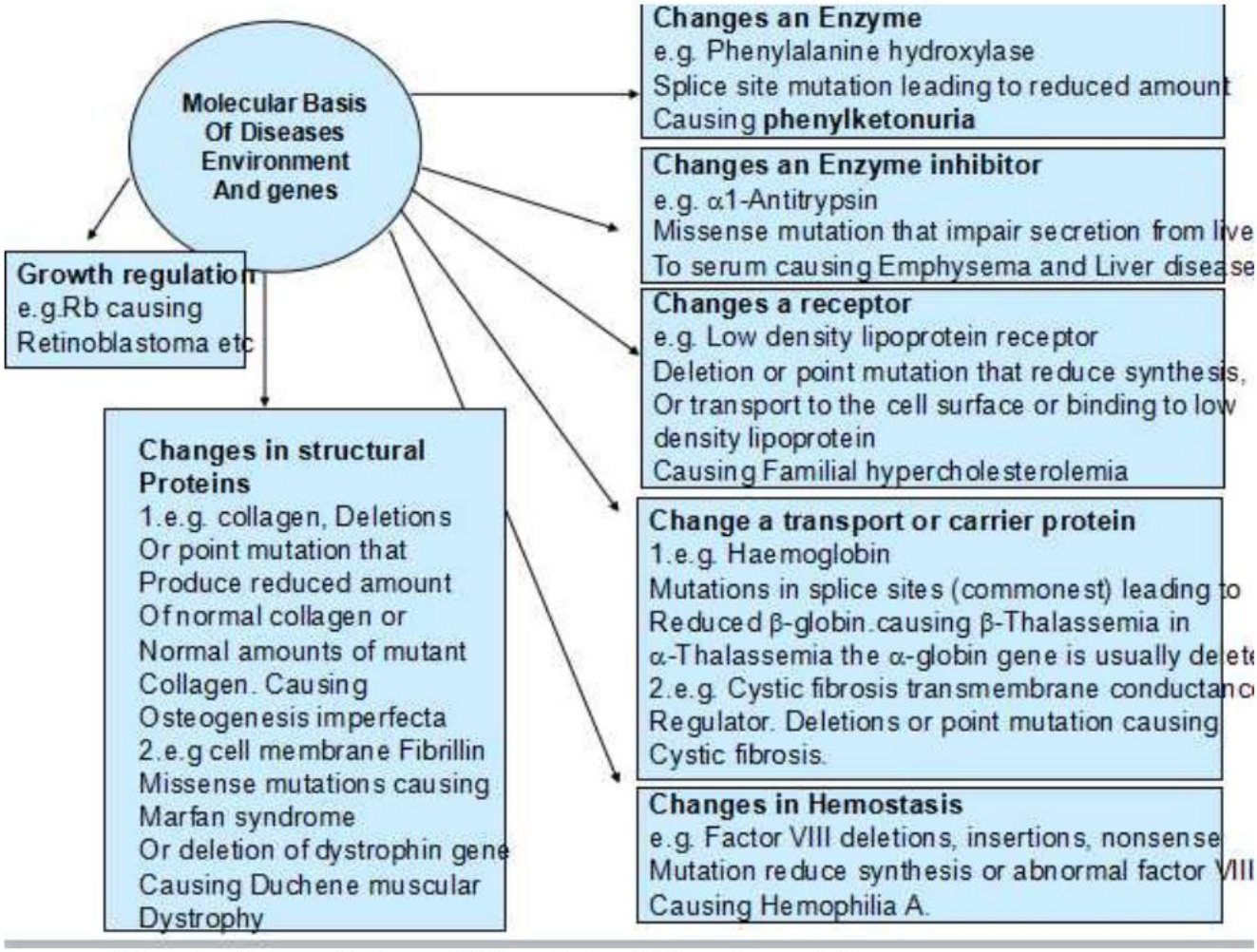
What is Disease?

- A pathological condition of a part, organ, or system of an organism resulting from various causes, such as infection, genetic defect, or environmental stress, and characterized by an identifiable group of signs or symptoms.

- A condition or tendency, as of society, regarded as abnormal and harmful.

- Obsolete. Lack of ease; trouble.
- Once you become proficient, you will think:
- What caused the disease? Endogenous reaction/defect or exogenous factor(s)
- How do I diagnose it
- What is the molecular basis for the disease?

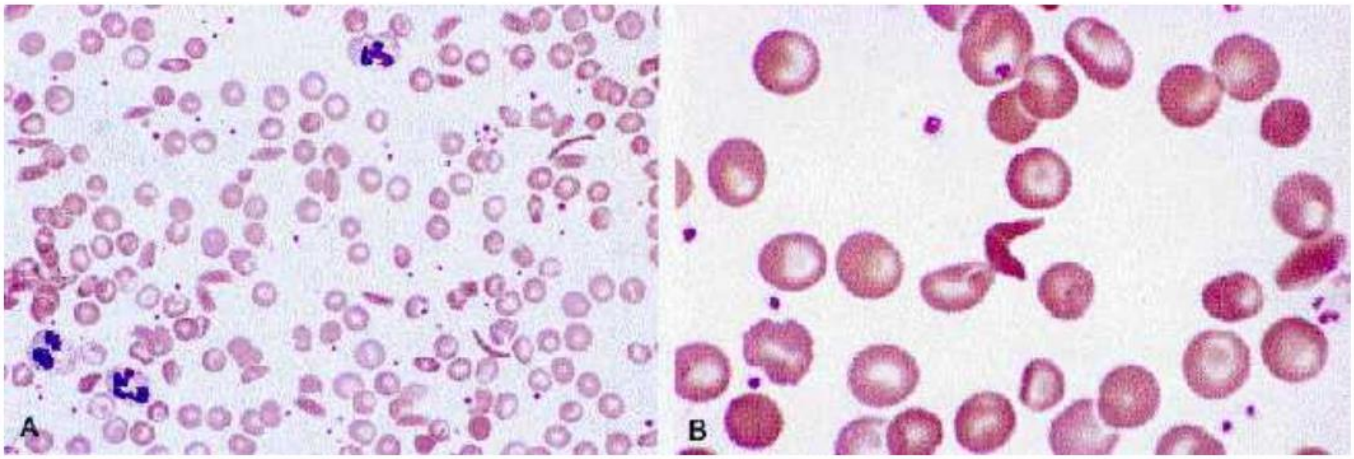


- Why do diseases behave differently in different people?

What is Molecular Pathology?

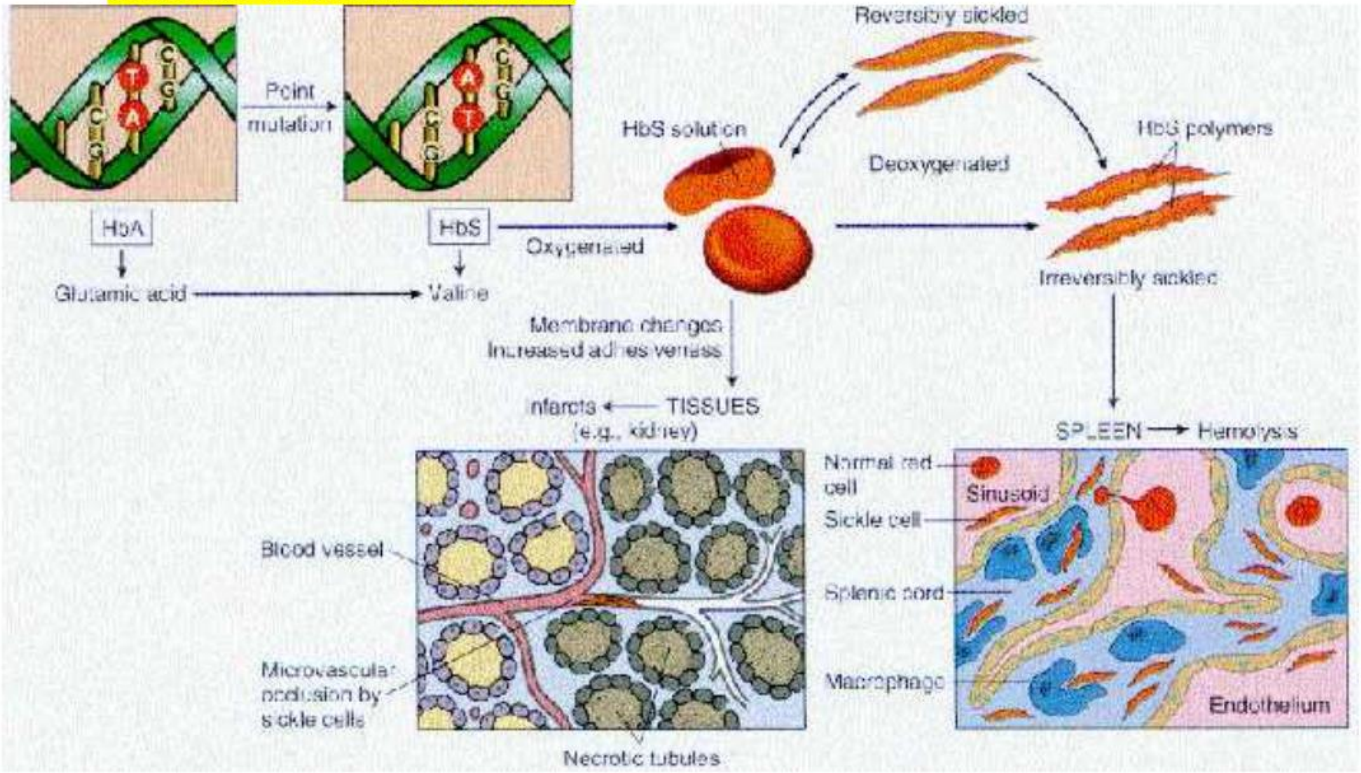
MOLECULAR BASIS OF DISEASE

- Pathology: is the study of diseases.
- Molecular biology: the study of molecules in biological systems that are responsible for normal biological traits or behaviors i.e.: DNA replication, transcription and translation in normal cells.
- Molecular pathology: an evolving field that examines and identifies the molecules involved in specific diseases.
- Integrates knowledge and techniques applied in molecular biology to pathology.



Example of Diagnostic powers of Molecular Pathology

SICKLE CELL ANAEMIA:



CONTRIBUTION OF BIOCHEMISTRY TO MEDICINE

- Chemistry is a science of matter.
- Biochemistry focuses on the studies of biological matter.
- Previously, biochemistry was referred to as 'biological chemistry' or 'physiological chemistry'.
- Medical biochemistry will be regarded as biochemistry (and molecular biology) applied to human organism in health and disease.
- Medical biochemistry seeks to advance the understanding of chemical structures and processes that constitute health and disease, and underlie transformations between these two states.

The Scope of Medical Biochemistry

- The Chemistry of Structures Comprising Human Organism.
- Eg:aminoacids and proteins, simple carbohydrates and lipids. Complex carbohydrates and complex lipids. Components of the extracellular matrix. Components of blood and plasma. Biological membranes.

- Key Chemical Processes in the Human Body.
- Eg: The nature of enzymes. Membrane transport mechanisms. Membrane receptors and signal transduction. Oxygen transport. Blood coagulation. The immune response and biochemical mechanisms of hormone action. Structure and function of neurotransmitters. Cellular homoeostasis, growth, differentiation and cancer. The process of ageing

Nutrition and Metabolism:

- Eg:
- Assimilation of nutrients,
- The function of the gastrointestinal tract, and processes of intestinal absorption.
- Macro and micronutrients: vitamins and minerals.
- Bioenergetics and oxidative metabolism.
- Mitochondrial respiratory chain.
- Main metabolic pathways: glycolysis, storage and synthesis of carbohydrates, the tricarboxylic acid cycle (Krebs cycle), oxidative metabolism of lipids, and biosynthesis and storage of fatty acids.
- Biosynthesis of cholesterol and steroids. Lipoproteins and lipid transport. Biosynthesis and degradation of amino acids.
- Oxidations and the role of free radicals

Integrative Aspects of Metabolism:

- Eg:Glucose homoeostasis and the metabolism of body fuels.
- Calcium and bone metabolism.
- Nutrition and energy balance.
- The metabolic role of the liver.
- Muscle
- metabolism (its energy metabolism and mechanism of contraction).

- Water and electrolyte homoeostasis and kidney function.
- The acid-base balance.

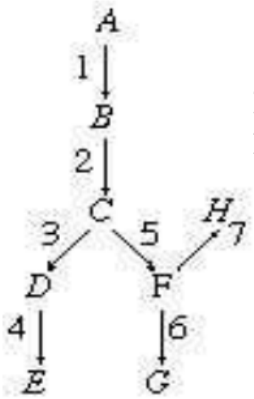
- Elements of Molecular Biology:
- Eg:Nucleic acids and molecular genetics. DNA, RNA and protein synthesis. Regulation of gene expression. Recombinant DNA technology. Genomics, proteomics and metabolomics.

ENZYMES :Role in defining disease

- Each enzyme is able to promote only one type of chemical reaction.
- The compounds on which the enzyme acts are called substrates.
- Enzymes operate in tightly organized metabolic systems called pathways.

- A seemingly simple biological phenomenon—the contraction of a muscle, for example, or the transmission of a nerve impulse—actually involves a large number of chemical steps in which one or more chemical compounds (substrates) are converted to substances called products; the product of one step in a metabolic pathway serves as the substrate for the succeeding step in the pathway.

- The role of enzymes in metabolic pathways can be illustrated diagrammatically. The chemical compound represented by A (FIGURE) is converted to product E in a series of enzyme-catalyzed steps, in which intermediate compounds represented by B, C, and D are formed in succession. They act as substrates for enzymes represented by 2, 3, and 4. Compound A may also be converted by another series of steps, some of which are the same as those in the pathway for the formation of E, to products represented by G and H
- The letters represent chemical compounds; numbers represent enzymes that catalyze individual reactions.
- The relative heights represent the thermodynamic energy of the compounds;
- e.g., compound A is more energy-rich than B, B more energy-rich than C.
- Compounds A, B, etc., change very slowly in the absence of a catalyst but do so rapidly in the presence of catalysts 1, 2, 3, etc.



- Enzymes identified with hereditary diseases:

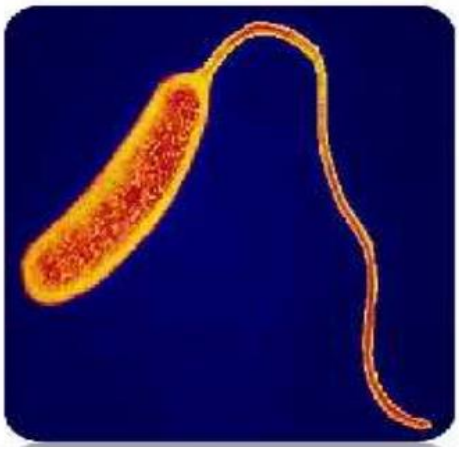
disease name	Defective enzyme
albinism	tyrosinase
phenylketonuria	Phenylalanine hydroxylase
fructosuria	fructokinase
methemoglobinemia	Methemoglobin reductase
galactosemia	galactose-1-phosphate uridyl transferase

CHOLERA

- Cholera is an infection of the small intestine caused by the bacterium Vibrio cholerae.
- The main symptoms are watery diarrhea and vomiting. This may result in dehydration and in severe cases grayish-bluish skin.
- Transmission occurs primarily by drinking water or eating food that has been contaminated by the feces (waste product) of an infected person, including one with no apparent symptoms.
-

Morphology and Identification

- V.cholrae is a comma shaped curved rod 2 – 4 µm long'
- It is actively motile by means of polar flagellum.
- On prolonged cultivation, vibrio's may become straight rods that resemble the gram-negative enteric bacteria.



Filippo Pacini - 1854

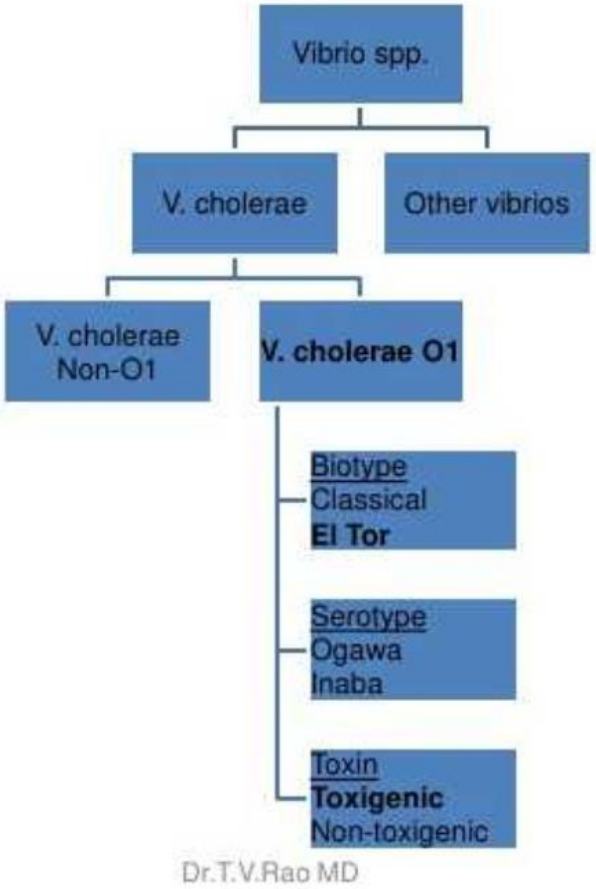
- Filippo Pacini, would gain prominence for his discovery of *Vibrio cholera*, but not until 82 years after his death, when the international committee on nomenclature in 1965 adopted *Vibrio cholerae Pacini 1854* as the correct name of the cholera-causing organism. Until then, many credited Robert Koch (1843-1910) with this seminal discovery.

Dr.T.V.Rao MD 6

Robert Koch Isolates V.cholrae 1883

- The German physician Robert Koch, like most of the scientific community, was unaware of Pacino's work at the University of Florence. Yet both independently came to a similar conclusion. Since Koch's findings eventually became accepted by his scientific peers, and were widely know in the popular press, he became the acknowledged discoverer of the cholera organism.

Vibrio Cholerae



- The severity of the diarrhea and vomiting can lead to rapid dehydration and electrolyte imbalance, and death in some cases.

- The primary treatment is oral rehydration therapy, typically with oral rehydration solution, to replace water and electrolytes.
- If this is not tolerated or does not provide improvement fast enough, intravenous fluids can also be used.
- Antibacterial drugs(azithromycin, ciprofloxacin) are beneficial in those with severe disease to shorten its duration and severity.
- The primary symptoms of cholera are profuse diarrhea and vomiting of clear

fluid.

- These symptoms usually start suddenly, half a day to five days after ingestion of the bacteria
-
- The diarrhea is frequently described as "rice water" in nature and may have a fishy odor.
- An untreated person with cholera may produce 10 to 20 litres of diarrhea a day.
- Cholera has been nicknamed the "blue death" because a person's skin may turn bluish-gray from extreme loss of fluids.

Mechanism

- When consumed, most bacteria do not survive the acidic conditions of the human stomach.
- The few surviving bacteria conserve their energy and stored nutrients during the passage through the stomach by shutting down much protein production.
- When the surviving bacteria exit the stomach and reach the small intestine, they need to propel themselves through the thick mucus that lines the small intestine to get to the intestinal walls where they can thrive.
- V. cholerae bacteria start up production of the hollow cylindrical protein flagellin to make flagella, the cork-screw helical fibers they rotate to propel themselves through the mucus of the small intestine.
- Once the cholera bacteria reach the intestinal wall they no longer need the flagella to move.
- The bacteria stop producing the protein flagellin to conserve energy and nutrients by changing the mix of proteins which they express in response to the changed chemical surroundings.
-
- On reaching the intestinal wall, V. cholerae start producing the toxic proteins that give the infected person a watery diarrhea.
-
- This carries the multiplying new generations of V. cholerae bacteria out into the drinking water of the next host if proper sanitation measures are not in place.

Cholera toxin-choleragen-enterotoxin-enteric cell

- is protein complex secreted by the bacterium Vibrio cholerae.
- CTX is responsible for the massive, watery diarrhea characteristic of cholera infection.

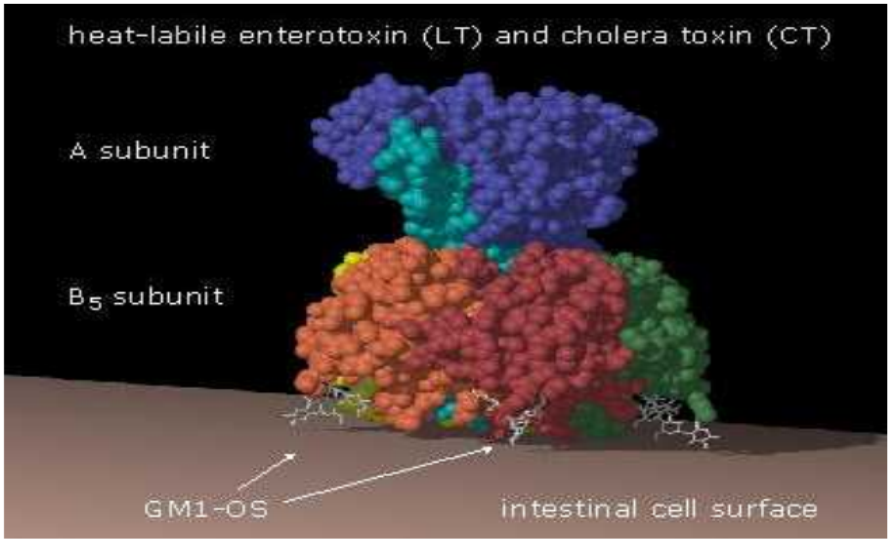
- The cholera toxin (CTX or CT) is an oligomeric complex made up of six protein subunits: a single copy of the A subunit (part A), and five copies of the B subunit (part B), connected by a disulfide bond. (denoted as AB5).
- The five B subunits form a five-membered ring that binds to GM1 gangliosides(kind of sphingolipid) receptor (mono sialotetra hexosyl ganglioside) on the surface of the intestinal epithelial cells (enterocytes)

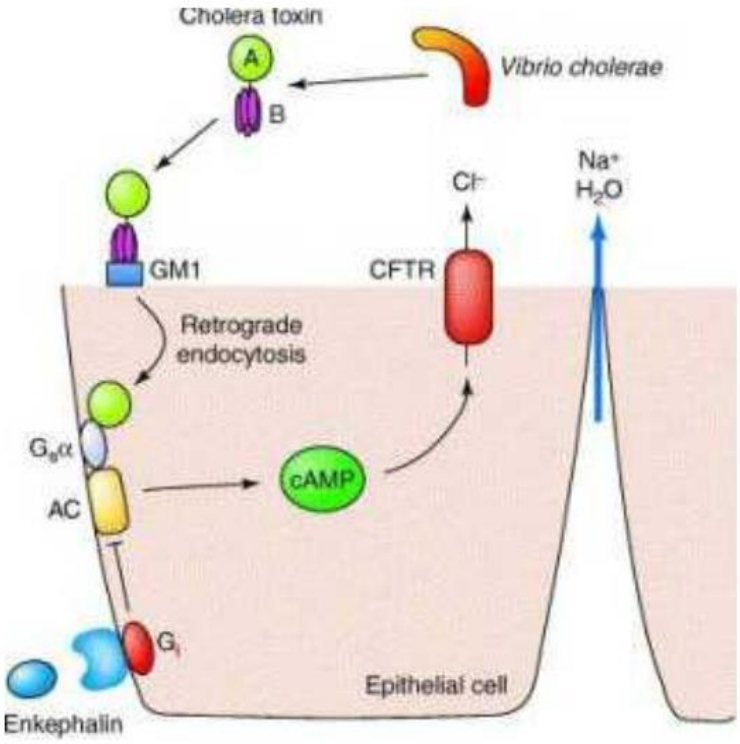
- B subunit never enter into the cell (larger subunit) do only binding.
- Endocytosis Triggering the CTA (CTA1 & CTA2)toxin ENTRY INTO THE CELL.
- The endosome is moved to the Golgi apparatus, where the A1 protein is recognized by enterocytes of intestine.

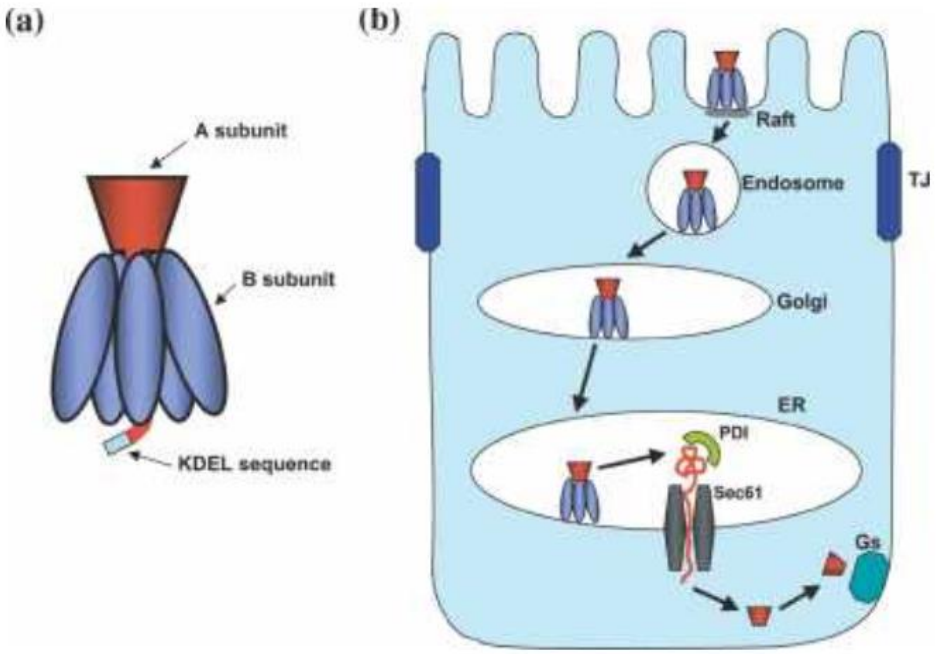
STRUCTURE OF CHOLEROGEN

-
- The three-dimensional structure of the toxin was determined using X-ray crystallography by Zhang et al. in 1995.
-
- The five B subunits—each weighing 11 kDa, form a five-membered ring.
-
- The A subunit which is 28 kDa, has two important segments. The A1 portion of the chain (CTA1) is a globular enzyme payload that ADP-ribosylates G proteins, while the A2 chain (CTA2) forms an extended alpha helix which sits snugly in the central pore of the B subunit ring.
-
- This structure is similar in shape, mechanism, and sequence to the heat-labile enterotoxin secreted by some strains of the Escherichia coli bacterium

- The high cAMP - cyclic-adenosine monophosphate levels activate the cystic fibrosis transmembrane conductance regulator (CFTR), causing a dramatic efflux of ions and water from infected enterocytes, leading to watery diarrhoea
-



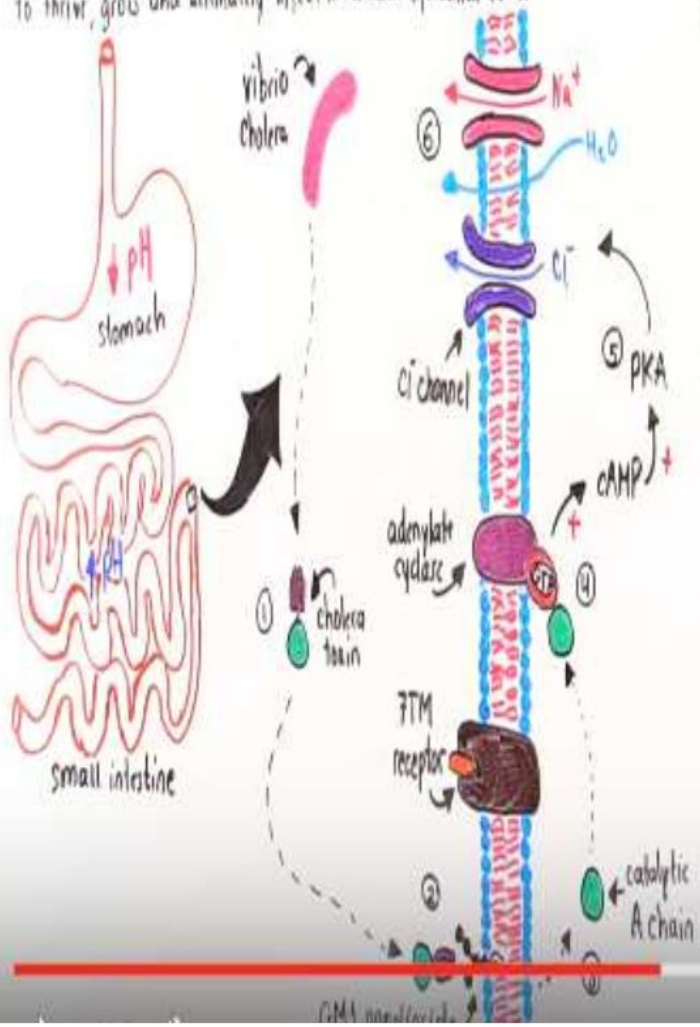

Cholera toxin
A
B
Vibrio cholerae
GM1
CFTR
Cl⁻
Na⁺
H₂O
Retrograde endocytosis
$G_s\alpha$
AC
cAMP
G_i
Epithelial cell
Enkephalin


(a)
A subunit
B subunit
KDEL sequence
(b)
Raft
Endosome
TJ
Golgi
ER
PDI
Sec61
Gs

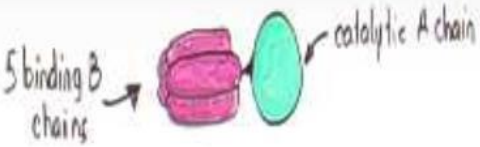
Cholera and G-Protein Coupled Signaling

1) are rod-shaped gram-negative bacteria that can infect humans
2) can use O_2 to produce ATP via aerobic cellular respiration.
3) are acid-labile (cannot survive under acid conditions)
4) affect G-protein coupled signal transduction pathways.

- Upon drinking contaminated water or eating contaminated food, cholera will make its way into the stomach. Although the acidic environment will kill off the majority of the bacterial cells, some may survive and pass into the small intestine. The basic environment of the small intestine will stimulate the cells to thrive, grow and ultimately infect intestinal epithelial cells.



① Once inside the lumen of the small intestine, cholera secretes the cholera toxin called cholerogen. This is a protein that consists of two types of chains that form a heteromeric structure.



② The cholerogen uses its B units to bind onto a membrane sphingolipid called the GM1 ganglioside.

③ Once bound, the catalytic A chain moves into the epithelial cell via endocytosis.

④ The catalytic A unit then binds to G_s protein in its GTP-state that is attached to adenylate cyclase. The A chain catalyzes the addition of an ADP-ribose component onto an arginine residue of the G_s-protein. This covalent modification stabilizes the G_s protein in its GTP-state, thereby trapping it in the active state.

⑤ The G_s-protein trapped in the active state continuously stimulates adenylate cyclase to form cAMP from ATP. The cAMP in turn activates protein kinase A (PKA). The PKA:

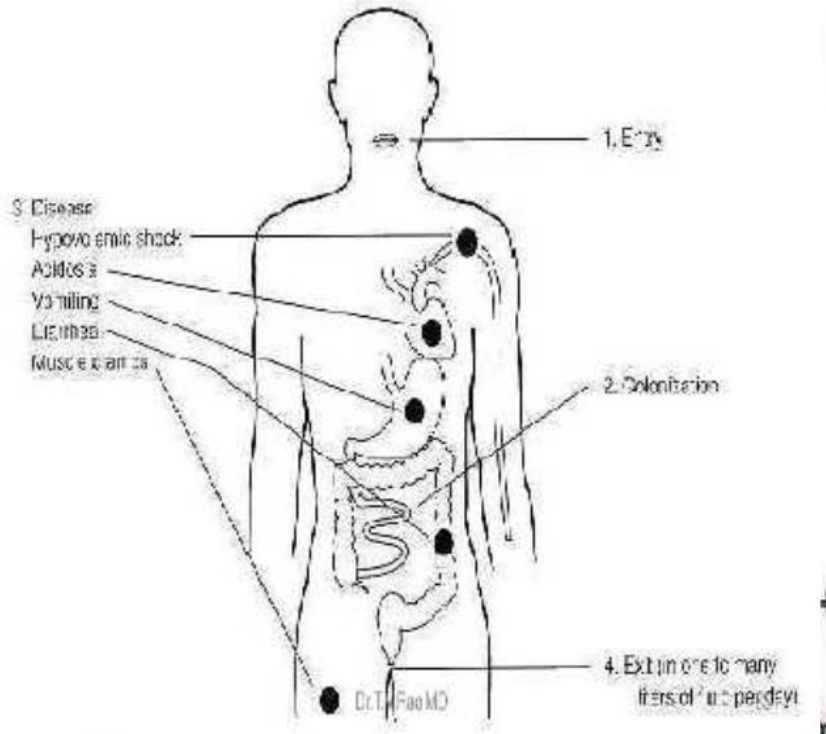
(a) phosphorylates and deactivates the Na^+-H^+ antiporter, which inhibits the Na^+ reabsorption by cells.

(b) opens up Cl^- channels, letting Cl^- out of the cell.

⑥ The net loss of Na^+ and Cl^- ions from the cells drives the water out into the lumen. This leads to the loss of large volumes of H_2O and electrolytes (watery diarrhea) through the small intestine. This dehydrates the individual and will lead to death if not treated.

Clinical events in Cholera

of Cholera Toxin



JAUNDICE

- Jaundice is a yellowish discoloration of the skin, mucous membranes and of the white of the eyes
- Caused by elevated levels of bilirubin in the blood (hyperbilirubinemia).
- The term jaundice is derived from the French word jaune, which means yellow.
- Jaundice is not a disease but visible sign of an underlying disease process.
- Jaundice is typically seen when the level of bilirubin in the blood exceeds 2.5-3 mg/dL (milligrams per deciliter).

Jaundice in adults

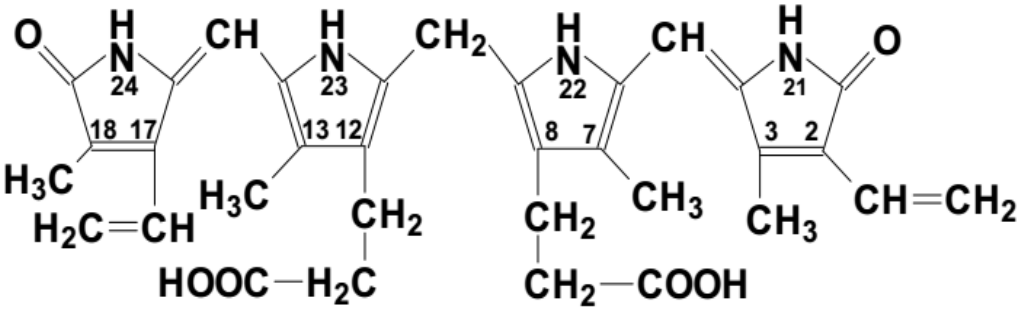
- Jaundice in adults can be caused by a variety of medical conditions, some of which are serious and potentially life-threatening.
- Any adult who develops jaundice needs to undergo a comprehensive medical evaluation in order to determine its cause.
- Neonatal jaundice, a condition seen in newborns, is most often a benign condition that improves without serious after effects.

What is Bilirubin/ haematoidin ?

- Jaundice in adults is caused by various medical conditions that affect the normal metabolism or excretion of bilirubin.
- Bilirubin is mostly formed from the daily breakdown and destruction of red blood cells in the bloodstream, which release hemoglobin as they rupture.
- The heme portion of this hemoglobin molecule is then converted into bilirubin, which is transported in the bloodstream to the liver for further metabolism and excretion.
- In the liver, the bilirubin is conjugated (made more water soluble), and is excreted into the gallbladder (where it is stored) and into the intestines.
- In the intestines, a portion of the bilirubin is excreted in the feces, while some is metabolized by the intestinal bacteria and excreted in the urine.

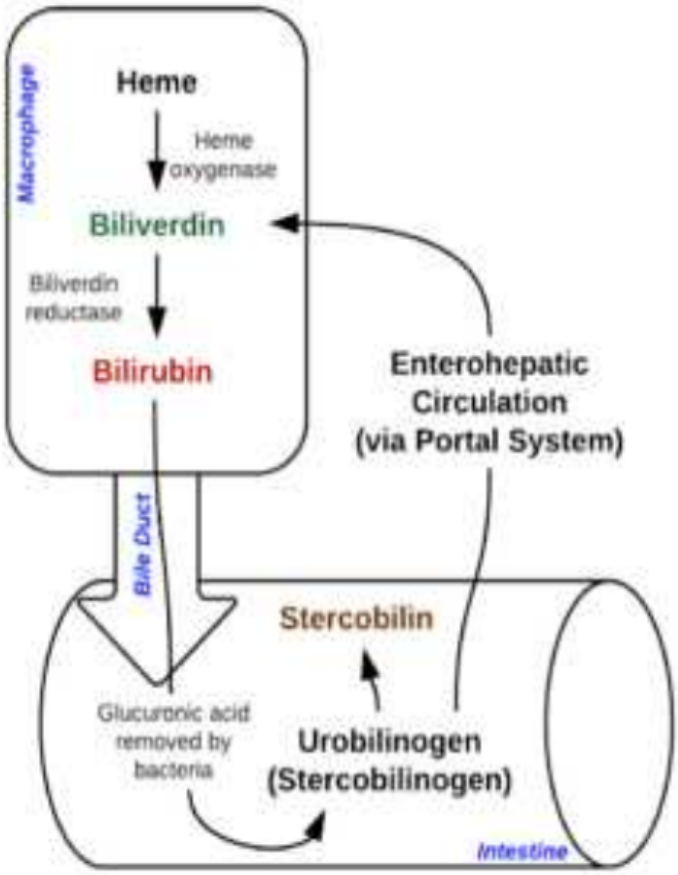
Bilirubin:

- Is a bile pigment.
- Results from the degradation of heme, one of the breakdown products of red blood cells.
- It is thought to be a toxin because it is associated with neonatal jaundice, possibly leading to irreversible brain damage due to neurotoxicity.

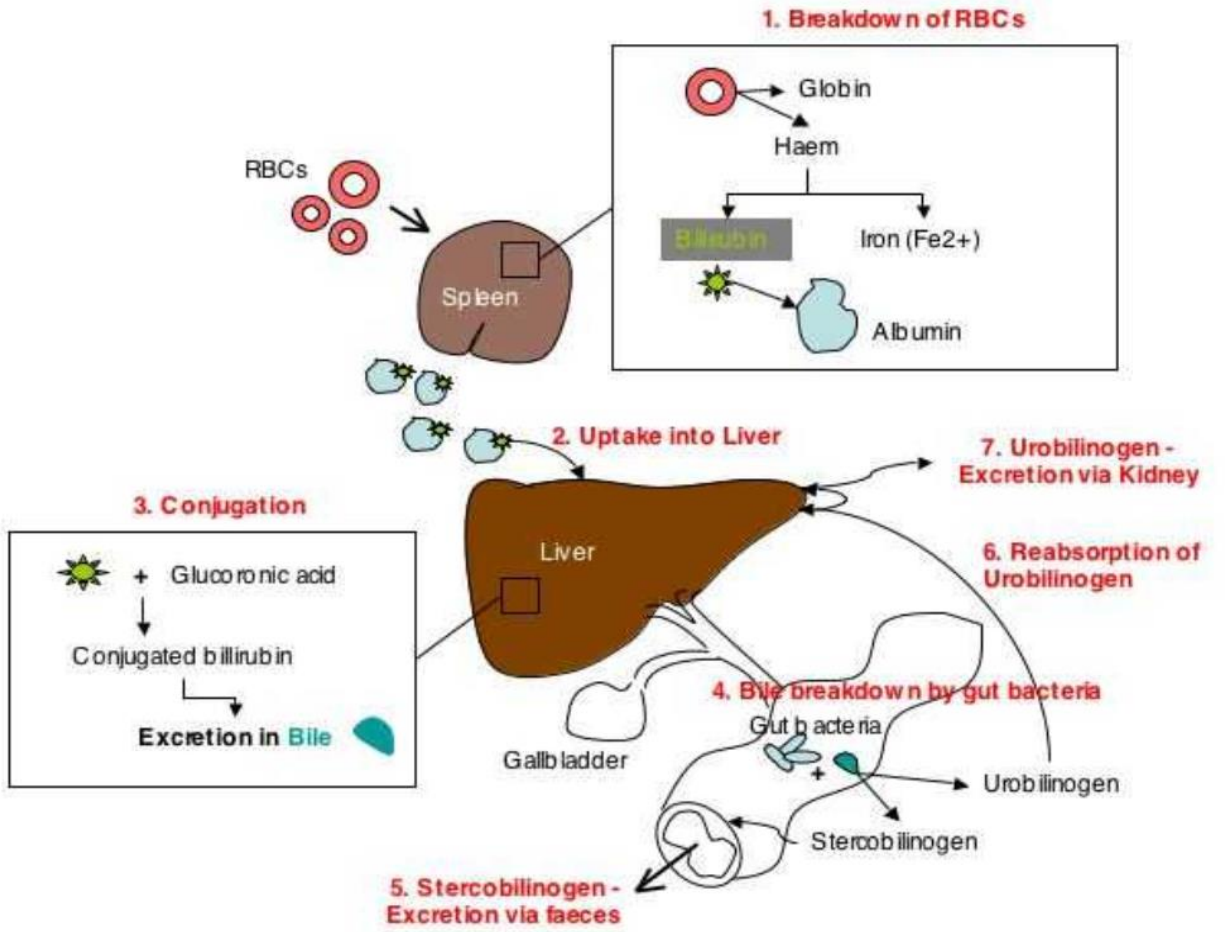


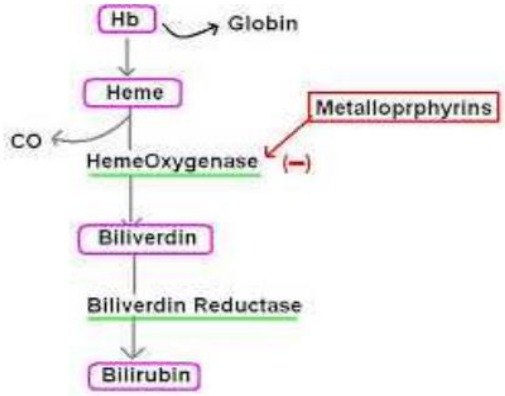
Bilirubin

- Bilirubin is created by the activity of biliverdin reductase on biliverdin, a green tetrapyrrolic bile pigment that is also a product of heme catabolism.



- Bilirubin, when oxidized, reverts to become biliverdin once again

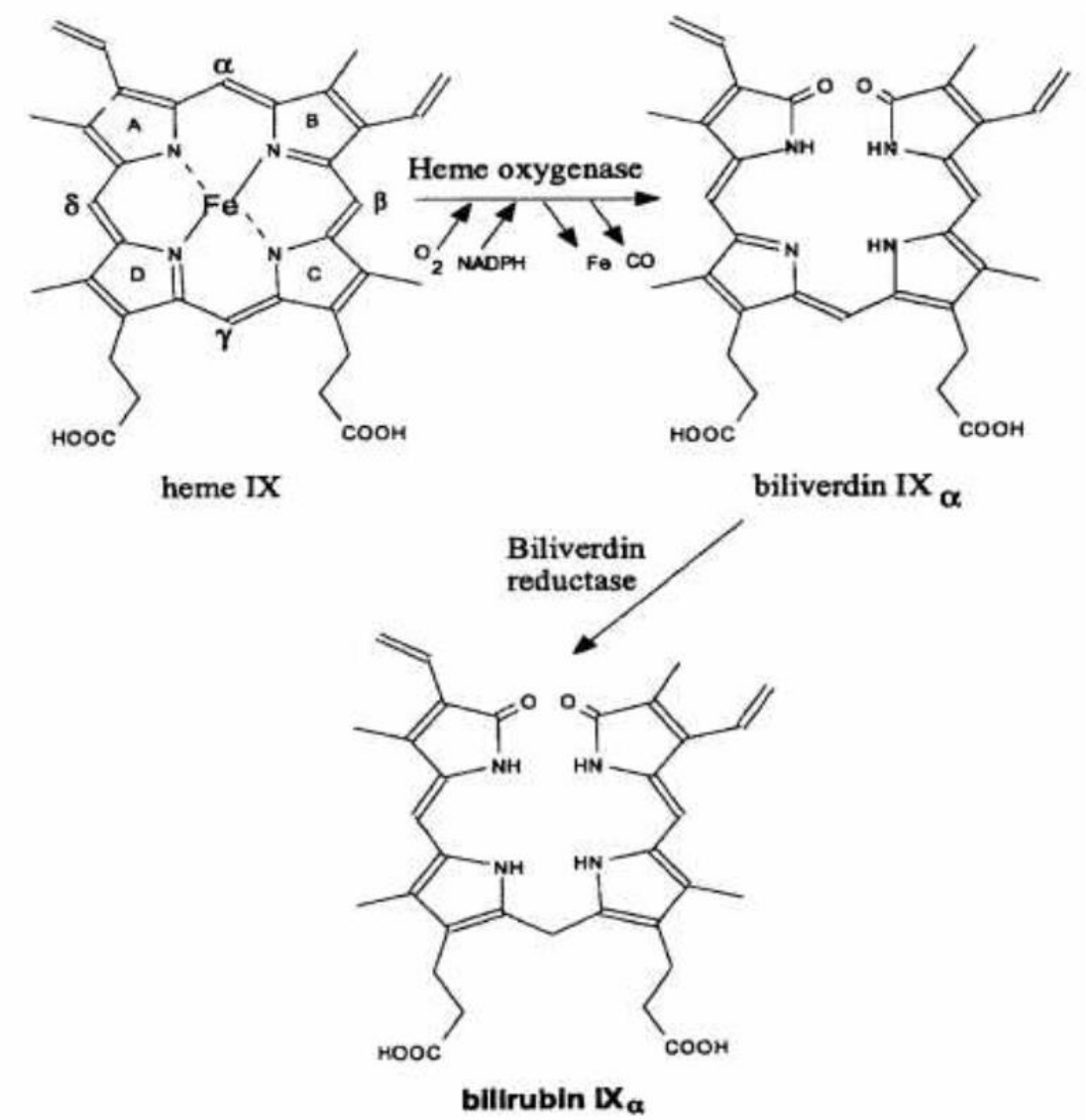




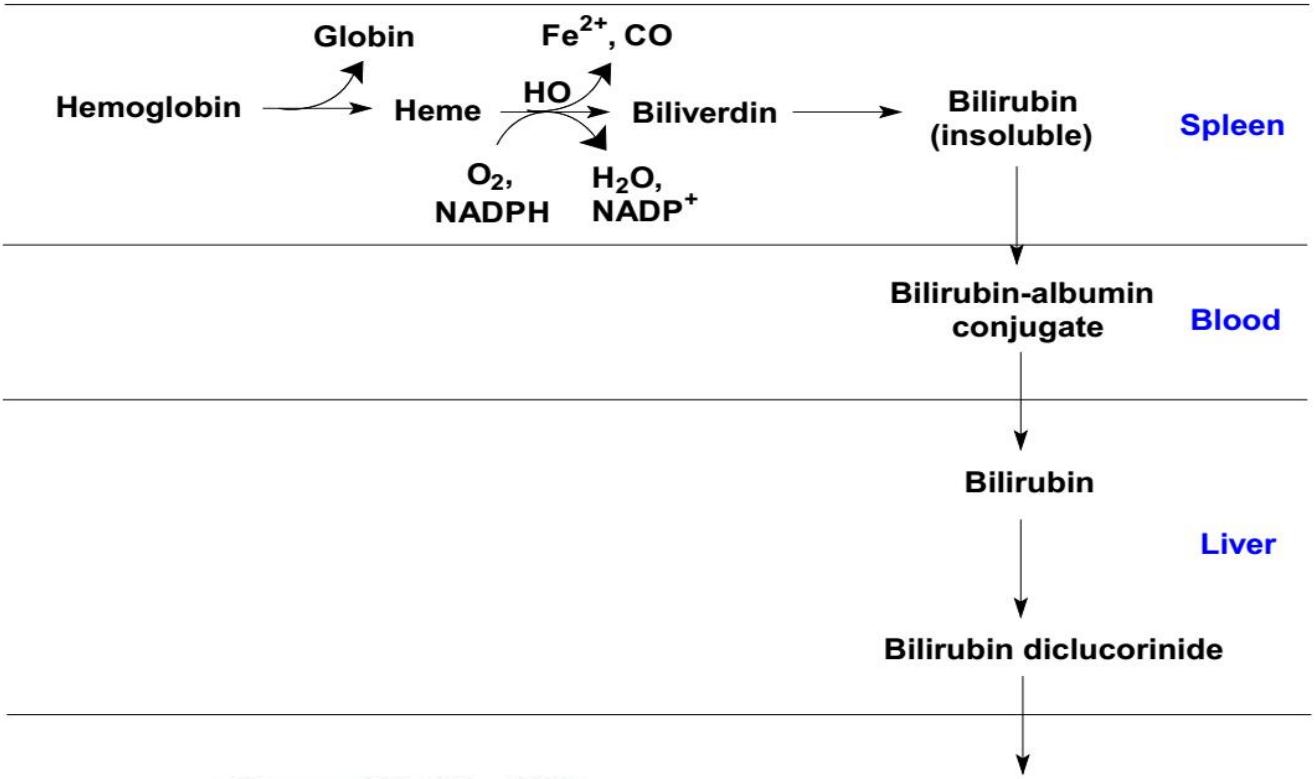
Formation of Bilirubin

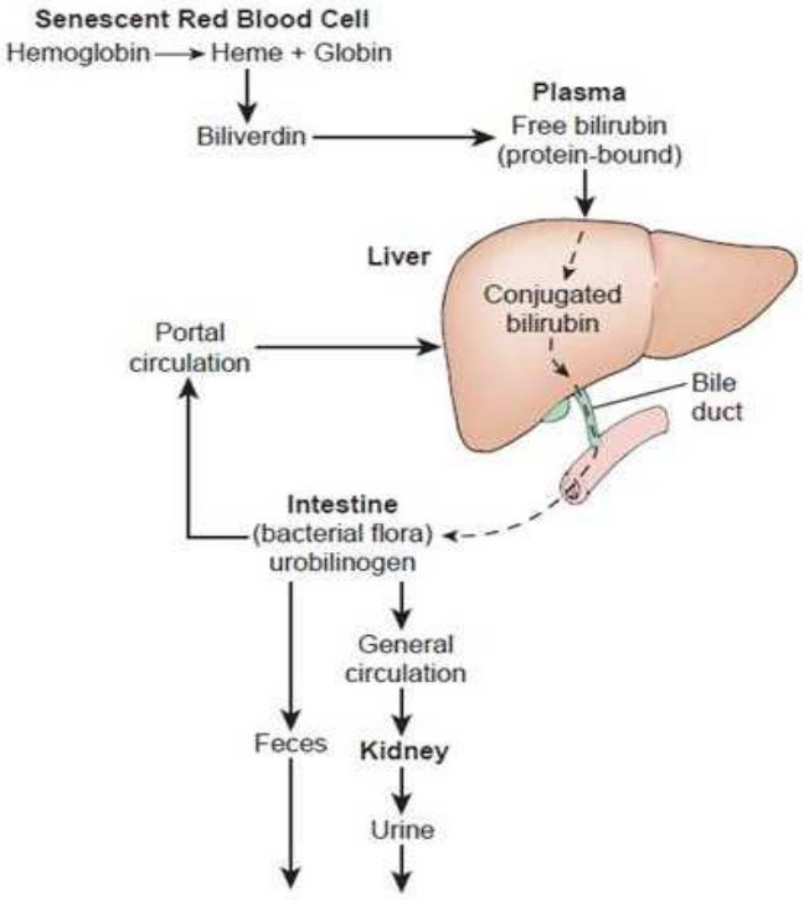
- Hemoglobin from senescent or hemolyzed red cells is broken down, releasing heme.
- Heme is then degraded in humans by the enzyme heme oxygenase (HO), which is the rate-limiting step in the formation of bilirubin.

- HO converts heme to biliverdin IX.
- Biliverdin is a hydrophilic compound that is reduced by biliverdin reductase into the hydrophobic compound bilirubin.



- HO catalyses an oxidase reaction opening the heme ring to convert one of the bridge carbons to carbon monoxide. This step releases iron from the now linear tetrapyrrole yielding biliverdin.
- Biliverdin reductase reduces the double bond on nitrogen inside one of four of the pyrrole rings leading to the formation of bilirubin.





The process of bilirubin formation, circulation and elimination

Unconjugated ("indirect"):

- Erythrocytes (red blood cells) generated in the bone marrow are disposed of in the spleen when they get old or damaged.
- This releases hemoglobin, which is broken down to heme as the globin parts are turned into amino acids.
- The heme is then turned into unconjugated bilirubin in the monocyte macrophages system of the spleen.

- This unconjugated bilirubin is not soluble in water, due to intramolecular hydrogen bonding.
- It is then bound to albumin and sent to the liver.

Conjugated ("direct"):

- In the liver, bilirubin is conjugated with glucuronic acid by the enzyme glucuronyltransferase, making it soluble in water: the conjugated version is also often called "direct" bilirubin.
- Much of it goes into the bile and thus out into the small intestine.
- Though most bile acid is resorbed in the terminal ileum to participate in enterohepatic circulation, conjugated bilirubin is not absorbed and instead passes into the colon
- There, colonic bacteria deconjugate and metabolize the bilirubin into colorless
- urobilinogen, which can be oxidized to form urobilin and stercobilin: these give stool its characteristic brown color.
- A trace (~1%) of the urobilinogen is resorbed into the enterohepatic circulation to be re-excreted in the bile: some of this is instead processed by the kidneys, coloring the urine yellow.

How Bilirubin getting distruped ?

- This disruption in the metabolism or excretion of bilirubin can occur at various stages, and it is therefore useful to classify the different causes of jaundice based on the where the dysfunction occurs.
- The causes of jaundice are generally classified as pre-hepatic (the problem arises before secretion to the liver), hepatic (the problem arises within the liver), and post-hepatic (the problem arises after bilirubin is excreted from the liver).

Pre-hepatic causes- the problem arises before secretion to the liver:

- ✓ Jaundice caused during the pre-hepatic phase is due to the excessive destruction (hemolysis) of red blood cells from various conditions.
- ✓ This rapid increase in bilirubin levels in the bloodstream overwhelms the liver's capability to properly metabolize the bilirubin, and consequently the levels of unconjugated bilirubin increase.

Conditions which can lead to an increase in the hemolysis of red blood cells include:

- Malaria,
- Sickle cell disease,
- Hereditary spherocytosis,
- Thalassemia,
- Glucose-6-phosphate dehydrogenase deficiency (G6PD),
- Drugs or other toxins, and
- Autoimmune disorders.

Hepatic causes- the problem arises within the liver:

- Jaundice caused during the hepatic phase can arise from abnormalities in the metabolism and/or excretion of bilirubin.
- This can lead to increase in both unconjugated and/or conjugated bilirubin levels.

Conditions with a hepatic cause of jaundice include:

- Acute or chronic hepatitis (commonly viral [Hepatitis A, B, C, D, E] or alcohol related),
- Cirrhosis (caused by various conditions),
- Drugs or other toxins,
- Crigler-Najjar syndrome,
- Autoimmune disorders,
- Gilbert's syndrome, and
- Liver cancer.

Treatment:

- supportive care,
- IV fluids in cases of dehydration,
- medications for nausea/vomiting and pain,
- antibiotics,
- antiviral medications,
- blood transfusions,
- steroids,
- chemotherapy/radiation therapy

- Jaundice caused by drugs/medications/toxins requires discontinuation of the offending agent. In cases of intentional or unintentional acetaminophen (Tylenol) overdose, the antidote N-acetylcysteine (Mucomyst) may be required.

- Antibiotics may be required for infectious causes of jaundice, or for the complications associated with certain conditions leading to jaundice (for example, cholangitis).

- Various medications may be used to treat the conditions leading to jaundice, such as steroids in the treatment of some autoimmune disorders. Certain patients with cirrhosis, for example, may require treatment with diuretics and lactulose.
- Individuals with cancer leading to jaundice will require consultation with an oncologist, and the treatment will vary depending on the type and extent (staging) of the cancer.

- Surgery and various invasive procedures may be required for certain patients with jaundice. For example, certain patients with gallstones may require surgery. Other individuals with liver failure/cirrhosis may require a liver transplant.

Post-hepatic causes- the problem arises after bilirubin is excreted from the liver:

- Jaundice from a post-hepatic cause arises from a disruption (an obstruction) in the normal drainage and excretion of conjugated bilirubin in the form of bile from the liver into the intestine.

- This leads to increased levels of conjugated bilirubin in the bloodstream.

Conditions that can cause post-hepatic jaundice include:

- Gallstones,
- Cancer (pancreatic cancer, gallbladder cancer and bile duct cancer),
- Strictures of the bile ducts,
- Cholangitis,
- Pancreatitis,
- Parasites (for example, liver flukes).

Toxicity- Neonatal jaundice

- Unconjugated hyperbilirubinaemia in a newborn can lead to accumulation of bilirubin in certain brain regions (particularly the basal nuclei) with consequent irreversible damage to these areas manifesting as various neurological deficits, seizures, abnormal reflexes and eye movements.
- This type of neurological injury is known as kernicterus.
- Aside from specific chronic medical conditions that may lead to hyperbilirubinaemia, neonates in general are at increased risk since they lack the intestinal bacteria that facilitate the breakdown and excretion of conjugated bilirubin in the feces (this is largely why the feces of a neonate are paler than those of an adult).
- Instead the conjugated bilirubin is converted back into the unconjugated form by the enzyme β-glucuronidase (in the gut, this enzyme is located in the brush border of the lining intestinal cells) and a large proportion is reabsorbed through the enterohepatic circulation
- Infants with kernicterus may have a fever or seizures.High pitched crying is an

effect of kernicterus.

Treatment

- Phototherapy: Exposing infants to high levels of colored light changes trans-bilirubin to the more water soluble cis-form which is excreted in the bile
- Sunlight: Natural sunlight also serves as an effective form of treatment as it also contains the optimal wavelengths. In one study it what is 6 times more effective than bilirubin lamps due to the difference in light intensities
- Exchange transfusions: Much like with phototherapy the level at which exchange transfusions should occur depends on the health status and age of the newborn. It should however be used for any newborn with a total serum bilirubin of greater than 428 μmol/l .
- Exchange transfusions performed to lower high bilirubin levels are an aggressive treatment

Any thing good about Bilirubin level

- Recent research has indicated that in the absence of liver disease, individuals with high levels of total bilirubin may experience various health benefits exceeding those with lower levels of bilirubin.
- Studies have found higher levels of bilirubin in elderly individuals are associated with higher functional independence.
- Studies have also revealed that levels of serum bilirubin are inversely related to risk of certain heart diseases.

INBORN ERRORS OF METABOLISM

- Inborn errors of metabolism comprise a large class of genetic diseases involving disorders of metabolism.

- The majority are due to defects of single genes that code for enzymes that facilitate conversion of various substances (substrates) into others (products).

- In most of the disorders, problems arise due to accumulation of substances which are toxic or interfere with normal function, or to the effects of reduced ability to synthesize essential compounds.

- Inborn errors of metabolism are now often referred to as congenital metabolic diseases or inherited metabolic diseases.

- The term inborn error of metabolism was coined by a British physician, Archibald Garrod (1857–1936), in the early 20th century (1908).

- He is known for work that prefigured the "one gene-one enzyme" hypothesis, based on his studies on the nature and inheritance of alkaptonuria. His seminal

text, Inborn Errors of Metabolism was published in 1923.