport employs chemical energy like ATP to move substances against their concentration gradient.
- This type of transport is commonly found in the small intestine wall and root hair cells
- Active transport is performed by a special type of protein molecules of the cell membrane called the transport proteins or pumps. They consume energy in the form of ATP molecules.

Types of active transport

- Primary

  Examples- Ca pump, Na pump, K pump, H pump, Cl pump
- Secondary- 2 types

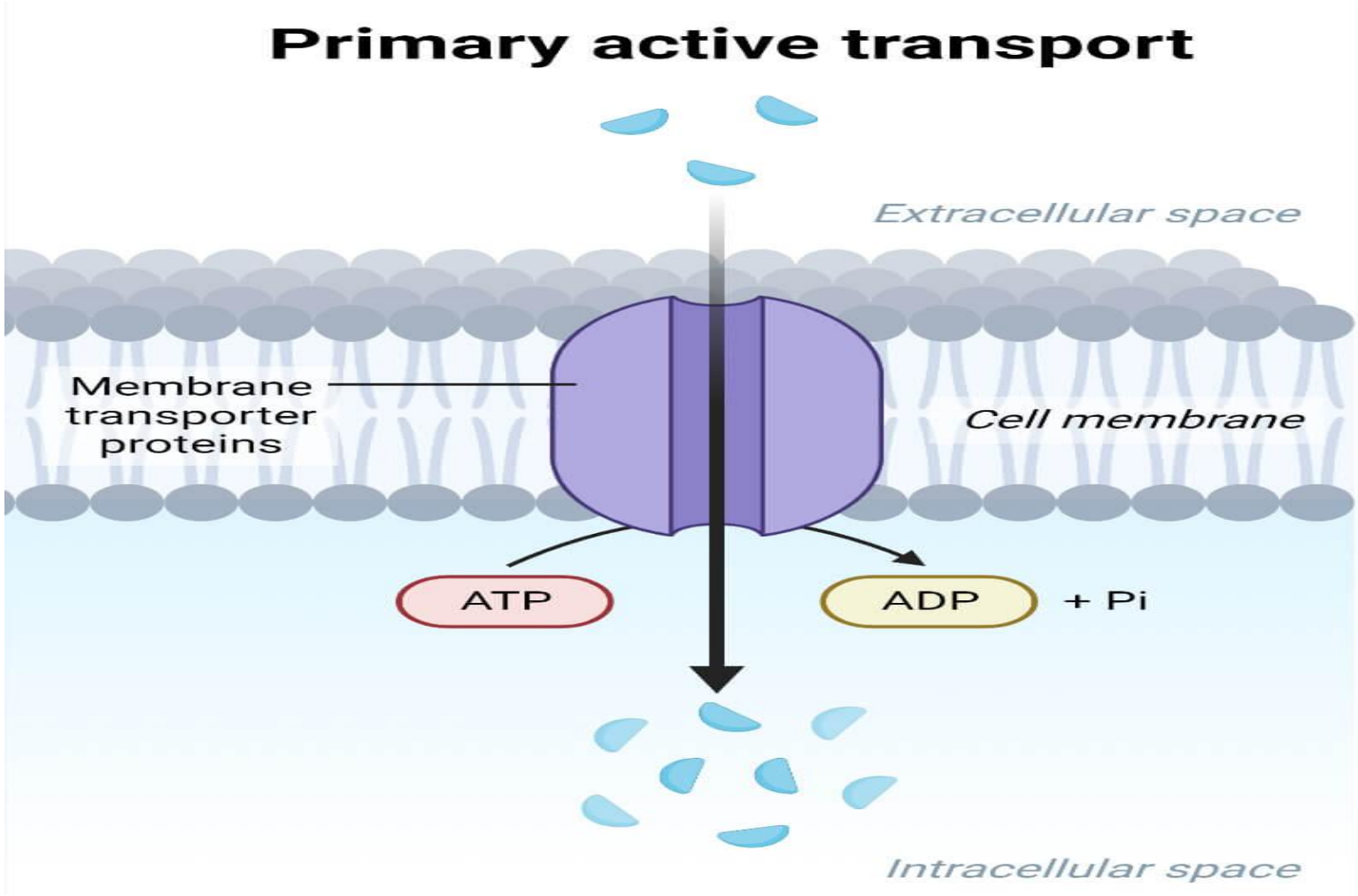
  Co transport (Symport)

  Counter transport (Antiport)

Primary Active Transport

- Primary active transport is the transport of molecules against a concentration gradient by the use of energy from ATP.
- This category of active transport directly employs the use of metabolic energy to translocate substances across the membrane, hence also called as ‘Direct Active Transport.’
- The transportation of molecules uses energy released from ATP, where Adenosine triphosphate (ATP) is broken down to ADP (adenosine diphosphate).
- This transport mode is used to move metal ions like K+, Na+, Cl-, H+, Mg2+, and Ca2+.
- The enzymes utilized for the primary active transport of ions are ATPases that facilitate the movement of charged ions across ion channels or pumps.

- Active transport is performed by a special type of protein molecules of the cell membrane called the transport proteins or pumps. They consume energy in the form of ATP molecules



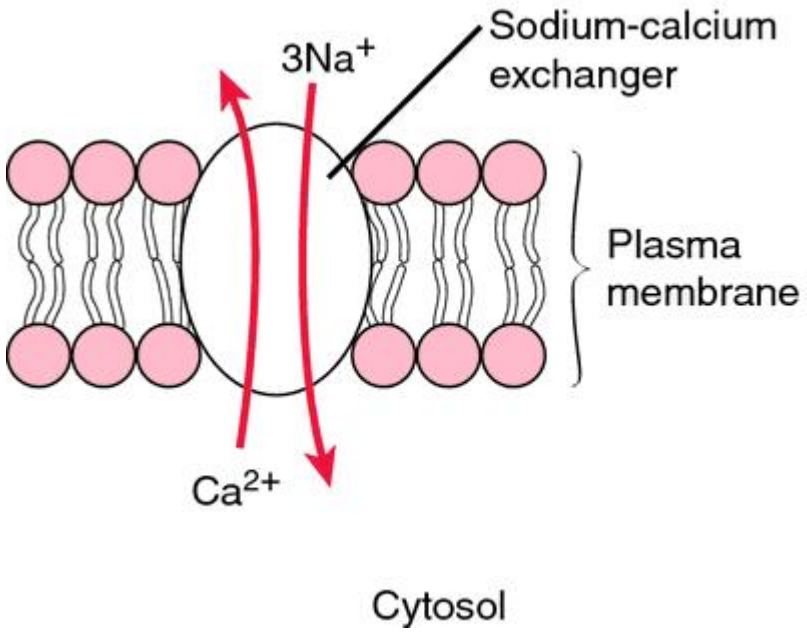
Secondary active transport

- Secondary active transport is the transport of two different molecules across a transport membrane using energy in other forms than ATP.
- Secondary active transport transports two molecules across the membrane at a time; the concentration gradient of the driving ion provides energy for the transport of driven molecule in secondary active transport.
- Secondary active transport allows one solute to move downward (along its electrochemical potential gradient) in order to generate enough entropic energy to drive the transport of the other solute upward (from a low concentration region to a high concentration region). This is also known as coupled transport.
- There are two types of coupled transport – antiport and symport.
- Antiport transport involves the movement of two ion or other solute species in opposite directions across a membrane.
- Symport transport involves the movement of two species in the same direction

- In **symport**, both driving and driven molecules are transported in the same direction.
- The Na^+/glucose cotransporter (SGLT1) is an example for symports in which both sodium and glucose are transported into the cell. It is found in kidney proximal tubules and the small intestine.
- Transport of lactose by lactose permease by E. coli

- Example 2 –
- Glucose symporter (SGLT1) (Sodium-dependent glucose cotransporters or sodium-glucose linked transporter)–
- It can be found in the cells of the intestinal epithelium, nephrons of the kidney, brain, and in heart.
- Two molecules of Na+ ions are imported down its concentration gradient into the cell per glucose (or galactose) molecule against the gradient.

- In **antiport**, driving and driven ions are transported in the opposite directions. The Na^+/Ca^{2+} exchanger (NCX) in muscle cells transports sodium ions into the cell while calcium ions are transported out of the cell.
- Antiport: sodium-calcium exchanger. The electrochemical gradient of Na^+ is used to pump Ca^{2+} out of the cell and thereby regulate the cytosolic Ca^{2+} level.

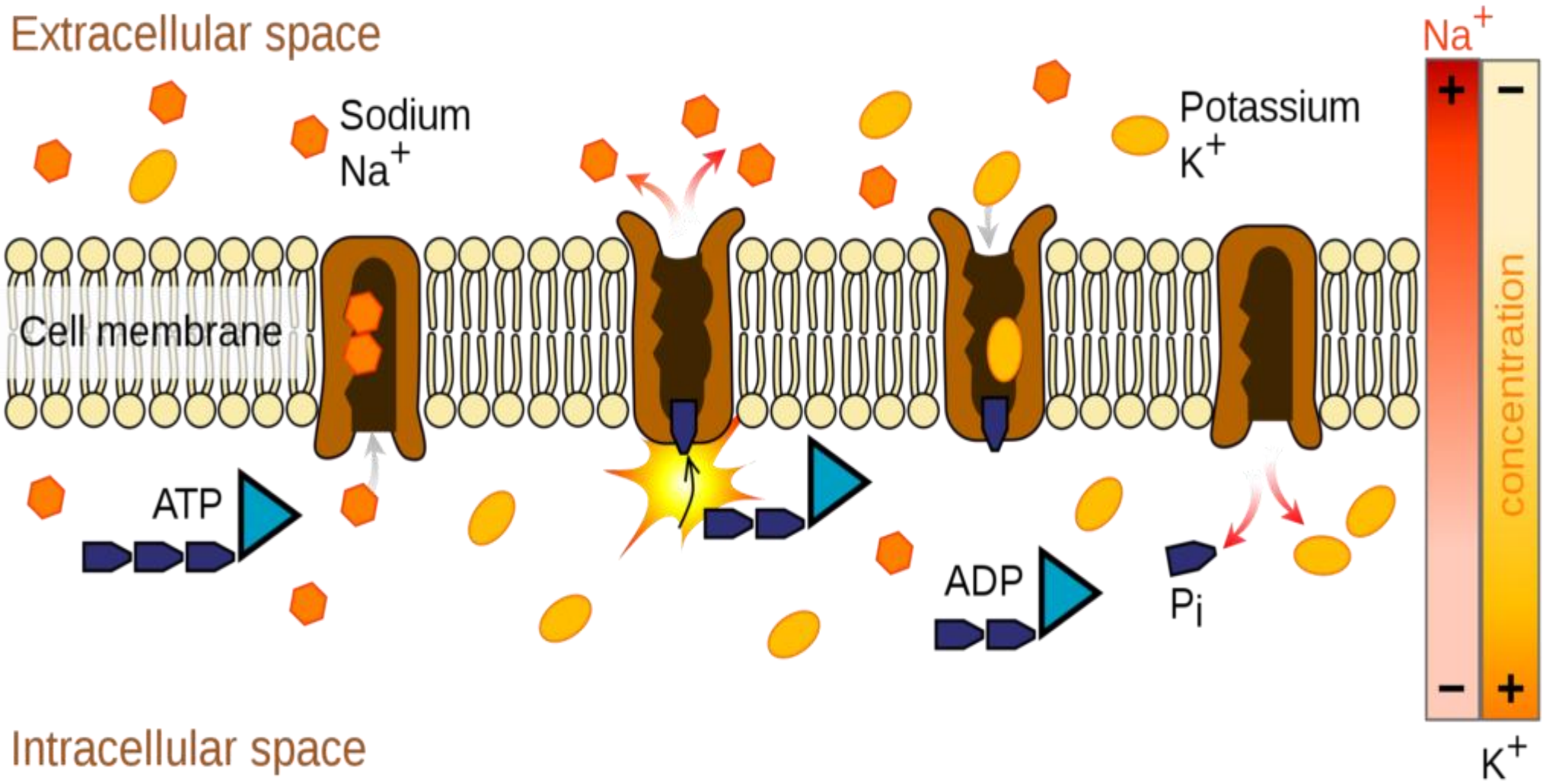


P-ATPase

- It is also known as E1-E2 ATPases because of their ability to interconvert between two conformations (E1 and E2). They are ion and lipids pumps found in bacteria, archaea, and eukaryotes.
- The “P-type” denotes their ability to autophosphorylate the components within the pump.
- Structurally, they contain four domains responsible for their functions, which include the Phosphorylation (P) domain, Nucleotide-binding (N) domain, Actuator (A) domain (domain which helps movement of other domain), and Regulatory (R) domain.
- Examples include the sodium-potassium pump (Na^+/K^+-ATPase), calcium pump (Ca^{2+}-ATPase, proton-potassium pump (H^+/K^+-ATPase), and proton pump (H^+-ATPase) of plants and fungi.

The sodium-potassium pump (Na^+/K^+-ATPase)

- The carrier protein opens to the cell interior, allowing sodium ions to adhere to the high-affinity pump.
- When sodium binds to the carrier protein, it induces the phosphorylation of the pump via ATP hydrolysis.
- The chemical modification to the pump due to phosphorylation causes it to undergo a conformational change. This change allows the pump to lose its affinity towards sodium, thus, releasing the sodium ions to the cell exterior or extracellular area.
- Though the conformational change in the pump causes sodium to lose its affinity, it creates a high-affinity environment for potassium ions on the pump. Thus, potassium ions bind to it and release the attached phosphate group.
- The phosphate released induces the pump to reassume its earlier confirmation, causing it to re-open inside the cell.
- This new change in conformation changes the affinity of the pump from potassium to sodium ions. And the cycle continuously repeats itself.


Extracellular space
Sodium
Na^+
Potassium
K^+
Na^+
Cell membrane
ATP
ADP
P_i
concentration
Intracellular space
K^+

- This pump employs a direct utilization of ATP to bring about the conformational changes to the protein and allow passage of three Na+ ions out of cells while bringing two K+ ions into the cell.

2. F-ATPase

- It is also known as ATP synthase or ATP phosphohydrolase (H+-transporting). These ATPases/synthases are found in mitochondrial inner membranes (in oxidative phosphorylation as Complex V) and chloroplast thylakoid membranes.

- It drives ATP synthesis by allowing the passive flux of protons across the membrane down their electrochemical gradient. The energy created during the transfer of protons releases the newly formed ATP from the active site of the F-ATPase.

- But under low driving force conditions, these ATP synthases function as ATPases, generating a transmembrane ion gradient at the expense of ATP hydrolysis.

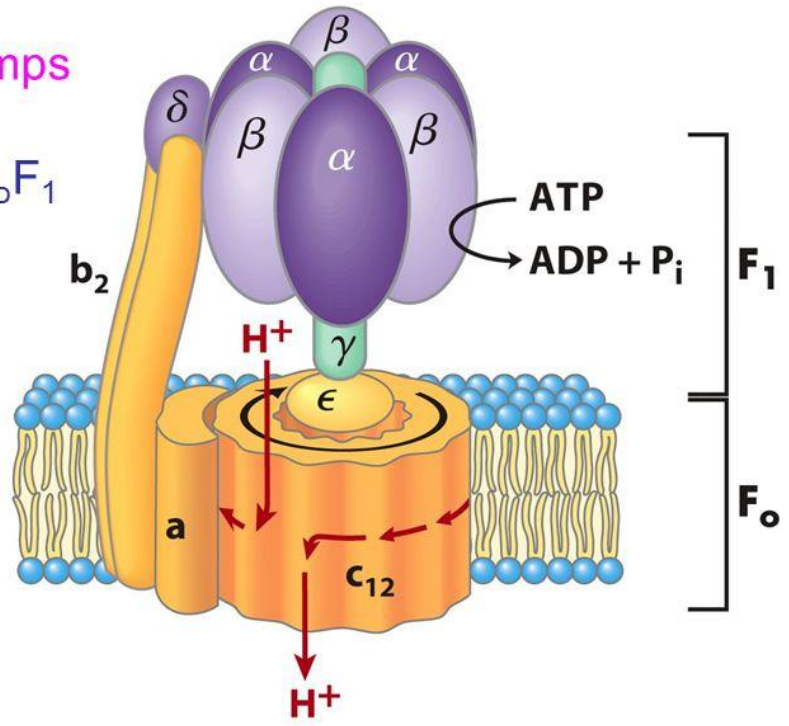
- Structurally, it’s composed of two domains:

- Fo Domain: It is integrated into the membrane and composed of three integral proteins classified as a, b and c. It’s responsible for ion translocation across the membrane.

- F1 Domain: It is composed of 5 polypeptide units – α (in 3 copies), β (in 3 copies), γ, δ, and ε. It carries the catalytic sites for ATP synthesis and hydrolysis.

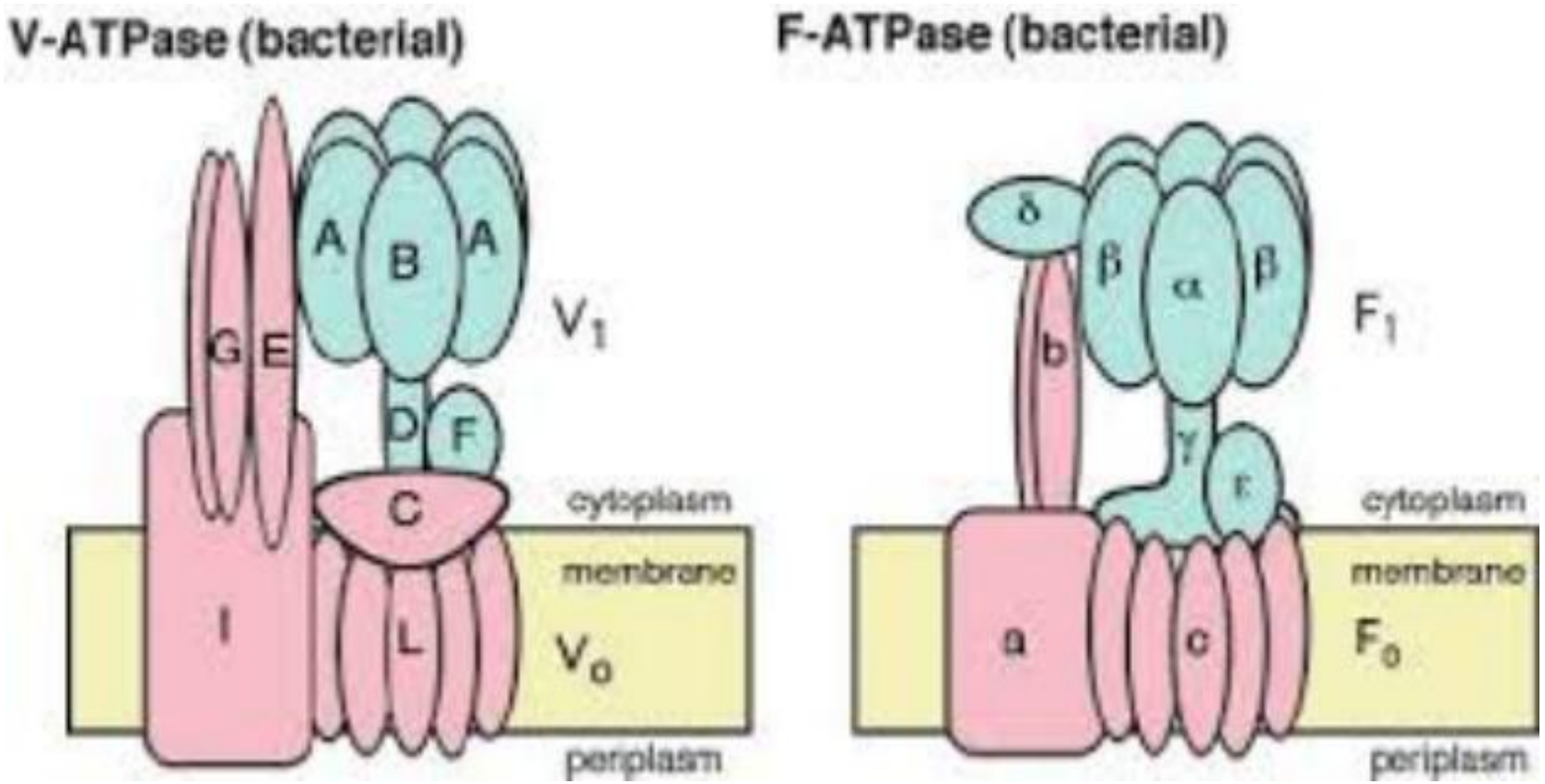
F-Type ATPase Are Reversible, ATP-driven Proton Pumps

Structure of the F_oF_1 ATPase/ATP synthase

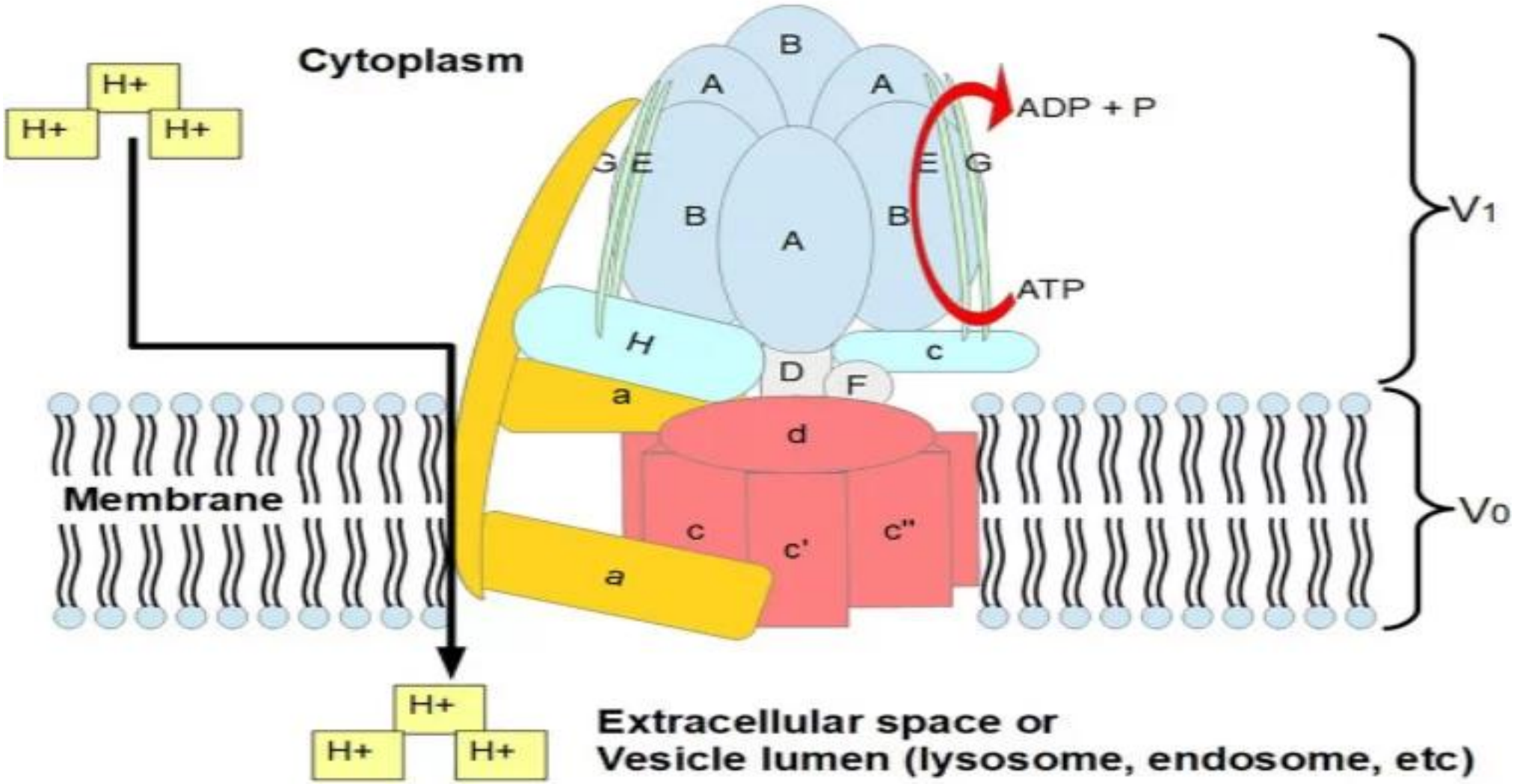


V ATPase

- The vacuolar proton-translocating ATPases
- They are responsible for making the organelle more acidic than the cytoplasm around them.
- This enzyme employs the energy obtained from ATP hydrolysis to drive the transport of protons across biological membranes.
- V-ATPases are found within the membranes of many organelles, such as endosomes, lysosomes, and secretory vesicles, where they play a variety of roles crucial for the function of these organelles.
- Example, the proton gradient across the yeast vacuolar membrane generated by V-ATPases drives calcium uptake into the vacuole through an H+/Ca2+ antiporter system


V-ATPase (bacterial)
A
B
A
G
E
D
F
C
I
L
V_1
V_o
cytoplasm
membrane
periplasm
F-ATPase (bacterial)
δ
β
α
β
b
γ
ε
a
c
F_1
F_o
cytoplasm
membrane
periplasm

STRUCTURE OF V-ATPase PROTON PUMPS



- The yeast V-ATPase is the best characterized. There are at least thirteen subunits identified to form a functional V-ATPase complex, which consists of two domains. The subunits belong to either the V_o domain (membrane associated subunits, lowercase letters on the figure) or the V_1 domain (peripherally associated subunits, uppercase letters on the figure).
- The V1 includes eight subunits, A-H, with three copies of the catalytic A and B subunits, three copies of the subunits E and G, and one copy of the C, H, D and F,
- The Vo domain contains six different subunits, a, d, c, c', c", and e
- The V_1 domain is responsible for ATP hydrolysis, whereas the V_o domain is responsible for proton translocation.